

ICCBH

6TH INTERNATIONAL CONFERENCE
ON CHILDREN'S BONE HEALTH

22-25 JUNE 2013

ROTTERDAM, NETHERLANDS



Programme and Abstracts



IN ASSOCIATION WITH
 **ECTS**
European Calcified Tissue Society

INSIDE FRONT COVER



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Differentiating Unusual Causes of Rickets and Hypercalcemia

Sunday, June 23, 18:00–20:00
Willem Burger Zaal
De Doelen Conference Centre
Rotterdam, Netherlands

Presented as a satellite symposium during the 6th International Conference on Children's Bone Health, Rotterdam, Netherlands, 22–25 June, 2013

A buffet dinner for attendees will be served at 20:15 – Arcadis Zaal, Conference Centre

18:00	Introduction & Welcome/ Case Introduction	Craig B Langman (Chair) <i>(Chicago, USA)</i>
18:10	New Insights on Vitamin D Metabolism: Role of 24-hydroxylase	Martin Konrad <i>(Münster, Germany)</i>
18:40	Hormone Independent Hypercalcemia: Jansen's Metaphyseal Chondrodysplasia	Harald Jüppner <i>(Boston, USA)</i>
19:10	Hypophosphatasia: The Impact of Alkaline Phosphatase Deficiency	Nick Bishop <i>(Sheffield, UK)</i>
19:40	Case Discussion: Pathogenesis and Differential Diagnosis	All Faculty & Audience
20:00	Close	Craig B Langman

This symposium is open only to healthcare professionals registered for ICCBH

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ALEXION

Organisers & Supporters



ORGANISERS

Organising Committee

Chair: Maria Luisa Bianchi (Milan, Italy)
Nick Bishop (Sheffield, UK)
Craig B Langman (Chicago, USA)
Coen Netelenbos (Amsterdam, Netherlands)

Programme Organising Committee

Chair: Maria Luisa Bianchi (Milan, Italy)
Nick Bishop (Sheffield, UK)
Annemieke Boot (Groningen, Netherlands)
Harald Jueppner (Boston, USA)
Craig B Langman (Chicago, USA)
Mary Leonard (Philadelphia, USA)
Roman Lorenc (Warsaw, Poland)
Coen Netelenbos (Amsterdam, Netherlands)
Aenor Sawyer (San Francisco, USA)
Eckhard Schönau (Cologne, Germany)

Local Organising Committee

Co-chairs: Sabine de Muinck Keizer-Schrama
(Rotterdam, Netherlands)
Coen Netelenbos (Amsterdam, Netherlands)

Annemieke Boot (Groningen, Netherlands)
Hans van Leeuwen (Rotterdam, Netherlands)
André Uitterlinden (Rotterdam, Netherlands)
Carola Zillikens (Rotterdam, Netherlands)

Meeting organisation

Janet Crompton
Conference Organiser
iccbh@ectsoc.org
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SPONSORS & OTHER SUPPORTERS

We are extremely grateful to the following organisations for their support.

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European Calcified Tissue Society (ECTS)

International Bone and Mineral Society (IBMS)

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International Osteoporosis Foundation (IOF)

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SATELLITE SYMPOSIUM



VITAMIN D: SHIFTING THE STATUS

Danone Baby Nutrition presents **Vitamin D: Shifting The Status**, a symposium for health professionals.

Presented as a satellite symposium during the 6th International Conference on Children's Bone Health, Rotterdam, Netherlands, 22-25 June 2013

VENUE:
**WILLEM BURGER ZAAL
3RD FLOOR
DE DOELEN**

WHEN:
**MONDAY 24TH JUNE
5.30PM - 7.00PM**

KEY SPEAKERS AND TOPICS:

Chair: Professor Nick Bishop, UK

Dr Zulf Mughal, UK
Global burden of vitamin D deficiency

Dr Mairead Kiely, Ireland
Food based strategies to increase vitamin D intake in young children

Dr Catherine Gordon, USA
Vitamin D deficiency in adolescence

inpractice
Practical advice for healthcare professionals from



Award Winners



The New Investigator Awards listed opposite were made by the Programme Committee based on scores achieved during the abstract review process. Certificates will be awarded during the Awards Ceremony on Tuesday 25 June.

On-site awards (Best Oral, Best Oral Poster and Best Poster) will be announced during the Awards Ceremony on Tuesday 25 June.

We are grateful to the following organisations who have made contributions towards these awards:

Alexion

American Society for Bone and Mineral Research (ASBMR)

European Calcified Tissue Society (ECTS)

International Bone and Mineral Society (IBMS)

New Investigator Awards

Pawel Abramowicz (Bialystok, Poland), P40

Justine Bacchetta (Bron, France), OP12

Rajbir Batra (Oxford, UK), OC5

Andrew Biggin (Sydney, Australia), OP13

Alison Boyce (Washington DC, USA), OC21

Andrea Brum (Rotterdam, Netherlands), OC11

Lauren Burt (Calgary, Canada), OP11

Moir Cheung (London, UK), OP1

Andrea Del Fattore (Rome, Italy), OC8

Sarah Foster (Sheffield, UK), OC22

Saber Ghadakzadeh (Toronto, Canada), OC25

Monica Grover (Houston, USA), OP15

Holger Henneicke (Sydney, Australia), P108

Heike Hoyer-Kuhn (Cologne, Germany), OC19

John Kemp (Bristol, UK), OC10

Mikhail Kostik (Saint-Petersburg, Russia), P143

Carolina Medina-Gomez (Rotterdam, Netherlands), OC7

Rebecca Moon (Southampton, UK), OP6

Rikke Petersen (Copenhagen, Denmark), P111

Simon Schoenbuchner (Cambridge, UK), OC16

Rachana Srivastava (Houston, USA), OC24

Mariël Lizet te Winkel (Rotterdam, Netherlands), OC28

Kebashni Thandrayen (Johannesburg, South Africa), OC2

Sara Vandewalle (Ghent, Belgium), P96

Amanda Whitaker (San Francisco, USA), P154

Wing Sze Yu (Hong Kong, China), OP8

Disclosure of Interests



The following disclosures have been submitted by the authors:

- TC Nick Bishop**
Advisory role: Alexion, Amgen, GSK, Merck
Honoraria: Danone
Research funding: Alexion
- IS2 Kassim Javaid**
Advisory role: Consilient
Research funding: NIHR Systematic review of vitamin D treatment during pregnancy
- IS6 Maja Di Rocco**
Advisory role: Genzyme, Shire
Honoraria: Genzyme, Shire, Actelion, BiMarin
- IS10 Craig Munns**
Advisory role: Aventis, Sanofi
- IS11 Catherine Gordon**
Advisory role: Pfizer, Johnson & Johnson
- IS13 Frank Rauch**
Research funding: Novartis, Alexion
- OC11** This work is financially supported by Arcarios BV
- OC15** HHT is the owner of Visiana, which holds and markets the BoneXpert medical device for automated determination of bone age. The other authors have nothing to disclose.
- OC23** Dr Boskey owns stock in Amgen. Amgen supplied the drugs for this study.
- OC29** Dr de Boer receives research funding from Abbott Laboratories. The remaining authors have nothing to disclose.
- LB1 Renaud Winzenrieth** is a senior scientist at Med-Imaps.
- P8** CH received a scholarship from the Interdisciplinary Centre for Clinical Research IZKF Wuerzburg, Germany, BM is supported by Bundesministerium für Bildung und Forschung BMBF, Berlin, Germany. JL and CH received a study grant for a phase two study on Asfotase alpha treatment for severe forms of HPP.
- P80 M Ominsky:** employee of Amgen Inc.
- P83** All authors are employees of Ablynx nv.
- P133** AZ: consulting fees (Protalix Biotherapeutics); options in Protalix Biotherapeutics and Scientific Advisory Board membership; honoraria (Shire HGT, Actelion, Pfizer). AZ's institution: support (Genzyme [ICGG Gaucher Registry], Shire HGT [GOS], Medison). DH: consulting fees, travel and research grants, and honoraria for speaking (Shire HGT, Genzyme, Protalix, Amicus). DE: reimbursement for travel expenses to meetings (Shire HGT). LS: research grants (Genzyme, Shire HGT, BioMarin); consultant fees (Shire HGT, BioMarin). PH: consulting support (Shire HGT); speakers' honorarium and travel support (Shire HGT); advisory boards (Shire [HOS]); consulting support (BioMarin); advisory boards, research support, and travel and speakers' honoraria (BioMarin); speakers' honorarium (Genzyme). WR: clinical trial support (TKT, Genzyme, Shire, Hyperion); speaker fees (Ucyclyd). PG: consulting fees (Shire HGT, Genzyme, Actelion). NM: travel funding (Genzyme, BioMarin, Shire); grant funding (Genzyme, BioMarin, Shire); seat on North American Advisory Board and publications committee for Shire. CP: research grants (Genzyme, Shire HGT). DZ, EC: Shire HGT employees.
- P181** **Jose Simon Camelo Jr:** Works full time at the University of São Paulo, participates in the ICGG Gaucher Registry and receives fees from Genzyme for lectures and educational activities. **Juan Francisco Cabello:** A member of the ICGG Gaucher Registry Latin American Board. **Guillermo G. Drelichman:** A member of the ICGG Gaucher Registry Latin American Board and receives fees from Genzyme as a lecturer and for educational activities. **Marcelo M. Kerstenetzky:** A member of the ICGG Gaucher Registry Latin American Board and receives fees from Genzyme as the Registry coordinator for Brazil, for lectures and educational activities. **Isabel C. Sarmiento:** A member of the ICGG Gaucher Registry Latin American Board and received fees from Genzyme as the Registry coordinator for Colombia.
- P182** HHT is the owner of Visiana, which holds and markets the BoneXpert medical device for automated determination of bone age. The other authors have nothing to disclose.
- P186** Renaud Winzenrieth is a senior scientist at Med-Imaps.
- P201** Miguel Galban is unpaid consultant for Pega Medical Inc.

SCIENTIFIC PROGRAMME

Abstracts are reproduced as submitted by the authors.

All parties will make reasonable efforts to present educational subject matter in a scientific, balanced and unbiased way. However, participants must always use their own judgment and professional opinion when considering future application of this information, particularly as it may relate to patient diagnostic or treatment decisions.

Statements and descriptions are informational only and are not made or given as a warranty. The views, opinions and statements made at the conference are solely those of the presenters and may not reflect the views of the Organisers. Furthermore, presenters may have vested interests in the concepts and products they discuss.



Overview Timetable



SATURDAY 22 JUNE

12:00-19:30 **Registration desk open**

14:00-17:00 **Training course:**

Bone - Bedside to Bench and Back

14:00 What does a basic bone scientist need to know about clinical bone disease in children?

Nick Bishop (Sheffield, UK)

14:30 What does a clinical bone scientist need to know about basic bone biology?

Harald Jüppner (Boston, USA)

15:00-17:00 **Approaches to ...**

15:00 Hypogonadal bone diseases

Convenor: Coen Netelenbos (Amsterdam, Netherlands)

Speakers: Wolfgang Högl (Birmingham, UK) & Annemieke Boot (Groningen, Netherlands)

16:00 Phosphate metabolism and bone diseases

Convenor: Craig B Langman (Chicago, USA)

Speakers: Rachel Gafni (Bethesda, USA) & Farzana Perwad (San Francisco, USA)

17:00 **Break**

17:15-17:30 **Welcome**

17:30-18:00 **Keynote Lecture:**

The past, the present and the future of paediatric bone research

Francis Glorieux (Montreal, Canada)

18:00-19:00 **Welcome Reception**

SUNDAY 23 JUNE

08:00-19:00 **Registration desk open**

08:30-10:30 **The Fracturing Child: Epidemiology**

08:30 Bone mass and other determinants of fractures in children and adolescents

Emma Clark (Bristol, UK)

09:00 Epigenetic influences on childhood fractures

Kassim Javaid (Oxford, UK)

09:30 Oral Communications

10:30-10:35 **Charles Siemenda Award**

10:35-11:00 **Coffee**

11:00-13:00 **The Fracturing Child: Biology**

11:00 Bone structure and fractures

Salman Kirmani (Rochester, USA)

11:30 The biology of bone revealed through bone biopsy

Katherine Wesseling-Perry (Los Angeles, USA)

12:00 Oral Communications

13:00-13:30 **Lunch**

13:30-14:30 **Posters (odd numbers presented)**

14:30-15:45 **Oral Posters**

15:45-16:15 **Coffee**

16:15-17:45 **Rare diseases**

16:15 Acrodysostosis

Agnès Linglart (Paris, France)

16:45 Gaucher

Maja Di Rocco (Genoa, Italy)

17:15 Osteogenesis Imperfecta

Gerard Pals (Amsterdam, Netherlands)

17:45-18:00 **Break**

18:00-21:00 **Dinner symposium**

Differentiating unusual causes of rickets and hypercalcemia

Supported by Alexion Pharmaceuticals

Overview Timetable



MONDAY 24 JUNE

08:00-19:00 **Registration desk open**

08:30-10:30 **The Fracturing Child: Diagnostics**

08:30 Non-invasive assessment of bone structure and strength using QCT and MRI

Mary Leonard (Philadelphia, USA)

09:00 DXA and Vertebral Fracture Assessment

Judith Adams (Manchester, UK)

09:30 Oral Communications

10:30-11:00 **Coffee**

11:00-13:00 **The Fracturing Child: Therapeutics**

11:00 Medical therapies: present and future

Craig Munns (Sydney, Australia)

11:30 Other therapeutic options: nutrition, vitamin D, physical activity

Catherine Gordon (Boston, USA)

12:00 Oral Communications

13:00-13:30 **Lunch**

13:30-14:30 **Posters (even numbers presented)**

14:30-15:30 **Chronic diseases 1**

14:30 IBD, bone, fractures and inflammation

Susanne Schmidt (Gothenburg, Sweden)

15:00 Muscle-bone interaction in pediatric bone diseases

Frank Rauch (Montreal, Canada)

15:30-16:00 **Coffee**

16:00-17:30 **Chronic diseases 2**

16:00 Oral Communications

17:00 Type I Diabetes

Susanne Bechtold (Munich, Germany)

17:30-19:00 **Satellite symposium**

Vitamin D: Shifting the Status

Supported by Danone Baby Nutrition

Immediately following satellite, buses to

Conference Dinner

19:30-23:00 **Conference Dinner**

Paddlesteamer De Majesteit

TUESDAY 25 JUNE

08:00-12:30 **Registration desk open**

08:30 **Presentation of Awards**

08:40 **Late Breaking Abstracts**

09:00-10:20 **Paediatric cancer and bone: Round Table**

09:00 Bone and endocrine side-effects in childhood Hodgkins lymphoma and acute lymphoblastic leukemia

Marry van den Heuvel-Eibrink (Rotterdam, Netherlands)

09:30 Childhood cancer and fractures

Carmen Wilson (Memphis, USA)

10:00 Round table discussion

10:20-10:45 **Coffee**

10:45-12:05 **Obesity as a bone disease: Round Table**

10:45 Bone as an endocrine organ

Paul Baldock (Sydney, Australia)

11:15 Obesity and skeletal health

Paul Dimitri (Sheffield, UK)

11:45 Round table discussion

12:05 **Close and final remarks**



14:00-17:00

**Training course:
Bone - Bedside to Bench and Back**

Chairs: Coen Netelenbos (Amsterdam, Netherlands)
Craig B Langman (Chicago, USA)

14:00

What does a basic bone scientist need to know about clinical bone disease in children?
Nick Bishop (Sheffield, UK)

14:30

What does a clinical bone scientist need to know about basic bone biology?
Harald Jüppner (Boston, USA)

15:00

Approaches to:
1. Hypogonadal bone diseases
2. Phosphate metabolism and bone diseases
Includes case discussions

15:00-16:00

Hypogonadal bone diseases
Convenor: Coen Netelenbos (Amsterdam, Netherlands)
Speakers:
Wolfgang Högler (Birmingham, UK)
Annemieke Boot (Groningen, Netherlands)

16:00-17:00

Phosphate metabolism and bone diseases
Convenor: Craig B Langman (Chicago, USA)
Speakers:
Rachel Gafni (Bethesda, USA)
Farzana Perwad (San Francisco, USA)

17:00-17:15

Break

17:15-17:30

Welcome

Maria Luisa Bianchi (Milan, Italy)
Sabine de Muinck Keizer-Schrama (Rotterdam, Netherlands)

17:30-18:00

**Keynote Lecture:
The past, the present and the future of pediatric bone research**

Chair: *Maria Luisa Bianchi (Milan, Italy)*
Francis Glorieux (Montreal, Canada)

18:00-19:00

Welcome Reception

WILLEM BURGER ZAAL FOYER



08:30-10:30

The Fracturing Child: Epidemiology

Chairs: Catherine Gordon (Boston, USA)
Zulf Mughal (Manchester, UK)

08:30

IS1 **Bone mass and other determinants of fractures in children and adolescents**
Emma Clark (Bristol, UK)

09:00

IS2 **Epigenetic influences on childhood fractures**
Kassim Javaid (Oxford, UK)

09:30-10:00

Oral Communications: Epidemiology

09:30

OC1 **The Amalgamated Paediatric Bone Density Study (The ALPHABET Study) - The collation and generation of UK based reference data for paediatric bone densitometry**
Nicola Crabtree (Birmingham, UK)

09:40

OC2 **Fracture patterns and bone mass in South African adolescent-mother pairs: The Birth to Twenty Cohort**
Kebashni Thandrayan (Johannesburg, South Africa)

09:50

OC3 **Pediatric differences in Bone Mineral Density according to ethnic background in children: the Generation R Study**
Carolina Medina Gomez (Rotterdam, Netherlands)

10:00

OC4 **Maternal antenatal 25(OH)-vitamin D status is associated with offspring muscle strength at 4 years of age**
Rebecca Moon (Southampton, UK)

10:10

OC5 **Novel musculoskeletal phenotypes during childhood for epidemiological studies**
Rajbir Batra (Oxford, UK)

10:20

OC6 **Role of bone-associated loci identified in GWAS meta-analyses in the context of longitudinal pediatric BMD in European Americans**
Babette Zemel (Philadelphia, USA)

10:30-10:35

Charles Slemenda Award

10:35-11:00

Coffee

11:00-13:00

The Fracturing Child: Biology

Chairs: Rachel Gafni (Bethesda, USA)
Gerard Pals (Amsterdam, Netherlands)

11:00

IS3 **Bone structure and fractures**
Salman Kirmani (Rochester, USA)

11:30

IS4 **The biology of bone revealed through bone biopsy**
Katherine Wesseling-Perry (Los Angeles, USA)

12:00-13:00

Oral Communications: Biology

12:00

OC7 **The PPP6R3/LRP5 locus influences lean mass in children of different ethnic background and highlights pleiotropic effects and muscle-bone interactions**
Carolina Medina-Gomez (Rotterdam, Netherlands)

12:10

OC8 **Generation of the first mouse model of Autosomal Dominant Type II Osteopetrosis (ADO2) harbouring the pG213R-clc7 mutation**
Andrea Del Fattore (Rome, Italy)

12:20

OC9 **EPO signaling and hematopoietic expansion as causes of osteoporosis in a thalassemia mouse model**
Maria Vogiatzi (New York, USA)

12:30

OC10 **Phenotypic dissection of bone mineral density facilitates the identification of skeletal site specificity on the genetic regulation of bone**
John Kemp (Bristol, UK)

12:40

OC11 **Connectivity map-based discovery of novel compounds that induce osteoblast differentiation**
Andrea Brum (Rotterdam, Netherlands)

12:50

OC12 **Improvement of collagen synthesis in fibroblasts of Brtl model for Osteogenesis Imperfecta following lentiviral-shRNA-mediated down-expression of mutant Col1a1 allele**
Valérie Trichet (Nantes, France)



13:00-13:30

Lunch

13:30-14:30

Posters (odd numbers presented)

14:30-15:10

Oral Posters

Chairs: Maria Luisa Bianchi (Milan, Italy)
Craig B Langman (Chicago, USA)

- 14:30
OP1 Skeletal effects of hypothyroidism are mediated by thyroid hormone receptor
Moira Cheung (London, UK)
- 14:35
OP2 High FSH serum levels may support the altered bone remodeling in Turner Syndrome patients
Giacomina Brunetti (Bari, Italy)
- 14:40
OP3 Calcimimetics as adjunct therapy in XLH: 18 months experience
Uri Alon (Kansas City, USA)
- 14:45
OP4 A new start-codon in IFITM5 causes Osteogenesis Imperfecta Type V
Oliver Semler (Cologne, Germany)
- 14:50
OP5 Abstract withdrawn
- 14:55
OP6 Children with nephrotic syndrome have increased tibial bone area but similar volumetric bone mineral density to healthy controls
Rebecca Moon (Southampton, UK)
- 15:00
OP7 Bone Mineralization Density Distribution, bone formation rate and bone turnover markers in a cohort of children with CKD before and after growth hormone treatment
Nadja Fratzl-Zelman (Vienna, Austria)
- 15:05
OP8 Quantitative measurement of abnormal bone quality and strength with bone micro-architecture and rod-plate configuration in osteopenic adolescent idiopathic scoliosis (AIS)
Wing Sze Yu (Hong Kong, China)

15:10-15:45

Oral Posters

Chairs: Nick Bishop (Sheffield, UK)
Coen Netelenbos (Amsterdam, Netherlands)

- 15:10
OP9 Anticalciuric effect of recombinant PTH in patients with activating mutations of the calcium-sensing receptor causing autosomal dominant hypocalcaemia-hypercalciuria (ADHH)
Agnès Linglart (Paris, France)
- 15:15
OP10 Metacarpal width, length, medullary diameter and cortical thickness in hypophosphatemic rickets
Signe Beck-Nielsen (Esbjerg, Denmark)
- 15:20
OP11 Artistic gymnasts display micro-architectural advantages over trampolining and tumbling gymnasts: A high resolution peripheral quantitative computed tomography study
Lauren Burt (Calgary, Canada)
- 15:25
OP12 The bone/vessels interplay in teenagers with chronic kidney disease
Justine Bacchetta (Bron, France)
- 15:30
OP13 Long-term effects of bisphosphonate therapy in children with osteogenesis imperfecta
Andrew Biggin (Sydney, Australia)
- 15:35
OP14 Inflammation and glucocorticoid therapy impair skeletal modeling during growth following Crohn Disease diagnosis
Mary Leonard (Philadelphia, USA)
- 15:40
OP15 Dissecting the role of osteoblast derived nitric oxide in bone remodeling
Monica Grover (Houston, USA)

15:45-16:15

Coffee



16:15-17:45

Rare diseases

- Chairs: Paul Baldock (Sydney, Australia)
Frank Rauch (Montreal, Canada)
- 16:15
IS5 Acrodysostosis
Agnès Linglart (Paris, France)
 - 16:45
IS6 Gaucher
Maja Di Rocco (Genoa, Italy)
 - 17:15
IS7 Osteogenesis Imperfecta
Gerard Pals (Amsterdam, Netherlands)

17:45-18:00

Break

18:00-21:00

**Dinner Symposium:
Differentiating unusual causes of rickets and hypercalcemia**

SUPPORTED BY ALEXION

18:00-20:00

Scientific presentations

WILLEM BURGER AUDITORIUM (3RD FLOOR)

Welcome/Introduction/Case Introduction
Craig B Langman (Chicago, USA)

New insights on vitamin D metabolism: the role of 24-hydroxylase
Martin Konrad (Münster, Germany)

Hormone independent hypercalcemia: Jansen's metaphyseal chondrodysplasia
Harald Jüppner (Boston, USA)

Hypophosphatasia: the impact of alkaline phosphatase deficiency
Nick Bishop (Sheffield, UK)

Case Discussion: pathogenesis and differential diagnosis
All Faculty & Audience

20:00-21:00

Dinner

ARCADIS ZAAL (1ST FLOOR)



08:30-10:30

The Fracturing Child: Diagnostics

Chairs: Paul Dimitri (Sheffield, UK)
Craig Munns (Sydney, Australia)

08:30
IS8 Non-invasive assessment of bone structure and strength using QCT and MRI
Mary Leonard (Philadelphia, USA)

09:00
IS9 DXA and Vertebral Fracture Assessment
Judith Adams (Manchester, UK)

09:30-10:30

Oral Communications: Diagnostics

09:30
OC13 Level of calcium intake modifies the correlation between parathyroid hormone and 25-hydroxyvitamin D: A proposal of adequate 25-hydroxyvitamin D levels in children
María Loreto Reyes (Santiago, Chile)

09:40
OC14 What DXA measurement sites are best for bone health assessment in children? Effect of inter-machine differences on bone outcomes from the Bone Mineral Density in Childhood Study (BMDCS)
Babette Zemel (Philadelphia, USA)

09:50
OC15 Bone Health Index: Swiss children have less in the bank than a generation ago
Hans Henrik Thodberg (Holte, Denmark)

10:00
OC16 Longitudinal analysis of volumetric density, size and strength towards the end of skeletal maturation in Gambian males habituated to low calcium intake
Simon Schoenbuchner (Cambridge, UK)

10:10
OC17 Determinants of bone mineral density in long-term adult survivors of childhood cancer
Saskia Pluijm (Rotterdam, Netherlands)

10:20
OC18 Trabecular bone score (TBS) applied to normal children's lumbar spine DXA scans
Judith Adams (Manchester, UK)

10:30-11:00

Coffee

11:00-13:00

The Fracturing Child: Therapeutics

Chairs: Francis Glorieux (Montreal, Canada)
Farzana Perwad (San Francisco, USA)

11:00
IS10 Medical therapies: present and future
Craig Munns (Sydney, Australia)

11:30
IS11 Other therapeutic options: nutrition, vitamin D, physical activity
Catherine Gordon (Boston, USA)

12:00-13:00

Oral Communications: Miscellaneous

12:00
OC19 New therapeutic approach in OI VI - suppression of bone resorption using the RANKL antibody denosumab
Heike Hoyer-Kuhn (Cologne, Germany)

12:10
OC20 Exercise completed when young provides lifelong benefit to cortical bone structure and estimated strength
Stuart Warden (Indianapolis, USA)

12:20
OC21 A randomized, double-blind, placebo-controlled trial of alendronate treatment for fibrous dysplasia of bone
Alison Boyce (Washington DC, USA)

12:30
OC22 Prolidase deficient mice are osteoporotic in early life
Sarah Foster (Sheffield, UK)

12:40
OC23 Switching from alendronate to RANKL blockade alters bone properties after 14 weeks of therapy in the oim/oim mouse
Cathleen Raggio (New York, USA)

12:50
OC24 Fibroblast growth factor 23 plasma levels are elevated with early chronic kidney disease and positively associated with steroid based versus steroid free immunosuppression in pediatric renal transplant patients
Rachana Srivastava (Houston, USA)



13:00-13:30

Lunch

13:30-14:30

Posters (even numbers presented)

14:30-15:30

Chronic diseases

Chairs: Wolfgang Högler (Birmingham, UK)
Carmen Wilson (Memphis, USA)

14:30
IS12 IBD, bone, fractures and inflammation
Susanne Schmidt (Gothenburg, Sweden)

15:00
IS13 Muscle-bone interaction in pediatric bone diseases
Frank Rauch (Montreal, Canada)

15:30-16:00

Coffee

16:00-17:30

Chronic diseases

Chairs: Mary Leonard (Philadelphia, USA)
Agnès Linglart (Paris, France)

16:00-17:00

Oral Communications: Chronic diseases

16:00
OC25 Wnt/ β -catenin: A candidate pathway for bone repair in Neurofibromatosis Type-1
Saber Ghadakzadeh (Toronto, Canada)

16:10
OC26 Vertebral fractures in the 3 year period following steroid initiation among children with chronic illnesses
Paivi Miettunen (Calgary, Canada)

16:20
OC27 Serum vitamin D level can affect the treatment outcome of whole-body vibration (WBV) for low bone mass in girls with Adolescent Idiopathic Scoliosis (AIS)
Tsz Ping Lam (Hong Kong, China)

16:30
OC28 Bone mineral density at diagnosis determines fracture rate in children treated according to the DCOG-ALL9 protocol.
Mariël Lizet te Winkel (Rotterdam, Netherlands)

16:40
OC29 Mineral metabolism, cortical volumetric bone mineral density and fracture risk in childhood chronic kidney disease
Michelle Denburg (Philadelphia, USA)

16:50
OC30 Alteration of Wnt/Beta-Catenin signaling in HIV-infected youths: A mechanism leading to impaired bone health?
Stefano Mora (Milan, Italy)

17:00
IS14 Type I Diabetes
Susanne Bechtold (Munich, Germany)

17:30-19:00

Satellite Symposium: Vitamin D: Shifting the Status

SUPPORTED BY DANONE BABY NUTRITION

Chair: Nick Bishop (Sheffield, UK)

Global burden of vitamin D deficiency
Zulf Mughal (Manchester, UK)

Food based strategies for increasing vitamin D status in young children
Mairead Kiely (Cork, Ireland)

Vitamin D deficiency in adolescence
Catherine Gordon (Boston, USA)

19:10 Buses depart for Conference Party from Conference Centre entrance immediately following Satellite Symposium

19:30-23:00

Conference Party

SUPPORTED BY DANONE BABY NUTRITION

PADDLESTEAMER DE MAJESTEIT

Buses return to the Conference Centre from 22:30, calling at the Hilton, Manhattan and Bilderberg hotels. It is a short walk from the disembarkation point to the Intel, Maritime and StayOK hotels.



08:30-08:40

Presentation of Awards

Chairs: Maria Luisa Bianchi (Milan, Italy)
Coen Netelenbos (Amsterdam, Netherlands)

08:40-09:00

Late Breaking Abstracts

Chairs: Nick Bishop (Sheffield, UK)
Craig B Langman (Chicago, USA)

08:40

Influence of age and gender on spine bone density and TBS microarchitectural texture parameters in infants

Renaud Winzenrieth (Pessac, France)

08:50

WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta identify a key WNT ligand regulating bone mass

Outi Mäkitie (Helsinki, Finland)

09:00-10:20

Paediatric cancer and bone: Round Table

Chairs: Sabine de Muinck Keizer-Schrama (Rotterdam, Netherlands)/Roman Lorenc (Warsaw, Poland)

09:00

IS15 Bone and endocrine side-effects in childhood Hodgkins lymphoma and acute lymphoblastic leukemia

Marry van den Heuvel-Eibrink (Rotterdam, Netherlands)

09:30

IS16 Childhood cancer and fractures

Carmen Wilson (Memphis, USA)

10:00

Round Table discussion

10:20-10:45

Coffee

10:45-12:05

Obesity as a bone disease: Round Table

Chairs: Aenor Sawyer (San Francisco, USA)
Eckhard Schönau (Cologne, Germany)

10:45

IS17 Bone as an endocrine organ

Paul Baldock (Sydney, Australia)

11:15

IS18 Obesity and skeletal health

Paul Dimitri (Sheffield, UK)

11:45

Round Table discussion

12:05

Closing Remarks



OP1-OP15 - As listed on page 12

P1 Abstract withdrawn

P2 Abstract withdrawn

P3 Abstract withdrawn

P4 Abstract withdrawn

P5 Camptodactyly-arthropathy-coxa vara-pericarditis syndrome: Clinical and molecular genetic findings
Sulaiman Al-Mayouf (Riyadh, Saudi Arabia)

P6 The relationship between bone health and body composition profile in patients with galactose metabolic disorders: implications for practice
Artemis Doulgeraki (Athens, Greece)

P7 Osteometric parameters of mature rats mandible molars when implanted in the tibia biogenic hydroxyapatite, saturated with iron
Morozov Vitaly (Luhansk, Ukraine)

P8 Compound heterozygosity of two functional null mutations in the ALPL gene associated with deleterious neurological outcome in an infant with hypophosphatasia
Christine Hofmann (Würzburg, Germany)

P9 A 6-month intervention study with vibration therapy in severely disabled children: effects on bone, biochemical markers and acceptance
Diana Swolin-Eide (Gothenburg, Sweden)

P10 Osteoporosis in young patients with neurological impairments
Yasser Yaghi (Saida, Lebanon)

P11 Abstract withdrawn

P12 DXL measurements in children 2-10 years
Ann-Charlott Söderpalm (Göthenburg, Sweden)

P13 Bone turnover compensates for the delayed growth in small for gestational age neonates
Roxane Tenta (Athens, Greece)

P14 Bone status and body composition analysis in young patients with phenylketonuria and hyperphenylalaninemia
Artemis Doulgeraki (Athens, Greece)

P15 Osteogenesis Imperfecta-bone mass acquisition under bisphosphonates treatment and additional gain in BMD in time of rhGH treatment for growth delay
Corina Galesanu (IASI, Romania)

P16 Bone size and bone mineral content in adolescents and young adults with eating disorders
Sheila Shepherd (Glasgow, UK)

P17 The optimal dose of vitamin D in growing girls during academic years: a randomized trial
Samane Tefagh (Yazd, Iran)

P18 Influence of anthropometric parameters on assessment of paediatric bone mineral density and bone mineral content
Thomas Hangartner (Dayton, USA)

P19 Positive correlation of serum vitamin D status with bone density and bone quality among adolescent girls in Hong Kong
Tsz Fung Cheung (Hong Kong, Hong Kong)

P20 Assessment of vitamin D nutrition status and dietary calcium intake in children 2-5 years of age
Priyanka Kureel (New Delhi, India)

P21 Bone disease in children with Geroderma Osteodysplasticum (GO) - A 25 year experience from a single tertiary centre
Jaya Sujatha Gopal-Kothandapani (Manchester, UK)

P22 Association of volumetric bone mineral density, bone morphometry and trabecular bone micro-architecture with leptin and soluble leptin receptor in adolescent idiopathic scoliosis
Elisa MS Tam (Hong Kong, Hong Kong)

P23 Abstract withdrawn

P24 Effects of endurance training on somatic growth in a rat model of chronic kidney disease (CKD) related growth retardation
Daniel Landau (Beer Sheva, Israel)

P25 Maternal bone density and rickets in Nigerian children
Thomas Thacher (Rochester, USA)

P26 Improvement in valgus deformity in hypophosphatemic rickets due to primary de Toni-Debré-Fanconi syndrome treated with phosphate, calcitriol and alkali therapy
Sasigarn Bowden (Columbus, USA)

P27 Randomized-controlled study in kidney transplanted children: early withdrawal of corticoids: effect on bone health recovery
Maria Loreto Reyes (Santiago, Chile)



- P28** Vitamin D insufficiency and its correlation with low bone mass in Adolescent Idiopathic Scoliosis (AIS)
Tsz Ping Lam (Hong Kong SAR, China)
- P29** Coping with Osteogenesis Imperfecta; What kind of difficulties are families living with?
Damla Goksen (İzmir, Turkey)
- P30** The effect of psychoeducation in families with Osteogenesis Imperfecta
Sukran Darcan (İzmir, Turkey)
- P31** Craniofacial consequences of high dose zoledronic acid injections in onco-pediatric patients
Frederic Lezot (Nantes, France)
- P32** The effect of vitamin D supplementation on calcium excretion in Thalassemia
Maria Vogiatzi (New York, USA)
- P33** Patients with mutations in PHEX or FGF23 share FGF23 excess but present distinct bone and mineral metabolism features
Agnès Linglart (Le Kremlin Bicêtre, France)
- P34** Reliability of pQCT scan protocol of second metatarsal for children with Juvenile Idiopathic Arthritis
David Greene (Strathfield, Australia)
- P35** Six monthly intravenous zoledronic acid in childhood osteoporosis
Andrew Biggin (Westmead, Australia)
- P36** Vitamin D deficiency and its relationship to parathyroid hormone in morbidly obese adolescents prior to bariatric surgery
Marisa Censani (New York, USA)
- P37** Reduction in bisphosphonate side effect profile using short term steroid cover
Andrew Biggin (Westmead, Australia)
- P38** Abnormal bone quality in both cortical and trabecular compartments in Adolescent Idiopathic Scoliosis (AIS)
Wing Sze Yu (Hong Kong, Hong Kong)
- P39** Prevalence of vitamin D deficiency in adolescent Muslim girls attending a school in the UK which adheres to a conservative dress code
M Zulf Mughal (Manchester, UK)
- P40** Altered bone metabolism in obese children: the pathogenic role of adipocytokines and inflammation
Pawel Abramowicz (Bialystok, Poland)
- P41** Management of a new case of neonatal hypocalcaemic hypercalcemia related to mutation of the calcium-sensing receptor gene with bone abnormalities
Jean-Pierre Salles (Toulouse, France)
- P42** The effect of glucocorticoids on bone indices in children with rheumatic and oncological medical conditions
Jennifer Harrington (Toronto, Canada)
- P43** Bone quality in young thalassaemic patients
Alberto Argentiero (Mesagne, Italy)
- P44** A homozygous mutation in the DNA binding domain of human vitamin D receptor causes vitamin D resistant rickets
Bram van der Eerden (Rotterdam, Netherlands)
- P45** Bone mineral density impairment in Marfan syndrome: a hidden and neglected issue
Giuliana Trifiro (Milan, Italy)
- P46** Interactions of adipokines and bone metabolism in patients with severe juvenile idiopathic arthritis
Kati Markula-Patjas (Tampere, Finland)
- P47** Vitamin D deficiency and structural and functional state of bone tissue in schoolchildren of Ukraine
Vladyslav Povoroznyuk (Kyiv, Ukraine)
- P48** Pubertal induction with testosterone of a boy with bilateral anorchia guided by the development of his monozygotic twin brother
Eva Van Caenegem (Ghent, Belgium)
- P49** Some features of regulation of the bone tissue metabolisms at the newborns who have transferred infectious influence during intrauterine development
Gulsym Manasova (Kyiv, Ukraine)
- P50** Bioelectrical impedance as a screening tool for low bone mass in Indian children and adolescents
Veena Ekbote (Pune, India)
- P51** Mild visual impairment in a 13 year old child with Osteoporosis-Pseudoglioma Syndrome
Maira Cheung (London, UK)
- P52** Incidence of fractures in 2 to 18 year old affluent Indian children: a multicentre study
Veena Ekbote (Pune, India)



- P53** The Influence of anthropometry and body composition on children's bone health: The Childhood Health, Activity and Motor Performance School (The CHAMPS) study, Denmark
Malene Heidemann (Odense C, Denmark)
- P54** Association of calcium and dairy intake with growth in Indian children and adolescents
Veena Ekbote (Pune, India)
- P55** Body composition, anthropometric parameters and bone densitometry in young Ukrainian male
V Luzin (Luhansk, Ukraine)
- P56** Effect of puberty on the muscle-bone relationship in Indian children and adolescents
Anuradha Khadilkar (Pune, India)
- P57** Heterozygous mutation in GALNT3 in a case of Hyperphosphataemic Familial Tumoral Calcinosis
Katie Knight (London, UK)
- P58** Puberty is critical for the development of bone mineral density impairment in patients with congenital adrenal hyperplasia
Stefano Mora (Milan, Italy)
- P59** The rapid effect of vibration on bone formation and resorption in the growing skeleton
Nick Bishop (Sheffield, UK)
- P60** Failure of free, public vitamin D supplementation program for Quebec infants - temporal trends and significant predictors
Celia Rodd (Montreal, Canada)
- P61** Elevated FGF23 levels in premature infants without excessive phosphaturia
Celia Rodd (Montreal, Canada)
- P62** The effect of the ketogenic diet on the developing skeleton
Peter Simm (Parkville, Australia)
- P63** Bone status of Indian children and adolescents with Type 1 Diabetes Mellitus
Lavanya Parthasarathy (Pune, India)
- P64** The level of vitamin D and calcium in urban pregnant women in Russian Federation
Olga Ershova (Yaroslavl, Russia)
- P65** Improvement in morphological properties of trabecular bones and longitudinal growth in tibia for growing rats through an impact stimulation
Sinae Eom (Seoul, Republic of Korea)
- P66** Effect of resistance training on peripheral bone mineral density and muscle strength in adolescents with motor difficulties
Beth Hands (Fremantle, Australia)
- P67** Dual Energy X-Ray Absorptiometry in predicting clinical bone disease in adults with childhood onset end-stage renal disease
Maike van Huis (Amsterdam, Netherlands)
- P68** Engineered tridimensional hydroxyapatite scaffold to support bone resorption
Andrea Del Fattore (Rome, Italy)
- P69** sRANKL/OPG in children with Idiopathic Hypercalciuria
Anna Challa (Ioannina, Greece)
- P70** Isolated bilateral zeugo-autopodal segments agenesis of the lower limb: Unusual malformation case report
Antoine Christiaens (Brussels, Belgium)
- P71** The microarchitecture of bone in osteochondromas
Heleen Staal (Maastricht, Netherlands)
- P72** Abstract withdrawn
- P73** Histomorphometric parameters of the alveolar ridges of the mandible in immature rats after thymectomy
V Luzin (Luhansk, Ukraine)
- P74** Assessment of bone mass and bone metabolism in children with chronic inflammatory bowel diseases
Vesna Kusec (Zagreb, Croatia)
- P75** Vitamin D Receptor Gene FokI Polymorphism and bone mass accrual in Indian girls
Anuradha Khadilkar (Pune, India)
- P76** Final height and bone health in young adults, transplanted in childhood
Maria Van Dyck (Leuven, Belgium)
- P77** A novel mutation in CRTAP gene in a patient with severe Osteogenesis imperfecta type VII
Ilkka Vuorimies (Helsinki, Finland)
- P78** Variable relationship of fat mass and bone with pubertal staging in obese and normal weight Indian children and adolescents
Anuradha Khadilkar (Pune, India)



- P79** High sedentary lifestyle practices has adverse influence on musculoskeletal health in adolescent boys and girls - findings from a population-based study in Malaysia
Pey Sze Teo (Kubang Kerian, Malaysia)
- P80** Osteogenesis imperfecta and short stature: effect of sclerostin antibody treatment in oim/oim mice
Mickaël Cardinal (Brussels, Belgium)
- P81** Reference point indentation testing detects age-related changes in tissue mechanical properties in mice
Peter Grabowski (Sheffield, UK)
- P82** The influence of sclerostin serum levels on bone mineral density and body composition in patients with Rett syndrome and healthy adolescent girls
Carla Caffarelli (Siena, Italy)
- P83** Anti-RANKL Nanobody® ALX-0141 shows sustained biomarker inhibition in a Phase I study in healthy postmenopausal women
Pieter Schoen (Zwijnaarde, Belgium)
- P84** Comparison of socioeconomic status on bone mass assessed by Quantitative ultrasound of hand phalanges of boys from 7 to 15 years old
Luiz Carlos de Barros Ramalho (Campinas, São Paulo, Brazil)
- P85** Comparison of socioeconomic status on bone mass assessed by quantitative ultrasound of the phalanges in girls from 7 to 15 years old
Juan Eduardo Samur-San-Martin (Campinas, Brazil)
- P86** Assessing bone quality and fracture resistance in children using microindentation
Peter Grabowski (Sheffield, UK)
- P87** Serum homocysteine levels in children and adolescents with impaired bone health
Stepan Kutilek (Pardubice, Czech Republic)
- P88** Children and adolescents with cystic fibrosis have normal volumetric BMD and geometry at the radius, but low muscle area at the forearm
Ondrej Soucek (Prague, Czech Republic)
- P89** Painful vertebral fractures during pregnancy: be aware of a potentially underlying genetic cause
Carola Zillikens (Rotterdam, Netherlands)
- P90** Intravenous pamidronate in the treatment of severe idiopathic infantile hypercalcemia
Stepan Kutilek (Pardubice, Czech Republic)
- P91** Hypovitaminosis D in chronically ill children in Greece and its management
Ioanna Paspali (Athens, Greece)
- P92** Bone mineralization in children with chronic gastroduodenitis
Larisa Scheplyagina (Saint-Petersburg, Russia)
- P93** Long term efficacy of low dose pamidronate treatment in Osteogenesis Imperfecta and its effects on growth
Damla Goksen (İzmir, Turkey)
- P94** Bone anomalies in Noonan syndrome
Ioana Bodescu (Iasi, Romania)
- P95** Abstract withdrawn
- P96** Cross-sectional associations of sex steroids with bone maturation, bone mineral density and bone geometry in boys
Sara Vandewalle (Ghent, Belgium)
- P97** Vitamin D status and bone health in survivors of childhood lymphoblastic leukemia
Michael M Schündeln (Essen, Germany)
- P98** Effects of Denosumab on bone biochemistry and calcium metabolism in a girl with juvenile Paget's Disease
Corinna Grasemann (Essen, Germany)
- P99** Potential association between vitamin D deficiency and hypertension in adolescents: a pilot study
Jerzy Konstantynowicz (Bialystok, Poland)
- P100** Preliminary evidence of reduced Volumetric Trabecular Bone Mineral Density in children with Idiopathic Hypercalciuria. A Peripheral Quantitative Computed Tomography (pQCT) study
Erato Atsali (Athens, Greece)
- P101** Rapid bone mass recovery after parathyroidectomy for primary hyperparathyroidism in a 15-year-old boy
Cristina Tau (Buenos Aires, Argentina)
- P102** Influence of nutritional status in the bone mass assessed by Quantitative Ultrasonography (QUS) of boys
Vinícius Justino de Oliveira Barbeto (Campinas, Brazil)



- P103** Celiac disease and bone disorders: diagnosis age influence
Oscar Brunetto (Buenos Aires, Argentina)
- P104** Immunohistochemical localization of bone morphogenetic proteins and their receptors in human osteochondromas
Araceli Cuellar (Sacramento, USA)
- P105** Bisphosphonate treatment in non-ambulatory patients with spastic quadriplegic cerebral palsy and other neuromuscular disorders: effectiveness of pamidronate vs zoledronic acid
Sasigarn Bowden (Columbus, USA)
- P106** Acquired hypophosphatemic rickets in a 13 year-old boy presenting with knee pain and valgus deformity
Sasigarn Bowden (Columbus, USA)
- P107** Bone cross-sectional geometry and volumetric density at the distal radius in female adolescents with anorexia nervosa
Jean De Schepper (Jette, Belgium)
- P108** Antenatal glucocorticoid injections do not aggravate stress-induced bone loss in young adult mice
Holger Henneicke (Concord, Australia)
- P109** Vertebral morphology assessment in children: can a modified algorithm-based qualitative technique be used reliably?
Ese Adiotomre (Leeds, UK)
- P110** Replacing conventional spine radiographs with dual energy x-ray absorptiometry (DXA) in children with suspected reduction in bone density
Ese Adiotomre (Leeds, UK)
- P111** Vitamin D status and Association to bone health in 781 healthy 8-11 year old Danish school children - preliminary results from the OPUS School Meal Study
Rikke Petersen (Frederiksberg C, Denmark)
- P112** Abstract withdrawn
- P113** Everyday chemicals modulate bone properties during development
Maria Herlin (Stockholm, Sweden)
- P114** Association between parameters of bone mass measured by dual energy X-ray absorptiometry and quantitative ultrasound of proximal phalanges in children and adolescents with congenital adrenal hyperplasia
Ezequiel M Gonçalves (Campinas, Brazil)
- P115** Bone mineral density in late adolescence of transsexuals treated with GnRH-analogues in their teens
Martine Caris (Amsterdam, Netherlands)
- P116** Vitamin D dependent rickets type 1 (VDDR-1): two years after discontinuation of treatment
Victoria Kougia (Thessaloniki, Greece)
- P117** Impact of age and pubertal development on bone mass assessed by quantitative ultrasound of the proximal phalanges in boys and girls aged 10 to 14 years
Tathyane Krahenbuhl (Campinas, Brazil)
- P118** Variation in response to vitamin D therapy in a series of consecutive children referred to a paediatric bone disease service
Fawaz Arshad (Sheffield, UK)
- P119** Depressive symptoms and bone mineral density in a cohort of Portuguese adolescents: no association
Teresa Monjardino (Porto, Portugal)
- P120** Osteoblasts communicate with their neighbouring cells via extracellular vesicles
Jess Morhayim (Rotterdam, Netherlands)
- P121** Tibial metaphyseal shape varies between children according to history of fracture
Paul Arundel (Sheffield, UK)
- P122** Fasting total Ghrelin levels are increased in patients with adolescent idiopathic scoliosis (AIS)
Jean-Pierre Salles (Toulouse, France)
- P123** Inadequately treated hypophosphatemic rickets (HR) in 14-year-old boy: a case report
Athanasios Athanasakos (Athens, Greece)
- P124** Adipokines and bone turnover throughout adolescence: an exploratory approach in a cohort of girls
Teresa Monjardino (Porto, Portugal)
- P125** Growth plate modifications in lysophosphatidic acid (LPA) LPA1 receptor-invalidated mice
Jean-Pierre Salles (Toulouse, France)



- P126** The role of severity of growth hormone deficiency on clinical and instrumental features and response to treatment in children
Anzhalika Solntsava (Minsk, Belarus)
- P127** Mechanosensitive TRP channels are essential for Ca²⁺ signaling in osteoclastogenesis
Yu-Mi Yang (Seoul, Republic of Korea)
- P128** Bone mineral density in survivors of childhood lymphoma
Eryk Latoch (Bialystok, Poland)
- P129** Radiographic evidence of rapid healing of vitamin D deficient rickets after 2 weeks of therapy
Sasigarn Bowden (Columbus, USA)
- P130** "Bone in bone" sign in Juvenile Osteoporosis (IJO) in a 13-year-old girl
Athanasios Athanasakos (Athens, Greece)
- P131** Ghrelin differentiates human osteoblasts via GHS-R1a receptor
Jean-Pierre Salles (Toulouse, France)
- P132** Studies on bone and osteoclasts in patients with Shwachman Diamond Syndrome
Miep Helfrich (Aberdeen, UK)
- P133** Linear growth over 2 years of velaglycerase alpha therapy in children with type 1 Gaucher disease previously treated with imiglycerase
Ari Zimran (Jerusalem, Israel)
- P134** Cessation of ambulation results in a dramatic loss of trabecular bone density in boys with Duchenne Muscular Dystrophy (DMD)
Nicola Crabtree (Birmingham, UK)
- P135** Is Vertebral Fracture Assessment by DXA more useful in a high fracture risk paediatric population than in a low risk screening population?
Nicola Crabtree (Birmingham, UK)
- P136** Time to low bone mass occurrence in children diagnosed with acute lymphoblastic leukemia
Katarzyna Muszynska-Roslan (Bialystok, Poland)
- P137** Influence of nutritional status in the bone mass assessed by Phalangeal Quantitative Ultrasound of girls aged 7 to 10 years old
Fabio Bertapelli (Campinas, Brazil)
- P138** Rare mutations associated with osteoclast-poor osteopetrosis provide molecular insights into receptor activator of NFκβ signalling
Julie Crockett (Aberdeen, UK)
- P139** How to cope with a case of heterotopic ossifications
Grazia Morandi (Cesole di Marcara, Italy)
- P140** Defects of SERPINF1 cause progressively deforming recessive Osteogenesis Imperfecta with normal collagen I
Giacomo Venturi (Verona, Italy)
- P141** Novel splicing mutation in FKBP10 gene in a patient with moderate/severe form of osteogenesis imperfecta
Giacomo Venturi (Verona, Italy)
- P142** A case of geleophysic dysplasia
Claudia Piona (Verona, Italy)
- P143** Fractures in juvenile idiopathic arthritis children: role of disease activity and genetic factors
Mikhail Kostik (Saint-Petersburg, Russia)
- P144** Long term bone sequelae following severe meningococcal septicaemia
Shaila Sukthankar (Manchester, UK)
- P145** The recurrent IFITM5 c.-14C>T transition which causes osteogenesis imperfecta type V occurs at a highly methylated CPG dinucleotide: a novel mutational hot-spot?
Elena Monti (Verona, Italy)
- P146** A case of familial cherubism
Evelina Maines (Verona, Italy)
- P147** A case of gorham-stout syndrome with Chylothorax
Claudia Piona (Verona, Italy)
- P148** Low bone mineral density in a group of girls with Rett syndrome
Steven Bachrach (Wilmington, USA)
- P149** Bone mineral content in healthy Danish children assessed by DXA-scanning and by computerised determination from hand radiographs
Anders Schou (Fredericia, Denmark)
- P150** Markers of bone turnover in obese children - relationship to the nutritional status and oxidative stress level
Pawel Matusik (Katowice, Poland)



- P151** Severity of spine deformity in children and adolescents with idiopathic scoliosis (IS) is associated with nutritional status and body composition
Pawel Matusik (Katowice, Poland)
- P152** Zinc supplementation improves bone density in young adults with thalassemia
Ellen B Fung (Oakland, USA)
- P153** Low urinary citrate: a risk factor for fragility fractures in children and adolescents
Jarzy Konstantynowicz (Bialystok, Poland)
- P154** Smad4 regulates growth plate chondrocyte proliferation, columnar organization and proteoglycan synthesis
Amanda Whitaker (San Francisco, USA)
- P155** Assessment of bone density in MPS IV (Morquio Disease)
Heidi Kecskemethy (Wilmington, USA)
- P156** Reflection analysis of infant scans results may improve infant DXA bone density and body composition result that contain motion
John Shepherd (Fairfax, USA)
- P157** Growth and bone health after hematopoietic stem cell transplantation or tyrosine kinase inhibitors in children with chronic myeloid leukemia: a single institution experience
Kimberley Dilley (Chicago, USA)
- P158** Exploring vertebral abnormalities in patients with thalassemia and sickle cell disease
Ellen Fung (Oakland, USA)
- P159** Novel SLC34A3 mutation causing mild hypophosphataemia, hypercalciuria and nephrolithiasis but no clinical or radiological evidence of rickets
Caroline Steele (Manchester, UK)
- P160** A familial case of Osteogenesis Imperfecta: study of genotype-phenotype correlation.
Maria Luisa Bianchi (Milan, Italy)
- P161** Autoimmune hepatitis and bone density in children
Maria Luisa Bianchi (Milan, Italy)
- P162** Low bone mass and fractures in young patients with chronic diseases
Maria Luisa Bianchi (Milan, Italy)
- P163** Phenotype-genotype correlation and role of ancillary investigations in atypical and rare forms of Osteogenesis Imperfecta
Meena Balasubramanian (Sheffield, UK)
- P164** Vitamin D deficiency in Moscow children and adolescents
Dmitry Shilin (Moscow, Russia)
- P165** Morquio disease in two sisters: Clinical case
Liliana Mejia de Beldjenna (Cali, Colombia)
- P166** Severe metabolic bone disease of prematurity following a brief period of continuous veno-veno haemofiltration (CVVH)
Nick Bishop (Sheffield, UK)
- P167** Recurrent fractures and low bone mass in a patient with new mutation of LRP5 gene
Agnieszka Rusinska (Lodz, Poland)
- P168** Osteogenesis imperfecta - diagnostic difficulties due to clinical symptoms diversity - the results of own studies
Agnieszka Rusinska (Lodz, Poland)
- P169** Milk, childhood and postmenopausal osteoporosis
Antonio Bazarrara-Fernandez (La Coruña, Spain)
- P170** Achondroplasia: medical and orthopedic management in a pediatric patient
Liliana Mejia de Beldjenna (Cali, Colombia)
- P171** Vitamin D levels and signs of metabolic bone disease in adolescents with idiopathic scoliosis
Andreas Kouklinos (London, UK)
- P172** Longitudinal assessment of spinal bone mineral density (BMD) in children with neurofibromatosis type 1 (NF1) using Dual Energy Absorptiometry (DXA) and Quantitative Computed Tomography (QCT)
M Zulf Mughal (Manchester, UK)
- P173** Vitamin D Prescription: A review of British National Formulary for Children recommendations, and a proposal
Andreas Kouklinos (London, UK)
- P174** Elite child athlete is our future: bone lumbar spine adaptation in Egyptian children monofin athletes
Magdy Abouzeid (Alexandria, Egypt)
- P175** Correlation analysis of bone vibration frequency and its mass-volume ratio
Amaka Offiah (Sheffield, UK)



- P176** One case of pseudohypoparathyroidism, clinical characterisation, follow up and treatment
Juan Javier Lamoglia (Cali, Colombia)
- P177** The bone mineral density of children with CF
Ciara McDonnell (Dublin, Ireland)
- P178** Hypomagnesaemia with hypercalciuria secondary to mutations in the Claudin gene: a single centre experience
Shaila Sukthankar (Manchester, UK)
- P179** The impact of selective serotonin reuptake inhibitors on bone mineral density in the pediatric and young adult population
Maria Vogiatzi (New York, USA)
- P180** Beyond brittle bones: a preliminary report from the Osteogenesis Imperfecta Adult Natural History Initiative
Laura Tosi (Washington DC, USA)
- P181** Long-term imiglucerase/algucerase treatment in Latin American children with type 1 Gaucher disease: Lessons from the International Collaborative Gaucher Group (ICGG) Gaucher Registry
Jose Camelo (Arlington, USA)
- P182** Reference curves for Bone Health Index for Han children from five large cities in China, and a comparison to Asian-American children
Hans Henrik Thodberg (Holte, Denmark)
- P183** Juvenile idiopathic osteoporosis - a case study
Ciara McDonnell (Dublin, Ireland)
- P184** High FGF23 measurements in a child with vitamin D dependent rickets type I: cause or consequence?
Ciara McDonnell (Dublin, Ireland)
- P185** An unusual presentation of progressive osseous heteroplasia in a 7-year-old female child
Dirk Schrandt (Maastricht, Netherlands)
- P186** Bone mass, bone microarchitecture (TBS) and anthropometric measurements during childhood growth in Spanish girls
Renaud Winzenrieth (Pessac, France)
- P187** Juvenile idiopathic osteoporosis responsive to intravenous Alendronate
Miguel Angel Guagnelli (Barcelona, Spain)
- P188** Abstract withdrawn
- P189** Clinical features of temporary brittle bone disease
Colin Paterson (Dundee, UK)
- P190** Vitamin D deficiency rickets in neonates
Colin Paterson (Dundee, UK)
- P191** Long-term follow-up in Stuve-Wiedemann Syndrome: A case report
Paola Sabrina Buonomo (Rome, Italy)
- P192** The assesement of the vitamin D supply in the population of Polish children at the age of 9-12 years - multicentre research- preliminary report
Joanna Golec (Lodz, Poland)
- P193** The effect of levothyroxine therapy on vitamin D and bone mineral density
Behzat Ozkan (Izmir, Turkey)
- P194** Celiac disease underlying rickets in an adolescent
Behzat Ozkan (Izmir, Turkey)
- P195** A case of progressive generalised osteolysis - a fibrous dysplasia or something else?
Veselin Boyadzhiev (Varna, Bulgaria)
- P196** Bigger but not stronger? Growth hormone treatment in Turner Syndrome may confer no benefit to HR-pQCT determined bone micro-architecture
Munier Nour (Calgary, Canada)
- P197** Parathyroid hormone administered by continuous subcutaneous infusion is more effective than when given by intermittent injection
Jeremy Allgrove (London, UK)
- P198** Severe hypercalcemia in an infant with idiopathic infantile hypercalcemia caused by mutation in CYP24A1 gene
Grazia Morandi (Verona, Italy)
- P199** Levels of 25(OH)vitamin D in children and adolescents with Type 1 Diabetes Mellitus and in healthy controls in Bulgarian population
Olga Slavcheva (Sofia, Bulgaria)
- P200** Low 25-hydroxyvitamin D levels in Scheuermann's disease: a preliminary study of nineteen patients
Edward Southern (Slidell, USA)
- P201** Guided growth with hinge plates for lack of extension and fixed flexion of the knee
Miguel Galban (Caracas, Venezuela)

ABSTRACTS





IS1 Bone mass and other determinants of fractures in children and adolescents

Emma Clark

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There is evidence from case-control and prospective cohort studies that low bone volumetric density is a risk factor for fractures in children and adolescents, and the size of effect is similar to that seen in postmenopausal women. Bone density and size is important even in childhood fractures due to moderate or severe trauma. However, there are determinants that may influence fracture risk through other pathways than bone fragility. These include gender, ethnicity, obesity and physical activity. Understanding how all the determinants of fractures in childhood and adolescence interact may allow us to intervene and reduce the burden of fractures in this age group.

IS2 Epigenetic influences on childhood bone accrual

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Fragility fractures including hip fracture are a significant global burden. There is a growing body of evidence that the early environment influence an individual's risk of fracture. Evidence from longitudinal studies have demonstrated the relationship between measures of body size in early life with later bone mass and risk of fragility fracture. These observations have been extended by parent / offspring cohorts with detailed examination of the maternal environment and specific effects on foetal and neonatal bone size and post natal trajectories. The mechanism for persisting effects on an individual's bone phenotype are likely to involve epigenetic changes of key regulators of bone mass. Current work has focused on CpG methylation of the Vitamin D/RXR and eNOS pathways and offer potential insights as well as surrogate outcomes and therapeutic targets for future studies.

IS3 Bone Structure and Fractures

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The incidence of distal forearm fractures in children is increasing, and peaks during the adolescent growth spurt. Advances in bone imaging have allowed us and other groups to obtain non-invasive "virtual bone biopsy" data in growing children using high resolution peripheral quantitative computed tomography (HRpQCT). We studied changes in bone structure at the distal radius in individuals ranging from age 6 till 21 years using HRpQCT. Transient regional deficits in cortical strength due to increased cortical porosity were observed during the pubertal growth spurt, mirroring the peak incidence of forearm fractures. In males during this period of rapid growth, we observed that a rise in serum Osteocalcin (OCN) was associated with increasing serum Testosterone (T), which in turn correlated with increasing periosteal circumference. This supports the evidence for a novel bone-testis axis, where OCN may further stimulate testicular T production, which, in turn, contributes to an increase in bone size. Serum sclerostin (S) levels were found to be higher in boys compared to girls, and declined in both sexes after puberty. In both males and females, serum S levels were inversely related to cortical porosity, suggesting that changes in S production may play a role in defining cortical structure. We then went on to perform a fracture case-control study to directly compare bone structure in children aged 8 to 14 years with and without a distal forearm fracture. We found

that in children with a distal forearm fracture due to mild trauma, there was cortical thinning and deficits in trabecular microstructure not only at the distal radius, but also at the tibia. No such differences were found between cases and controls in children who fractured due to moderate trauma, indicating that fractures caused by mild trauma are due to underlying skeletal deficits. Our population-based cohort studies indicate that a distal forearm fracture in boys, but not girls, is associated with an increased risk for fragility fractures as older adults. Further work needs to be done to clarify these sex-differences, and to show if lifestyle and nutritional interventions will prevent these deficits in bone quality.

IS4 The biology of bone revealed through bone biopsy

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Children with long-standing CKD display clinical symptoms of bone disease, including bony deformities and fractures, which contribute to long-standing disability. Bone biopsy is the only available method for assessing all three recommended areas of bone histology (turnover, mineralization, and volume) and new techniques in human bone tissue analysis have shed light on the progression of renal ROD throughout the course of CKD, including its early stages, as well as on the alterations in cell biology that accompany ROD.

Recent studies have identified that bone expression of fibroblast growth factor 23 (FGF23), dentin matrix protein 1 (DMP1) and sclerostin (SOST) increase early in the course of chronic kidney disease (CKD) and are linked to abnormalities in bone turnover and mineralization skeletal mineralization, thus defining osteocytes as endocrine cells which generate hormones that affect bone healthy. In contrast to patients with normal kidney function, FGF23 processing and osteocyte biology appear to change with a progressive decline in kidney function. Indeed, although circulating FGF23 undergoes cleavage in patients with normal kidney function and in those with mild CKD, the majority of circulating FGF23 in dialysis patients is in its full-length form. Changes in circulating mineral ion and hormone concentrations may play a significant role in osteocytic protein expression as CKD advances. Current data suggest that increasing PTH levels suppress osteocytic SOST expression and that circulating phosphorus and PTH both increase FGF23 concentrations. Vitamin D sterols, the most common therapy for controlling secondary hyperparathyroidism and bone turnover, also alters osteocytic protein expression. The effect of these changes on long-term outcomes, including on the systemic effects of altered mineral metabolism in CKD (i.e. cardiac morbidity and mortality), remain to be determined.

IS5 Acrodysostosis

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Acrodysostosis refers to a group of rare chondrodysplasia that share severe brachydactyly, short stature and nasal hypoplasia. Through a candidate gene approach or exome sequencing, heterozygous mutations in *PRKARIA* or in *PDE4D*, respectively, have been identified in patients with acrodysostosis. *PRKARIA* encodes the regulatory subunit of the protein kinase A (PKA), which allows, upon binding of cAMP, phosphorylation of target proteins by the catalytic subunit of PKA. *PDE4D* is a cAMP-specific phosphodiesterase. Interestingly, patients with *PRKARIA* mutations present with resistance to hormones that signal through G-protein coupled receptors including PTH, TSH and epinephrine resistance. *PDE4D* mutations have been identified in patients with acrodysostosis yet, in most patients, no hormone resistance. In addition, impaired cognitive function is more prevalent in patients with acrodysostosis due to *PDE4D* mutations. We propose that acrodysostosis results from the deficient action of PTHrP, hence PKA,



because the chondrodysplasia is highly reminiscent of bone features observed in patients with mutations in *PTHLH*, the gene encoding PTHrP and *PHP1A*/pseudoPHP syndromes caused by inactivating loss of function mutation in *Gsa*, the alpha subunit of the G-protein necessary for the signaling of GPCRs. Our *in vitro* studies indicate that *PRKARIA* mutants are expressed when transfected in a cell model, and prevent the dissociation of the catalytic subunit of PKA. The impact of the *PDE4D* mutations on the protein function remains unsolved. Further investigation of the growth pattern, chondrodysplasia and hormone resistance in patients with acrodysostosis is required to decipher the roles of key components of the cAMP pathway in endocrine diseases.

IS6 Gaucher disease

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Gaucher Disease (GD) is a lysosomal storage disorder due to deficiency of glucocerebrosidase, leading to glucocerebroside storage mainly in macrophages, but also in other cells (lymphocytes, osteoblasts, neurons).

Clinically important bone manifestations of GD include severe acute "bone crisis" (acute avascular osteonecrosis), medullary infarction, osteopenia or osteoporosis, osteolytic lesions, pathologic fractures, defective bone remodelling (Erlenmeyer flask deformity) and growth failure in children. At diagnosis nearly 100% of patients exhibit symptomatic or imaging evidence of at least one of these skeletal manifestations.

Decreased bone mineral density has generally been attributed to increased bone resorption, possibly due to osteoclastogenesis mediated by T cell via TNF-alpha or by macrophages via other cytokines. However biomarkers of osteoclast function are inconsistently increased in GD patients and no significant clinical response arises from inhibition of bone resorption with bisphosphonates. Recently osteoblast dysfunction mediated by accumulating glycolipid through inhibition of protein kinase C has been demonstrated in GD. Nonetheless poor osteoclast-osteoblast signalling from osteoclasts via reduced sphingosine 1 phosphate production may also play a causal role.

Avascular necrosis and medullary infarction are generally related to bone marrow infiltration by macrophages, causing vascular occlusion, compression and increased intraosseous pressure. However the inflammatory mediators secreted by macrophages may also play a causal role. The biomarkers *PARC/CCL18* and *chitotriosidase*, which are directly related to burden of storage in macrophages, are associated with prevalent osteonecrosis, and, in particular, with osteonecrosis occurring despite treatment.

The golden standard of treatment in GD is enzyme replacement therapy with macrophage targeted recombinant glucocerebrosidase. Enzyme replacement therapy reverses haematological and visceral complications, but bone improvement occurs slowly and incompletely. In particular the achievement of age- and sex-adjusted normal bone mineral density takes a longer period of time and require higher doses of ERT than other complications of GD. Other GD therapies, like substrate inhibitors, which are not macrophage targeted seems to have better efficacy to bone mineral density.

IS7 Osteogenesis imperfecta

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Osteogenesis imperfecta (OI) is a genetic disorder, leading to fragility of the bones. The clinical variability is extreme, ranging from relatively mild to perinatally lethal. Secondary features such

as short stature, blue sclerae, dentinogenesis imperfecta and hearing loss may also exist in affected individuals. OI is most often caused by

mutations in the collagen type I genes *COL1A1* and *COL1A2*, that show a dominant mode of inheritance. The least severe OI cases are usually caused by a reduction in the production of collagen type I protein, due to an effective null allele or deletion of one allele of *COL1A1*, leading to haploinsufficiency. The more severe forms of OI are often caused by missense mutations in the type I collagen genes, that affect the formation of the triple helix of the collagen type I protein. Incorporation of aberrant collagen in the extracellular matrix leads to a dominant negative effect.

In recent years, several genes have been discovered that convey recessive forms of OI. Many of these genes encode proteins, involved in processing, modification or transport of type I collagen.

In our laboratory, some 25% of the >1200 OI cases that were tested by DNA analysis were negative for mutations in all known OI causing genes. So it is evident that several hitherto unknown genes are involved in OI. These will most likely be identified by exome sequencing in the near future.

Severe OI is evident at birth, but milder cases may not be noticed immediately. Therefore, in young children, OI may lead to suspicion of non-accidental injury, which may have devastating effects on families. Differential diagnosis, based on radiological and molecular findings, is essential in cases of suspected child abuse, if suspicion is mainly based on recurrent unexplained fractures.

The primary defect in most OI cases is the production of insufficient amounts of collagen type I, or deposition of structurally abnormal collagen type I in the extracellular matrix. Collagen fibers in the correct orientation are essential for proper bone mineralization. The therapy that is currently used is based on bisphosphonate inhibition of osteoclasts to reduce bone turnover. This leads to reduction of bone fragility by increasing the bone mineral density, but the resulting harder bone is still brittle. Bisphosphonate therapy does not target the primary defect of reduced, or structurally abnormal collagen. Long term administering of these drugs may eventually have adverse effects, such as complete loss of osteoclasts. Consequently, new therapeutic targets are needed for OI.

IS8 Non-invasive Assessment of Bone Structure and Strength using QCT and MRI

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Skeletal development is characterized by sex-, race- and maturation specific increases in bone strength. Studies using conventional QCT in the spine and femur, and peripheral QCT (pQCT) in the extremities provided insight into differences in compartment volumetric BMD (vBMD) and cortical dimensions but were limited by inadequate resolution to assess microarchitecture. For example, pQCT studies demonstrated that cortical vBMD was greater in females, while cortical section modulus was greater in males and these differences in structure were more pronounced in later Tanner stages (Leonard, JCEM 2010). In contrast, high resolution (HR-pQCT) scanners have a voxel size of 82 µm (conventional pQCT scanners have a voxel size of 400 µm and a slice thickness of 2.3 mm) and provide estimates of trabecular microarchitecture and cortical porosity. HR-pQCT images can be used as input for micro-finite element (µFE) analysis to estimate bone strength. These methods have been used to identify changes in cortical porosity and the proportion of load borne by cortical bone during mid to late puberty that mirror the timing and sex differences in distal forearm fractures in epidemiologic studies (Kirmani, JBMR 2009). Similarly, a study comparing the tibia and radius suggested that more rapid modeling at the distal radial metaphysis results in a greater dissociation between growth and mineral accrual than observed at the distal tibia with transitory low cortical thickness and vBMD in boys but not in girls (Wang, JBMR 2010). To our knowledge, no prospective studies have examined associations between HR-pQCT results and subsequent fractures. However, Chevalley, et al reported that fractures in healthy females during childhood were associated with lower trabecular thickness and µFE measures of bone stiffness and failure load



at age 20 years (JCEM 2012). Micro-MRI also has sufficient resolution for in vivo assessment of bone microarchitecture; however, relatively long scan times (e.g. 20 minutes) and the lack of automated methods for the quantitative analysis of microstructural parameters have limited use in children. Of note, MR spectroscopy measures of marrow adipose tissue has been used in children and may provide an index of adipocyte vs. osteoblast differentiation in the mesenchymal stem cell pool.

IS9 DXA and vertebral fracture assessment

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Vertebral fractures (VF) in adults are the most common osteoporotic fracture, are powerful predictors of future fracture risk (hip X2; spine X5) and their prevalence increases as bone mineral density (BMD) declines. The most common imaging method for diagnosis is spinal radiography, but they can be identified fortuitously also on other imaging techniques performed for various clinical indications (1). Midline reformations of multi-detector CT (MDCT) scans of thorax and abdomen are particularly sensitive to identify VF (1,2). There is underreporting of VF in adults (3,4) which stimulated the Vertebral Fracture Initiative of the International Osteoporosis Foundation (http://www.iofbonehealth.org/vertebral-fracture-teaching-program). For assessment of VF grading the semi-quantitative method (SQ) method is most widely applied (5). Vertebral fracture assessment (VFA) from DXA images is being used increasingly in adults with improvement in spatial resolution to 0.35mm (6).

In children the epidemiological study of VF is much less extensive and relation to low BMD less clearly defined (7). VF may occur in children in relation to trauma (8) and in various diseases and therapies which compromise bone strength. Spinal radiographs are the most common imaging technique used to identify VF in children and studies applying the SQ method of grading have indicated the prevalence is higher than previously perceived (9). This might in part be that clinicians have been reluctant to perform spinal radiographs because of the high dose of ionising radiation involved (500-600 microSv for lateral projection) (10). DXA VFA has several advantages with the entire spine being depicted on a single image, the X-ray beam being parallel to the vertebral endplates, so avoiding the biconcavity of endplates ('bean can' effect) caused by the divergent X-ray beam in radiographs and most importantly a low radiation dose (3-10 microSv) (10). Single (SE) and dual energy (DE) VFA images are obtained, but differently between scanner manufacturers; simultaneously in a single pass with Lunar General Electric (Madison, MI, USA) and separately by Hologic (Bedford, MA, USA). Former has advantages in children with DE images obtained more rapidly. In adults DE images are superior to SE images to visualize the thoracic vertebrae. An initial report in 2007 of DXA VFA in children was disappointing (11), but further improvements in image quality give VFA the potential for routine application to identify VF in children.

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IS10 Medical therapies: present and future

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Bisphosphonates are the mainstay of medical therapy in the fracturing child with osteoporosis. The majority of the data in children pertains to intravenous pamidronate use in children and adolescents with osteogenesis imperfecta (OI), where pamidronate has been associated with improvements in bone mineral density, cortical thickness, vertebral shape, pain, mobility and height¹. Side-effects of pamidronate including acute phase response to the initial dose and retardation of bone healing have also become apparent. To date, there have been no reports of osteonecrosis of the jaw. The best functional outcomes occur when bisphosphonates are given as part of a multidisciplinary approach to treatment.

More recently, bisphosphonates have been used to treat other primary and secondary osteoporotic disorders e.g. immobility and glucocorticoid. Zoledronate is a third generation bisphosphonate with a potency 100-200 times that of pamidronate. Even though both pamidronate and zoledronate have a similar mechanism of action, zoledronate has potential advantages over pamidronate in the management of paediatric bone disorders due to its shorter infusion time and longer duration of action. Zoledronate has been shown to be effective in the management of osteogenesis imperfecta² and secondary osteoporosis³. The optimal regimen for intravenous bisphosphonate use in both the acute and maintenance phase of treatment remains to be developed.

Oral bisphosphonates do not appear to be as beneficial as intravenous bisphosphonates in children. Although they result in increased bone density, they do not improve bone pain or alter bone histomorphometry⁴. Larger studies await publication. Further the use of bisphosphonates in primary fracture prevention in children is yet to be investigated.

Biological agents hold promise for the future. Denosumab (RANKL inhibitor) use in children has been reported but it would appear unlikely it will be used widely. Anti-sclerostin antibodies and Dickkopf-1 (DKK1), two Wnt pathway inhibitors, however are potential treatments for primary and secondary osteoporosis with their potent effects on periosteal bone formation⁵.

In summary, bisphosphonates have improved the life of children with significant bone fragility. Their use in primary fracture prevention and the utility of new agents such as anti-sclerostin antibodies and DKK1 require further investigation.

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IS11 Other Therapeutic Options: Nutrition, Vitamin D, and Physical Activity

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The childhood and adolescent years represent a critical period for bone acquisition. Extrinsic factors such as diet and physical activity represent modifiable variables that may have a significant impact on a young adult's peak bone mass. This lecture will consider dietary supplementation with specific nutrients as a strategy to augment bone density during the childhood and teenage years. An overview will be provided, as well as data reviewed from supplementation trials in the



pediatric age group. Calcium and vitamin D will be discussed as traditional approaches to increase bone mass, as well as data from trials of vitamin K and magnesium, with discussion on these and other less common nutrients. Lastly, physical activity will be discussed as part of the skeletal therapeutic armamentarium for children and adolescents. Different types of activities will be reviewed (weight bearing vs. not), as well as the use of vibrating plates for pediatric chronic disease groups. These platforms represent a unique means by which high frequency, low magnitude mechanical stimulation can be provided to change bone turnover to increase bone mass in children.

IS12 Bone mineral density and fractures in pediatric inflammatory bowel disease

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The term "inflammatory bowel disease" (IBD) describes a chronic and relapsing inflammation. Up to 25% of all patients with IBD develop the disease during childhood and adolescence. IBD is considered one of the most common chronic childhood diseases in the Western world. Besides epidemiologic data, a short overview about disease presentation, diagnostic criteria and current treatment strategies will be given.

The etiology of IBD is still unknown but it is considered to be multifactorial. It has been hypothesized that a yet unidentified trigger may, in a genetically susceptible individual with an altered intestinal microbial flora and in association with particular environmental factors, activate an aberrant immune response, which results in a chronic intestinal inflammation. Key players in the inflammatory process are cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) that are released from the inflamed mucosa. Possible links between autoimmune disease and bone metabolism will be highlighted.

The association between IBD and bone mineral density (BMD) was first described in the 1960s. Since the introduction of new methods of BMD measurement in the early 1990s, several studies have concluded that low BMD is common in both adults and children who suffer from IBD. However, only few studies have investigated the clinical relevance of low BMD, the occurrence of fractures, in IBD patients. An overview over studies that are available to date will be given. In addition, current guidelines and recommendations regarding evaluation, treatment and follow-up of low BMD in IBD patients will be reviewed.

IS13 Muscle-bone interaction in pediatric bone diseases

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Muscle size and function are closely correlated with skeletal development. Examining the relationship between muscle and bone is thus of central interest in clinical bone research. Surprisingly, however, there is little information on how to evaluate the functional muscle-bone relationship in clinical studies. Many past studies on muscle-bone interaction seem to have analyzed muscle and bone measures that were convenient to collect but did not evaluate a specific model of the muscle-bone relationship. Recently, Anliker et al (Med Sci Sports Exerc 2011;43:2102-9) have proposed an approach to examine the relationship between bone and muscle function that is based on the mechanostat model. According to this model, bone strength adapts to the largest physiological forces to which it is exposed. The proposed approach relates tibia characteristics, as assessed by peripheral quantitative computed tomography to results of muscle performance tests on a force plate ('mechanography'). Bone mineral content at the 14% site of the tibia (measured from the distal articular surface) is used as a surrogate parameter of bone strength, as this is the cross-sectional location where bone mineral content is at its minimum. As the largest physiological forces on bones result from eccentric muscle contraction,

the approach uses peak force during forefoot hopping as a measure of muscle function ('functional muscle-relationship'). In a study on 30 individuals with X-linked hypophosphatemic rickets (XLH), we found that muscle force was significantly lower in XLH patients than in age- and sex-matched controls. The XLH cohort had statistically significant higher bone mineral content, due to a larger bone cross-sectional area. Thus, patients with XLH had increased bone mass and size at the distal tibia despite muscle function deficits. Viewed from the perspective of the mechanostat model, these results suggest that the bones of individuals with XLH are more sensitive to mechanical forces than those of healthy controls.

IS14 Chronic diseases - Type I Diabetes

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Numerous studies in adult patients with type 1 diabetes (T1D) described an association with reduced bone mineral density, altered bone geometry and osteoporosis. Epidemiologic data on hip fractures demonstrate an increased risk in a large adult population with T1D. Diabetes is therefore categorized as adversely affecting the skeleton.

In children and adolescence observations have been more controversial regarding bone mineral content, bone mineral density and markers of bone turn-over. Several studies have documented a lower bone mineral density (BMD) or bone mass, altered bone strength and postponed attainment of peak bone mass. However, other studies found normal levels of bone mass and BMD. The methods used for measuring bone quality have varied making comparison of results from individual studies difficult. The majority of studies are cross-sectional using Dual-Energy X-ray Absorptiometry (DXA) of the spine. The clinical impact of possibly lower bone mineralization in children with T1D has to be discussed since fracture rate data are lacking. Analyzing bone histomorphometry and micro CT in young adults with T1D normal results were seen. However, with diabetes associated complications lower bone mass was present.

Low rates of bone formation along with reduced trabecular bone structure and strength have been shown in rat and mice models of T1D but it is unclear whether this could be transferred to humans. The mechanisms behind impaired bone metabolism in T1D are not clear. Lack of insulin, IGF-1 and further osteoanabolic factors (e.g. amylin), chronic hyperglycemia, inflammation and increased concentrations of AGE (Advanced Glycation End products) as well as diabetic complications like microangiopathy or neuropathy were reported. Further a smaller muscle mass and an intrinsic bone disease were discussed.

The extent of diagnostic and therapeutic activities in patients with T1D in respect to generalized bone disease or diabetic osteopenia should be based on individual conditions and risk profile. Encouraging patients to optimize glycemic control in the long run, to follow a healthy life style and to increase muscle mass by emphasizing physical activity may help to prevent the reported decrease of bone mass and elevated fracture risk later in the course of the disease.

IS15 Osteogenic complications during and after childhood cancer

Marry van den Heuvel-Eibrink

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Childhood cancer has become curable in the majority (>70%) of patients. This is mainly due to rising intensity of treatment, including (combinations of) surgery, chemotherapy, radiotherapy and stem cell transplantation. In addition, intensive international collaboration for rare



subgroups, enhanced stratification for treatment regimens and optimised supportive care has contributed to the improved survival of paediatric cancer that was accomplished over the last decades.

Decreased bone mineral density and subsequent increased fracture risk is an important consequence of childhood cancer treatment in which substantial dosages and long-term administration steroids is required. Not only the total cumulative dosage(TCD), but also the type of steroids has been shown to be relevant. In addition, therapeutic agents, used for solid tumors such as ifosfamide are detrimental for bone turnover, directly or due to renal toxicity (Renal Fanconi). Recently, the use of the promising drug Imatinib, has shown to cause serious bone toxicity. Apart from treatment, the diseases itself(bone marrow infiltration), lymphokine production, immobilisation and general illness are obvious contributing factors for impaired bone mineral density. In addition, similar treatment can cause different bone toxicity in different individuals, suggesting a role for genetic variation, which has been confirmed by identifying several single nucleotide polymorphisms(SNPs) that predispose to a higher risk for osteopenia in childhood cancer.

Another important osteogenic complication of childhood cancer treatment is avascular bone necrosis or osteonecrosis(ON), which is especially observed in children suffering from Acute Lymphoblastic Leukemia(ALL). Risk factors for developing this serious invalidating side effect are female gender and pubertal age. Also it has been show that treatment factors are important, and especially the individual combined Steroids and Asparaginase pharmacokinetics. seems to be the most important factor for the development of ON. In addition, also for ON, genetic variance is relevant. Avoidance of ON in a teenager with disease as ALL is a challenge, as treatment adjustment carries the risk of impaired survival.

As survival rates improve, the absolute number of childhood cancer survivors increases substantially, and late sequelae become increasingly important. Interestingly, avascular bone necrosis has been shown to be reversible in a substantial number of children that have survived childhood ALL. Long term effect studies as based on national survivor cohorts are currently being designed. Although it has been shown that most childhood cancer survivors reach normal peak bone mass in young adolescence, whether the risk of osteoporosis is increased in survivors reaching menopause. to be determined as the first cohorts of childhood cancer survivors now reach that era.

IS16 Fractures among long-term survivors of childhood cancer

Carmen Wilson

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Improvements in diagnosis, multi-modal therapy and supportive care over the past several decades have resulted in substantial reductions in mortality rates for childhood cancer. Approximately 80% of children diagnosed with cancer are now expected to survive for at least five years after initial diagnosis. Nevertheless, improvements in survival rates have not come without cost, with survivors at risk of skeletal morbidities as a result of disturbances in normal bone metabolism during childhood or adolescence. Deficits in bone mineral density (BMD) are among the most commonly reported skeletal morbidity among long-term survivors of childhood cancer. Reductions in BMD may develop as a consequence of the side effects of childhood cancer, such as nutritional deficiencies and reduced physical activity levels, or as a result of the anti-cancer therapies, such as methotrexate and corticosteroids, received in childhood. Among survivors, BMD deficits may also occur as a result of gonadal failure following exposure to pelvic radiation or alkylating agents, or as a consequence of hypothalamic pituitary endocrinopathies following radiation to the central nervous system. In the general population, reductions in BMD can significantly increase the risk of fracture, which may result in acute or chronic pain, impaired mobility and physical function, as well as an increased risk of mortality. Failure

to accrue sufficient bone mass during childhood may place childhood cancer survivors at an increased risk for fracture and skeletal morbidity later in life. This presentation will review the literature and findings from two large cohort studies, the Childhood Cancer Survivor Study and the St. Jude Lifetime Cohort Study, regarding the current state of knowledge of fractures and associated risk factors among childhood cancer survivors. A brief discussion of the methodological challenges encountered when conducting research among cancer survivors will also be included. This presentation will conclude with a short discussion of several innovative approaches and interventions being explored in an effort to ameliorate or reverse bone loss among individuals treated for childhood cancer.

IS17 Bone as an endocrine organ

Paul Baldock

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Our understanding of skeletal biology has revealed bone as a tissue under complex regulatory control, with numerous systems influencing bone development and remodeling. In contrast, the regulatory output from bone tissue is very minimal. However, skeletal research is currently undergoing a period of marked expansion. One aspect in particular is the relationship between bone and fat metabolism. In addition to well-defined responses to weight bearing, emerging evidence indicates that bone and adipose tissue are co-regulated and interdependent. Signals from fat cells are known to regulate bone mass, with prominent adipokines such as leptin and adiponectin. Interestingly, signals produced by bone cells are now being identified that are capable of regulating fat cells, both directly and through central hypothalamic signalling, thereby providing feedback from bone to the regulatory elements of energy homeostasis, within the adipocyte and the brain.

Osteocalcin, a protein secreted by osteoblasts, has emerged as a bone-specific endocrine signal, capable of feedback control of energy homeostasis. Osteocalcin null mice are obese, hyperglycaemic, glucose intolerant and hypoinsulinaemic. Importantly, opposing changes were evident following treatment with exogenous Ocn. Increasing Ocn levels reduced fat mass and improved insulin sensitivity in wild type mice. In subsequent studies investigating energy and glucose homeostasis, osteocalcin has been demonstrated to beneficially regulate energy metabolism through secretion of the undercarboxylated form of osteocalcin (ucOC), acting to increase the insulin sensitising adipokines adiponectin, and directly increasing insulin production in the beta cell. Recently, our laboratory has produced data suggesting a novel central loop for OC signalling, also capable of regulating adipose and glucose homeostasis. To date human data are emerging with varied results, with several recent studies in children conflicting with this view.

In conclusion, the link between energy and bone homeostasis is far more complex than a response to weigh bearing, with multiple axes of control, involving both central and direct signalling pathways. Feedback signals must exist for this bone/fat cross talk to be controlled, and osteocalcin has emerged as a candidate for that role. Further research, particularly in children, is required to define this novel axis.

IS18 Obesity and skeletal health

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Child and adolescent obesity has reached epidemic proportions worldwide. The impact of excess fat on musculoskeletal health is of significant concern. Abnormal mechanical loading of the lower limbs in obese children may lead to anatomic alterations and an increased prevalence of slipped capital femoral epiphysis and tibia vara. Obese children are also over-represented in fracture groups and excess fat may



result in low bone mass relative to body size, although this effect may be confined to adolescence. Paradoxically, obese adults have a higher bone mass and fracture less, although this observation may be bone site-specific. The factors underpinning this paradox are poorly understood. Changes in bone microarchitecture may differ in relation to the relative proportion of subcutaneous and visceral fat. Fat-induced alterations in hormonal factors and cytokines during growth and pubertal development may play a pivotal role in disturbing osteoblast and osteoclast function and thus bone accrual. These changes may be more prevalent in obese children who have obesity-related metabolic risk factors. Reduced levels of physical activity and calcium intake during childhood and adolescence may further exacerbate poor bone mass accrual in obesity.

Despite a considerable body of bone densitometry data there remains controversy about the affect of obesity in children and adults. DXA is unable to capture changes in bone compartments and adjustments are required during DXA analysis to account for body size in children. Longitudinal analysis using novel scanning techniques including High Resolution peripheral Quantitative Computed Tomography (HRpQCT) and skeletal MRI may help to overcome the limitations in using DXA and provide information about obesity-mediated alterations in skeletal microstructure, geometry and strength during growth. Understanding the effects of fat mass on skeletal health during growth is vital in informing future health strategies to optimise peak bone mass and prevent fracture across all ages.

OC1 The Amalgamated Paediatric Bone Density Study (The ALPHABET Study) - The Collation and generation of UK based reference data for paediatric bone densitometry

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Understanding normal patterns of bone growth is important for optimising bone health in children and reducing osteoporotic fractures in later life. Recently published guidelines for bone assessment in children state that to predict fractures a technique should identify children at risk of clinically significant fractures and that dual-energy absorptiometry (DXA) is the preferred method of assessment. Despite these guidelines there is still inconsistency and lack of consensus regarding the management of paediatric bone disease across centres, both nationally and internationally. The major inconsistencies arise from lack of robust reference data and clarity regarding the diagnostic application of these data. The aim of this project was to create a large robust but modifiable reference data set for the assessment of bone status in children by collating all currently available UK measurements of bone density in healthy children.

Seven centres provided data on just over 3000 healthy children aged between 5 and 20 years. DXA scans were acquired from either GE Lunar (DPX-L, Prodigy & iDXA) or Hologic Discovery. To account for the known differences between Hologic and GE scanners and between different generations of machines in vivo and in vitro cross calibration was performed. To ensure consistency all scans were analysed using the latest version of software (Apex 4.0; Hologic, Encore 14.0 GE). To test for significant historic changes in software versions and ensure sustainability of the reference data for future software iterations a subset of 100 Hologic and 100 GE scans were randomly selected from the database.

Analysis between software versions was stable for lumbar spine and hip scans. However, significant changes were recorded for the latest versions

of whole body analysis. Standardisation of the data was achieved using transformation equations generated from the in-vivo cross calibration and published data (Shepherd 2011). Overall, 3030 whole body, 2823 lumbar spine and 1221 hip scans were included in the dataset from which gender, age and size specific reference curves were generated.

In summary, the newly generated curves provide robust reference data which enables successful interpretation of paediatric DXA scans and permits clinicians to follow internationally agreed guidelines on the interpretation of paediatric DXA.

This work was funded by an Arthritis Research-UK Grant

KW is funded by Medical Research Council Grant Code U105960371

OC2 Fracture patterns and bone mass in South African adolescent-mother pairs: The Birth to Twenty Cohort

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Differences in fracture rates and bone mass in families and individuals of different ethnic origins may be due to differing lifestyles and/or genetic backgrounds. This study aimed to assess the associations of bone mass and fracture prevalence in adolescents with maternal bone mass and fracture history, and sibling fracture history.

Data from 1389 adolescent-biological mother pairs from the Birth to Twenty (Bt20) longitudinal study were obtained. Questionnaires were completed by adolescents on fractures until 17/18 years of age. Caregivers completed questionnaires on fractures occurring at any age in the adolescent's sibling/s. Biological mothers completed questionnaires on their own fractures prior to the age of 18 years. Anthropometric and bone mass data on adolescents-biological mother pairs was collected.

White adolescents reported more than double the fracture prevalence of that of other ethnic groups (white (W): 42% vs. black (B): 20% and mixed ancestry (MA): 20%; both p<0.001). White mothers reported a higher prevalence of fractures before the age of 18 years than the other groups (W: 31% vs. B: 6%; p<0.001 and MA: 16%; MA > B: p<0.01). An adolescent's risk of fracture was higher if a sibling had a history of fracture (OR = 1.5; 95% CI 1.02-2.21; p=<0.05), and if they were white and male, and decreased with increasing maternal lumbar spine BMC (24% reduction in fracture risk for every unit increase in maternal BMC Z-score). Adolescent height, maternal bone area (BA) and bone mineral content (BMC), and white ethnicity were positive predictors for adolescents' bone mass.

In conclusion we had demonstrated firstly, a strong familial component in fracture patterns among South African adolescents and their siblings, and secondly a significant influence of maternal bone mass on their adolescents' fracture rates; a novel finding across all ethnic groups.

OC3 Paediatric differences in Bone Mineral Density according to ethnic background in children. The Generation R Study.

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Aim: Differences in fracture risk between ethnic groups have been documented. The basis for these differences is yet incomplete and the age at what ethnic differences appear is uncertain. Assessment of bone health in pediatric populations could bring insights on factors compromising bone accrual. We describe here differences in total body



bone mineral density (TB-BMD) in a unique setting of children of the same age, measured with the same device (iDXA) different ethnic background and in a well-defined geographic region.

Methods: The Generation R study is a prospective multiethnic birth cohort in Rotterdam, The Netherlands including in this study 6,134 children visiting the research center at 6 years. Up to 45% of the children were of non-Dutch background and belonging to 15 ethnic groups (Dutch Central Office of Statistics) and regrouped into European, Asian and African descent. Differences in TB-BMD were assessed by multivariate regression with multiple comparisons of least-squares (LS) means using the Dutch/ European population as reference, adjusting for age, gender, (followed by) fat mass, lean mass and height.

Results: TB-BMD was highest in groups of African descent and lower in groups of Asian descent as compared with Europeans when adjusting for gender and age. After adjustment for body height and lean mass, BMD levels in Asians were equal to Dutch and Europeans, while differences in children of African descents remained significantly higher even after correction for diverse lifestyle variables.

Conclusion: Ethnic differences in bone mass are already present in childhood. Lower BMD in Asian children (as compared to Dutch & Europeans) results from smaller skeletal frame size and adaptation to loading (i.e. lean mass); while the higher BMD in African children is independent of body size or loading. These findings provide further understanding into the differences in fracture risk observed at a given BMD value across ethnicities.

OC4

Maternal antenatal 25(OH)-vitamin D status is associated with offspring muscle strength at 4 years of age

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Objectives: Serum 25(OH)-vitamin D [25(OH)D] concentration is known to influence muscle function in postnatal life. Maternal 25(OH)D status during pregnancy has been implicated in the fetal programming of bone and fat mass, but little is known about its role in determining offspring muscle development. We investigated the associations between maternal serum 25(OH)D concentration in pregnancy and offspring muscle mass and strength at 4 years.

Materials and Methods: A prospective mother-offspring birth cohort, the Southampton Women's Survey (Southampton, UK), was studied. Maternal serum 25(OH)D status was measured at 34 weeks gestation. At 4 years, offspring hand-grip strength (Jamar Dynamometer) and body composition by dual energy Xray Absorptiometry (Hologic Discovery) were assessed. Offspring physical activity (PA) was assessed over seven days using accelerometry (Cambridge Neurotechnology Actiheart).

Results: 326 mother-offspring pairs were included. Maternal serum 25(OH)D concentration in late pregnancy was positively associated with offspring height-adjusted hand grip strength ($\beta=0.12$ SD/SD, $p=0.02$), which persisted after adjustment for a number of maternal confounding factors (including maternal height, pre-pregnancy body mass index, gestational weight gain, walking speed in late pregnancy and smoking status), duration of breastfeeding and child's physical activity at 4 years ($\beta=0.12$ SD/SD, $p=0.03$). Maternal 25(OH)D was also positively associated with offspring percent lean mass ($\beta=0.11$ SD/SD, $p=0.05$), but not total lean mass ($\beta=0.02$ SD/SD, $p=0.67$). This however did not persist after adjustment for confounding factors ($\beta=0.07$ SD/SD, $p=0.24$).

Conclusions: Maternal 25(OH)D status during pregnancy is positively associated with offspring grip strength at four years. These results are

consistent with a role for antenatal 25(OH)-vitamin D exposure in offspring muscle development.

OC5

Novel musculoskeletal phenotypes during childhood for epidemiological studies

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Objectives: Bone, muscle and fat share common mesenchymal origins yet current methods separately examine lean, fat and/or bone content. We hypothesized specific musculoskeletal phenotypes derived from relative contributions of each tissue.

Methods: Design: We obtained information from the 6-year follow-up of mother-offspring pairs within the Southampton Women's Survey, a prospective population-based cohort study of 12,583 initially non-pregnant women.

Measurements: Fat, lean and bone mineral content of the offspring's upper limbs were measured by DXA (Hologic Discovery, Massachusetts, USA). The following maternal characteristics were used: pre-pregnancy height and weight; early pregnancy triceps skinfold thickness; late pregnancy smoking status, strenuous activity and serum 25(OH)-vitamin D concentration (Diasorin, Minnesota, USA).

Statistics: Two-stage variable clustering was used to identify statistically independent phenotypes based on arm fat, lean and bone mineral content using log-likelihood and Bayesian Information Criterion. We tested predictors of the clusters adjusting for the child's sex and age at time of DXA scan using logistic regression.

Results: 1050 offspring (median age 6.8 years; 52% boys) had whole body DXA. Using the above methods, four independent phenotypes were identified (Figure 1): two proportionate phenotypes - Big (17%) and Small (28%); and two disproportionate - Fatty bone (19%) and Lean bone (36%). Within proportionate offspring, Big phenotype had significantly higher maternal weight ($p=0.001$), height ($p<0.001$) and smoking during pregnancy ($p=0.024$) compared to Small. Within disproportionate offspring, increased maternal triceps skinfold ($p=0.001$) and lower 25(OH)-vitamin D concentrations ($p=0.012$) were independent determinants of Fatty vs. Lean bone phenotypes.

Conclusion: We have identified novel composition phenotypes that have distinct early life predictors. These phenotypes may offer insights into aetiopathogenesis of growth and inform future research into the impact of early environment on clinical outcomes in later life.

Figure 1: DXA-derived Fat, Lean and Bone Mineral Content composition for each of the 4 upper limb phenotypes.

Phenotypes	Upper limb Fat	Upper limb Lean	Upper limb Bone Mineral Content
Proportionate	Big (17%)	++	++
	Small (28%)	-	--
Disproportionate	Fat bone (19%)	+	average
	Lean bone (36%)	-	+

++ and -- for each of upper limb fat, lean and bone mineral content indicate comparison of each phenotype to the total sample.



OC6

Role of bone-associated loci identified in GWAS meta-analyses in the context of longitudinal pediatric BMD in European Americans

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Objective: With recent genome wide association studies (GWAS), approximately 70 loci have been robustly and reproducibly associated with adult bone density and/or osteoporosis. However, to date no systematic effort has investigated which of these loci operate early in life. We investigated whether these single nucleotide polymorphisms (SNPs) are associated with childhood areal bone mineral density (aBMD). In addition we determined if any of the associations were age dependent.

Methods: The Bone Mineral Density in Childhood Study (BMDCS) was a multi-center, multi-ethnic longitudinal study that enrolled ~2,000 subjects, (ages 5 to 19). aBMD was measured annually (up to seven years) using Hologic DXA devices. Scans were analyzed centrally and adjusted for inter-site differences and longitudinal drift. Additional measures included growth, puberty stage, and dietary intake and physical activity. Blood or saliva was collected at the final visit and genotyped on the Illumina Human OmniExpress BeadChip, involving in excess of 700,000 markers. Principal component analysis and self-report were used to restrict the cohort to subjects of European ancestry. Longitudinal mixed effects models were used to test for associations between spine BMD Z-score and all SNPs previously identified in adulthood, while accounting for sibships and multiple observations on the same subject. All models included adjustment for height and BMI Z-score, age, sex, puberty, calcium intake and physical activity. SNPs were included in the model as an additive trait. A SNP x age interaction term was also included to assess whether the associations changed relative to age. Significance was determined at a nominal P-value of 0.05.

Results: The following GWAS-established adult bone density loci were associated with spine aBMD: *LACTB2(+)*, *GPATCH1(+)*, *DHH(+)*, *WLS(-)*, *IDUA(+)*, *LRP5(+)*, *SPTBN1(+)* and *STARD3NL(+)*. In addition, significant SNP x age interactions were identified for: *ARHGAP1*, *SP7*, *GPATCH1*, *JAG1*, *MEF2C*, *FOXL1*, *PKDCC*, *SMG6*, *C17orf53*, *C16orf38/CLCN7*, *CYLD*, *INSIG2*, *WNT16*, *CTNBN1*, *NTAN1*, *FAM9B*, *LRP4*, *SPTBN1*, *TNFRSF11B*, *FUBP3*, *KCNMA1*, *SOX9*, *KLHDC5/PTHLH* and *C6orf97/ESR1*.

Conclusions: These findings suggest that multiple variants originally associated with adult bone density are in fact exerting their effects early on in life, and become increasingly expressed as children increase in age.

Support: R01 HD058886 and U54 RR024134

OC7

The PPP6R3/LRP5 Locus Influences Lean Mass in Children of Different Ethnic Background and Highlights Pleiotropic Effects and Muscle-bone Interactions

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Aim: Lean and bone mass have considerably high phenotypic and genetic correlations with a shared heritability estimate ranging between 30-40% in adults. A genome-wide association study (GWAS) on total body lean mass and a bivariate GWAS on lean mass & BMD were ran in a cohort of children to identify genes with pleiotropic effects on muscle mass and peak bone mass attainment.

Methods: Subjects are part of the Generation R study, a prospective multiethnic birth cohort in Rotterdam, The Netherlands; we included 4,096 children (mean age=6.2,SD=0.50 years) with total body DXA measurements (GE-Lunar iDXA) and genomewide genotyping (Illumina 660K). The univariate and bivariate GWAS were adjusted for age, sex, height, fat percent and 20 genomic principal components using bivariate PLINK. A $P<5 \times 10^{-8}$ was considered genome-wide significant (GWS).

Results: Genomic inflation factors were close to unity indicating adequate correction for stratification. In the univariate analysis we identified a GWS association with lean mass ($\beta=0.13$, $P=2.9 \times 10^{-8}$) for a SNP mapping to 11q13.2, in the *PPP6R3/LRP5* locus. The SNP explained 0.8% of the variation in lean mass and was nominally significantly associated with BMD ($\beta=0.10$, $P=7.6 \times 10^{-5}$) explaining 0.4% of BMD variation. The association with lean mass was reduced after additional correction for bone mineral content ($\beta=0.08$, $P=0.001$), explaining 0.2% of the phenotypic variance. In the bivariate GWAS this SNP was also associated at GWS level ($P=4.8 \times 10^{-8}$) showing positive correlations of the bivariate trait with both lean mass (0.96) and BMD (0.68).

Conclusion: While *LRP5* (an ubiquitously expressed gene member of the Wnt signaling pathway) is known to play a key role in bone mechanosensing with GWAS showing association with BMD and fracture in elderly adults; we showed that genetic variation in the *PPP6R3/LRP5* locus exerts pleiotropic effects on muscle mass and peak bone mass acquisition of children. Given the high regional LD it is difficult to establish from which gene the GWAS signal is arising. *PPP6R3* is a gene of unknown function ubiquitously expressed across tissues among others in bone and muscle. Replication in additional children cohorts is underway while the exact same SNP has been found associated at genome significant level in a bivariate GWAS of bone strength and lean mass in two large consortia of adult individuals. Such pleiotropic effects on muscle mass and BMD observed in children are still evident later in life.

OC8

Generation of the first mouse model of Autosomal Dominant Type II Osteopetrosis (ADO2) harbouring the pG213R-clc7 mutation

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ADO2 is a rare osteosclerotic disorder due to heterozygous missense mutations of *CLC7* gene encoding the type 7 chloride channel. Our two labs (L'Aquila and Indianapolis) independently generated the first C57



black 6 (B6) mouse model of ADO2 by inserting the pG213R-*clc7* mutation. We created pG213R-*clc7* KI mice using a gene targeting approach. Homozygous mice showed lack of tooth eruption and died within 30 days of age with severe osteopetrosis and central nervous system degeneration. Compared to WT, heterozygous B6 ADO2 mice showed increase of whole body aBMD (4%, $p < 0.05$) and much greater change at distal femur for BV/TV and Trab.N (75% and 65%, $p < 0.01$). Histomorphometric analysis revealed 2-fold increase of osteoclast number in the proximal tibia compared to WT mice. Bone marrow monocytes from B6 ADO2 mice showed 2-fold increase of TRAcP-positive mononuclear cells and of osteoclast formation, and 80% reduction of resorption pits, confirming cell autonomous impairment of bone resorption. Since the penetrance of the disorder in human is approximately 66% and severity varies considerably, we cross-bred B6 ADO2 with mice of different genetic backgrounds (129, D2, Balb/c and CD1). Compared to WT, the whole body aBMD and BMC at 12 weeks of age were very high in ADO2 mice on 129 background (8% and 12%, $p < 0.01$). ADO2 mice on D2 background also had significantly higher whole body aBMD (4%, $p < 0.02$). The BV/TV was significantly higher at distal femur in ADO2 mice on 129, D2 and Balb/c backgrounds. CTX/TRAcP ratio was significantly lower in all ADO2 backgrounds, except the D2. Our results demonstrate that we have generated the first animal model of ADO2 that will help us to study the penetrance and to test innovative therapies to treat this incurable disease.

OC9

EPO signaling and hematopoietic expansion as causes of osteoporosis in a thalassemia mouse model

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Thalassemia is a chronic anemia associate with high rates of osteoporosis. To determine how erythropoiesis leads to bone loss in thalassemia, we used the *th3/+* thalassemia mouse to study the role of erythropoietin (EPO) and hematopoietic progenitors (HP), since they are both increased in thalassemia.

Methods: Bone marrow mesenchymal stem cells (MSCs) cultures and MSC cocultures with various HP from wild type (wt) and *th3/+* mice were differentiated into osteoblasts. Osteogenesis was determined by Colony Forming Units for Osteoblasts (CFU-O) and qPCR for osteogenesis genes (Runx2, BSP, osterix, osteocalcin).

Results: Compared to wt, bone formation was decreased in *th3/+* animals. MSC cultures from *th3/+* showed decreased osteogenesis, indicating that altered MSC fate leads to decreased osteogenesis in thalassemia. Treatment of MSC cultures with EPO antibody (EPO-Ab) or soluble EPO receptor (sEPOR) reversed the decreased osteogenesis in *th3/+*, demonstrating that EPO signaling guides MSC fate. Furthermore, increased EPO and EPOR expression and changes in JAK2/Stat signaling were seen in thalassemia MSC.

We then isolated various HP and co-cultured them with MSC from wt animals. Lin-Sca+ ckit+ (LSK) precursors from *th3/+* mice led to decreased osteogenesis, while mature erythroid cells (Ter119+) had no effect. Treatment of LSK-MSC co-cultures with EPO-Ab or sEPOR normalized the suppressed osteogenesis in *th3/+*, indicating a role of EPO signaling in LSK-MSK interactions. Consistently, EPOR and EPO expression in thalassemia LSK was increased.

Bone Morphogenic Proteins (BMPs) potentiate both erythroid expansion and osteogenesis. BMP-2,-4,-6 expression in LSK was decreased in *th3/+* compared to wt, and reversed with treatment with EPO-Ab or JAK2 inhibitor. In LSK-MSC cocultures, the effect of EPO-Ab on osteogenesis was lost with additional treatment with noggin, a BMP inhibitor, suggesting that EPO affects LSK-MSC interaction in thalassemia by regulating BMP expression in LSK.

Overall, in *th3/+* thalassemia mice, we observed i) decreased osteogenesis as a result of altered MSC differentiation ii) EPO produced

by thalassemic MSC decreases osteogenesis via direct EPO signaling, supporting an extra-hematopoietic action of EPO. iii) LSK but not mature erythroid cells interact with MSCs in *th3/+* and decrease osteogenesis iii). EPO guides MSC-LSK interactions by regulating BMP expression in hematopoietic progenitors.

OC10

Phenotypic dissection of bone mineral density facilitates the identification of skeletal site specificity on the genetic regulation of bone

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Heritability of bone mineral density (BMD) varies at skeletal sites, possibly reflecting different relative contributions of environmental and genetic influences. To quantify shared genetic influences across different sites, we estimated the genetic correlation of BMD at the upper limb (UL), lower limb (LL) and skull (S) obtained from whole body DXA scans, using bivariate genome-wide complex trait analysis (GCTA). The study (n=9395) combined data from the Avon Longitudinal Study of Parents and their Children (n=5299, mean age=9.9 years) and the Generation R study (n=4096, mean age=6.2 years). GCTA estimates indicated that LL- and UL-BMD shared a high proportion of common genetic architecture ($r_g=0.78$), compared to UL- and S-BMD ($r_g=0.58$) and LL and S-BMD ($r_g=0.43$). To explore the basis for these differences, genome-wide association analyses (GWAS) (with meta-analysis) were performed to identify genetic signals associated with specific skeletal regions. A novel variant was identified within the *RIN3* gene, independent of that previously reported in association with BMD, which was specifically associated with LL-BMD ($P < 5 \times 10^{-8}$). Several genetic variants previously reported to be associated with BMD differed in their associations with BMD at different sub-regions. Specifically, effect sizes of variants which were independent, but proximal, revealed considerable degrees of site specificity at the *WNT16* (7q31.31) and *CENPW* (6q22.32) loci. *WNT16*: rs13223036 showed stronger associations with S-BMD ($\beta=0.17$, $P=1.5 \times 10^{-28}$) and UL-BMD ($\beta=0.19$, $P=1.3 \times 10^{-34}$) compared to LL-BMD ($\beta=0.02$, $P=0.2$); rs2908004 was more strongly associated with UL-BMD ($\beta=0.18$, $P=1.4 \times 10^{-32}$) compared to S-BMD ($\beta=0.09$, $P=3.6 \times 10^{-9}$) and LL-BMD ($\beta=0.10$, $P=3 \times 10^{-11}$). *CENPW*: rs2130604 was associated with S-BMD ($\beta=0.11$, $P=3.3 \times 10^{-11}$) more strongly than with UL-BMD ($\beta=0.04$, $P=0.02$) and LL-BMD ($\beta=0.02$, $P=0.28$). Our results suggest that BMD at different skeletal sites are to a certain extent under distinct genetic influences. Allowing for these differences may help to uncover new genetic influences on BMD, by providing greater power due to stronger site specific genetic effects.

OC11

Connectivity Map-based discovery of novel compounds that induce osteoblast differentiation

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Osteoporosis is a common skeletal disorder characterized by low bone mass leading to increased bone fragility and fracture susceptibility. Little is currently known about what specific factors stimulate osteoblast



differentiation from human mesenchymal stem cells (hMSCs). Therefore, the aim for this project is to determine novel factors and mechanisms involved in human bone production which can be targeted to treat osteoporosis, using gene expression profiling and bioinformatic analyses, including the Connectivity Map, as an in silico approach.

Gene expression profiling was performed on hMSCs differentiated towards osteoblasts using Illumina microarrays. Osteogenic hMSC differentiation was assessed by analyses of alkaline phosphatase activity (ALP) and mineralization by calcium assay and alizarin red staining. Gene expression was determined by qPCR. Immunofluorescent analysis was performed to examine changes in the cytoskeleton. Kegg analysis was performed to determine enriched pathways.

The gene signature of osteogenic hMSCs (top significantly regulated genes 6 hours after induction by dexamethasone) was uploaded into Connectivity Map (www.broadinstitute.org/cmap/). This identified parabendazole as a compound with a statistically significant correlating gene signature to osteogenic hMSCs. Parabendazole stimulated osteogenic hMSC differentiation as indicated by increased ALP and mineralization, which interestingly occurs independent of the presence of glucocorticoids. Moreover, strong upregulation of glucocorticoid receptor target genes by glucocorticoids, is absent in parabendazole-treated cells. Parabendazole caused profound cell morphological and cytoskeletal changes including strong inhibition of microtubules. Kegg analysis of the gene signature indicated TGF-beta signalling, mineral absorption, and MAPK signalling pathways were enriched.

By combining genomic and bioinformatic tools against the backdrop of highly characterized human osteogenic differentiating hMSCs we have identified a novel bone anabolic candidate that induces osteoblast differentiation independent of glucocorticoid stimulation. In combination with the Kegg analysis we will identify important cellular processes and signalling cascades that can be manipulated to stimulate bone formation.

This work was supported by EC FP-7 program Interbone

OC12

Improvement of collagen synthesis in fibroblasts of Brtl model for Osteogenesis Imperfecta following lentiviral-shRNA-mediated down-expression of mutant Colla1 allele

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Objectives: The Brtl mouse, a unique model for the autosomal dominant forms of *Osteogenesis Imperfecta* was used to prove the feasibility of a lentiviral-shRNA-based strategy to improve collagen quality by targeting the mutant *Colla1* allele at the point mutation responsible for the causative substitution Gly349Cys. The ability to specifically suppress the mutant allele should convert the moderate Brtl outcome to the mild one caused by quantitative defect.

Methods: A model of human embryonic kidney cell lines which express the *firefly* luciferase gene combined with either wild-type or mutant Brtl *Colla1* exon 23 sequences enabled the identification of two siRNAs which were effective specifically against mutant sequence and not active against the wild type allele. The corresponding shRNA subcloned in a lentiviral vector were evaluated *ex vivo* in Brtl fibroblasts at transcript and protein levels, respectively measured by allele specific qPCR and collagen quantification after fluorescent labeling and SDS-PAGE in non reducing conditions.

Results: No effect was detected on cell proliferation, but a preferential reduction of the mutant allele transcript (up to 52%) associated to a decrease of the mutant protein (about 40%) was obtained. Interestingly a down regulation of Hsp47, a specific collagen chaperone known to be

up regulated in some *Osteogenesis Imperfecta* case, was also detected. Brtl fibroblasts stably expressing the shRNA of interest and down-regulating the mutant *Colla1* were seeded in alginate to measure engraftment in mice and collagen synthesis *in vivo*.

Conclusion: Our data support the use of cautiously designed, lentivirus delivered-shRNAs as a strategy to specifically suppress the mutant allele making appealing the modification of mesenchymal stem cells of *Osteogenesis Imperfecta* patients for autologous transplantation.

OC13

Level of calcium intake modifies the correlation between parathyroid hormone and 25-hydroxyvitamin D: A proposal of adequate 25-hydroxyvitamin D levels in children.

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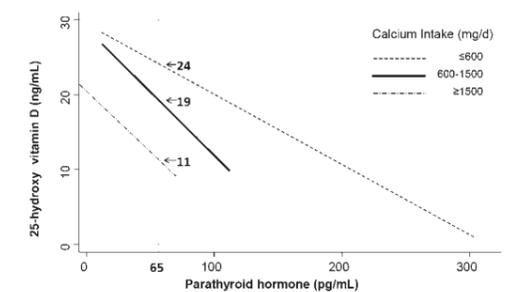
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Background: The “adequate” level of 25-hydroxyvitamin D (25OHD) in children remains unclear. For bone outcomes, parathyroid hormone (PTH) is an important functional biomarker. Prior studies have shown too weak a correlation between 25OHD and PTH levels to determine adequate 25OHD level.

Objective: To determine adequate 25OHD level(s) in children using a normal PTH value of 65 pg/mL while adjusting for calcium intake.

Design: We performed a cross-sectional study in 403 children; 237 were healthy (age 10.2 ± 4.4 years; 51% male) and 166 were chronically ill (9.9 ± 4.2 years; 53% male). Diseases in ill children were cancer, osteogenesis imperfecta, connective tissue disorders, and motor disabilities.

Results: Serum 25OHD <20 ng/ml was present in 41% of healthy and 66% of ill children, while 25OHD <10 ng/ml was present in 3.0% of healthy and 11.4% of ill children. PTH was negatively correlated with both 25OHD and calcium intake ($r = -0.11$, $p = 0.02$; and $r = -0.25$, $p = 0.01$, respectively). The 25OHD-PTH correlation became stronger when adjusted for calcium intake: For calcium <600 mg/day: $r = -0.45$ ($p = 0.02$), 600-1500 mg/day: $r = -0.30$ ($p < 0.001$) and >1500 mg/day: $r = -0.27$ ($p < 0.001$). Serum 25OHD levels that maintain a PTH concentration of <65 pg/mL were: 24 ng/mL at calcium intake <600 mg/day, 19 ng/mL at 600-1500 mg/day, and 11 ng/mL at >1500 mg/day.



Conclusions: PTH can help define adequate 25OHD levels for bone outcomes when the 25OHD-PTH correlation is adjusted for calcium intake in children. Based on the serum 25OHD level that maintains PTH <65 pg/mL, we propose adequate 25OHD levels for three ranges of calcium intake. To improve generalizability of these findings, we encourage replication of our analysis in diverse populations.



OC14

What DXA measurement sites are best for bone health assessment in children? Effect of inter-machine differences on bone outcomes from the Bone Mineral Density in Childhood Study (BMDCS)

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Objective: Total body less head (TBLH) and spine are the recommended DXA sites for bone health assessment in children and adolescents. However, inter-machine differences will affect use and interpretation of results in clinical care and research applications. We examined BMC and areal-BMD (aBMD) at 4 skeletal sites among healthy children to identify the magnitude of inter-machine differences in Z-scores.

Methods: BMDCS evaluated healthy participants, ages 5-20 years, as previously described. All five centers used Hologic devices and scans were analyzed centrally. Data from the first study visit for subjects with complete data for the distal 1/3 radius, hip, spine and and TBLH, and covariates (demographic information, height and BMI Z-score, race, puberty stage, calcium intake and physical activity) were included in the analysis. BMDCS Z-scores for BMC and areal-BMD were calculated, and differences among study centers assessed by ANCOVA adjusting for covariates. Logistic regression assessed the probability of having a BMC or aBMD Z-score ≤ -1.5 among centers after adjusting for covariates.

Results: 1889 subjects (48% female, 24% African American, 17% Hispanic, 47% Non-Hispanic white, 12% other) were evaluated. There were significant differences in Z-scores adjusted for covariates among study centers for all measures except total hip aBMD Z-score. Differences from the group mean in adjusted Z-scores were lowest for spine and hip measures (-0.14 to 0.14 sds), and highest for TBLH aBMD (-0.19 to 0.53 sds). After adjusting for covariates, the probability of having a Z-score ≤ -1.5 (expected probability .067) was not significantly different between centers for most skeletal sites, except for TBLH aBMD (probabilities ranged from 0.02 [95% CI: 0.1 to 0.04] to 0.09 [95% CI: 0.07 to 0.15]).

Conclusions: Spine aBMD and hip measurements showed relatively good agreement between centers. There was wide variation in DXA whole body scan results obtained on healthy children measured on comparable Hologic DXA devices. This can potentially result in misdiagnosis of children with low bone status in clinical care and research. The recommendation of whole body scans as an optimal measurement site in children should be reconsidered.

Support: R01 HD058886, U54 RR024134, N01-HD-1-3331

OC15

Bone Health Index: Swiss children have less in the bank than a generation ago

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Objective: The aim of this study is to compare the Bone Health Index (BHI) for healthy Swiss children born in 1955 with healthy Swiss children born a generation later.

Method: BHI is derived from the cortical thickness in the three middle metacarpals. It is determined with the BoneXpert medical device, which automatically analyses a standard bone age hand radiograph. The measurement result is independent of the sharpness of the image. The

image data are from two longitudinal studies performed on healthy children at Zurich University Hospital. The first study recruited 232 children who were followed longitudinally with annual hand X-rays until age 20 years. The second study includes 200 children which have a participant in the first study as one of their parents, and these children were imaged biannually until adulthood.

Results: Reference curves of BHI are presented. The shapes of the curves are similar in the two generations, but the level of BHI is different. To quantify the level we form the average BHI over the bone age range 7-14 years for boys and 6-14 years for girls. This BHI level is found to be 1.5% lower in the modern generation.

Conclusion: The two generations exhibit the same adult height and the same tempo of maturation. In contrast, the BHI showed a secular trend of minus 1.5%. The stringent design of the study minimises other factors, which could explain the difference. Secular trends in bone health are difficult to study, because DEXA scanners were not available a generation ago, and even if they were, it would be difficult to ensure compatible calibration given the change of technology in these machines. In contrast, plain X-rays were a mature technology already 100 years ago, and large archives of hand X-rays have been collected for the purpose of bone age determination around the world and over the time. With the BHI method these become a valuable resource for studies of secular trends, population differences and relationship between children's bone health and fracture incidence later in life.

OC16

Longitudinal analysis of volumetric density, size and strength towards the end of skeletal maturation in Gambian males habituated to low calcium intake

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To understand differences in bone health between and within populations, it is crucial to characterise bone development during childhood and adolescence. Peak height velocity at age 16 and young adult height at age 23.5 years were recently reported in Gambian males accustomed to low calcium intake.¹ Our study aims to describe bone accrual after peak height velocity in the same population.

We used peripheral quantitative computed tomography to measure the 66% radius biennially, at mean(SD,n) ages 19.4(0.9,60), 21.7(1.0,59) and 23.6(0.9,51) years. Outcomes were cortical volumetric bone mineral density (vBMD), bone mineral content (BMC), total and cortical cross-sectional area (CSA), and stress-strain index (SSI). We used random intercept and random slope models to assess the relationship between each outcome and changes in age, height and weight. Random intercept models assume that all individuals' slopes share the same gradient, but allow their intercepts to vary. Random slope models allow for between-individual variation in gradients as well as in intercepts.

Random slope models were a significantly better fit than random intercept models for BMC, vBMD and total CSA against age (likelihood-ratio tests $p < 0.05$), indicating individual differences in the rate at which these bone measures changed. For all other variables, random intercept models were sufficient. Annual change in SSI and cortical CSA was similar for all individuals. No bone measure showed significant differences between individuals' gradients when modelled against height or weight. Slope coefficients in all models were significant and positive ($p < 5 \times 10^{-5}$).

Individual differences in the rate of age-related change in BMC, vBMD and total CSA may reflect differences in deceleration of bone development in older individuals. Alternatively, differences in maturational stage may modify the effect of age on bone. Anthropometric measures are more directly associated with bone development than chronological age i.e. somatic and skeletal growth are co-ordinated but not determined by chronological age alone. This may reflect biomechanical responses in the skeleton, but could equally be



due to joint control of growth and bone development by genetic, hormonal or nutritional factors. The age of cessation of bone accrual remains to be determined, and the implications for population bone health require further investigation.

¹Prentice *et al.* (2012) *AJCN* 96:1042-50.

Funding: UK Medical Research Council (Grant codes U105960371 and U123261351)

OC17

Determinants of bone mineral density in long-term adult survivors of childhood cancer

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Objectives: Osteopenia is a complication of childhood cancer treatment but it is unknown to which extent this occurs in adult survivors, and which subgroups are at risk. We examined bone mineral density (BMD) and assessed the relative importance of potential determinants of low BMD in very long-term adult survivors of childhood cancer.

Methods: The single-center cohort study included 410 adult CCSs (median age at diagnosis: 6.6 years (range: 0-17 years) with a median follow-up time of 15.2 years (range 5-39 years). Total body BMD (BMD_{TB}) and BMD of the lumbar spine (BMD_{LS}) were measured with dual X ray absorptiometry (DXA). In a subsample of 188 survivors, a sequential DXA scan was performed (median time after first DXA: 3.2 years).

Results: Survivors had a lower BMD_{TB} (median standardised difference score (SDS): -0.60; $p < 0.001$) and BMD_{LS} (SDS: -0.40; $p < 0.001$) as compared to healthy peers. In the subsample, BMD_{TB} and BMD_{LS} significantly increased between the first and second DXA (mean SDS: BMD_{TB}=0.08; $p=0.003$; BMD_{LS}: 0.06; $p=0.03$). We identified higher age (st. b=-0.17), having had a brain tumor (st. b=-0.20) or sarcoma (st. b=-0.13), cranial (st. b=-0.23) or total body irradiation (st. b=-0.16), use of glucocorticoids (GC) during treatment (st. b=0.22), and lower BMI at follow-up (st. b=0.53) as important determinants of low BMD_{TB}. BMD_{LS} was only associated with BMI (st. b=0.22) and use of cyclophosphamide (st. b=-0.18).

Conclusions: Long-term childhood cancer survivors have a lower BMD_{TB} and BMD_{LS} as compared to healthy peers, but improvement in BMD may still occur. Furthermore, we showed that patients who have had a brain tumor or sarcoma, those who were irradiated, who were treated with glucocorticoids and who have low BMI, are at risk of osteopenia.

OC18

Trabecular bone score (TBS) applied to normal children's lumbar spine DXA scans

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Trabecular bone score (TBS) extracts a texture parameter from pixel grey-level variations in DXA lumbar spine images. The TBS is claimed to be a measure of trabecular structure and was validated in an in-vitro study of vertebral bodies with micro-CT (1). TBS has shown the potential for fracture prediction in adults (2). However, data are sparse

regarding the reliability and usefulness of TBS (3) and the method has not previously been applied in children.

Methods: The Manchester Children's DXA BMD reference database (Hologic QDR Discovery) was published and is widely applied in UK (4). The LS-DXA (n= 463; males; age mean [SD] range; n=252, 15.0 [5.6], 5.2-25yrs; females n=211; 12.6 [3.9], 5.4-20.7yrs) have been retrospectively analyzed and TBS L1-4 extracted. The relationships between TBS and age, BMD (g/cm²), bone mineral apparent density (BMAD), height, weight and BMI were examined. Linear regression analysis explored predictors of TBS; model 1 included LSBMD, age, BMI, model 2 LSBMAD, age, BMI. Significant predictors are reported.

Results: There was a significant correlation between TBS and age in females ($r=0.63$, $p < 0.001$) and males ($r=0.67$, $p < 0.001$), and between TBS and BMI (females $r=0.41$, $p < 0.001$; males $r=0.41$, $p < 0.001$). For both males and females mean TBS was lower in the younger (<13yrs) relative to the older (>13years) age groups ($p < 0.001$), but not significantly different between children in annually adjacent year groups. TBS was lower ($p < 0.001$) in children with a lower (<20kg/m²) versus a higher BMI (>20kg/m²). Correlation between BMAD and TBS was $r = 0.61$, $p < 0.01$ in females and $r = 0.62$, $p < 0.01$ in males. Predictors of TBS were: females: 1) L1-L4 aBMD ($p < 0.001$), and BMI ($p = 0.002$); 2) L1-L4 BMAD ($p < 0.001$), age ($p < 0.001$), BMI ($p = 0.086$); Males: 1) L1-L4 BMD ($p < 0.001$), BMI ($p < 0.001$); 2) L1-L4 BMAD ($p < 0.001$), age ($p < 0.001$). BMI ($p = 0.004$).

Conclusions: TBS is related to age, aBMD and BMAD in growing children. The dichotomy of the relationship with age and BMI may be due to technical limitations in the method. Our findings require further investigation in this and other populations. A critical evaluation of the tool and whether it improves our understanding of children's bone health is required.

1) *J. Clin. Densitom* 2011;14:302-312.

2) *J Bone Miner Res* 2011;26:2762-2769.

3) *Osteoporos. Int.* 2012;23:1489-1501.

4) *Arch Dis Child.* 2007;92:53-59.

KW is funded by Medical Research Council Grant Code U10596037

Acknowledgement for access to specialist TBS analysis software: <http://www.medimaps.fr/>

OC19

New Therapeutic Approach in OI VI - Suppression of Bone Resorption using the RANKL Antibody Denosumab

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Background: Osteogenesis imperfecta (OI) as a rare disease is characterized by reduced bone mass, increased fracture rate, bone deformities and skeletal pain.

Currently patients are treated with intravenous bisphosphonates regardless of the underlying mutation.

Recently the gene causing OI type VI was described (SERPINF-1, altered RANKL-pathway). This leads to a new understanding of the underlying pathophysiology and offered a new therapeutic approach.

OI type VI is not caused by a reduced synthesis of collagen in the osteoblasts but by an increased activity of osteoclasts leading to a higher proportion of bone resorption.

Objective: Suppression of bone resorption in children with OI type VI treated with the RANKL antibody denosumab?

Methods: This case serie includes 4 patients (male=4, median age 10.0 years) with confirmed mutation in SERPINF-1 and reduced response to bisphosphonates (measured by inadequate reductions of urinary deoxypyridinolin (DPD) level as parameter for osteoclastic suppression)



who were treated with denosumab 1mg/kg body weight s.c. every 12 weeks. DPD levels were determined under treatment as a marker of bone resorption. Bone mineral density (BMD [g/cm²]) was determined after a completed one year course of treatment by DEXA of the lumbar spine.

Results: Urinary DPD/creatinin dropped to the normal range after each injection (tab.1).

BMD of the lumbar spine increased during the first year of treatment more than during bisphosphonate treatment. Levels of serum calcium were stable under oral substitution (tab.1).

Conclusions: Denosumab was well tolerated and laboratory parameters and BMD measurement provided evidence that this treatment reversibly reduced bone resorption in OI VI. A prospective trial to evaluate efficacy and safety is needed for further proof of the effect of denosumab in patients with OI caused by mutations in COL1A1/ COL1A2.

Days after denosumab injection	Calcium [mmol/l] (2.20-2.65)				DPD/Crea [nM/mM]			
	Pat 1	Pat 2	Pat 3	Pat 4	Pat 1	Pat 2	Pat 3	Pat 4
Day 1	2.34	2.24	2.21	2.35	70.8	33.3	66.9	41.5
Day 2	2.22	2.16	2.13	2.26	58.9	28.7	81.7	45.2
Day 7	2.34	2.10		2.24	23.2	25.1	26.3	19.7
Day 14	2.27	1.99		2.36	23.7	13.4	26.0	20.4
Day 29	2.26	2.21			25.2	22.7	25.1	16.8

Tab. 1: Changes of Calcium and DPD levels during the first treatment cycle

OC20

Exercise Completed When Young Provides Lifelong Benefit to Cortical Bone Structure and Estimated Strength

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Objectives and methods: Exercise induces greatest bone gains during growth, yet reduced bone strength is an age-related phenomenon. This raises the question of whether exercise-induced bone changes when young persist into adulthood. Previous work has suggested exercise-induced gains in bone mass are lost with aging. However, exercise during growth primarily influences bone structure rather than mass to increase strength and mechanisms exist for the maintenance of exercise effects on structure. The current studies used Major/Minor League Baseball (MLB/MiLB) players to explore whether exercise-induced gains in humeral bone structure and strength accrued when young persist lifelong. MLB/MiLB players are a unique model as the unilateral upper extremity loading associated with throwing enables the contralateral side to serve as an internal control site and former MLB/MiLB players were consistently exposed to extreme loading reducing secular variations in exercise levels between generations. Dominant-to-nondominant (D-to-ND) differences in humeral cross-sectional properties in MLB/MiLB players in the current studies were normalized to matched controls to correct for side-to-side differences due to elevated habitual loading associated with arm dominance.

Results: Exercise when young induced significant skeletal benefits, with active MLB/MiLB players having nearly double the estimated ability to resist torsion (polar moment of inertia, I_p) in the humerus of their dominant arm. The cortical bone mass and area benefits of exercise observed in active MLB/MiLB players were lost in former MLB players following 40-49 years of detraining (age=68.4±4.9 yrs) as a result of elevated medullary expansion and endocortical trabecularization. However, 42% of the total bone area benefit persisted following 50+ years of detraining (age=81.4±6.4 yrs) and contributed to the maintenance of 24% of the benefit on I_p. In MLB players who continued to exercise during aging, medullary expansion and endocortical trabecularization were reduced and there was maintenance of the cortical bone mass and area benefits of exercise.

Conclusions: These cumulative data indicate: 1) the extreme plasticity of the growing skeleton to exercise; 2) that exercise when young has

lifelong benefits on cortical bone size and estimated strength, but not bone mass, and; 3) exercise continued during aging maintains the bone mass benefits of exercise.

OC21

A randomized, double-blind, placebo-controlled trial of alendronate treatment for fibrous dysplasia of bone

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Fibrous dysplasia (FD) is a benign skeletal disease caused by activating mutations of G_sα. These mutations lead to formation of abnormal and mechanically unsound bone and fibrotic tissue. Clinical sequelae include deformity, fracture, and pain. Studies in bisphosphonates have shown improvement in bone pain and inconsistent effects on FD mineralization; however interpretation has been limited by a lack of controlled trials. Objective: To determine the efficacy of oral alendronate for treatment of FD.

Methods: Forty subjects with FD (15 children, median age 10, range 6-16, and 23 adults, median age 40, range 20-57) were enrolled in a randomized, double-blind placebo-controlled trial of alendronate (1 subject in each group withdrew prior to treatment). The co-primary efficacy endpoints were the change from baseline in N-telopeptide and osteocalcin at 18 months. Secondary endpoints included effects on pain, and bone mineral density at FD and non-FD sites. Subjects received drug or placebo for 6 months, followed by 6 months off, 6 months on, and 6 months off. Dosing was stratified as follows: 40 mg/day for subjects >50 kg, 20 mg for 30-50 kg, and 10 mg for 20-30 kg.

Results: Compared to placebo at baseline, at 18 months alendronate use was associated with a decrease in the resorption marker N-telopeptide (p=0.001), with no significant effect on the formation marker osteocalcin (p=0.7). Subjects in the alendronate group did not show significant changes in pain as compared to placebo. At FD sites there was no significant change in bone mineral density at affected femora or humeri. At non-FD sites there was a significant increase in bone mineral density (p=0.003). Three subjects in the placebo and three in the alendronate group sustained fractures during the study period. One alendronate-treated subject with an undisclosed history of reflux developed an esophageal stricture. Conclusions: Alendronate at four times the osteoporosis dose decreased bone resorption markers, but did not improve FD mineralization or bone pain. These data support findings from previous open-label studies which demonstrated a lack of effect of bisphosphonates on FD mineralization, and suggest that oral bisphosphonates may be ineffective for FD-related bone pain.

OC22

Prolidase deficient mice are osteoporotic in early life

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Background: Proline and hydroxyproline account for approximately 25% of aminoacids in collagen. Prolidase (Peptidase D [EC 3.4.14.9]), cleaves iminodipeptides with a C-terminal proline or hydroxyproline, playing a major role in collagen catabolism. Mice with prolidase deficiency (PD) present with varied phenotypes including reduced size



compared to wild-type littermates. We measured structural and mechanical properties of bones in PD mice.

Methods: Whole femurs from 28 day old male (n=6) and female (n=10) mice with PD (HOMO) and wild type mice (WT: male, n=6; female, n=10) and male 2 month old mice (HOMO: n=6, WT: n=6) were scanned on a Skyscan μ-CT 1172 using published protocols. Bone mineral density was calculated by linear extrapolation using a 2mm phantom rod pair containing 0.25 and 0.75g.cm⁻³ calcium hydroxyapatite. Data were analysed by two-way ANOVA with Tukey post-hoc analysis for genotype and gender effects. Mechanical properties of whole femurs were analysed using microindentation (Biodent).

Results:

1 month old mice	HOMO			WT		
	Mean	SEM	N	Mean	SEM	N
BY/TV	5.95	0.71	9*	10.20	0.72	10
FEMALE	7.05	1.06	6	15.48	1.63	6
MALE						
Tb.Tb						
FEMALE	0.033	0.0006	9*	0.034	0.0007	10
MALE	0.033	0.0009	6	0.035	0.0004	6
Tb.N						
FEMALE	1.690	0.165	9*	2.109	0.212	10
MALE	2.149	0.315	6	4.433	0.435	6
Femur length						
FEMALE	10.324	0.0786	9*	10.985	0.1653	9*
MALE	9.960	0.4999	6	11.420	0.3435	6
CLBV						
FEMALE	0.359	0.004	10	0.388	0.015	10
MALE	0.110	0.007	6	0.121	0.004	6
2 month						
Femur length						
MALE	13.66067	0.255952	6	14.49217	0.141096	6

PD resulted in significant reductions in trabecular bone volume (p<0.0001), trabecular number (p<0.0001), trabecular thickness (p=0.018), cortical bone volume (p=0.004) in 28 day old mice only and femoral length at 28 days (p=0.0005) and 2 months (p=0.017). There was no effect of genotype on mechanical properties.

Conclusions: PD in mice reduced trabecular bone volume, thickness and number as well as cortical bone volume and femoral length in comparison to wild type mice at 28 days. This indicates that delayed collagen recycling through PD affected bone structure at early stages of development. The catch up seen by 2 months potentially reflects reduced need for rapid collagen turnover. There was no clear effect of PD on bone material properties measured by microindentation. The prolidase deficiency phenotype is essentially osteoporotic in early life.

OC23

Switching from Alendronate to RANKL Blockade alters Bone Properties after 14 Weeks of Therapy in the oim/oim Mouse

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Objective: The purpose of this study was to determine whether osteogenesis imperfecta (OI) patients entering adulthood should continue with bisphosphonate therapy or would benefit from switching to a RANKL blockade therapy. To address this question, we used a mouse model of type III OI.

Methods: Animal studies were performed under IACUC approval. OI (oim/oim) and wild-type (WT) mice were treated from 2-26 weeks with 1) Saline; 2) Alendronate (ALN; 0.21 mg/kg/dose weekly); or 3) ALN from 2-14 weeks followed by 1.5 mg/kg/dose biweekly RANK-Fc from 14-26 weeks (ALN+RANK-Fc). There were 20 mice per group. Fracture number was calculated from high-resolution faxitrons obtained in the anterior-posterior and medial-lateral planes at 14 weeks and sacrifice. Femurs (n=4-10/group) were analyzed by microcomputed tomography (micro-CT) using a Scanco μCT 35 system (Scanco Medical, Basserdorf, Switzerland). A subset of the femora were embedded in poly methyl

methacrylate and section at 2 μm for Fourier transform infrared imaging (FTIRI) analysis (n=3-5/group). Nine images of cortical and trabecular bone were taken from each femur using a Perkin Elmer Spotlight Imaging Spectrometer and ISys (Malevern Instruments) software. Results are statistically significant p<0.05 by two-way ANOVA (SigmaStat).

RESULTS: Fractures: At 14 weeks, ALN-treated oim/oim mice had fewer fractures compared to saline. At 26 weeks, both treatment groups had fewer fractures compared to saline. There were no differences between treatment groups at either time-point and no new fractures after 14 weeks in either group.

Micro-CT: There were no differences in cortical bone in any parameters studied. Trabeculae number was increased in oim/oim for both treatment groups compared to saline. ALN+RANK-Fc increased trabeculae number compared to ALN. Trabeculae spacing decreased and bone volume fraction increased in both treated oim/oim groups compared to saline. Changes in the WT mirror oim/oim.

FTIRI: oim/oim trabecular bone: ALN treatment increased mineral:matrix ratio compared to saline and both treatment groups increased collagen maturity compared to saline. oim/oim cortical bone mineral:matrix ratio was increased in ALN compared to ALN+RANK-Fc treatment. WT cortical bone: ALN+RANK-Fc treatment resulted in more perfect crystals compared to saline.

DISCUSSION: Based on our current data, treatment with ALN or ALN+RANK-Fc comparably reduces fracture number, increases bone quantity, and alters bone quality in oim/oim mice. Mechanical testing will be essential in determining the implication of these changes on patient life.

OC24

Fibroblast Growth Factor 23 (FGF23) Plasma Levels Are Elevated with Early Chronic Kidney Disease (CKD) and Positively Associated with Steroid Based (SB) versus Steroid Free (SF) Immunosuppression in Pediatric (ped) Renal Transplant (Tx) Patients (pts)

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Background: FGF23 is a circulating phosphaturic hormone that also suppresses renal 1α-hydroxylase activity. In adult pts plasma FGF23 increases in early stages of CKD, even before serum phosphorus changes. In a study of 984 adult renal transplant pts, higher FGF23 levels were independently associated with increased risk of all-cause mortality and allograft loss during 39 months follow-up.² Few studies have focused on FGF23 & CKD in ped renal Tx pts.

Aim: Determine plasma FGF23 & evaluate potential associations with renal function & mineral metabolism parameters in ped Tx pts.

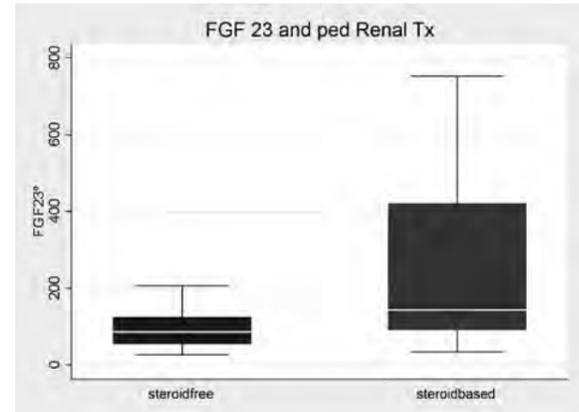
Methods: Cross-sectional study of 59 ped Tx pts (21F; median age 15.2y, range 3.9-21.8y; 25 Hispanic/17white/16 black/1 other) with CKD stages 1-3 (mean±SD Schwartz eGFR 97±25 ml/min/1.73m²); median time since Tx 1.2y (range 0.16-11.1y). Immunosuppression was 47 pts steroid free (SF) & 12 steroid based (SB). Serum Cr, Ca, P, iPTH, 1,25vitD; plasma C-terminal FGF23 by ELISA; & urine Ca, P, Cr were measured. 12/59 pts (20.9%) were on P supplements for hypophosphatemia for age. 13/59 pts (25%) were prescribed calciferol, & 4/59 pts (7%), calcitriol.

Results: 17/59 pts (28.8%) had plasma FGF23>140 RU/ml (normal±2SD=69±39 in healthy controls in 2 ped studies). Plasma FGF 23 levels were not associated with gender, age, ethnicity, time since Tx, tubular reabsorption P (TRP), or serum P, iPTH or 1,25vitD. Elevation of plasma FGF23 was the only mineral metabolism abnormality associated with lower eGFR to CKD stage 2-3 (Table). Plasma FGF23 was higher in pts receiving SB vs. SF (206±207 RU/mL vs. 95±67 RU/ml, p=0.004).



eGFR (ml/min/1.73m ²)	Schwartz Serum (mg/dl)	Ca Serum (mg/dl)	PiPTH (pg/ml)	25vit D (ng/ml)	1,25vit D (pg/ml)	TRP (%)	FGF23 (RU/ml)
eGFR ≥90; n=35	9.9 (9.6,10.2)	4.1 (3.6,4.6)	62 (35,104)	19 (12,23)	67 (55, 85)	81 (87, 89)	73 (49,113)
eGFR 30-89; n=24	9.7 (9.4,9.9)	4.4 (3.2,4.7)	63(39,163)	22 (18,27)	47 (39, 84)	80-113* (80, 88)	(93,164)

Values as median (interquartile range); *p=0.0003, CKD 1 vs CKD 2-3



Conclusions: In ped renal Tx pts with CKD stages 2-3 plasma FGF23 is the earliest mineral metabolism marker of decreasing GFR. Elevated FGF23 is strongly associated with steroid-based transplant immunosuppression, suggesting an effect of steroids on FGF23 metabolism in ped renal Tx pts. Longitudinal study of this pediatric renal Tx population will be needed to assess whether early elevation of plasma FGF23 predicts increased risk for worsening graft function/loss.

OC25
Wnt/ β-catenin: A Candidate Pathway for Bone Repair in Neurofibromatosis Type-1

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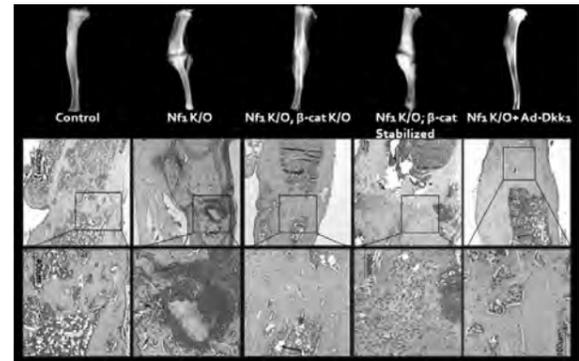
Objectives: Skeletal abnormalities occur in half of the individuals with neurofibromatosis type-1 (NF1), usually in the first two years of life; the most difficult to manage is congenital pseudarthrosis of tibia (CPT, tibial non-union) which may lead to the amputation of the limb. Previous work in our lab identified that high levels of β-catenin early in fracture repair, result in a phenotype similar to CPT. There is data showing higher than normal β-catenin levels in CPT samples from NF1 patients, therefore we hypothesized that Wnt/ β-catenin pathway mediates the effect of NF1 mutation on bone by altering the differentiation of mesenchymal stromal cells (MSCs) into osteoblasts.

Methods: Nf1^{fl/fl} mice were crossed with conditional β-catenin over-expressing and knock-out models. Adenovirus containing Cre was injected at the site of tibial fractures to both knock-out (k/o) Nf1 and modulate β-catenin levels. Fracture healing was evaluated using radiological, histological and biomechanical tests.

Results: Nf1^{-/-} tibial fractures were associated with higher cellular β-catenin levels early after fracture and did not heal after 21 days, exhibiting a fibro-cartilaginous phenotype at the site of fracture. Nf1^{-/-}; β-catenin k/o fractures showed repair and union with significantly decreased fibro-cartilaginous tissue. Nf1^{-/-}; β-catenin stabilized fractures did not heal and the amount of fibro-cartilaginous tissue increased significantly. Utilizing Dkk-1, as a specific Wnt/ β-catenin signaling inhibitor, at the site of Nf1^{-/-} fractures resulted in markedly improved bone formation and union of fractures.

Consistent with our *in-vivo* mouse models, human NF1 CPT samples contained increased β-catenin levels. Unlike wild-type bone marrow (BM) MSCs, cultured human NF1 and mouse Nf1^{-/-} BMSCs showed impaired osteoblastic differentiation which was either rescued or further inhibited by β-catenin signaling down- or up-regulation, respectively.

Conclusion: These data indicate that the level of β-catenin needs to be precisely controlled for normal osteoblastogenesis during fracture repair, and that in NF1 patients, this level is abnormally elevated, inhibiting osteoblastogenesis and bone formation. This study provides important insights into the pathways regulating impaired fracture healing in relation to NF1 patients and will assist the clinical management of non-unions healing.



OC26
Vertebral Fractures in the 3 Year Period Following Steroid Initiation among Children with Chronic Illnesses

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Objectives: To describe the incidence of vertebral fractures in steroid-treated children.

Methods: Fractures were assessed prospectively each year for 3 years according to the Genant semi-quantitative method. Proportions of children with incident fractures were determined annually over the study period. To examine associations with baseline clinical factors, the 3-year total number of incident fractures was analyzed using multivariable Poisson regression.

Results: 404 children were enrolled at a median age of 6.2 years, range 1-17; 50% boys; 188 (46%) had leukemia, 136 (34%) rheumatic conditions, and 80 (20%) nephrotic syndrome. The baseline study visit occurred at a median of 18 days following steroid initiation (inter-quartile range 11-24 days). Overall, the prevalence of vertebral fractures at baseline was 11% (95% Confidence Interval (CI) 8-14), and 19% of children (95% CI 15-24) had at least one incident fracture over the 3 years. Among those with incident fractures, 23/52 children (44%) had ≥1 moderate or severe fracture; in addition, 53/130 incident fractures (41%) were moderate or severe. Disease-specific results for baseline fracture prevalence and 3 year incidence were as follows: Leukemia: 16% (95% CI 11-21) and 25% (95% CI 19-33); rheumatic disorders: 7% (95% CI 4-12) and 14% (95% CI 9-22); nephrotic syndrome 8%



(95% CI 4-16) and 11% (95% CI 4-25). The annual proportion of children with incident fractures peaked at 12 months and declined thereafter (p=0.04). In Poisson multivariable modeling assessing *baseline* clinical factors, the following were associated with higher fracture incidence: prevalent fractures (incidence Rate Ratio (RR) 6.3, 95% CI 3.2-12.4), female gender (RR 1.8; 95% CI 1.0-3.3), pre-pubertal status (RR 2.1; 95% CI 0.8-5.4), and lower BMD Z-scores (RR 1.4; 95% CI 1.1-1.7).

Conclusions: Within 3 years of steroid initiation, 19% of children had incident vertebral fractures. Fracture incidence peaked at 12 months, and almost half of the incident fractures were moderate or severe. Of the clinical factors measured at *baseline*, prevalent fractures were most strongly associated with fracture incidence over the study period.

Funded by CIHR FRN 64285

OC27
Serum Vitamin D Level can Affect the Treatment Outcome of Whole-body Vibration (WBV) for Low Bone Mass in Girls with Adolescent Idiopathic Scoliosis (AIS)

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Objectives: AIS was associated with low bone mass which, apart from being an important health issue that could persist into adulthood, was also a significant prognostic factor for curve progression in AIS. We have performed a randomized controlled trial on WBV and reported its effect on increasing femoral neck areal bone mineral density (aBMD) mainly at the dominant leg. The objective of this study was to evaluate the role of Vit-D in moderating the anabolic bone effect of WBV.

Methods: This was a study nested within a randomized controlled trial, with enrolment of 122 AIS girls (15-25 years old) with BMD Z-scores < -1. They were randomly allocated to the Treatment or Control group. The Treatment group received WBV by standing on a low-magnitude high-frequency WBV platform 20 mins/day, 5 days/week (acceleration 0.3g, frequency 35 Hz). The Control group received observation alone. The study period was one year. aBMD at bilateral femoral necks was measured with Dual-Energy X-ray Absorptiometry at baseline and at 12-month. Serum 25(OH)Vit-D level by liquid chromatography tandem mass spectrometry was measured at 6-month within the treatment period.

Results: The mean age was 17.8(SD=1.5) years old and mean Cobb angle was 29.4(SD=8.8) degrees. Subgroup analysis for those with serum 25(OH)Vit-D>40nmol/L revealed not only the positive effects of WBV were greater at both sides, treatment effects were explicitly also noted at the non-dominant leg(Table 1). In addition, the positive correlation between serum 25(OH)Vit-D and percentage increase in femoral neck aBMD that was not present in the Control group was explicitly detectable in the Treatment group at the non-dominant leg(p=0.004).

Conclusion: The results strongly suggested the treatment effect of WBV could be enhanced through its synergistic factor interaction with Vit-D. The study carried significant clinical implication in that Vit-D insufficiency could affect negatively the treatment outcome of WBV for low bone mass in girls with AIS.

Funding Source: General Research Fund, Research Grants Council of Hong Kong (Project no: 467808 and 468809).

Table 1: Percentage change in femoral neck aBMD from baseline to 12-month follow up for the Treatment and Control group (SD: standard deviation, p from Independent samples t-test, *:p<0.05)

	Percentage change				
	Treatment group		Control group		p
	Mean	SD	Mean	SD	
All cases: n=61 (Treatment group), n=61 (Control group)					
Dominant femoral neck aBMD (g/cm ²)	2.15	4.32	0.14	3.67	0.007*
Non-dominant femoral neck aBMD (g/cm ²)	2.11	3.95	1.97	3.29	0.835
For those with serum 25(OH)Vit-D level <= 40 n=46 (Treatment group), n=42 (Control group)					
Dominant femoral neck aBMD (g/cm ²)	1.50	4.11	-0.17	3.37	0.039*
Non-dominant femoral neck aBMD (g/cm ²)	1.40	3.36	2.26	3.39	0.238
For those with serum 25(OH)Vit-D level > 40 n=15 (Treatment group), n=19 (Control group)					
Dominant femoral neck aBMD (g/cm ²)	4.14	4.48	0.83	4.28	0.035*
Non-dominant femoral neck aBMD (g/cm ²)	4.27	4.91	1.33	3.040	0.040*

OC28
Bone mineral density at diagnosis determines fracture rate in children treated according to the DCOG-ALL9 protocol

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Objectives: To elucidate the incidence and risk factors of skeletal toxicity in children with ALL treated with the dexamethasone-based DCOG-ALL9 protocol.

Methods: Prospectively, the cumulative incidence of fractures was assessed in 672 patients and compared between different subgroups using the Log-Rank test. Serial measurements of bone mineral density of the lumbar spine (BMD_{LS}) were performed in 399 ALL patients using dual energy X-ray absorptiometry (DXA). We evaluated risk factors for a low BMD using multivariate regression. Osteoporosis was defined as having a BMD_{LS} ≤ -2 SDS combined with clinical significant fractures.

Results: The cumulative incidence of fractures at 3 years was 17.8%. At diagnosis, mean BMD_{LS} of ALL patients was lower than that of healthy peers (mean BMD_{LS} = -1.10 SDS, P<0.001), and this remained significant lower during and after treatment (8 months: BMD_{LS} = -1.10 SDS, P<0.001; 24 months: BMD_{LS} = -1.27 SDS, P<0.001; 36 months: BMD_{LS} = -0.95 SDS, P<0.001). Multivariate linear regression analysis showed that age at diagnosis, weight and B-cell-immunophenotype were associated with a lower BMD_{LS} at diagnosis. After correction for weight, height, gender and immunophenotype, stratification to the HR protocol arm and older age had a significant independent larger decline of BMD_{LS} during treatment (HR group: b=-0.52, P<0.01 and age: b=-0.16, P<0.001). Cumulative incidences of fractures between ALL risk groups and age groups were not significantly different. Patients who developed fractures had a lower BMD_{LS} during treatment than those without fractures. Treatment-related bone loss was similar in patients with and without fractures (respectively: ΔBMD_{LS} = -0.36 SDS and ΔBMD_{LS} = -0.12 SDS; interaction group time, P=0.295). Twenty of the 399 patients (5%) met the criteria of osteoporosis.

Conclusion: This large prospective study shows that a low absolute value of BMD_{LS} both at diagnosis and during treatment, rather than the



treatment-related decline of BMD_{LS}, determines the markedly increased fracture risk of 17.8% in children with ALL.

OC29

Mineral Metabolism, Cortical Volumetric Bone Mineral Density and Fracture Risk in Childhood Chronic Kidney Disease

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Background and Objectives: The relations among cortical volumetric bone mineral density (CortBMD) and comprehensive measures of mineral metabolism have not been addressed in chronic kidney disease (CKD). The aim was to identify determinants of CortBMD in childhood CKD. A secondary objective was to assess if CortBMD was associated with subsequent fracture.

Design/participants: This prospective cohort study in children, adolescents and young adults (ages 5-21 yr) with CKD stages 2-5D included 171 participants at enrollment and 89 one year later.

Outcomes: Serum measures included vitamin D [25(OH)D, 1,25(OH)₂D, 24,25(OH)₂D], vitamin D-binding protein (DBP), intact parathyroid hormone (iPTH), fibroblast growth factor 23 (FGF23), calcium, and phosphorus. Tibia pQCT measures of CortBMD were expressed as sex-, race-, and age-specific Z-scores based on 675 controls. Multivariable linear regression identified independent correlates of CortBMD-Z and change in CortBMD-Z. Cox regression analysis, adjusted for sex and age, was used to determine if baseline CortBMD-Z was associated with fracture (hazard ratio for time to first fracture).

Results: Lower calcium ($\beta=0.31/1$ mg/dl, $p=0.01$) and 25(OH)D ($\beta=0.18/10$ ng/ml, $p=0.04$) and higher PTH ($\beta=-0.02/10\%$, $p=0.002$) and 1,25(OH)₂D ($\beta=-0.07/10\%$, $p<0.001$) were independently associated with lower baseline CortBMD-Z. The correlations of total, free and bioavailable 25(OH)D with CortBMD did not differ. Higher baseline 1,25(OH)₂D ($p<0.05$) and greater increases in PTH ($p<0.001$) were associated with greater declines in CortBMD-Z (Table). Greater increases in calcium concentrations were associated with greater increases in CortBMD-Z in growing children (interaction $p=0.009$). The hazard ratio for fracture was 1.75 (95% CI: 1.15, 2.67; $p=0.009$) per SD lower baseline CortBMD-Z.

Conclusions: Greater PTH and 1,25(OH)₂D and lower calcium concentrations were independently associated with baseline and progressive cortical deficits in childhood CKD. Lower CortBMD-Z was associated with increased fracture risk.

Final multivariate model of determinants of change in CortBMD-Z*

Covariate	Beta	95% CI	p-value
Change in tibia length*	-1.21	-2.06, -0.37	0.006
Change in calcium*	-0.78	-1.58, 0.01	0.053
Change in calcium by change in tibia length interaction	0.45	0.12, 0.79	0.009
Change in PTH*	-0.26	-0.37, -0.14	<0.001
Baseline 1,25(OH) ₂ D*	-0.07	-0.13, -0.0003	0.049

Also adjusted for age, study site, baseline CortBMD-Z, baseline calcium, and change in renal function. *Natural log transformed.

OC30

Alteration of Wnt/Beta-Catenin Signaling in HIV-Infected Youths: A Mechanism Leading to Impaired Bone Health?

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Objectives: Impairment of bone mass accrual and alterations of bone metabolism is a common finding in HIV-infected youths. In particular, previous studies demonstrated higher bone formation and bone resorption rates in HIV-infected children and adolescents. Wnt ligands promote bone formation by stimulating osteoblast differentiation and their survival. Recent studies demonstrated that Sclerostin (Scl) and dickkopf factor 1 (DKK-1), Wnt antagonists, are important negative regulators of bone formation. The aim of the study was to measure serum concentration of Scl and DKK-1 in young HIV-infected patients.

Methods: We studied 86 HIV-infected patients whose ages ranged from 5.7 to 27.9 years. Their mean CD4 number was 674 (SD 274) and their CD4 % was 33.2 (9.5). Patients were all on highly active antiretroviral therapy (HAART), but 7 who were naïve to antiretroviral treatment. Scl and DKK-1 were measured in serum by enzyme immunoassay. Bone mass was measured by dual-energy x-ray absorptiometry at the lumbar spine and in the whole skeleton. We also measured Scl and DKK-1 concentration in 105 healthy youths (age range 4.5 to 17.7 years). The values of young adult patients (>20 years) have been compared with published reference data.

Results: HIV-infected patients had lower than normal bone mineral density measurements (spine $P<0.005$, and whole skeleton $P<0.03$). Serum concentration of Scl of HIV-infected children and adolescents were lower than in controls (25.0 [11.5] vs. 34.2 [16.3] pmol/L, respectively; $P=0.003$). Similarly, DKK-1 concentration was lower in these patients than in controls (19.2 [12.5] vs. 22.5 [14.1] pmol/L, respectively; $P=0.05$). HIV-infected young adults showed both Scl and DKK-1 serum concentration (19.1 [12.1] and 15.6 [7.4] pmol/L, respectively), lower than reference ($P<0.001$). The serum concentration of both analytes of patients naïve to antiretroviral treatment was not different from that of HAART-treated patients. No correlations were found between Scl, DKK-1 and bone mineral measurements.

Conclusion: Our data confirm the alteration of bone metabolism pathways in HIV-infected individuals, possibly leading to reduced bone mineral density. The increased concentration of Wnt antagonists is consistent with the increased bone formation markers observed in previous studies.

OP1

Skeletal effects of Hypothyroidism are mediated by Thyroid Hormone Receptor

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Childhood hypothyroidism results in delayed skeletal maturation and impaired growth. Thyroid hormones act via temporo-spatially regulated thyroid hormone receptors α (TR α) and β (TR β).

In the skeleton, TR α is the predominant receptor and we hypothesise that the skeletal effects of hypothyroidism are mediated by TR α .

To investigate this we assessed the response of wild type (WT), TR α knockout (TR $\alpha^{0/0}$) and TR β knockout (TR $\beta^{-/-}$) mice to hypothyroidism. Adult mice from each genotype were rendered/remained hypothyroid (hypo) or euthyroid (eu) for six weeks before skeletal phenotyping.

WT hypo mice had increased bone mineral content (BMC) by 26kV point projection digital x-ray microscopy analysis compared to eu



controls. ($p<0.001$, $n=6$). Trabecular bone volume (BV/TV) by μ CT (4.3 μ m voxel) was elevated in WT hypo mice compared to controls (15.6 \pm 1.3% vs 10.9 \pm 1.1%, mean \pm SEM, $n=5-6$, $p<0.05$ t-test). Bone formation rate (BFR) in WT hypo mice was reduced compared to controls (0.04 \pm 0.03 μ m³/ μ m²/day vs 0.45 \pm 0.04 μ m³/ μ m²/d mean \pm SEM, $p<0.001$ students t-test, $n=4$). TR $\beta^{-/-}$ hypo mice also had increased BMC ($p<0.001$, $n=6$) and BV/TV (13.4 \pm 0.8% vs 7.7 \pm 0.8% $p<0.001$, $n=5-6$) and BFR was similarly reduced compared to controls (0.03 \pm 0.01 μ m³/ μ m²/d vs 0.33 \pm 0.06 μ m³/ μ m²/d, $p<0.01$, $n=4$). In contrast to WT and TR $\beta^{-/-}$ mice, TR $\alpha^{0/0}$ hypo mice had similar BMC ($p=ns$, $n=6$) and BV/TV (12.1 \pm 0.5% versus 13.1 \pm 0.4%, $p=ns$, $n=5-6$) compared to controls. BFR was reduced in TR $\alpha^{0/0}$ hypo mice compared to controls (0.18 \pm 0.07 μ m³/ μ m²/d, 0.53 \pm 0.04 μ m³/ μ m²/d, $p<0.001$ $n=4$). In summary, WT and TR $\beta^{-/-}$ mice had similar responses to hypothyroidism resulting in increased BMC and BV/TV and reduced BFR. In contrast, TR $\alpha^{0/0}$ mice showed no change in BMC or BV/TV. BFR in TR $\alpha^{0/0}$ hypo mice, although reduced compared to controls, was fourfold higher than WT hypo or TR $\beta^{-/-}$ hypo mice.

In conclusion, these studies indicate that TR α has a key role in the skeletal response to hypothyroidism.

Funded by a MRC clinical research training grant.

OP2

High FSH serum levels may support the altered bone remodeling in Turner Syndrome patients.

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Objective: Turner Syndrome (TS) is a chromosomal aberration characterized by total or partial loss of one of the two X-chromosomes, and affects about 1 in every 2,500 girls. TS patients can develop the bone disease with decreased bone density and selective reduction in cortical bone thickness, which probably contributes to the increased fracture risk. However, the mechanisms underlying the bone disease remain poorly understood. Thus, the aim of this study was to investigate the osteoclastogenic potential of unfractionated peripheral blood mononuclear cells (PBMCs) and T cell-depleted PBMC cultures from TS patients (mean age 10.44 \pm 3.48) with high or normal FSH serum level and controls.

Methods: PBMCs and T-cell depleted culture from patients and controls were cultured in presence/absence of M-CSF and RANKL, or neutralizing antibodies anti-RANKL and anti-TNF-alpha. At the end of the culture period, mature multinucleated OCs were identified as TRAP+ cells. By real-time PCR we studied gene expression in freshly isolated T cells and monocytes. ELISA were performed in sera and media.

Results: Spontaneous formation of active resorbing osteoclasts, without adding M-CSF and RANKL, occurred in PBMC cultures from TS patients with elevated FSH serum levels. Conversely, M-CSF and RANKL were essential to trigger and sustain osteoclastogenesis in PBMCs from controls as well as TS patients with normal FSH serum levels. T-cell depleted PBMC cultures from TS patients with high FSH serum level showed only a partial reduction of spontaneous osteoclast formation, suggesting that both monocytes and T cells have an important role supporting the elevated osteoclastogenesis. In fact, in these patients, the percentage of circulating osteoclast precursors (OCPs) is increased and monocytes expressed elevated c-fms, IL-1, TNF-alpha and RANK levels, whereas T cells showed high RANKL amounts. Moreover, elevated amount of RANKL were detected in PBMC culture media and sera from TS patients with high FSH circulating levels by ELISA. Finally, functional anti-RANKL and anti-TNF-alpha antibodies significantly inhibited osteoclastogenesis.

Conclusion: We showed for the first time the high osteoclastogenic potential of PBMCs from young TS patients with high FSH circulating

levels. This condition could contribute to the bone disease that become evident in their adult life.

OP3

Calcimimetics as adjunct therapy in XLH: 18 months experience

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Objective: based on our preliminary findings, indicating a potential for Cinacalcet [due to its suppression of PTH (CJASN 2008;3:658)], to allow the use of lower doses of oral phosphate (OP) and calcitriol in treating XLH, the objective of this study was to examine its long-term effect as adjunct treatment in children with XLH.

Methods: Eight children (F5), ages 7.8-20.9y (median 13.9), who were already treated by OP and calcitriol were enrolled in the study. Cinacalcet was added at 30mg once daily to those weighing \leq 30kg and 60mg to those $>$ 30kg. Clinic visits were done every 3m; dose of medications adjusted to maintain serum P similar to baseline, PTH within its normal range and Ca⁺⁺ $>$ 1.00 mmol/l. In case of hypercalciuria ($>$ 4mg/kg/24h) a thiazide/amiloride preparation was added. Blood was checked for creatinine, Ca⁺⁺, P, Alk. Phosph., PTH and FGF23; and urine for TP/GFR and Ca excretion. Radiographs of lower joints were done every 6 months and renal ultrasound annually.

Results: Follow-up lasted 21.2 \pm 5.3m (median 18).

	Start	End	p
Calcitriol dose (ng/kg/24hr)	22.0 \pm 12.0	12.5 \pm 8.9	<0.001
K-Phos dose (mg/kg/24hr)	40.4 \pm 24.4	23.3 \pm 20.7	<0.01
PTH (pg/ml)	45.0 \pm 38.8	21.9 \pm 16.3	<0.05
Ca ⁺⁺ (mmol/l)	1.20 \pm 0.06	1.06 \pm 0.07	<0.005
P (mg/dl)	3.01 \pm 0.31	3.10 \pm 0.41	NS
Alk. Phos. (u/l)	242 \pm 160	196 \pm 120	0.06
FGF23 (RU/ml)	272 \pm 71	197 \pm 82	<0.005
TP/GFR (mg/dl)	2.24 \pm 0.33	2.49 \pm 0.41	<0.005
Urine Ca (mg/kg/24hr)	1.9 \pm 1.4	1.1 \pm 0.7	NS

Rickets stayed healed in all. Nephrocalcinosis grade 1 was present at baseline and did not change in 1 child, and in another developed after 2 years into the study. Serum creatinine was normal in all. At the end of the study, 7 children were receiving Cinacalcet 30mg, and the oldest 60mg. 3 children had their growth plates closed at start. Height SDS improved in 2 patients, were unchanged in 2 and decreased in 1. One child was on thiazide/amiloride at baseline and in 3 it was added during the study. No side effects were observed and none of the patients discontinued the study.

Conclusions: The addition of the calcimimetic: (1) resulted in significant decreases in PTH and FGF23 and increase in TP/GFR enabling significant decreases in calcitriol and OP doses (2) requires careful attention to serum and urine calcium. Further studies are warranted exploring the beneficial effects of calcimimetics in the treatment of children with XLH

OP4

A new start-codon in IFITM5 causes Osteogenesis Imperfecta Type V

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Background: Osteogenesis imperfecta (OI) is a rare disease characterized by increased fracture rate and bone deformities. Patients are classified by phenotype and most are affected by mutations in COL1A1/2.

Patients with OI type V present with specific clinical symptoms including hyperplastic callus formation, only mildly decreased height,



metaphyseal lines and a calcification of the membrane interossea of the forearm. The disease causing mutation for OI type V was unknown till now.

Objective: Identification of the mutation causing OI type V.

Patients/ Methods: In one patient (age 4.8 years) with clinical signs of OI type V we performed “whole exome sequencing” to identify the underlying mutation. Additionally, the phenotypical healthy parents were analyzed for „*de novo* mutations“. A second patient with clinically proven OI type V (15.1 years of age) and his unaffected parents were analyzed per Sanger sequencing.

Results: Using whole exome sequencing we identified a „*de novo* mutation“ in the 5’-UTR region of IFITM5, a bone-specific gene. The mutation was 14 bp above the regular startcodon. Exactly the same mutation was found in our second patient. Therefore this mutation could be described as the causative mutation for OI type V. This mutation leads to a new startcodon. Further functional analysis could proof that this codon is predominantly used in vitro. The additional 5 aminoacids (M-A-L-E-P) will be added to the den N-terminus and change its function as a transmembrane receptor interacting with the osteoblastogenesis.

Conclusions: Using whole exome sequencing and Sanger sequencing we were able to identify the molecular reason for OI type V. The dominant mutation leads to the creation of a new startcodon, which prolong the resulting transmembrane receptor IFITM5 by 5 aminoacids. By now for all clinically defined types of OI the molecular reasons are known.

OP5

Abstract withdrawn

OP6

Children with nephrotic syndrome have increased tibial bone area but similar volumetric bone mineral density to healthy controls

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Objectives: An increased fracture risk is reported in children requiring recurrent courses of glucocorticoids. Reduced bone mineral density (BMD), particularly in the trabecular compartment, has also been demonstrated in a number of childhood diseases treated with glucocorticoids. The differential contribution of glucocorticoids and underlying inflammatory disease to bone demineralisation is poorly understood. Childhood nephrotic syndrome (NS) often follows a relapsing-remitting course, requiring multiple steroid courses, but with low systemic inflammation during remission. We therefore used peripheral quantitative computed tomography (pQCT) to investigate compartmental volumetric BMD and bone geometry in NS, and evaluated the influence of treatment factors on bone outcomes.

Methods: Children with NS (n=29, 55% male, age 10.7±3.1years) were compared to healthy controls (n=29, 55% male, age 11.0±3.0years). Body composition was assessed by whole body DXA. pQCT scans were obtained at metaphyseal (4%) and diaphyseal (66%) sites of the tibia. Lifetime cumulative glucocorticoid exposure was calculated from medical records. Bone outcomes were adjusted for age, gender, height, ethnicity and pubertal status using linear regression.

Results: Children with NS had similar height standard deviation score (SDS) to controls (p=0.28), but were heavier (0.65±1.28SDS vs -0.04±0.89SDS, p=0.022) and had greater body fat SDS (0.31±1.01 vs -0.52±1.10, p=0.008). Tibial trabecular and cortical vBMD were similar between the two groups but bone cross-sectional area (CSA) was significantly greater in children with NS at both the metaphysis

(954±234 mm² vs 817±197mm², p=0.002) and diaphysis (511.0±1.4mm² vs 442.1±1.4mm², p=0.006). Endosteal and periosteal circumferences were greater in children with NS than controls (both p<0.01), resulting in reduced cortical thickness (2.4±0.7mm vs 2.8±0.7mm, p=0.018), but similar cortical CSA (p=0.22). The differences in cortical geometry were not statistically significant when weight was included as a confounding factor. There were no associations between cumulative steroid exposure, duration of NS or number of relapses and any bone parameter.

Conclusions: Tibial bone CSA is increased in children with NS. We speculate this is a compensatory response to increased body weight. Defects in trabecular BMD were not identified in this cohort of children with NS.

OP7

Bone Mineralization Density Distribution, bone formation rate and bone turnover markers in a cohort of children with CKD before and after growth hormone treatment.

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Background and Methods: A specific skeletal complication in children with CKD is growth impairment. The effects of recombinant human growth hormone (rhGH) therapy on bone turnover and bone material quality are not well understood. Bone matrix mineralization density distribution (BMDD) as assessed by quantitative backscattered electron microscopy (qBEL) is an important determinant of bone material quality and reflects bone turnover (average tissue age) and mineralization kinetics of the individual bone packets of the sample.

We have recently evaluated the BMDD in cancellous and cortical bone in paired transiliac biopsies (before/after one year rhGH) in short children on dialysis (AJKD *in press*) and in the present study we extended the cohort to 20 pediatric patients (CKD stage 4: n=2; end-stage renal disease treated by dialysis: n=18). Bone histomorphometry was performed and all biopsies were classified according to the new TMV system.

Results: Prior treatment, strong associations between the mean bone matrix mineralization and bone turnover were found: bone matrix mineralization was higher while BFR/BS was lower compared to reference data.

After rhGH-treatment, the mean height acquisition was about 8.5 cm, serum ALP levels and bone turnover indices were significantly increased compared to baseline, and bone matrix mineralization in cancellous and cortical compartments was decreased towards normal range. A striking exception was the case of an adolescent with difficulties to adhere to therapy, who developed severe hyperparathyroidism and osteitis fibrosa concomitantly with abnormally low bone matrix mineralization.

Conclusion: our data show that bone turnover rate is a strong predictor of the BMDD in young patients with CKD and growth deficiency. At baseline, our cohort had low bone turnover and, increased bone matrix mineralization, indicating further normal mineralization kinetics in these patients with CKD. The data suggest that rhGH treatment in children with CKD does not only increase height but also bone turnover, which appears beneficial for bone matrix mineralization.



OP8

Quantitative Measurement of Abnormal Bone Quality and Strength with Bone Micro-architecture and Rod-plate Configuration in Osteopenic Adolescent Idiopathic Scoliosis (AIS)

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Objectives: Multiple studies have documented the presence of systemic osteopenia in AIS and in simulated animal models. Osteopenia was also found to be associated with severe curves and as one of the prognostic factor in predicting curve progression in AIS. The objective of this study was to compare the bone quality and its association with osteopenia between AIS and normal controls.

Methods: AIS (n=234) and non-AIS girls (n=211) between 11-13 years old were recruited. aBMD of bilateral femoral necks was measured by DXA. Subjects were classified into the osteopenic (Z-score≤-1) and non-osteopenic (Z-score>-1) subgroup. Trabecular Bone Micro-architecture and Structural Model Index (SMI), which measures the degree of rod/plate-like configuration of trabeculae, were measured using HR-pQCT.

Results: In AIS, osteopenic group showed a lower Trabecular Thickness, greater Trabecular Separation and SMI, reflecting more rod-like trabeculae when compared to the normal BMD AIS subjects. In contrast, among the controls, only lower BV/TV and Trabecular Thickness were found between the osteopenic and normal BMD subgroups. Two-way ANOVA showed a significant interaction between AIS and osteopenia on Trabecular Thickness.

Conclusions: We revealed a unique alteration of trabecular bone micro-architecture in osteopenic AIS. Besides, there is an interaction between osteopenia and AIS on Trabecular Thickness, which indicates an important role of trabecular bone micro-architecture in the etiopathogenesis of AIS. The % difference in SMI between osteopenic and non-osteopenic AIS (15.6%) was highest among all the parameters. One of the clinical significance was the call for developing a composite prognostic factor incorporating both BMD and SMI for more accurate prediction of curve occurrence and progression in AIS in clinical practice.

This study was supported by Research Grants Council of the Hong Kong S.A.R., China (Project no: 468809 and 468411)

OP9

Anticalciuric Effect of Recombinant PTH in Patients with Activating Mutations of the Calcium-Sensing Receptor Causing Autosomal Dominant Hypocalcaemia-Hypercalciuria (ADHH)

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Background: Most patients with hypoparathyroidism are controlled under conventional treatment with calcium and vitamin D analogues. However, this treatment may be difficult to manage, especially in patients with ADHH who have an increased risk of nephrocalcinosis and chronic renal insufficiency. ADHH is caused by activating mutations in the calcium-sensing receptor (CaSR) resulting in suppressed PTH secretion and decreased calcium reabsorption within the thick ascending limb of loop of Henle. The CaSR modulates urinary calcium reabsorption through a PTH-independent mechanism.

Aim: Evaluate the efficacy of rPTH¹⁻³⁴ as an alternative to vitamin D analogue therapy for ADHH patients, in particular regarding the prevention of hypercalciuria and nephrocalcinosis.

Patients: Four patients, three toddlers (8, 18 and 30 months old; P1, P2, P3) and one young adult (19 years old, P4) with ADHH and CaSR mutations, received rPTH¹⁻³⁴ by continuous subcutaneous infusion via an insulin pump. The observed duration of therapy was 2 to 8 months (ongoing in all patients), with a mean daily dose of rPTH¹⁻³⁴ 0.54, 0.57 and 0.37µg/kg/day in the toddlers and 0.20 µg/kg/day in the adult patient. Three additional patients received rPTH¹⁻³⁴ (n=1, 48 months 0.75µg/kg/day, twice daily injections) of rPTH¹⁻⁸⁴ for 6 and 19 months, (n=2, 100µg/day). Additional treatments received were adjusted calcium supplements and cholecalciferol (none received vitamin D analogues after switching to rPTH).

Results: On rPTH therapy mean serum calcium levels increased in P1, P3 and P4 respectively from 1.4±0.15 to 2.1±0.25 mmol/L (p<0.05), 1.5±0.3 to 1.8±0.5mmol/L (ns) and from 2.2±0.3 to 2.38±0.3 mmol/L (ns). P2 decreased her calcemia from 2.13±1.8 to 1.95±0.1 mmol/L (p<0.05). All four patients showed a decrease in mean urinary calcium excretion respectively from 1.8±0.9 to 0.5±0.5mM/mM (ns), 2.4±0.5 to 1.1±0.4 mM/mM (p<0.05), 1.3 to 0.6±0.26mM/mM (ns) and from 4.8±3.4 to 3±0.9mmol/L (ns) in P1, P2, P3 and P4. When the 3 toddlers are analyzed as one group, the mean calcium level increased from 1.8±0.4 to 1.95±0.2 mmol/L (ns) and the mean urinary calcium excretion decreased from 2.1±1 to 1±0.5 mM/mM (p<0.05).

Conclusion: Our data show that rPTH allows the maintenance of serum calcium at near-normal levels in ADHH and correction of the clinically severe manifestations of hypocalcaemia. More importantly, even with near-normal blood calcium, rPTH had a significant anticalciuric effect, i.e. decreased significantly the urinary calcium excretion in ADHH patients, likely preventing or delaying renal damage. Treatment was safe and well tolerated.

OP10

Metacarpal width, length, medullary diameter and cortical thickness in Hypophosphatemic Rickets

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Hand X-rays from patients with hypophosphatemic rickets (HR) were assessed to evaluate if HR influences the dimensions of the metacarpal bones and the distribution between cortical and cancellous bone. In addition, we aimed to test the hypothesis that HR caused by mutation in *DMP1* has a greater impact on bone dimensions than HR caused by mutations in *PHEX*.

Hand X-rays from 17 children with HR were evaluated. Three children had HR caused by a *DMP1* mutation, and the remaining 14 had a verified *PHEX* mutation. BoneXpert® (Visiana) was used to calculate the dimensions of the second, third and fourth metacarpal bones. HR patients were compared with age- and gender matched healthy children from Switzerland. For the whole HR group, data are reported as mean Z-scores (95% CI) and p value, followed by the mean Z-score in subgroups of patients with a *PHEX* mutation followed by patients with a *DMP1* mutation.

Overall, patients with HR had significantly broader metacarpal bones, +2.5 (95% CI 1.7–3.2) SD, p<0.001, (2.0; 4.6) SD, with a wider medullary diameter of +2.4 (95% CI 1.8-2.1) SD, p<0.001, (2.1; 4.2) SD and a reduced cortical thickness of -0.6 (95% CI -1.3 to -0.3) SD, p<0.02, (-0.8; 0.3) SD. The metacarpal length of + 0.7 (0.2-1.2) SD, p=0.3, (0.4; 1.9) SD, was not different compared with controls (Fig 1).

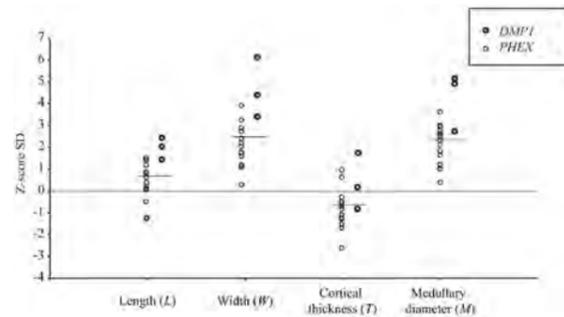


Figure 1: Distribution of metacarpal dimensions in children with PHEX and DMP1 mutations, respectively.

Children with HR have significantly broader metacarpal bones, a wider medullary diameter, and decreased cortical thickness. The metacarpal bones in children with a *DMP1* mutation appear longer and broader with a wider medullary diameter compared to children with a *PHEX* mutation. Our data suggest that *DMP1* mutations affect bone size more severely than *PHEX* mutations.

OP11

Artistic Gymnasts Display Micro-architectural Advantages Over Trampolining and Tumbling Gymnasts: A High Resolution Peripheral Quantitative Computed Tomography Study

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Participation in high impact sports during growth increases bone quality. Gymnasts have previously displayed increased bone mass and strength at both the upper and lower limbs compared with controls. However, it is not yet understood how bone microarchitecture is affected by gymnastics participation, and if this differs based on gymnastics discipline. Therefore, the objective of this study was to investigate the influence of gymnastics discipline on bone microarchitecture in a youth cohort using high-resolution peripheral quantitative computed tomography (HR-pQCT).

Seven artistic and 18 trampolining and tumbling (T&T) gymnasts were recruited. Gymnasts were male and female 16-24 years. HR-pQCT was used to determine bone microarchitecture, specifically total volumetric bone mineral density (Tt.BMD), cortical BMD (Ct.BMD), trabecular BMD (Tb.BMD), total area (Tt.Ar) and cortical thickness (Ct.Th) of the radius and tibia. Finite element analysis estimated apparent bone strength. Muscle strength was determined by grip strength and Biodex dynamometers. The gymnastics-specific non-dominant limb was used for all procedures. Independent sample T-tests and two-way ANOVAs compared group means.

At the radius, artistic gymnasts had bigger bones (Tt.Ar +30%), greater bone density (Tt.BMD +18%; Tb.BMD +36%), higher Ct.Th (+12%) and superior bone strength (+57%) than T&T gymnasts (p<0.05). While there was a trend for artistic gymnasts to have enhanced microarchitectural parameters at the tibia, significant differences between groups did not emerge (p>0.05). Furthermore, gymnasts displayed enhanced bone quality compared to our normative population. There were no significant muscle strength differences between gymnastics groups, and no interaction effect for gymnastics discipline and sex (p>0.05).

Compared with T&T gymnasts, artistic gymnasts had bigger, stronger and denser bones at the radius. Trends for advantageous microarchitectural parameters displayed in these gymnasts are likely the

result of the increased mechanical loading observed at the wrist. While both gymnastics disciplines are associated with high ground reaction forces on landings, the trampoline likely dissipates the ground reaction forces observed at the wrist of T&T gymnasts. In addition, many T&T skills do not involve wrist contact with the trampoline or sprung floor. In conclusion, gymnastics discipline influences bone microarchitecture on a skeletal site-specific basis at the radius, but not the tibia.

OP12

The bone/vessels interplay in teenagers with chronic kidney disease

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Background: Bone fragility and vascular calcifications are to the two main morbidities of the mineral and bone disorders associated with chronic kidney disease (CKD), resulting from a combination of abnormalities such as impaired growth hormone axis, vitamin D deficiency, hyperparathyroidism, increased FGF23 levels, hypogonadism, denutrition and drug toxicity.

Methods: In a single-centre ancillary study of the longitudinal prospective European 4C study (Cardiovascular Comorbidities in Children with CKD), we evaluated bone and cardiovascular status in 22 teenagers with pre-dialysis CKD. We used the tridimensional non-invasive high resolution peripheral quantitative computed tomography (HR-pQCT, SCANCO®), as well as pulse wave velocity (PWV), carotid intima-media thickness (IMT) and ambulatory blood pressure (BP) monitoring (ABPM). Statistical analyses were performed with non-parametric tests (SPSS).

Results: At a mean age of 14.1±2.7 yrs (skeletal age 12.8±2.5 yrs, 16 boys), average height and BMI were 152±15 cm and 18.9±2.5 kg/m² corresponding to SDS of -0.8±1.2 and 0.0±1.0, respectively. Median serum phosphorus was 1.41(0.90-2.58) mmol/L for an average GFR of 32±10 mL/min/1.73m². PTH and 25OH-vitamin D levels were 107 (29-359) pg/mL and 66±18 nmol/L, respectively. Bivariate analyses between bone and cardiovascular data showed strong positive associations between trabecular densities/thickness and BP (24-hr and daytime ABPM results for systolic, diastolic and mean BP), the Spearman correlation coefficient ranging from 0.46 to 0.65 (p<0.05). Trabecular thickness was also strongly associated with PWV (r=0.598, p=0.003). In contrast, IMT results were not associated with any bone parameter.

Conclusion: For the first time, these results suggest a significant interplay between trabecular bone and BP in teenagers with pre-dialysis CKD: the greater the trabecular thickness, the greater the PWV and ABPM parameters, and thus the greater the arterial stiffness. These data seem conflicting with previously published studies in adults showing increased vascular calcifications with decreased bone densities as assessed by DXA. In pediatric CKD however, one may hypothesize that the use of calcium therapies may be efficient for improving bone quality while deleterious for vessels.

OP13

Long-term effects of bisphosphonate therapy in children with osteogenesis imperfecta

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Objectives: To evaluate the clinical outcomes of intravenous bisphosphonate treatment in children with mild-moderate osteogenesis



imperfecta (OI) who had progressed from active bisphosphonate treatment to maintenance therapy for greater than two years.

Methods: A retrospective review was conducted on 17 patients with mild-moderate OI. Clinical data, fracture history, biochemistry, dual energy x-ray absorptiometry (DXA) parameters, vertebral measurements, bone age and metacarpal cortical thickness were collected at three time points: Before treatment, following active treatment with high dose bisphosphonates and after establishment on a low dose maintenance treatment phase. Active treatment was defined as zoledronic acid 0.05mg/kg six-monthly or pamidronate 6-9mg/kg/year. Maintenance treatment was defined as zoledronic acid 0.025mg/kg six-monthly or pamidronate less than 4mg/kg/year.

Results: The mean age at commencement of active treatment was 4.8±2.6 years and progression to maintenance therapy was 10.0±2.6 years. Mean time on maintenance therapy was 4.1±1.4 years. Height Z-scores did not change significantly over time but weight z-scores during active and maintenance treatment were higher than pre-treatment levels (see Table). There was a significant reduction in fracture rate when active treatment was commenced. This improvement was maintained during maintenance treatment. Biochemical analysis of bone homeostasis revealed a significant reduction in bone turnover markers between active and maintenance treatment.

DXA showed a significant improvement in bone mineral density (BMD), bone mineral content (BMC) and BMC for lean-tissue mass Z-scores. Vertebral height increased in both normal lumbar vertebrae (L1-L4) and fractured thoracic and lumbar vertebrae from pre-treatment to active therapy and was maintained during maintenance treatment. Assessment of hand x-rays showed that 2nd metacarpal cortical thickness and relative cortical area increased over the treatment periods.

	Pre-treatment	Active	Maintenance
Height Z-score	-1.4 ± 1.6	-1.3 ± 1.7	-1.7 ± 2.4
Weight Z-score	-1.2 ± 1.6	0.1 ± 1.5 *	0.0 ± 1.6 *
Fracture rate (number/year)	1.5 ± 1.1	0.7 ± 0.7 *	0.7 ± 0.9 *
Calcium (nmol/L)	2.43 ± 0.10	2.35 ± 0.07	2.30 ± 0.07
Alkaline phosphatase (U/L)	277 ± 63	210 ± 46 *	164 ± 74 *
Phosphorus (mmol/L)	1.61 ± 0.17	1.50 ± 0.13 *	1.40 ± 0.15 *
Osteocalcin (nmol/L)	7.4 ± 3.7	7.8 ± 6.2	3.4 ± 3.0 *
25-OH-Vitamin D (nmol/L)	75 ± 32	69 ± 22	62 ± 17
Urine Deoxypyridinoline:Cr ratio (nM/mM)	115 ± 75	117 ± 89	57 ± 52
Total BMD Z-score	-0.9 ± 1.2	-0.6 ± 1.2 *	-0.8 ± 1.8
L1-L4 BMD Z-score	-2.5 ± 1.2	-0.5 ± 1.3 *	-0.3 ± 1.1 *
Total BMC Z-score	-1.1 ± 0.6	-0.7 ± 1.5 *	-0.9 ± 1.9 *
BMC for LTM Z-score	-1.4 ± 1.1	0.2 ± 1.7 *	0.5 ± 1.5 *
Vertebral height (anterior:length ratio)	0.70 ± 0.06	0.76 ± 0.07 *	0.76 ± 0.09 *
2 nd Metacarpal relative cortical area	8.6 ± 4.0	14.6 ± 4.9 *	21.8 ± 6.5 *

Table: Mineral homeostasis, DXA data and bone morphometry

Values represent mean±SD, asterisk represents p<0.05 compared to pre-treatment values

Conclusion: Maintenance intravenous bisphosphonate therapy preserved the beneficial effects of a high dose active treatment regimen. Further studies are required to determine the optimal bisphosphonate treatment regimen in the management of children with OI.

OP14

Inflammation and Glucocorticoid Therapy Impair Skeletal Modeling during Growth Following Crohn Disease Diagnosis

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Objectives: Examine changes in volumetric BMD and cortical structure following Crohn Disease (CD) diagnosis, and identify associations with growth, glucocorticoid exposure and disease activity.

Methods: Prospective cohort study in 76 CD participants, ages 5-21 years. Tibia pQCT scans were obtained at diagnosis, 6, 12 and a median of 42 months later. Sex, race and age-specific Z-scores were generated

based on >650 controls. Cortical dimension Z-scores were adjusted for tibia length-for-age Z-score. Generalized estimating equations were used to identify correlates of changes in Z-scores.

Results: Disease activity improved markedly over the study interval (Table). Trabecular BMD Z-scores increased significantly: improvements were greater over the first 6 months and in younger children (p=0.005), and were associated with improvements in disease activity (p<0.001). Cortical BMD Z-scores decreased significantly: greater increases in tibia length were associated with greater increases in cortical area Z-scores and declines (improvements) in endosteal circumference Z-scores (both p<0.001). Increases in cortical area Z-scores were associated with declines in cortical BMD Z-scores (p<0.001). Greater glucocorticoid doses and disease activity were significantly associated with lesser gains in cortical area, and these associations were more pronounced with greater linear growth (interaction p<0.05). Despite minimal disease activity at the final visit, trabecular and cortical BMD and cortical area Z-scores were significantly reduced, compared with controls (all p<0.001).

Conclusions: These data suggest glucocorticoids and inflammatory disease activity independently impair cortical bone accrual relative to increases in tibia length, reflecting the unique vulnerability of the growing skeleton. In contrast, the greater improvements in trabecular BMD in younger participants, and the positive association between growth and accrual of cortical dimensions suggest a window of opportunity for recovery in the absence of significant glucocorticoid exposure and disease activity.

Table. Results at baseline and LTFU

	Baseline	LTFU	p value
Paediatric CD Activity Index, N (%)			<0.001
No active disease (< 10)	1 (2)	41 (85)	
Mild (11-30)	14 (29)	7(15)	
Moderate to severe (> 30)	33 (69)	0 (0)	
Trabecular BMD-Z	-1.47 ± 1.19*	-1.01 ± 1.21*	0.0001
Cortical BMD-Z	-0.25 ± 1.06	-1.06 ± 1.06*	<0.0001
Cortical Area-Z	-0.83 ± 1.18*	-0.57 ± 1.10*	0.03
Periosteal Circumference-Z	-0.22 ± 0.92	-0.22 ± 0.83	0.98
Endosteal Circumference-Z	0.60 ± 0.91*	0.38 ± 0.92*	0.004

* p <0.05 compared to reference participants. Table limited to the 51 that completed the LTFU visit. Z-scores presented as mean - SD

OP15

Dissecting the Role of Osteoblast Derived Nitric Oxide in Bone Remodeling

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Background: Bone accrual during adolescence is the main contributor to peak bone mass. Bone remodeling is a balance between bone formation and resorption as directed by osteoblast and osteoclast activity, respectively. Nitric oxide (NO) is a potent regulator of bone remodeling via mediating effects of cytokines, estrogen and mechanical strain. NO is synthesized from the conversion of L-arginine to L-citrulline by the enzyme Nitric Oxide Synthase (NOS). Citrulline can be reconverted to arginine by the enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). ASL is the sole mammalian enzyme responsible for endogenous L-arginine production and routing exogenous arginine to NOS for NO synthesis. Deletion of ASL abolishes arginine dependent NO production in the cell. Multiple studies have attempted to determine the role(s) of NO in bone remodeling. However, as of yet no study has been able to dissect its role in a cell specific manner.



Hypothesis: NO produced by osteoblasts stimulates osteoblast proliferation and increases bone mass in homeostasis and under hormonal stress.

Research Design and Methods: Using Cre-Lox technology, we created an osteoblast specific knockout of *Asl* in the mouse model. Bilateral ovariectomy was performed at 12 weeks of age to induce estrogen deficiency. Osteoblast activity and bone remodeling was evaluated at 12, 20 and 24 weeks using bone turnover markers, μ CT and histomorphometry. *Asl* cKO female mice were compared to their sham-operated and *Asl^{Flox/Flox}* littermates at each time point.

Preliminary Results: At 12 weeks of age, *Asl* cKO female mice have significantly lower trabecular bone density (BV/TV), trabecular number (Tb.N.) and higher trabecular separation (Tb.Sp.) in lumbar spine when compared to *Asl^{Flox/Flox}* littermate female controls ($p < 0.05$).

Discussion: Our preliminary results show that *Asl* cKO female mice have lower bone mass in lumbar spine at baseline suggesting an inherent role of NO in normal bone mineralization. Evaluation of bone remodeling in the *Asl* cKO female mice under an estrogen deficient state is ongoing. The translational value of the *Asl* cKO model is potential development of pharmacologic and genetic manipulation of ASL as an effective regulator of NO metabolism, ultimately leading to improved bone mineralization.

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Camptodactyly-arthropathy-coxa vara-pericarditis syndrome: Clinical and molecular genetic findings

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Objective: To describe the clinical, laboratory, radiological and genetic findings of camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome in Saudi children

Methods: Medical records of all children with CACP syndrome seen between June 1990 and June 2012 at KFSHRC-Riyadh were reviewed. The data included: gender, age at disease onset, referral diagnosis, clinical and radiological features and molecular genetic studies as well as functional status at the last follow up visit.

Results: 22 patients (15 boys) with mean age at diagnosis of 3.7 years were identified. Juvenile idiopathic arthritis (JIA) was the referral diagnosis in the majority. Camptodactyly and large joints arthropathy were present in all cases. Camptodactyly was observed in the neonatal period in all patients, while other joint involvement was observed through the 1st year of life. Two children had evidence of pericarditis. All patients had normal inflammatory markers and negative rheumatoid factor. Radiological findings included coxa vara with short femoral neck and flat, irregular femoral heads and intra-osseous cysts. Synovial biopsy from 3 patients revealed proliferating synovial epithelium with moderate fibro-collagenous densities and multinucleated giant cells. A locus responsible for causing CACP syndrome has been reported previously in 8 patients of our cohort; it has been assigned to 1q25-q31.

Furthermore; in 7 newly diagnosed patients from 4 unrelated families; 5 novel mutations were found. No genotype/phenotype association was observed. All patients referred to us while they were on NSAIDs, 10 patients used prednisone and methotrexate; and 2 patients treated with etanercept. Treatment in all patients was ineffective apart from mild pain relief.

Conclusion: CACP syndrome is not uncommon disorder in Saudi Arabia. Pericarditis is rarely seen in our patients. Our data suggests that CACP syndrome may be easily confused with JIA, causing a delay in diagnosis and probably unnecessary treatment with anti-rheumatic drugs including biologic agents.

P6

The relationship between bone health and body composition profile in patients with galactose metabolic disorders: implications for practice

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Objectives: To evaluate bone health and its possible correlations to body composition parameters in young patients with galactose metabolic disorders, aiming to suggest appropriate lifestyle interventions.

Methods: We studied twenty two patients, aged 5-16 years with galactose metabolic disorders, detected by neonatal screening. Fourteen suffered from classic galactosemia and eight from other galactose metabolic disorders (i.e. epimerase or galactokinase deficiency or homozygosity in Duarte type 2). All subjects had normal growth and were under strict dietary control, using a non-soya product, which is a casein-based, lactose-free feed, enriched with vitamins and minerals. They were followed-up closely (every six months). Bone mineral density and body composition were assessed with dual X-ray absorptiometry and the results were compared to the reference population of the pediatric software of the apparatus. We evaluated areal bone mineral density of the lumbar spine and total body, lean tissue mass, body fat percentage, fat mass index and bone strength (bone mineral content/lean tissue mass ratio).

Results: In both groups, bone strength and median bone mineral density Z-scores were within normal limits at both sites. However, classic galactosemia patients appeared sarcopenic (median lean tissue mass Z-score = -1.93, $p < 0.05$), whereas subjects with other enzymatic defects had low-normal muscle mass. Also, nearly half of all patients were either overweight or obese. In classic galactosemia, spine and total body bone mineral density were strongly correlated to muscle mass ($r = 0.81$ and $r = 0.9$ respectively, $p < 0.05$). In patients with other galactose metabolic disorders, bone mineral density of total body was positively correlated to body mass index ($r = 0.78$, $p < 0.05$). Finally, an almost linear relationship was found between body fat percentage and fat mass index.

Conclusion: Adequate dietary control and close follow up favour our patients' bone health. However, it appears that there is an imbalance between muscle and fat mass, especially in patients with classic galactosemia. Given the observed strong correlation between bone and muscle mass, counseling on exercise is needed, in order to prevent sarcopenia, combat fatness and preserve bone quality.

P7

Osteometric parameters of mature rats mandible molars when implanted in the tibia biogenic hydroxyapatite, saturated with iron

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Objectives: The aim of the study was to examine experimentally the possibility smoothing of adverse effects of «fracture syndrome» in the parameters of the growth of the molar row of the mandible with implant



in the proximal tibial shaft biogenic hydroxyapatite, saturated with iron at concentrations of 0.05%, 0.15%, 0.50%.

Methods: For the experiment were collected 168 white mature male rats were divided into 4 groups: 1st group, animals that in the proximal tibial shaft was applied the defect, 2nd, 3rd, 4th groups, the animals that formed in the bone defect was implanted biogenic hydroxyapatite, saturated with iron at concentrations of 0.05%, 0.15%, 0.50% respectively. Observation periods were 7, 15, 30, 60, 90 and 180 days. After the time of observation rats were euthanized under ether mask anesthesia, extract of mandibles and using calipers measure the length and height of the molar row of the mandible. The results obtained were processed in the program «STATISTICA 5.5».

Results: The height of the molar row in the group of animals with implanted biogenic hydroxyapatite, saturated with iron in a concentration of 0.05% was more parameters comparison group to 90 days at 5.03%, with 0.15% - length of the molar row increased from 30 to 90 per day for 4.85%, 2.74%, 3.00%, and the height to only 90 days 3.91%, with 0.50% - length of the molar row increased to 60 days at 2.31%, and the height - decreased to 180 days at 7.88% ($p < 0.05$).

Conclusion: Thus, we can assume that the implantation of biogenic hydroxyapatite, saturated with iron in a concentration of 0.15% is preferable to smooth the negative manifestations of the «fracture syndrome» in relation to the growth parameters of the molar row of the mandible than 0.05% and 0.50%.

P8

Compound heterozygosity of two functional null mutations in the ALPL gene associated with deleterious neurological outcome in an infant with hypophosphatasia

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Background: Hypophosphatasia (HPP) is a heterogeneous rare, inherited disorder of bone and mineral metabolism caused by different mutations in the *ALPL* gene encoding the isoenzyme, tissue-nonspecific alkaline phosphatase (TNAP). Prognosis is very poor in severe perinatal forms with most patients dying from pulmonary complications of their skeletal disease. TNAP, a ubiquitous enzyme, is mostly known for its role in bone mineralization. TNAP deficiency, however, may also result in neurological symptoms such as neonatal seizures. The exact biological role of TNAP in the human brain is still not known and the pathophysiology of neurological symptoms due to TNAP deficiency in HPP are not understood in detail.

Presenting Problem and clinical management: In this report, we describe the clinical features and functional studies of a patient with severe perinatal HPP which presented with rapidly progressive encephalopathy caused by new compound heterozygous mutations in the *ALPL* gene which result in a functional *ALPL* «knock out», demonstrated *in vitro*. In contrast, an *in vitro* simulation of the genetic status of his currently asymptomatic parents who are both heterozygous for one mutation, showed a residual *in vitro* AP activity of above 50%. Interestingly, in our patient, the fatal outcome was due to progressive encephalopathy which was refractory to antiepileptic therapy including pyridoxine, rather than hypomineralization and respiratory insufficiency often seen in HPP patients. The patient's cranial MRI showed progressive cystic degradation of the white matter and nearly complete destruction of the cerebrum. To our knowledge, this is the first MRI-based report of a deleterious neurological clinical outcome due to a progressive encephalopathy in an infant harboring a functional human *ALPL* «knock out».

Conclusion: This clinical course of disease suggests that TNAP is involved in development and may be responsible for multiple functions

of the human brain. According to our data, a certain amount of residual TNAP activity might be mandatory for normal CNS function in newborns and early childhood.

P9

A 6-month intervention study with vibration therapy in severely disabled children: effects on bone, biochemical markers and acceptance

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Objectives: To study acceptance and the effects on bone during a 6-month whole body vibration (WBV) therapy in severely disabled children.

Methods: Nineteen patients, age 5-16 years, with severe motor disabilities completed the 6-month WBV therapy standing on a self-controlled dynamic platform with vibration, jumps and rotation. The WBV was performed twice per week at 40-42 Hz. Bone mass and biochemical markers were measured at start and after 6 and 12 months.

Results: WBV was perceived as positive by children and staff. Fractures were reported during the study and 58% of the children had sustained fractures earlier in life. Total body bone mineral density (BMD) (head excluded) and total body bone mineral content (BMC) (head excluded), increased over the study period ($p < 0.05$). Total body BMD Z-scores were low in the group (range -4.4 to -0.8) at start and did not change. Their BMI, fat and lean mass were unchanged during the WBV. Markers of bone and mineral metabolism did not change significantly, except for a small decrease in serum calcium. CTX, a marker of bone resorption, was increased in 8 of 19 patients. Some patients normalized their CTX levels after WBV, which could indicate a positive response to WBV. However, the actual CTX values did not change significantly.

Conclusion: WBV appears to be safe and well-tolerated. Total body BMD and BMC (head excluded) increased; however, the Z-scores were low and unchanged over the study period. The low BMD Z-scores could be an effect of increased bone resorption and decreased bone formation. Larger study groups, and possibly longer vibration treatment periods, are needed to elucidate the potential benefits of WBV on bone mass in children with severe motor disabilities.

P10

Osteoporosis in young patients with neurological impairments

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Aim: Osteoporosis and resulting spontaneous fractures in young patients with neurological impairments living outside institutions have not received much attention. The aim of this study was to determine the degree of demineralization in children and teens with such disabilities living in South Lebanon, an under privileged region.

Subjects and Methods: We reviewed 40 patients attending outpatient clinics in a referral rehabilitation center in South Lebanon. All patients were under 20 years of age and their impairment ranged from paraplegia to spastic palsy and polio. All patients underwent CUS measurements, serum Vitamin D testing and calculation of daily dietary calcium intake.



Results: We observed bone loss in almost all patients, Their mean serum vitamin D level was 19.32ng/ml while their mean daily calcium intake was 429.25mg/day.

Conclusion: Younger patients with neurological disabilities are super fast bone loser population and this could lead to high fracture rate, their management should include supplementation with calcium and Vitamin D and nutritional intervention to improve dietary calcium intake and malnutrition as well as intensive rehabilitation programs that include standing using various devices and walking with orthotics for weight bearing the skeleton.

P11 Abstract withdrawn

P12 DXL measurements in children 2-10 years

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Objectives: To generate pediatric reference values for calcaneal bone mineral density (BMD) in healthy 2 - 10-yr-old Swedish children.

Methods: Dual energy X-ray absorptiometry (DXA) in combination with a laser measurement of the heel thickness, DXL Calscan (Demetech AB), measures bone mass in the calcaneus and an apparent density (BMAD) is calculated. Healthy, Swedish children were included. The left foot was scanned in 117 2-yr-old, 110 4-yr-old and 107 7-yr-old children using the DXL technique. Half of the children were boys. More than 35 % of the children from each age group were followed for another 2 years. Height and weight were determined annually and questionnaires concerning general health were completed at every visit.

Results: The mean BMD in the 2-yr-olds was 0.170 ± 0.003 g/cm², in the 4-yr-olds 0.221 ± 0.003 g/cm² and in the 7-yr-olds 0.296 ± 0.005 g/cm². The 7-yr-old girls had a significantly higher BMD than the boys (p=0.026) but there were no significant gender differences in the calcaneal BMD in 2- and 4-yr-olds. BMD was significantly correlated with age (p<0.001, r=0.78). A weaker correlation was found between BMAD and age (p<0.001, r=0.23). Based on the data from the 2-yr follow-up; a total of 645 (328 girls/ 317 boys) measurements, reference curves (mean ± 2 SD) were produced for calcaneal BMD in girls and boys aged 2 - 10 yrs according to age and height.

Conclusion: Calcaneus can be an alternative for measurements when other bone mass measurement techniques are not possible to use. Gender differences are present in the calcaneal BMD at an early age. This study presents gender specific calcaneal BMD reference curves for children 2-10 yrs of age. These data will be valuable in future research and for evaluating the bone health in children with different disorders.

P13 Bone turnover compensates for the delayed growth in small for gestational age neonates

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Objectives: To investigate the possible relationship between neonatal anthropometric characteristics and bone turnover and growth markers in a sample of neonates and their mothers, taking into account the size for the gestational age.

Methods: A sample which consisted of 20 SGA (small for the gestational age), AGA (appropriate for the gestational age) and LGA

(large for the gestational age) randomly selected term neonates and their 20 mothers were analyzed twice, at birth and at exit. Elisa method was used to measure the OPG (Osteoprotegerin), RANK (Receptor activator of nuclear factor-kappaB), RANKL (Receptor activator of nuclear factor-kappaB Ligand), IGF-1 (Insulin-like growth factor 1), IGFBP3 (Insulin-like Growth Factor Binding Protein 3) and Leptin levels in neonate and maternal serum. Data was analyzed using SPSS Software (16.0).

Results: Birth weight and height were positively correlated with RANKL, IGF-1, IGFBP3 and negatively with the ratio OPG/RANKL. The multi-adjusted analysis showed that SGA neonates presented lower RANKL values (Beta = - 0.520, p=0.036) and higher OPG/RANKL ratio (Beta = 0.498, p=0.044) comparing to AGA neonates, while LGA neonates had higher RANK levels than AGA neonates (Beta = 0.593, p=0.022). Moreover, a positive association was shown between neonatal IGFBP3 and maternal IGF-1 values at birth (rho = 0.466, p=0.038) and at exit (rho = 0.558, p=0.011) as well as between neonatal and maternal RANK values at birth (rho = 0.464, p=0.039) and at exit (rho = 0.732, p<0.001). Leptin levels did not show any statistically significant results. The kind of birth, the number of birth and the maternal habits (smoking, alcohol and coffee consumption) did not alter these correlations.

Conclusion: These results reveal a remarkable up regulation of OPG/RANKL ratio in SGA neonates, pointing out the role of bone turnover in compensating for the delayed neonatal growth.

P14 Bone status and body composition analysis in young patients with phenylketonuria and hyperphenylalaninemia

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Objectives: To evaluate bone status and body composition in patients with phenylketonuria and hyperphenylalaninemia.

Methods: Eighty patients (48 with phenylketonuria, 32 with hyperphenylalaninemia), aged 5-18 years, early-diagnosed, underwent dual energy X-ray absorptiometry. Bone mineral density (lumbar spine, total body), bone strength (bone mineral content/lean tissue mass ratio), lean tissue mass, body fat percentage and fat mass index were measured. The above parameters were compared to reference population values.

Results: Compared to controls, all patients had bone strength and bone mineral density within normal range at both sites (Z-score between -2 and 2), with low-normal Z-scores at the spine (between -1 and -2) in 16% of patients with phenylketonuria and 31% of hyperphenylalaninemias. Nearly 20% of patients in both groups appeared sarcopenic (lean tissue mass Z-score <-2) and 33% had low-normal muscle mass. Regarding fatness, 33% of the subjects in both groups were either overweight or obese. Phenylketonuric, female teenagers with poor dietary compliance were most at risk of being fat. In the hyperphenylalaninemia group, no sex-related difference was detected; however, adolescents also tended to be heavier. A positive correlation was found between bone mineral density and muscle mass; also, between bone and fat mass. Moreover, Phenylalanine levels and fat indices were positively correlated. Between the two groups, a higher body fat percentage was recorded in the hyperphenylalaninemic subjects. There was no difference in muscle and fat mass.

Conclusion: Despite the muscle-fat imbalance in patients with phenylketonuria and hyperphenylalaninemia, bone status is relatively preserved. Adolescent patients with poor dietary compliance or low-normal bone mineral density should be targeted towards participation in more active exercise programs and more frequent follow up.



P15 Osteogenesis Imperfecta-bone mass acquisition under bisphosphonates treatment and additional gain in BMD in time of rhGH treatment for growth delay

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Objectives: Patients with Osteogenesis Imperfecta (OI) type IA have a mild phenotype with normal or near-normal height. The addition of recombinant human growth hormone (rhGH) to ongoing treatment with bisphosphonates can increase measures of BMD and growth. We studied growth rate bone density and bone metabolism in two girls affected by OI type IA and growth delay.

Material and methods: Eight children (six girls and two boys) with OI type IA were treated with Risedronate. Among them two girls with mean age 6.5years old presented growth delay. These girls were treated with rhGH at a dose 0.35 mg/kg/week for four years or more. Auxologic data were measured every three months, bone age was determined at the start and at every six months. At every three months IGFI, osteocalcin,alkalinphosphatase, calcium and phosphorus blood levels, and urinary hydroxyproline and calcium levels were determined. Bone mineral density (BMD) measurements were made at the start and repeated at 12,24,36,48 months at the lumbar spine and whole body by DXA.

Results: After 48 months, linear growth velocity increased from 4.5 cm/yr to 8 cm/yr in the first year, to 8.5cm/yr in the second, to 9.5 cm/yr in the third year, and 5cm/yr in the fourth. DXA-BMD at lumbar spine increased significantly from 0.359 g/cm² to 0.464 g/cm²(+29%) in the first year to 0.505 g/cm²(+9%)in the second and to 0.585 g/cm²(+15%) in the third and 0.649 g/cm²(+10%) in the fourth year. Whole body:BMD improved+ 36% in the first year, 4.4% in the second year, 9.5% in the third and 16% in the fourth year. Serum osteocalcin levels increased significantly after rhGH treatment.

Conclusion: rhGH treatment addition at bisphosphonates in OI type IA increases significantly the rate of linear growth velocity.The bone turnover increases, and BMD in lumbar spine and whole body increase also. The fractures did not increase. More children should be treated with rhGH to see the long term benefits of this treatment in final adult height and quality of bone in OI.

P16 Bone size and bone mineral content in adolescents and young adults with eating disorders.

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Objective: The incidence of eating disorders has increased in 15-19 year olds (1). There is growing concern as to its impact on bone health during adolescence where peak bone mass acquisition is of paramount importance. This paper describes bone size and size adjusted bone mineral content in an adolescent/young adult eating disorder population.

Method: A total of 68 patients (63F/5M, 90% anorexia nervosa and 10% atypical eating disorder), median age 15.4 years (range 10.9–19.8) median BMI SDS -1.2(range -4.7 to 0.8) attended the bone densitometry service between January 2009 and December 2012 for total body(TB) and lumbar spine(LS) DXA scans (Lunar Prodigy DXA scanner driven by Encore Paediatric Software Version 13.3). Bone size is reported as percent predicted bone area for age (ppBA-for-age), and for size adjustment, the extent of bone mineralisation within the bone is described as percent predicted bone mineral content for bone area (ppBMC-for-BA).

Results: Median ppBA-for-age was 89% (range 66-114) at TB site, with 59% of patients presenting with ppBA-for-age ≤90. When size adjusted, median ppBMC-for-BA was 99.5%(range 89-116), with only 3% being

in the ≤ 90% category at TB. At LS site, median ppBA-for-age was 95%(range 65-128), while median ppBMC-for-BA was 94%(range 73-131). TB ppBA-for-age correlated positively with BMI SDS(r=0.417,p=0.001), and negatively with age (r=-0.245,p=0.021). Median DXA software derived paediatric analysis centiles were 35th (range 0-91st) for height for age (shorter bones=less linear growth), 22nd (range 0-75th) for bone area for height (thinner bones=less periosteal expansion), 27th (range 0-95th) for lean mass for height (reduced muscle mass), and 52nd (range 1-99th) for BMC for lean mass centile (adequate BMC for reduced lean mass).

Conclusion: In the adolescent/young adult eating disorder population, bones are small compared to the normal healthy population, but not obviously pathological at this stage. Muscle mass is low, but bone mineral content appears preserved at the expense of a combination of periosteal expansion and linear growth. It is likely that bone pathology in anorexia nervosa begins when restricted caloric intake is prolonged following closure of the endplates, where little scope remains for size adaptation. Long-term follow-up in these patients is required.

References 1. Epidemiology of Eating Disorders: Incidence, Prevalence and Mortality Rates, Curr Psychiatry Rep (2012) 14:406–414

P17 The optimal dose of vitamin D in growing girls during academic years: a randomized trial

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Objectives: Vitamin D is an essential hormone for growth and development of bones in children. Prevalence of vitamin D deficiency is remarkable during childhood and adolescence throughout the world. The aim of this study was to specify the optimal dose of vitamin D in growing girls during an academic year in a sunny city.

Materials and methods: This randomized clinical trial study was carried out in Yazd in the center of Iran in 2007;120 junior high school girls (aged 12-15 years) were randomly divided into 4 groups. Sixty students in groups I and II were initially treated for vitamin D deficiency with 300,000 IU vitamin D3 and then randomly received 50,000 U/monthly or 100,000IU/3 months vitamin D3; 60 other students in groups III and IV received 50,000 IU/3 months and 100,000/3 months from the beginning of the academic year. Medication continued for the entire academic year; 1 month after the last dose, serum 25(OH)D levels were measured.

Results: The mean level of 25 (OH) D was 29.7 ± 4.60 ng/mL in group I and 30 ± 5.61 ng/mL in group II. Mean serum levels of 25 (OH) D were 15.2 ± 6 ng/mL and 23 ± 6.8 ng/mL for groups III and IV, respectively. (table)

Conclusion: This study shows that in the area with high prevalence of vitamin D deficiency (more than 50%), recommended doses of neither 400IU/day nor 800IU/day are sufficient to maintain optimal level in all. But after treatment of deficiency with 300000IU of vitamin D.both doses of 1000 or 2000IU/day would maintain serum 25(OH)D concentrations ≥20ng/ml in all of the samples during academic years.Yazd is one the sunniest provinces in the country and the Middle East. therefore this study could reveal the minimum requirement of vitamin D in other areas with less sunshine than our region.



Groups/ 25(OH)D level	300,000IU+ 50000IU every month Group I	300,000IU+ 100,1000IU Every months Group II	30000 IU every 3 months group III	10000IU Every 3 months group IV
Sever deficiency (25(OH)D<12)	0%	0%	13.5%	0%
Deficiency (25(OH)D=10-20)	0%	0%	73%	48%
Adequate (25(OH)D=20-30)	47%	57%	10.8%	35.5%
Ideal (25(OH)D=30-100)	53%	43%	2.7%	19.5%

P18
Influence of Anthropometric Parameters on Assessment of Paediatric Bone Mineral Density and Bone Mineral Content

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Objectives: Creation of reference curves for areal bone mineral density (aBMD) and bone mineral content (BMC) with consideration of relevant anthropometric variables.

Methods: Analysis of the dual-energy x-ray absorptiometry (DXA) data collected as part of the Bone Mineral Density in Childhood Study¹, including 2012 boys and girls, 5-22 y old, with a total of 10,525 visits, resulting in aBMD and BMC observations at the lumbar spine, hip (neck and total), forearm and whole body (total and sub-cranial). Multivariate statistics were used to rank order the influence of the independent variables age, gender, race (black/non-black), height, weight, percent body fat (%fat) and sexual maturity. Two different models were created for each aBMD and BMC parameter, the practical model containing age, gender, race, height and weight as well as the full model adding %fat. We compared the number of cases that fell below 2 standard deviations in our models with those below the same limit of the currently standard LMS model², which is based on age, gender and race, and of the height adjusted Z-scores³.

Results: For the 6 aBMD parameters, age, gender, weight and %fat were the most influential predictors, whereas height, race and maturity added little improvement to the models. In contrast, for the 6 BMC parameters, age, weight and %fat were the top predictors, but not gender. In comparing the overlap of subjects identified as below the normal limit of -2 standard deviations, between 56% and 84% of subjects identified as below normal based on the LMS model are not classified as being below normal in our practical model. Using the full model, the misclassification increases for all aBMD and BMC parameters, ranging from 61% to 92%. The height-adjusted Z-scores reduced the misclassifications to 41-59% in comparison to the practical model and to 55-73% in comparison to the full model.

Conclusion: The traditional comparison of paediatric BMD and BMC data against age-, gender- and race-matched controls can be refined if anthropometric parameters are taken into account.

¹ Sponsored by the National Institute for Child Health and Human Development

² Zemel BS et al., J Clin Endocrinol Metab 96:3160-3169, 2011

³ Zemel BS et al., J Clin Endocrinol Metab 95:1265-1273, 2011

P19
Positive correlation of serum vitamin D status with bone density and bone quality among adolescent girls in Hong Kong

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Objectives: Vitamin D is essential for bone modeling/remodeling but the relationship between vitamin D status, bone mineral density (BMD) and bone quality parameters remains controversial especially in adolescent population. The aims of this study was to evaluate the vitamin D status and its correlation with BMD and bone quality parameters among adolescent girls in Hong Kong where information is lacking.

Methods: 156 adolescent girls (11-15 years old) were recruited separately in both summer and winter. Serum 25(OH) VitD was measured by liquid chromatography-tandem mass-spectrometry. BMD of bilateral femoral necks and bone quality parameters (bone morphometry, compartmental volumetric BMD[vBMD] and trabecular micro-architecture) of the non-dominant distal radius were measured by dual-energy X-ray absorptiometry and high-resolution peripheral quantitative computed tomography respectively. Multivariate linear regression was used to detect any correlation between bone parameters and serum 25(OH)VitD levels and p<0.05 was considered statistically significant.

Result: Prevalence of significant vitamin D insufficiency (25<25(OH)VitD<50nmol/L) and deficiency (25(OH)VitD<25nmol/L) was 69.2% and 11.5% respectively. The mean serum 25(OH)VitD level in summer and winter were 43.9±12.0nmol/L and 34.9±10.0nmol/L respectively. After adjustment for age, Body Mass Index, Tanner staging, physical activity level, dietary calcium intake and season, the positive correlations between 25(OH)VitD and femoral BMD was statistically significant. The positive correlations between 25(OH)VitD and femoral BMD, distal radius trabecular vBMD, meta-trabecular vBMD and bone volume-over-tissue volume remained significant for those with 25(OH)VitD level>25nmol/L in summer. In contrast, no significant correlation was detected with the winter data.

Conclusion: Despite being a subtropical city located at a latitude of 22°N, Vitamin D insufficiency with associated low femoral BMD and abnormal key bone quality parameters was found to be highly prevalent among adolescent girls in Hong Kong. This could post an important bone health issue of significant public health concern. Further studies to investigate the association with lifestyle factors and related therapeutic measures targeted on vitamin D insufficiency are warranted.

Acknowledgments: The project was funded by General Research Fund, University Grants Council of Hong Kong (Project number: 468411, 468809).

P20
Assessment of vitamin D nutrition status and dietary calcium intake in children 2-5 years of age

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Objectives: To assess the vitamin D nutrition status of healthy children in the age group 2-5 years and to assess the daily dietary calcium intake of these children.

Methods: A total of 100 healthy children (age group 2-5 years) were studied in this cross sectional study. Dietary calcium and energy intake was estimated using 24 hour dietary recall method. Average sun exposure over last 3 days was assessed by calculating UV score.



Biochemical parameters assessed included serum calcium (total and ionic), inorganic phosphorous, alkaline phosphatase (ALP), parathormone (PTH) and 25 hydroxy vitamin D (25OHD).

Results: Diet of 32% children provided met the ICMR recommendation of daily dietary calcium intake ≥ 600 mg. 23% children had an adequate vitamin D nutrition status (serum 25OHD ≥20 ng/ml). Average sun exposure was 21.4 ± 19.8 min/day (range 5-120 min). Only 9% children had adequate calcium intake (≥600 mg/day) as well as optimal vitamin D status. 54% children did not have either adequate calcium intake or optimal vitamin D status. Serum PTH was elevated in 5% and ALP in 10% cases. Serum ALP was significantly higher in subjects with 25OHD<10 ng/ml. No correlation was observed between serum 25OHD and PTH levels.

Conclusion: Bone health status of children 2-5 years of age is sub-optimal, with prevalence of vitamin D deficiency in 77% & low dietary calcium intake in 68% subjects.

P21
Bone Disease in Children with Geroderma Osteodysplasticum (GO) – A 25 year experience from a single tertiary centre

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Geroderma Osteodysplasticum (GO) is a rare autosomal recessive connective tissue disorder characterised by progeria like facies, wrinkled lax skin, joint hypermobility, congenital dislocation of hips and propensity to fragility fractures. In the past 25 years, five patients (3 female; 2 male) diagnosed with GO were referred to our Paediatric metabolic bone service for assessment and management of secondary bone problems. All five children were born to consanguineous parents of Pakistani origin.

Four out of 5 patients had significant bone problems at presentation or developed them subsequently. Two patients had congenital dislocation of hips and two had talipes equinovarus. Four patients with radiologically apparent osteopaenia developed vertebral wedge fractures. Three children suffered non-vertebral fractures which included: the femur, the tibia, the clavicle, a metatarsal bone and a middle phalanx.

Trans-iliac bone biopsies in 2 children showed severe cortical and trabecular osteopaenia. Data on bone densitometry, which was performed in 3 children, is shown in the table below. Cyclical intravenous Pamidronate therapy resulted in reduced number of long-bone fractures and remodelling of vertebral fractures. In one patient who had serial bone densitometry performed, IV Pamidronate therapy resulted in an improvement in bone mineral density (BMD) values at axial and appendicular skeletal sites.

In our experience, severity of bone disease in children with GO is variable. It is characterised by cortical and trabecular osteopenia and IV bisphosphonate therapy appears to improve bone mineral density and reduce the fracture risk.

Age (yrs)	Lumbar spine bone mineral density Z score (g/ml ³)	Spine bone apparent Z score (mg/ml ³)	Lumbar spine volumetric BMD Z score (mg/ml ³)	Distal radial total HMD Z score (mg/ml ³)	Distal radial trabecular HMD Z score (mg/ml ³)
1 5	0.169	-1.62	1.3	0.29	
2 11	-3.726	-5.16	-0.03	-2.03	
3 5.4	0.498	-1.98	-2.35	-1.6	

P22
Association of Volumetric Bone Mineral Density, Bone Morphometry and Trabecular Bone Micro-Architecture with Leptin and Soluble Leptin Receptor in Adolescent Idiopathic Scoliosis

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Objectives: Low bone mass in adolescent idiopathic scoliosis (AIS) has been well reported, however the etiologies of the disease and this abnormal phenotype were still unknown. Leptin have profound effects on bone metabolism and skeletal growth, and was speculated to play a role in the etiopathogenesis of AIS. The objective of this study was to investigate the bone quality in AIS and its association with leptin and soluble leptin receptor (sOB-R).

Methods: This was a case-control study involving 94 newly radiologically diagnosed AIS girls (Cobb angle 12-48°) aged 12 to 14 years old and 87 age and gender-matched normal controls. Serum total leptin and sOB-R were assayed with ELISA. Non-dominant distal radius was scanned with High Resolution Peripheral Quantitative Computed Tomography for assessing bone quality in terms of bone morphometry, volumetric bone mineral density (vBMD) and trabecular bone micro-architecture.

Results: AIS girls had higher sOB-R (p=0.006), lower cortical vBMD (p=0.023), higher cortical bone perimeter (p=0.024) and higher trabecular area (p=0.026). Correlation analysis (Table 1) on leptin level indicated that while its correlations with cortical bone parameters were present in both AIS and controls, the correlations with trabecular bone parameters were only present significantly in AIS. For sOB-R, significant correlations were detected with cortical bone parameters only in controls.

Conclusion: This study showed that bone quality in AIS was deranged as compared with normal controls. In addition, the difference in correlation pattern between leptin, sOB-R and bone parameters indicated possible abnormalities in bone metabolism and disturbance on leptin signaling. The implication and how this is linked to the generalized low bone mass and the etiopathogenesis of AIS warrant further studies.

TABLE 1. Correlations of cortical and trabecular bone parameters with leptin and sOB-R in AIS and controls

	Leptin		sOB-R	
	Controls	AIS	Controls	AIS
Cortical vBMD	0.287**	0.362**	-0.229*	ns
Cortical Area	0.294**	0.367**	-0.248*	ns
Cortical Thickness	0.249*	0.385**	-0.239*	ns
Cortical Bone Perimeter	0.261*	ns	ns	ns
Trabecular vBMD	ns	0.303**	ns	ns
Meta Trabecular vBMD	ns	0.288**	ns	ns
Inner Trabecular vBMD	ns	0.296**	ns	ns
Trabecular Area	ns	-0.269**	ns	ns
Trabecular BV/TV	ns	0.303**	ns	ns
Trabecular Thickness	ns	0.254*	ns	ns

* p<0.05; **p<0.01; ns = not significant

This study was supported by Institut de France Fondation Yves Cotrel.



P23

Abstract withdrawn

P24

Effects of endurance training on somatic growth in a rat model of chronic kidney disease (CKD) related growth retardation

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Objectives: CKD in children is associated with suppressed body growth. Physical activity has been previously shown to increase expression of IGF-1 signaling in muscles of rats with CKD, but the effects of this intervention on bone tissue have not been investigated yet. The purpose of this study was to examine the effects of aerobic exercise on CKD related bone disease.

Methods: Twenty-day old / 50g male rats underwent a 2 step subtotal nephrectomy (Nx) or sham surgeries. The animals were divided into 2 running groups: control (Cr) and CKD (CKDr) (treadmill running, 20 m/min, 0.5 hr/day, 5 days/wk.) and 2 non running groups: control (C) and CKD. Food delivery was equal for all groups. The intervention lasted 4 weeks. We monitored somatic growth levels, kidney function and proximal tibiae epiphyseal growth plate (EGP) histomorphometry.

Results: Growth retardation (both longitudinal and weight gain) was well noticed in CKD Vs C. Exercise caused an increase in longitudinal growth and tibial length in Cr Vs C rats. However, CKDr rats did not grow better than CKD. CKDr rats consumed less food than CKD but grew to the same extent, hinting for better food efficiency. The EGP hypertrophic zone was wider and vascularization at the primary ossification center was decreased in CKD Vs C. EGP immunostainable IGF-I and VEGF as well as VEGF mRNA were reduced in CKD. However, total EGP width (both proliferative and hypertrophic zones) was increased in CKDr Vs CKD.

Conclusions: CKD induces growth retardation irrespective of food intake, associated with widened EGP hypertrophic zone but less vascularization, hinting for a chondrocyte maturation arrest. Endurance training did not rescue CKD somatic growth retardation, but longitudinal bone formation (as reflected by EGP histomorphometry) was better organized.

P25

Maternal bone density and rickets in Nigerian children

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Objectives: While nutritional rickets is traditionally associated with vitamin D deficiency, a number of other etiological factors have been proposed, including low calcium intake. Maternal nutrition can affect fetal and infant skeletal growth and development. Our aim was to determine the relationship between maternal bone density and rickets in Nigerian children.

Methods: We measured areal forearm bone mineral density (BMD) in 56 and 131 mothers of children with and without nutritional rickets, respectively. Active rickets was confirmed with radiographs of the wrists and knees. Pregnancy and lactation status were recorded, but bone density measurements were not performed in the first trimester of pregnancy. Using logistic regression, we assessed the association of maternal forearm bone density, controlling for parity, pregnancy and lactation status, duration of most recent breastfeeding, age of menarche, and maternal age with nutritional rickets.

Results: The median (range) age of mothers was 30 y (17-47 y), and parity was 4 (1-12). A total of 36 (19%) were pregnant and 55 (29%) were currently breast feeding. Mean (\pm SD) distal forearm BMDs were 0.321 \pm 0.057 and 0.314 \pm 0.052 g/cm² in mothers of children with and without rickets, respectively (P=0.43). Proximal 1/3 forearm BMDs were 0.719 \pm 0.071 and 0.713 \pm 0.070 g/cm², respectively (P=0.58). Bone mineral content and bone area were not significantly different between the two groups. Neither the distal nor proximal 1/3 forearm maternal BMD was associated with nutritional rickets in multivariate logistic regression (P=0.42 and 0.84, respectively). In the adjusted analysis, rickets was associated with shorter duration of breast feeding (OR 0.90 for each additional month; P=0.01) and use of lead-containing eye cosmetics by mothers (OR 4.78; P=0.01). Maternal age, parity, age of menarche, and body mass index were not associated with having had a child with rickets in multivariate analysis.

Conclusion: There was no relationship between maternal BMD and nutritional rickets in children. However, early discontinuation of breast feeding and use of lead-containing eye cosmetics may increase the risk of nutritional rickets in Nigerian children.

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Improvement in Genu Valgus Deformity in Hypophosphatemic Rickets Due to Primary De Toni-Debré-Fanconi Syndrome Treated with Phosphate, Calcitriol and Alkali Therapy

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Background: Primary de Toni-Debré-Fanconi syndrome is a metabolic disorder characterized by hypophosphatemic rickets or osteomalacia, renal tubular acidosis, renal glycosuria, generalized aminoaciduria. It is a non-FGF23-mediated hypophosphatemic disorder, with primary defect in proximal tubular dysfunction. The orthopaedic sequela of this rare disorder in the literature is scarce.

Presenting Problem: We present a clinical case of a 10 year old female with primary de Toni-Debré-Fanconi syndrome resulting in hypophosphatemic rickets treated with phosphorus, calcitriol and sodium citrate with a satisfactory orthopaedic outcome.

Clinical Management: The patient presented with genu varum with biochemical and radiographic rickets at age 1 year. She was initially diagnosed with vitamin D deficiency rickets and treated with ergocalciferol at various doses for 2 years with no improvement. She had complaints of polyuria, polydipsia, enuresis, and bone pain. Further investigations showed phosphaturia, glycosuria, proteinuria, and acidosis. Diagnosis of hypophosphatemic rickets due to primary de Toni-Debré-Fanconi syndrome was subsequently made, without evidence of cystinosis. Respiratory chain enzyme analysis identified a complex I mitochondrial deficiency as underlying cause. She was then started on low dose phosphate therapy, and sodium citrate at age 3 years with some improvement in bone pain but continued bone deformity. At her first evaluation at the Pediatric Metabolic Bone Clinic at age 5 years, she was noted to have swelling of her knees with genu valgus deformities of 24 degrees. Her phosphate dose was increased to 70 mg/kg/day and she was started on calcitriol 0.5 mcg/day. She had significant improvement in her genu valgum with normal growth. By age 10 years, her genu valgus deformities were 4 degrees with healing of rickets. She had no fractures. Her bone mineral density (BMD) at age 10 years showed normal lumbar BMD Z score at 0, while total body BMD Z score was low at -2.2.

Discussion: This patient had excellent improvement in bone deformity associated with hypophosphatemic rickets, despite late proper medical intervention. Non-FGF23-mediated hypophosphatemic rickets may have better response to medical therapy as compared to FGF23-mediated hypophosphatemic rickets in which bone deformity continues to progress on medical therapy and surgical correction is often required.



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Randomized-controlled study in kidney transplanted children. Early corticoids withdrawal. Effect on bone health recovery

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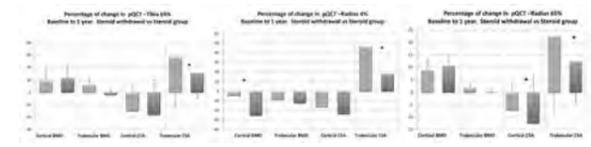
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Background: Glucocorticoid immune suppression in kidney transplanted children jeopardizes optimal bone health recovery. So far, there are no studies that evaluate the effect of transplant and corticoid in bone parameters separately.

Objective: To determine the effect of early corticosteroid withdrawal in bone parameters.

Methods: Randomized, controlled study; 2 groups: corticosteroid withdrawal (at the 6th day post-transplant, then tacrolimus/mycophenolate) (CW) and corticosteroids (C). Evaluations: PTH, 25OH and 1,25 (OH)₂ vitamin D, bone turnover markers, Klotho, FGF-23, IGF-1, IGFBP-3, pQCT (Radius 4%, tibia y radio 65%) at baseline and 12 months post-transplant.

Results: 30 patients: 14 CW and 16 SC Age: 7.8 \pm 4.3 yrs, 17 males. Significantly, there was a decrease in PTH, increase in IGF-1; IGFBP-3; 25OHD, bone specific alkaline phosphatase. Acid phosphatases increase only in C group. Figure: Percentage changes in pQCT variables (mean, SE). Rose bars: Corticoid withdrawal, blue bars: Corticoid group. *p<0.05



In all sites, cortical cross sectional area (CSA) decreases and trabecular CSA increases, significantly more in the C group. Cortical bone mineral density (BMD) decreases in both groups significantly at the 4% radius, and tends to increase at the 65% tibia and 65% radius. These increases correlate positively with IGF-1 and lean mass. In both groups, 4% radius changes, in trabecular and cortical BMD and CSA correlate with changes in height-SD, bone turnover markers and PTH. In 65% radius and tibia, increase in trabecular CSA correlated positively with height-SD, IGF-1 and 25OHD. In both groups, FGF-23 and Klotho decrease. Klotho correlates negatively with trabecular CSA in 4% radius and 25OHD.

Conclusions: Transplant produces significant changes in pQCT and turnover markers independently of corticoids. These changes are more favorable when corticosteroids are removed earlier.

Supported by Fondecyt 1080166. <https://register.clinicaltrials.gov>; IDr: NCT00707759.

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Vitamin D Insufficiency and Its Correlation with Low Bone Mass in Adolescent Idiopathic Scoliosis (AIS)

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Objectives: AIS is associated with both low bone mass and elevated serum bone alkaline phosphatase. The greater the latitude of the geographical region, the higher is the prevalence of AIS. These specific

features were compatible with the presence of either Vit-D insufficiency or abnormal physiology with Vit-D. It is important to evaluate these potentially treatable conditions regarding their roles in the etiopathogenesis of AIS. The objectives of this case-control study were to evaluate Vit-D status and its correlation with areal bone mineral density (aBMD) in AIS subjects and normal controls.

Methods: 215 AIS girls and 186 gender-matched non-AIS healthy controls (mean age 12.9 \pm 0.6 and 12.9 \pm 0.5 years old respectively, p=0.449) were recruited in the summer and winter season. aBMD at bilateral femoral necks was measured with Dual Energy X-ray Absorptiometry(DXA) and serum 25(OH)Vit-D was measured with liquid chromatography tandem mass spectroscopy.

Results: The mean aBMD at the right and left side for AIS subjects were 0.752 \pm 0.109, 0.745 \pm 0.107, and that for controls were 0.785 \pm 0.114 and 0.785 \pm 0.117 gm/cm² respectively. The mean 25(OH)Vit-D levels for AIS and controls were 41.6 \pm 14.4 and 39.5 \pm 11.5 nmol/L respectively (p=0.103). With multivariate linear regression analysis using aBMD as the dependent variable and after adjustment for age, body weight, armspan, season, physical activity and dietary calcium intake levels, the p-value of the regression coefficient for 25(OH)Vit-D level for the right and left side for controls were 0.055 and 0.047, while that for AIS were 0.804 and 0.466 respectively.

Conclusion: Both the AIS and control group had mean 25(OH)Vit-D levels at the insufficiency range. The positive correlation between Vit-D level and aBMD that was seen in normal controls was not present in AIS subjects, thus spelling out the possibility of certain degree of Vit-D resistance being present in AIS. Whether the lack of correlation is responsible for low bone mass that characterizes AIS and how this is related to the etiopathogenesis of AIS warrant further studies.

Funding source: Research Grants Council of the Hong Kong S.A.R., China(Project no: 468809 and 468411).

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Coping with Osteogenesis Imperfecta; What kind of difficulties are Families living with?

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Aim: Osteogenesis imperfecta (OI) the most common genetic disorder of bone is characterized by frequent, unpredictable fractures of long bones with progressive skeletal deformity. Although patients with OI are severely affected physically, this disorder may have a profound influence on patients and their families. This report reviews the extent of this influence which includes the emotional burdens, the social costs of immobilization and repeated hospitalization.

Methods: A total of 46 OI family members were included in this cross sectional report. The interviews were based on a questionnaire including a total of 16 open ended and 19 close ended questions. Descriptive statistical methods were used.

Results: The mean age of the study group was 35,52 \pm 6,65 years and 93,5 % of the were mothers. 37 % of the families were consanguineous. 95,7 % of the family members described OI as brittle bones but included emotional emphasis as 'very bad, damn, something that needs sacrifice, hard, disappointing, a disease that composes perturbation and hopelessness'. 91,3 % of them thought that their knowledge about OI and books in Turkish about OI were inadequate. When they were first diagnosed all of them felt anxiety, 95,7 % sadness, 89,1 % amazement, 87 % uneasiness, 87 % fear, 84,8 % disappointment, 60,9 % depressive. They coped with this situation by; trying to learn about the disease 67,4 % , taking social support from their relatives 65,2 % , with the help of religion 54,3 % , help of their doctor 45,7 % , with the help of the health care providers 37 % and by drifting apart from people 43,5 % . 56,5 % them had physical, 97,8 % psychological, 97,8 % social and 76,1 % had



economical problems. 82,6 % of the caregivers claimed inadequate information was given by the health care professionals.

Conclusion: Although medical treatment is of utmost importance it is necessary to regard other aspects as psychological, social and moral in patients and their families with OI.

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The effect of psychoeducation in families with osteogenesis imperfecta

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Aim: To investigate the effect of psychoeducation program in families with osteogenesis imperfecta (OI).

Methods: Sixteen family members of OI patients were included in the program. The research was designed as a semi structural, semi experimental pre and post test. The scales used were; Introductory Information Form, Burden Interview (BI), Coping Strategies Scale (CSS), Problem Solving Inventory (PSI), Psychosocial Adjustment to Illness Scale (PAIS). A ten session psycho education program was conducted between September-December 2012 with the caregivers of OI patients. SPSS 16 package program was used for the statistical analyses.

Results: The mean age of the caregivers' was 35.25±6.79 years. In the pre education period the participants described OI as; fragile bones (100 %) and the emotional impression was described as 'very bad, damn'. After psychoeducation these changed to 'a decrease in collagen' (68,8 %) and a disease that effects a family lifelong without a definite cure. The psychological effects of the disease decreased with education. Anger from 43.8 % to 6.3 %, sadness from 93.8 % to 6.3 %, anxiety from 68.8 % to 31.3 %, stress from 81.3 % to 6.3 %, intimidation from 50 % to 0 %. Similarly social isolation decreased from 50 % to 18 %, social scantiness from 87,5 % to 12,5 %, protective behavior from 93,5 % to 12,5 %. Besides all of these the participants reported a decrease; in neglecting the healthy child, channeling the anger to the other child or to the spouse and feeling of anger. The ambiguity in the progress of the disease decreased from 100 % to 56,3 %, lack of information from 75 % to 6,3 %, and the cosmetic anxiety from 18,8 % to 6,3 %. After psychoeducation 75 % of the participants stated positive modifications in their lives. In variance analyses the differences before and after psychoeducation was significant in burden interview scale; compliance subscale of coping strategies scale; orientation, professional surrounding, social surrounding and psychosocial adjustment subscales of PAIS-SR (p<0.05).

Conclusion: With the psychoeducation as their knowledge increased about OI, the feeling of anxiety have decreased. Positive modifications have been stated in social area and their family relations. With this education program the participants had the chance of evaluating their lives and making changes in their psychosocial environment.

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Craniofacial consequences of high dose zoledronic acid injections in onco-pediatric patients

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Background: High doses of zoledronic acid (ZOL), one of the most potent inhibitors of bone resorption; is currently evaluated in a phase III clinical trial in Europe for the treatment of malignant pediatric primary bone tumors. The impact of such an intensive treatment on the craniofacial skeleton is a critical question in the context of patients with

actively growing skeleton; in particular in the light of our previous studies evidencing that endochondral bone formation was transiently disturbed by high doses of ZOL.

Methods: Two protocols adapted from pediatric treatments were developed for newborn mice (A total of 5 or 10 injections of ZOL 50 µg/kg every two days). Their impact on skull bones and teeth growth was analyzed by microCT and histology up to 3 months after the last injection.

Results: ZOL administrations induced a transient delay of skull bone growth and an irreversible delay in incisor and first molar eruption and root elongation. Other teeth eruption was affected, but most were erupted by 3 months. All molars root histogenesis was severely impacted and massive odontogenic tumor-like structures were observed in all mandibular incisors.

Conclusion: High doses of ZOL irreversibly disturbed teeth eruption and elongation, and delayed skull bone formation. These preclinical observations are essential for the follow-up of onco-pediatric patients treated with ZOL.

P32

The effect of vitamin d supplementation on calcium excretion in thalassemia

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Objective: Transfusion dependent thalassemia (TM) patients have routinely been placed on vitamin D supplementation due to their increased risk of osteoporosis, as well as their high rates of vitamin D deficiency (serum 25 hydroxyvitamin D (25-OHD) < 11 ng/mL) and insufficiency (25-OHD < 30 ng/mL). Furthermore, recent studies have linked 25-OHD levels to hypercalciuria and nephrolithiasis in TM. The objective of this study is to determine the effect of vitamin D supplementation on vitamin D stores and calcium excretion in TM patients.

Methods: Prospective, single-blind, placebo-controlled study of TM patients followed in the transfusion clinic at Weill Cornell/New York Presbyterian Hospital. Patients with 25-OHD concentrations between 15-29 ng/mL were eligible for this 3-month study. Subjects were assigned in a block type of enrollment to the "high dose" (2,000 IU of vitamin D/day) group versus placebo group.

Results: 20 subjects were evaluated, with 10 assigned to each group. The "high dose" group consisted of 7 females/3 males, aged 14.6-45.7 years. The placebo group consisted of 5 females/5 males, aged 15.2-45.5 years.

After the 3 month study period, hypercalciuria developed more frequently in those treated in the "high dose" group. In the placebo group, the average 25-OHD level did not change significantly (baseline: 17.6 ng/mL, 3 month 17.0 ng/mL, p=0.65). 2/10 (20.0%) and 0/5 (0%) developed hypercalciuria or had worsening hypercalciuria, based on spot urine calcium/creatinine and 24 hour urine calcium excretion, respectively. In the "high dose" group, the average 25-OHD level increased from 20.1 ng/mL to 31.9 ng/mL (p<0.01). 5/10 (50.0%) and 3/7 (42.8%) developed hypercalciuria or had worsening hypercalciuria, based on spot urine calcium/creatinine and 24 hour urine calcium excretion, respectively.

Conclusion: Our findings suggest that "high dose" vitamin D supplementation results in high rates of hypercalciuria in TM patients. Further studies are necessary to determine the optimal dose of vitamin D supplementation and ideal 25-OHD level to minimize the risk of osteoporosis while preventing nephrolithiasis in TM patients.



P33

Patients with mutations in PHEX or FGF23 share FGF23 excess but present distinct bone and mineral metabolism features

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Mutations in PHEX and specific missense mutations of FGF23 result in elevated circulating FGF23 and hypophosphatemic rickets, respectively X-linked hypophosphatemic rickets (XLHR) and autosomal dominant HR (ADHR). FGF23, secreted by osteoblasts and osteocytes, regulates phosphate handling and vitamin D metabolism through its action on kidney. Extra renal effects of FGF23, including bone, have been very recently suspected mainly from overexpression or underexpression of FGF23 in mouse models. PHEX is expressed by osteoblasts, osteocytes and odontoblasts; its precise function in controlling circulating FGF23 level is still unclear.

Aim: Examine the role of PHEX on bone and mineral metabolism by comparing the phenotype of patients with elevated FGF23 levels due to PHEX or FGF23 mutations

Patients: 6 patients with FGF23 mutations and ADHR (4 children and 2 untreated adults); 23 patients with PHEX mutations and XLHR (18 children and 5 untreated adults); XLHR patients were matched to ADHR patients for age at start of treatment

Results: Children with ADHR were diagnosed earlier (1.5±0.0 yrs) than children with XLHR (2.3±0.2 yrs, p=0.03), with similar leg bowing (intercondylar distance 7.9±2.3 and 5.0±0.7, respectively). At diagnosis, they presented with bone demineralization and fractures in one patient, whereas none of the 18 XLHR patients had fractures nor bone demineralization. ADHR children had significantly higher alkaline phosphatases than XLHR children (649±103 and 2037±439, p=0.01, respectively). Phosphate, PTH and calciuria were similar in both groups. Follow-up revealed that, in opposition to XLHR, phosphate supplements and vitamin D analogs easily restored serum phosphate levels in ADHR. Final height of untreated ADHR adults appears higher than that of untreated XLHR.

Conclusion: Despite the limited number of patients, we pinpointed differences in the phenotypes of ADHR and XLHR. This suggests that the phenotype associated with PHEX deficiency does not uniquely result from FGF23 excess, yet advocates a direct role of PHEX on bone mineralization and growth.

P34

Reliability of pQCT scan protocol of second metatarsal for children with Juvenile Idiopathic Arthritis

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Juvenile Idiopathic Arthritis (JIA) is associated with low bone mass, poor bone strength, and an increased fracture risk. Children with JIA enter adulthood with suboptimal bone mass. In children with JIA, fracturing of the 2nd metatarsal is common due to poor bone strength. Currently no gold standard measure exists for bone quality in the foot. A reproducible protocol is required to assess key bone outcomes at the 2nd metatarsal using pQCT. Objective: To develop a pQCT scan protocol for the measurement of 2nd metatarsal bone outcomes. Methods: A custom-made foot plate standardised the optimal scanning position.

Eleven embalmed cadaveric lower leg specimens were scanned 6 times; 3 times with, and 3 times without repositioning. Sixty-six scans were obtained at 15% (distal end) and 50% (mid shaft) of the 2nd metatarsal. Voxel size and scan speed were reduced to 0.40 mm and 25 mm.sec⁻¹, respectively. The reference line was positioned at the most distal portion of the 2nd metatarsal. To maximise trabecular bone analysis at the 15% distal end, we selected a zero contour threshold, 650 mg.cm² inner threshold, peel mode 4, and 1% concentric peel. At 50% mid shaft, a 600 mg.cm² threshold and separation mode 2 were used. Results: Reliability of scans without repositioning: trabecular area (intra-class correlation coefficient [ICC] 0.86, 95%CI 0.63-0.96), trabecular density (ICC 0.96, 95%CI 0.90-0.99), Strength Strain Index [SSI] (ICC 0.99, 95% CI 0.99-1.0), cortical area (ICC 0.99, 95%CI 0.98-1.0). Reliability for scans after repositioning: trabecular area (ICC 0.96, 95%CI 0.90-0.98), trabecular density (ICC 0.98, 95%CI 0.95-0.99), SSI (ICC 0.99, 95% CI 0.98-1.0), cortical area (ICC 0.99, 95%CI 0.98-1.0). Conclusion: The scanning protocol generated excellent reliability for key bone outcomes measured at the distal and mid-shaft regions of the 2nd metatarsal. The pQCT protocol will now be applied to children with JIA for identifying insufficiency fractures.

P35

Six monthly intravenous zoledronic acid in childhood osteoporosis

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Objectives: Childhood osteoporosis can be treated with intravenous bisphosphonates in order to improve bone mass and density. The aims of this study were to evaluate the safety and efficacy of six-monthly zoledronic acid (ZA) in children with osteoporosis.

Methods: A retrospective cohort study of 27 patients (16 males and 11 females) were treated with six-monthly ZA (0.05mg/kg/dose) for a minimum of one year. 17 patients were immobile, 4 had steroid-induced osteoporosis, 2 had osteogenesis imperfecta and 4 had other diagnoses. 16/27 (59%) had long bone fractures and 12/27 (44.4%) had vertebral wedging at baseline. Mineral homeostasis, bone mineral density by DXA and vertebral morphometry were evaluated at baseline and 1 year.

Results: The median age at commencement of treatment was 12.3 years (range 8-15.8). Following the first infusion, 2/27 (7%) and 1/27 (4%) developed asymptomatic hypocalcemia at 48 hours and 72 hours, respectively. A fever above 38  C developed in 14/27 (52%), generalised aches/pains in 13/27 (48%) and nausea in 6/27 (22%). At 1 year there was a significant reduction in bone turnover and improvement in bone mineral density (BMD) (Table). Patients with vertebral wedging at baseline showed significant improvement in anterior, middle and posterior vertebral height ratios at 1 year. Only one patient fractured after starting ZA. There was normal growth.

	Baseline	1 year
Calcium (mmol/L)	2.38 (2.35-2.44)	2.36 (2.28-2.42)
Alkaline phosphatase (U/L)	188 (143-271)*	148.5 (127.25-205.5)*
Osteocalcin (nmol/L)	7.9 (4.35-11.35)*	2.3 (1.1-3.95)*
25-OH-VitD (nmol/L)	75 (67-94)	76 (57.5-86)
Parathyroid hormone (pmol/L)	3.5 (2.3-4.1)	3.65 (2.93-5.4)
Total body areal BMD Z-score	-0.56 (-1.7 to 0.35)*	-0.03 (-1.13 to 0.86)*
L2-4 areal BMD Z-score	-1.73 (-2.43 to -0.96)*	-0.37 (-1.44 to 0.09)*
Bone mineral content for lean tissue mass Z-score	-1.68 (-2.51 to -0.66)*	-0.10 (-0.9 to 1.35)*

Table: Mineral homeostasis and DXA data at baseline and 1 year values represent median (interquartile range), asterisk represents p<0.05 comparing baseline to 1 year

Conclusion: Six monthly ZA was associated with an acute phase reaction to the first dose and improvement in BMD, reduction in bone turnover and improved vertebral shape at 1 year.



P36
Vitamin D Deficiency and its Relationship to Parathyroid Hormone in Morbidly Obese Adolescents Prior to Bariatric Surgery

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Objectives: Obese adults commonly have vitamin D deficiency and secondary hyperparathyroidism prior to bariatric surgery. Whether similar metabolic abnormalities exist in morbidly obese adolescents is not known. This study investigated the prevalence of vitamin D deficiency and evaluated the relationship between vitamin D and parathyroid hormone (PTH) in morbidly obese adolescents undergoing evaluation for bariatric surgery.

Methods: A cross-sectional study of preoperative laboratory measures from 236 adolescents evaluated for bariatric surgery between March 2006 and June 2011

Results: Of 236 patients, 219 patients had 25-hydroxyvitamin D (25OHD) and PTH levels. Patients (76 boys, 143 girls; age: 15.9 +/- 1.2 years; 43% Caucasian, 35% Hispanic, 15% African-American) had mean BMI of 47.6 +/- 8.1 kg/m². Serum 25OHD levels (mean 20.7 +/- 9.8 ng/ml) were deficient (<20 ng/ml) in 53%; 8% had severe deficiency (<10 ng/ml); only 18% of patients were replete (>30 ng/ml). Findings varied by race, with 82% of African-Americans, 59% of Hispanics, and 37% of Caucasians vitamin D deficient. Race was the strongest predictor of 25OHD (p<0.0002). Greater BMI was associated with lower serum 25OHD (r = -0.28, P < 0.0001); this persisted after controlling for PTH (r=-0.22, p<0.002). PTH levels varied inversely with 25OHD (r = -0.24, p = 0.0003). Although only 8 patients (3.7%) had frankly elevated PTH levels (>55 pg/ml), 37 patients (16.9%) had PTH levels in the upper third of normal (>40 pg/ml), 89% of whom had 25OHD levels <30 pg/ml. African American race, BMI, and PTH explained 21% of the variance in 25OHD (p < 0.0001).

Conclusions: The vast majority of severely obese adolescent patients presenting for bariatric surgery have sub-optimal vitamin D levels. Those at greatest risk for deficiency were African-American and those with highest BMIs. Our results also suggest that the current laboratory normal range for PTH may significantly overestimate normal values for children and adolescents and underestimate the prevalence of secondary hyperparathyroidism with its subsequent negative effects on long-term bone health. This is particularly important prior to bariatric surgery, where weight loss itself and decreased calcium and vitamin D absorption in some procedures may place these patients at further risk.

P37
Reduction in bisphosphonate side effect profile using short term steroid cover

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Objectives: Bisphosphonate infusions are associated with numerous adverse effects including acute systemic inflammatory reactions and electrolyte abnormalities. The aims of this study were to evaluate the safety and efficacy of a three-day course of prednisone on children receiving their first dose of pamidronate or zoledronic acid.

Methods: A retrospective cohort of 166 patients (85 males) were commenced on pamidronate (16%) or zoledronic acid (84%) for treatment of osteoporosis. 58 patients (35%) received a three-day course of prednisone (1mg/kg/day) starting on the day of bisphosphonate treatment. All patients received supplementation with calcium (1g twice-daily) and calcitriol (250ng twice-daily) for three days. Mineral homeostasis was assessed on day two and three. Symptomatology

(including fever, nausea, vomiting, headache and malaise) was evaluated on day three.

Results: The mean age at commencement of treatment was 11±4 years for both groups (p>0.05). There was a significant decrease in serum calcium and phosphate on day two that persisted to day three post bisphosphonate infusion for both groups (Table 1). Alkaline phosphatase levels remained unchanged throughout. There were no differences in mineral homeostasis between those receiving prednisone and those who did not. There were no new cases of significant hypocalcemia (<1.9mmol/L) on day three that were not evident by day two.

	Baseline		Day 2		Day 3	
	No Steroid	Steroid	No Steroid	Steroid	No Steroid	Steroid
Ca (mmol/L)	2.35 ± 0.19	2.37 ± 0.13	2.18 ± 0.18*	2.26 ± 0.18*	2.17 ± 0.18*	2.22 ± 0.15*
ALP (U/L)	211 ± 77	232 ± 139	224 ± 73	231 ± 131	206 ± 78	214 ± 128
PO ₄ (mmol/L)	1.51 ± 0.22	1.52 ± 0.26	1.18 ± 0.25*	1.06 ± 0.30*	1.00 ± 0.24*	0.90 ± 0.25*

Table 1: Mineral homeostasis

Values represent mean ± SD, asterisk represents p<0.05 compared to corresponding baseline value

Patients receiving steroid cover had a significantly lower incidence of headache, nausea and malaise compared to those who did not (Table 2).

	No Steroid n=108 (%)	Steroid n=58 (%)	Relative Risk (95% CI)
Headache	30 (28%)	5 (9%)	0.3 (0.1-0.8)*
Nausea	36 (33%)	7 (12%)	0.4 (0.2-0.8)*
Malaise	71 (66%)	23 (40%)	0.6 (0.4-0.9)*
Vomiting	14 (13%)	5 (8%)	0.7 (0.3-1.8)
Fever	43 (40%)	28 (48%)	1.2 (0.9-1.7)

Table 2: Symptomatology

Asterisk represents p<0.05

There were no differences in mineral homeostasis or symptomatology between those receiving pamidronate and zoledronic acid.

Conclusion: A three-day course of prednisone at the time of initiating intravenous bisphosphonate therapy significantly reduced the incidence of headache, nausea and malaise. Steroid use did not affect bisphosphonate-induced hypocalcemia or hypophosphatemia. Biochemical monitoring for hypocalcemia was not necessary beyond 48 hours.

P38
Abnormal bone quality in both cortical and trabecular compartments in Adolescent Idiopathic Scoliosis (AIS)

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Introduction: AIS is associated with osteopenia which was found to be a significant prognostic factor for curve progression. However, in-depth quantitative assessment of bone quality was previously hampered by invasive nature of the investigations. The recently available high-resolution pQCT (HR-pQCT) allows a three-dimensional assessment of the bone quality in-vivo. This study aimed to evaluate and compare the bone quality in AIS girls and controls.

Methods: 234 untreated AIS and 211 non-AIS healthy girls between 11-13 years old were recruited. Bone quality, including Bone Morphometry, vBMD and Trabecular Bone Micro-architecture were measured at the non-dominant distal radius using HR-pQCT. To consolidate our understanding on the trabecular bone micro-architecture, Structural Model Index (SMI), which measures the degree of rod/plate-like configuration of trabeculae, was further determined in 73 AIS and 48 controls.

Results: Bone quality is altered in both cortical bone and trabecular bone in AIS. With multivariate linear regression analysis, after adjusted for age, arm span and sexual maturity, AIS was significantly associated with lower Cortical Area, Cortical Thickness, Cortical Bone vBMD, Trabecular Bone vBMD, BV/TV, Trabecular Number and greater Trabecular Separation and SMI.



Conclusion: The abnormal profile in bone quality suggested the presence of disturbed endocortical modeling, trabecular bone formation and bone mineralization and could contribute to a better understanding of the role of abnormal bone quality in the etiopathogenesis of AIS. Furthermore, Cortical Area, an important parameter in bone strength index associated with cross-sectional moment of inertial (CSMI), could be an important potential clinical parameter for further longitudinal studies on the prognostication of curve progression in AIS.

This study was supported by Research Grants Council of the Hong Kong S.A.R., China (Project no: 468809 and 468411)

P39
Prevalence of Vitamin D deficiency in adolescent Muslim girls attending a school in the UK which adheres to a conservative dress code

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Aim: To determine the prevalence of vitamin D deficiency among adolescent Muslim girls attending a school in the UK which adheres to a conservative dress code.

Methods: Fifty six (31%) out of 180 girls attending a Muslim High School for Girls [median age 13.2years, (IQR 12.5-13.8 years)] took part in this cross-sectional study. Seventy nine percent (n=45) were of South Asian origin, 3.5% were Black African origin (n=2), 1.8% was Middle Eastern origin (n=1) and 15.8% were other ethnic origin (n=9). The participant's serum concentration of calcium (Ca), phosphate (P), alkaline phosphatase (ALP), parathyroid hormone (PTH) and serum 25-hydroxyvitamin D (25OHD) were measured. Dietary intake of calcium and vitamin D were also estimated.

Results: Fifty-one out of 56(91%) (95% CI 86 to 95) girls were found to be vitamin D deficient (25OHD < 25nmol/L) and 4 out of 56 (7%) (95% CI 5 to 17) were vitamin D insufficient (25nmol/L < 25OHD < 50nmol/L). The median serum concentration of 25OHD was 12.8nmol/L, (IQR 10 to 17 nmol/L). Serum concentration of Ca, P, ALP and PTH were all within the normal limits. The median vitamin D intake was 69 i.u./day, (IQR 29 to 122 i.u./day), which is 17% of the recommended daily intake (400 i.u.). The median dietary calcium intake was 662mg (IQR 456 -860mg), which is four-fifth of the recommended daily intake (800 mg). The median serum 25OHD of subjects taken multivitamin (MV) supplements [median= 17.3nmol/L (IQR 13-26.3)] was (p<0.01) higher than that of subjects not taking MV supplements [11.3nmol/L (IQR 8.8-14)].

Conclusions: All subjects (98%) bar one had vitamin D deficiency (91%) and vitamin D insufficiency (7%). Those taking MV supplements had higher serum 25OHD concentrations, although optimum serum concentrations of >50 nmol/l were not achieved. Further studies are needed to determine the dose of vitamin D supplementation needed to optimise the vitamin D status of these groups of adolescent girls.

P40
Altered bone metabolism in obese children: The pathogenic role of adipocytokines and inflammation

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Objective: Associations between childhood obesity and disturbed bone metabolism have been extensively studied. Both RANK/RANKL/OPG and adipocytokines are involved in bone metabolism in obese individuals although published data remain inconsistent. Some reports show evidence of protective role of adiposity in the maintenance of

skeletal mass, whereas others fail to support this evidence or even demonstrate an increased fragility. The aim of the study was to assess bone metabolism in obese children and to evaluate influence of adipocytokines on their bone mass.

Methods: In 40 obese children aged 7.1-17.6 years and 30 non-obese controls, total and lumbar spine bone mineral content (BMC) and density (BMD) were determined using dual-energy X-ray absorptiometry (DXA). Serum lipids, total calcium (Ca_{total}), 25-hydroxycholecalciferol (25OHD), parathormone (PTH), leptin, adiponectin, osteocalcin (OC), osteoprotegerin (OPG), RANK-ligand (RANKL) and high-sensitivity CRP (hsCRP) were measured.

Results: Higher levels of leptin (p<0.001), hsCRP (p<0.001), Ca_{total} (p=0.012), PTH (p<0.001) were found in obese children. Serum adiponectin and OC were lower in obese individuals (p=0.005 and p=0.12, respectively). Total and spine BMD and BMC were higher in the obese than in controls. We found association between adiposity and hsCRP which correlated with leptin (r=0.55;p<0.001), RANKL (r=0.25;p=0.04), PTH (r=0.36;p=0.004), total cholesterol and triglycerides (r=0.38, 0.39; both p=0.001). Furthermore, PTH activity correlated positively with leptin and inversely with adiponectin levels. There was no difference in RANK/RANKL/OPG pathway activity between obese and controls, and no correlations were found between its activity and adipocytokines levels either. No differences were found in concentration of 25OHD between the groups.

Conclusions: These results support the role of inflammation in obesity. Chronic inflammation with altered adipocytokine levels may be responsible for inadequate bone metabolism in childhood obesity. Higher PTH and Ca_{total} concentrations may reflect parathyroid hyperactivity as a mechanism leading to bone resorption in obese individuals despite excessive BMD accrual. Coincidence of hypercalcemia and decreased adiponectin seems an additional risk factor of atherosclerosis during growth. More research is needed to understand the role of PTH in bone and fat relationships in children.

The study was supported by the Human Capital Programme (8.2.1.) organized by the European Social Fund (EU Structural Fund).

P41
Management of a new case of Neonatal Hypocalcaemic Hypercalcemia Related to Mutation of the Calcium-Sensing Receptor Gene with Bone Abnormalities

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Background: A 5-month-old girl was referred to our unit after a systemic screening for hip dislocation by x-rays revealed bilateral femoral bowing. She was the first child of healthy non-consanguineous parents, and her family history was unremarkable. Her parents had a normal physical examination, and normal laboratory findings. At presentation, her height was 64.0 cm (Z-score: 0.0) with a regular height velocity. Weight was 7.4 kg (Z-score: 1.0). On physical examination, there was bilateral bowing of the femurs. The remaining examination was unremarkable.

Presenting Problem: Laboratory investigations revealed hypercalcemia (total calcium 3.20 mmol/l), phosphatemia at lower limit (1.6 mmol/l), normal alkaline phosphatase level (643 UI/l), inappropriate level of intact PTH (85 pg/ml), and urinary calcium/creatinine ratio at upper limit (1.08 mmol/mmol). Serum 25-hydroxyvitamin D (25OHD) level was normal (23 ng/ml). A skeletal survey revealed bilateral femoral bowing without other bone abnormalities. Calcium-Sensing Receptor (CaSR) gene analysis found combined heterozygote mutations with a missense mutation resulting in amino-acid N592S substitution in the extracellular domain and a R648X nonsense mutation.

Clinical Management: Intravenous disodium pamidronate (2 infusions of 0.5 mg/kg and 1.0 mg/kg at months 6 and 7 respectively) was



administrated to control the excess of bone resorption and hypercalcemia. Tolerance was good. Calciuria decreased under treatment. Cholecalciferol was associated.

Discussion: We report here a new case of neonatal hypocalciuric hypercalcemia responsible for bone deformity with combined mutations of the CaSR gene. Inactivating mutations of the CaSR gene usually cause Familial Hypocalciuric Hypercalcemia, an autosomal dominant disorder characterized by hypercalcemia, inappropriately high PTH levels, and low urinary calcium excretion. Various managements have been proposed. Because of the bone presentation, instead of reduction of calcium intake and cholecalciferol administration, we indicated biphosphonate treatment in order to reduce hypercalcemia and the consequences of chronic hyperparathyroidism. Calcemia remained controlled and calciuria was reduced, indicating reduction of the bone turn-over. Like in other cases of hyperparathyroidism, the use of biphosphonates seemed to us logical as transient treatment in cases of CaSR mutations associated with bone lesions. Use of calcimimetics also should be further considered when the mutations potentially affect CaSR binding.

P42
The effect of glucocorticoids on bone indices in children with rheumatic and oncological conditions

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Children with chronic medical conditions are at increased risk for bone fragility from multiple mechanisms, related both to the underlying condition and its treatment, in particular glucocorticoids. The differential effects of the underlying medical disease on bone micro-architecture have not been well elucidated.

Objectives: To describe the bone micro-architectural characteristics in children with rheumatic and oncological disorders treated with glucocorticoids, and to determine associations between micro-architectural findings with clinical variables.

Methods: Trans-iliac biopsies were performed on 25 children (13.9±3.4 yrs, 10 males) on chronic glucocorticoids; 15 with either systemic lupus erythematosus or idiopathic juvenile arthritis, 10 with previous malignancy or transplant. Subjects presented with at least one vertebral compression fracture. Static histomorphometry results were compared between groups and to published controls.

Results: Mean duration of glucocorticoid exposure was 3.7(0.4-14.4) years with a cumulative prednisone dose of 0.27±0.26 mg/kg/day in the 12 months prior to biopsy. Lumbar spine bone mineral density (BMD) was reduced (Z score -3.3±1.4), with no difference between groups. On histomorphometry, there were significantly lower structural and formation parameters compared to controls, but no difference between groups.

	Total cohort	P value ¹	Rheumatic Group	Oncological Group	P value ²
Bone volume BV/TV(%)	21.0 (6.5)	0.02	21.4 (6.9)	20.3 (6.3)	0.71
Trabecular thickness (µm)	118.6 (55.2)	0.01	114.7 (33.2)	125.2 (83.9)	0.70
Trabecular separation (µm)	470.6 (123.8)	0.49	469.5 (133.1)	469.7 (114.4)	0.99
Osteoid volume (%)	1.9 (0.1)	0.02	1.8 (0.7)	2.0 (0.7)	0.75
Osteoid surface (%)	19.5 (9.5)	0.02	18.5 (7.8)	21.5 (12.9)	0.55
Osteoblast surface	4.6 (1.6)	0.01	4.6 (1.6)	4.6 (1.6)	0.99
Adipose volume (%)	25.5 (7.0)	n/a	24.3 (8.0)	28.3 (3.5)	0.30

¹compared to published controls (Glorieux et al, 2000); ²between groups. Data are mean(SD)

Glucocorticoid dose correlated with bone volume (BV/TV r=-0.5, p=0.01), osteoblast surface (r=-0.42, p=0.02) and bone adipose volume (r=0.47, p=0.02), while steroid duration was associated with osteoid volume (r=-0.42, p=0.03). Controlling for glucocorticoid dose, lower bone volume was associated with slower growth velocity (r=0.51, p=0.01). Lower bone formation (p=0.01) and greater bone adipose tissue (p=0.02) was seen in females compared to males. There were no

significant associations between lumbar spine BMD and histomorphometry parameters.

Conclusion: Children on chronic glucocorticoids have significant impairments in bone structural and formation parameters, independent of the underlying condition. These deficits relate primarily to cumulative glucocorticoid dose.

P43
Bone quality in young thalassaemic patients

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Osteoporosis is a leading cause of morbidity in patients affected by β-thalassaemia major (TM) and intermediate thalassaemia (TI). Appropriate supportive care and identification of long-term sequels of therapy are important in thalassaemic patients. As low bone mineral quality (BMQ) in patients can be considered a marker of possible degeneration to osteopenia and osteoporosis in adulthood, we evaluated bone features in a young population followed at “A. Perrino” Hospital in Brindisi. Fifty-five thalassaemic patients (29 males, 26 females; aged 18-45 years) were analyzed during 2012 and compared vs. a matched control population (55 healthy adults: 24 males, 31 females; aged 18-46 years). Seven patients were affected by TI while the rest was affected by TM. BMQ was assessed by quantitative ultrasound (QUS) technique at the phalanx level. The main values of phalangeal QUS are the amplitude-dependent speed of sound (AD-SoS, m/sec) and the bone transmission time (BTT, microsec). QUS values were significantly lower in cases than in controls (AD-SoS: 2119.4 ± 53.9 and BTT: 1.75 ± 0.3 in controls; AD-SoS 2031 ± 75.2 and BTT: 1.43 ± 0.3 in cases). AD-SoS was negatively associated with BMI (r = -0.36, p = 0.0067 in controls; r = -0.37, p = 0.0054 in cases), while BTT was correlated with gender in both cases (p < 0.01) and controls (p < 0.0001), showing lower values in females. Our results suggest that bone quality in thalassaemic young patients is influenced by many factors that were not present in control subjects, such as iron chelation therapy, delayed sexual maturation, growth hormone deficiency, parathyroid dysfunction, hypothyroidism and liver diseases.

P44
A homozygous mutation in the DNA binding domain of human vitamin D receptor causes vitamin D resistant rickets

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In this case report, we present a brother and sister with hereditary vitamin D resistant rickets (HVDRR). Both children presented at the age of 18 months with severe rickets and elevated serum levels of 1,25-(OH)₂D₃. They differ from each other in that the girl presented with hypophosphatemia instead of hypocalcemia. Besides, she developed alopecia earlier than the boy and needed more 1,25-(OH)₂D₃ supplementation. Interestingly, the boy does not require supplementation anymore since the age of 19.

We performed DNA sequencing of both patients along with their 3 siblings and their parents, who are first cousins. Both patients carry a homozygous point mutation (A133G) in the vitamin D receptor (VDR) gene, leading to an amino acid change in the DNA binding domain (K45E). Both parents were heterozygous for the same mutation. We collected skin fibroblasts from the boy. 1,25-(OH)₂D₃ was unable to inhibit their proliferation (in contrast to a healthy subject). Moreover,



1,25-(OH)₂D₃ failed to enhance 24-hydroxylase activity and VDR expression. Binding of 1,25-(OH)₂D₃ to the VDR, however, was not affected in the patient.

Since 1,25-(OH)₂D₃ plays a role in the innate and adaptive immune system, we screened the 1,25-(OH)₂D₃ response of peripheral blood mononuclear cells (PBMCs) from the boy, his parents and healthy controls. Both the T helper cell (Th) population-specific transcription of Tbet (Th1), Gata3 (Th2) and Rorty (Th17) as well as production of cytokines, such as interferon γ (Th1) and interleukins IL-4 (Th2), IL-10 (Th17) and IL-22 (memory T cells) was altered in control PBMCs. No transcriptional or translational changes were observed in the PBMCs from the boy further supporting the inability to respond to 1,25-(OH)₂D₃.

Collectively, these data show that the A133G mutation in the VDR prevents nuclear binding, which leads to unresponsive 1,25-(OH)₂D₃ downstream signalling. Although 1,25(OH)₂D₃ acts on the immune system and has been associated with autoimmune diseases such as diabetes and multiple sclerosis, the patient, who is 1,25-(OH)₂D₃ insensitive, has no immunopathies but long-term follow-up necessary to monitor this. The treatment independence of the boy may be related to enhanced calcium uptake capacity in the intestine between 15 and 25 years of age, an age range he currently is in. The intriguing differences in onset of alopecia between the boy and the girl as well as their respective reduced serum calcium and phosphate at 18 months of age remain to be scrutinized.

P45
Bone mineral density impairment in Marfan syndrome: A hidden and neglected issue

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Objectives: Marfan syndrome (MFS) is a connective disorder caused by mutations in FBN1 gene which encodes the extracellular matrix protein fibrillin 1. Pathogenesis relies on a dysregulation of activated TGF-β. Cardiovascular, ocular and skeletal systems are involved with a variable expressivity. Findings evolve with age, making the diagnosis in children more difficult. Skeletal involvement includes disproportionate long bone overgrowth, scoliosis, and chest deformity. Although several surveys investigated bone mineral density (BMD) in adult MFS patients, data on children are scarce. Aim of our study was to assess BMD in a large cohort of children with an ascertained diagnosis of MFS.

Methods: Sixty Caucasian patients with MFS diagnosis based on 2010 Ghent nosology (29 female and 31 males, mean age 10 [3.7] y), were investigated. Neither calcium or vitamin D supplementation, nor exercise restriction was performed. Height, as expected, was above mean values (1.5 [0.2] m, Z-score 1.9 [1.4]). We measured BMD by DXA (Discovery W- Hologic) at the lumbar spine and proximal femur. PTH and 25(OH)D₃ concentrations were measured in serum.

Results: BMD Z-score were lower than normal: -0.9 (1.1) at lumbar spine, -0.69 (1.3) total femur, -1.1 (1.4) at the femoral neck (FN). As DXA overestimates density of large bones, an accurate estimate of bone mass requires a correction of the potential confounding effects of body size. The adjustments for tall stature were made using height-for-age Z-score (HAZ). Corrected data showed worse values than those standardised only to age and gender: -1.8 (1.0) at lumbar spine, -1.5 (1.3) at total femur and -0.97 (1.7) at FN. All these data were significantly different from 0 (P<0.0001). Four fractures were reported (6.6%), above the mean incidence per year from literature. PTH levels were within normal range (44.3 [25.8] pg/mL), while 25(OH)D₃ levels were below international reference values (22.6 [7.8] ng/mL).

Conclusions: MFS children have low BMD values and a high prevalence of fractures. Bone density should be considered among the

skeletal features in MFS. Management guidelines should take into account bone density impairment.

P46
Interactions of adipokines and bone metabolism in patients with severe juvenile idiopathic arthritis

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Objectives: The skeleton and adipose tissue interact. Our aim was to evaluate the interactions between adipokines and bone metabolism, and their association with disease-related factors in patients with severe juvenile idiopathic arthritis (JIA).

Methods: The study included 49 patients (median age 14.8 years, median disease duration 10.2 years) with refractory polyarticular JIA and 89 sex- and age-matched healthy controls. The subjects underwent clinical examination, body composition measurement with DXA and analyses for leptin, adiponectin and bone turnover markers.

Results: Patients with JIA were shorter and more often overweight (p=0.001) or obese (p<0.001) than controls. They had significantly higher leptin levels, even when adjusted for fat mass (p<0.001), and similar adiponectin but slightly lower adiponectin-to-fat mass ratio (p=0.073) than controls. Concentrations of bone formation markers were similar, but the level of carboxyterminal telopeptide of type I collagen (ICTP) (p=0.006) and the ratios of ICTP to osteocalcin or alkaline phosphatase were higher in the patients, indicating increased bone resorption. In controls, the inverse association between leptin and bone turnover markers was largely dependent of fat mass. Conversely, in patients the inverse association between leptin and bone formation markers was strengthened and became significant when adjusted for fat mass. Leptin, adiponectin or bone markers did not associate with disease duration or activity. Adiponectin associated inversely with ICTP in the controls.

Conclusions: We observed high adiposity and thus increased risk for metabolic complications in a cohort of patients with severe JIA. This was accompanied with increased bone resorption. Serum leptin was higher in patients, even when adjusted for fat mass. In patients leptin tended to associate inversely with bone formation markers but it did not associate with disease activity.

P47
Vitamin D deficiency and structural and functional state of bone tissue in schoolchildren of Ukraine

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Introduction: Vitamin D is an essential material in bone metabolism, and regulation of body minerals. Vitamin D deficiency has various causes, including limitations in sunlight exposure (type of clothing, sunscreen usage, indoor activity), seasonal geographic latitude and altitude, atmospheric pollution, diet, and ageing.

The aim of the work was to determine the frequency of vitamin D deficiency among Ukrainian schoolchildren and its influence on bone mineral density.

Methods: There were examined 304 children aged 10-18 years. The boys consisted 55.0 %. The average age of boys was 12.9±0.2 and girls - 12.4±0.2 yr. old. The study was performed within two months -



October and November 2011, to exclude the influence of seasonal factors on the level of 25(OH)D. Researches included ultrasound densitometry of calcaneus by SAHARA (Hologic), blood chemistry, 25(OH)D and intact parathyroid hormone (iPTH) in plasma were determined by Elecsys 2010. Also, it was evaluated the average content of calcium and vitamin D in the diet form the products consumption frequency questionnaire.

Results: Vitamin D deficiency was founded in 92.2% of schoolchildren, and vitamin D insufficiency was diagnosed in 6.1% of cases. Secondary hyperparathyroidism was verified in 0.9% of children. The average level of consumption of calcium and vitamin D in children was below recommended data, and consisted (Me 649 [488.7; 691.86]) mg/day for calcium and (Me 68.69 [58.45; 117.3]) IU/day for vitamin D.

Children with vitamin D insufficiency had significantly higher data of structural and functional state of bone tissue in comparison with the data of pupils with severe deficiency of vitamin D: stiffness index 105.03±6.12 vs 93.7±2.51% (p<0.02); BMD 0.574±0.024 vs 0.528±0.019 (p<0.02) and speed of sound 1573.61±6.70 vs 1557.2±5.41 (p<0.01).

Conclusion: High level of vitamin D deficiency (92.2%), secondary hyperparathyroidism (0.9%), low data of ultrasound densitometry in severe vitamin D deficient children make doctors to research the effective methods of treatment and prophylactics of revealed disorders.

P48

Pubertal induction with testosterone of a boy with bilateral anorchia guided by the development of his monozygotic twin brother

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Introduction: We describe a monozygotic twin pair, of which one boy was diagnosed with anorchia. Both were followed-up till age 17.

Case Report: At birth, in one twin 46 XY boy (A), testes were not palpable while his brother (B) was unaffected. Stimulation with human chorionic gonadotrophin (hCG) and orchidopexia were unsuccessful at age 3. A second hCG-stimulation test was performed at age 8, where serum testosterone response failed to increase. No testicular tissue was detected by abdominal laparoscopy. At the age of 10.5, when the bone age was 11.6 years both in A and B, low dose testosterone substitution therapy (25 mg/2 weeks) was started. Before puberty induction, A and B had similar weight and height. During puberty, a slightly faster increase in weight (A/B 11-19%) and height (A/B 3-7%) was observed in A. A and B ended up with a similar and normal final height, weight, arm span and sitting height. Secondary sexual characteristics developed normally in both brothers. At the age of 17, bone mineral density, body composition (dual x-ray absorptiometry, DXA-scan), volumetric bone parameters at forearm and calf (peripheral quantitative CT-scan) were evaluated. We observed similar bone mineral density at the lumbar spine, total hip, distal radius and whole body (DXA, A-B <5%). Fat percentage was 14% in A vs. 11% in B. Trabecular (distal radius) and cortical volumetric bone parameters (mid and proximal tibia) were comparable (A-B <0.5%). However, at one cortical site (proximal radius), A had a smaller cortical bone size with a thicker cortex (all 10-20%).

Conclusion: Low dose testosterone substitution in bilateral anorchia, guided by the pubertal evolution in the healthy twin, led to a comparable pubertal development, final height and bone mineral density. Moreover, testosterone did not seem to be necessary for normal increase in length before puberty.

P49

Some features of regulation of the bone tissue metabolisms at the newborns who have transferred infectious influence during intrauterine development

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Perinatal infections appreciably defines the level's of the neonatal diseases and death rates. The Systemic inflammatory Response Syndrome at the infections can promote the softening of the bone's tissue in pregnant women and their fetuses.

Objectives: Definition of the connection of the level's calcium-regulated hormones and bone tissue remodeling marker's in blood at 32 (II) healthy and 48(I) pregnant women with perinatal infections, also in the funic blood at their fetuses with development of bone-muscular system of children.

Method: In dynamics were defined the blood levels of 25 (OH) vitamin D, parathyroid hormone (PTG), osteocalcin (OC), β-CrossLaps by immunoassay methods. At children' birth anthropometrical data, development of the bone-muscular system, quality of neonatal period's reflexes were estimated.

Results: At the third newborns from I-st group clinical attributes of insufficient mineralization of bone system are revealed. At women I-st group concentration of vitamin D (72,17±3,29 vs 84,22±5,08 mmol/l) was authentically smaller; in the funic blood was above (85,39±6,72 vs 51,40±3,01 mmol/l, p<0,01). Significant distinction in PTG levels between groups at women (25,46±2,20 vs 30,24±1,61pg/ml) and in funic blood (6,12±0,14 vs 6,14±0,12 pg/ml, p>0,05) is not revealed. Concentration of OC (90,37±5,96 vs 53,00±6,09 ng/ml, p<0,01) and β-Cross-Laps in the umbilical cord (0,85±0,03 vs 0,65 ±0,04 pg/ml, p<0,01) was above in II-nd group. At women with infection bones synthesis and resorption's speed were significant above (OK-24,46±1,79 vs 18,48±1,17ng/ml, β-CrossLaps - 0,80±0,07 vs 0,61±0,04pg/ml).

Conclusion. At women with perinatal infections and their newborns infringement of processes of regulation of the bone's tissue metabolism and the mineralization is observed.

P50

Bioelectrical impedance as a screening tool for low bone mass in Indian children and adolescents

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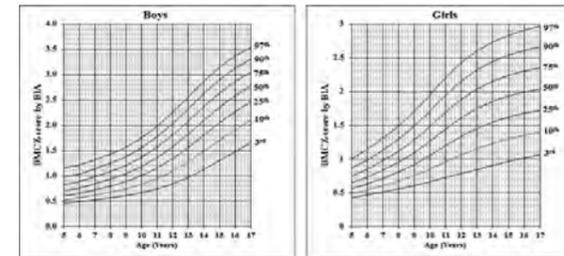
Objectives: 1) To assess bone (BMC) by BIA in apparently healthy Indian children 2) To generate percentile curves for BIA measured BMC by age/gender 3) To investigate relationship between BMC measured by BIA and DXA in separate subset.

Methods: In a multicentre study, 4,154 children (2298 boys), 5-18 years underwent BMC assessment by BIA. BMC for age percentiles were computed using LMS method. In 41 children (not from the multicentre study) total body BMC was measured by DXA and BIA. Z scores for BMC for age by DXA and BIA (derived from the present study) were computed. Pearson's correlation and Bland-Altman limits of agreement were derived between BMC by BIA and DXA. Receiver operating curve (ROC) analysis was performed for cut-off point for BIA Z score corresponding to Z score of -2 by DXA to discriminate between those with or without risk of low bone mass.

Results: Mean age of children was 10.7 ±3.3; height, weight and BMI for age Z scores were close to zero for all ages. Correlation coefficient between BMC by BIA and DXA was 0.83. Bland-Altman limits of agreement indicated that a BIA reading would be -1.52 below or 1.3 above the DXA, thus ROC analysis was performed. Cut-off yielding the



maximal sensitivity and specificity for predicting low bone mass by BIA was Z score of -1.3 (10th percentile) (sensitivity = 94.1%, specificity = 40%, AUC 0.85) (95% CI: 0.694-0.941).



Conclusions: A z score of -1.3 by BIA corresponds to -2 of DXA which may be a useful tool for screening for the risk of low bone status. Further studies are required to confirm our results.

P51

Mild visual impairment in a 13 year old child with Osteoporosis-Pseudoglioma Syndrome

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Background: Osteoporosis-pseudoglioma (OPPG) syndrome is an autosomal recessive disorder characterised by severe juvenile osteoporosis and congenital or infancy-onset visual loss. OPPG is caused by loss of function mutations in low-density lipoprotein receptor-related protein 5 (LRP5) gene. We present a 13 year old child with a homozygous mutation in LRP5 and low bone mass but without visual loss.

Presenting Problem/Clinical Management: This child presented with multiple low trauma fractures and baseline bone mineral density at the lumbar spine was 0.759g/cm² (age matched Z score of -2.0) prior to treatment with cyclical intravenous bisphosphonates. Ophthalmology assessment detected retinal folds and myopia (0.56 on the LogMAR scale). Functionally the patient was able to attend normal school, read and travel on public transport without support.

The LRP5 gene was analysed and found to be homozygous for the sequence variant c1517A>T predicted to result in a p.Asn506Ile amino acid change. Both parents were non affected heterozygous carriers.

Discussion: Mutations in the LRP5 gene are known to be associated with skeletal disorders: gain of function mutations lead to high bone mass; loss of function mutations results in OPPG and heterozygous mutations can result in primary osteoporosis. The homozygous mutation in this patient was in the highly conserved YWTD motif and supported the diagnosis of OPPG. Most patients with OPPG are blind by 15 years of age; however, this patient's visual impairment only caused mild functional compromise. Whilst further functional studies are needed, this unusual case of mild visual impairment in a patient with OPPG is important in furthering our understanding of genotype-phenotype correlations in this disease.

P52

Incidence of fractures in 2 to 18 year old affluent Indian children: A multicentre study

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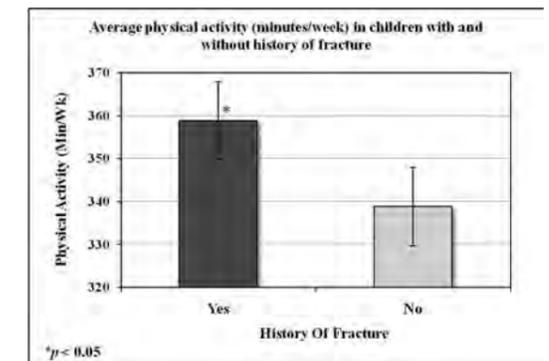
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Objective: Fractures represent a common injury during childhood and adolescence. Knowledge of epidemiology of fractures is crucially important for implementation of prevention strategies for target

population. Our objective was to evaluate incidence of fractures in Indian children and adolescents and to investigate association of fractures with physical activity.

Methods: Data on history of fracture, age, site of fracture and physical activity were collected from 9496 (5230 boys) apparently healthy children and adolescents from 5 major cities from the north, south, east and western regions of India (2011-2012). Height and weight were measured and BMI was computed; standard deviation scores were calculated using Indian reference data (Indian Pediatr. 46, 477-489, 2009)

Results: Mean age of the study children was 10.6 ± 3.6 years. The mean height for age, weight for age and BMI for age z-scores were -0.2 ± 1.2, -0.1 ± 1.4 and 0.0 ± 1.5 respectively. Of the children studied, 9.4% (890 children) had suffered at least one fracture (5.9% fractures were in boys). The incidence of fractures was significantly (p <0.05) greater in boys than in girls. Fractures had occurred between 2 to 5 years in 2% children, between 6 to 9 years in 26%, between 10 to 14 years in 53% and between 15 to 18 in 18% children. Of the fractures, 58% were in the upper limbs, 26% in the lower limbs and 16% were other fractures. After adjusting for age, as minutes of physical activity (sports; Football, Volleyball etc.) per week increased the odds of fracture increased by 1.1.



Conclusion: Fractures were commoner in boys; in upper limbs; 10-14 years was commonest age. Strategies for fracture prevention are important, especially with increased physical activity.

P53

The Influence of Anthropometry and Body Composition on Children's Bone Health. The Childhood Health, Activity and Motor Performance School (The CHAMPS) study, Denmark

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Adiposity, physical inactivity and sedentary behavior have become an increasing problem during the past decade and raise concerns about future health. Increased sedentary behavior may change the body composition by increasing the fat mass relative to the lean mass (LM). These changes may influence bone health. This study aimed at evaluating the influence of body mass index (BMI) and body fat percent (BF%) and LM on children's bone health represented by bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) during a two-year follow up period.

Methods: The study is a part of The CHAMPS study- DK. In this longitudinal observational cohort study children were DXA scanned at baseline and at a two-year follow up. Body composition (BC) represented by lean mass (LM), fat mass (FM) and bone mineral content (BMC), bone mineral density (BMD), and bone area (BA) were



measured, using total body less head (TBLH). The relationship between bone traits and anthropometry (height and body mass index) and BC were analyzed by multiple regression analyses.

Results: Of the invited children, 742/800 (93%) accepted to participate in the DXA scans. Of these, 682/742 (92%) participated at follow up. Mean (range) of age at baseline were 9.5 years (7.7-12.1). Mean (range) BMI, BF% and LM were 16.7 (12.3-28.5), 20 (6-43), 24.1 (16.3-40.1), respectively.

Conclusions: BMI is an important predictor of bone traits obtained by DXA in boys and girls. LM is a better predictor of bone traits in boys and BF% is a better predictor in girls. BMI do not detect gender differences in bone outcome as well as measurements of BC by DXA.

P54

Association of calcium and dairy intake with growth in Indian Children

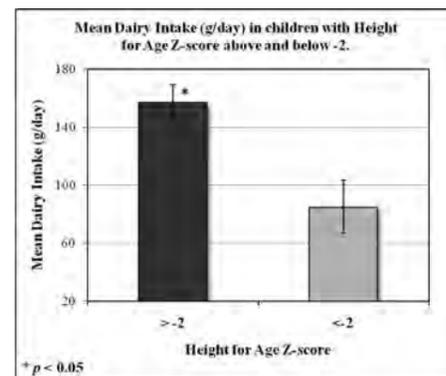
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Objective: Optimal intakes of calcium and milk are necessary in children and adolescents to facilitate not only mineralization but also growth in stature. Low intakes of calcium and also, of milk and milk products in Indian children have been reported. Hence, the objective was to study Indian children's growth with respect to their calcium and dairy intakes.

Methods: We studied 220 children (boys 104, age range 2-16 years). Data on their height, weight and dietary intakes (by 24 hour dietary recall for 2 non-consecutive week days and a Sunday) were collected. Height, weight and BMI for age z-scores were computed using contemporary Indian normative standards (Indian Pediatr. 46, 477-489, 2009)

Results: Mean height for age (HAZ) and weight for age (WAZ) z-scores were -0.8±1.2 and -1.2±1.5 respectively. Eighteen percent children had their HAZ below -2 and 27% had their WAZ below -2. Average daily energy intakes were 83 % of the age and gender matched RDA. Mean % RDA intake of protein was 115% and of calcium was 55%. The median daily calcium intake was 367(258-556) mg; of this, 30 % was from dairy sources and 70% was from plant foods. A significant ($p < 0.05$) positive correlation was found between the HAZ with the intake of protein ($r=0.27$), calcium ($r = 0.19$), phosphorus ($r = 0.23$) after controlling for energy intake. Further, a significant ($p < 0.05$) positive correlation of HAZ was found with the consumption of dairy products ($r = 0.14$).



Conclusion: Indian children studied had low intakes of calcium which was chiefly derived from non-dairy foods. Consumption of dairy foods is likely to be associated with better growth.

P55

Body composition, anthropometric parameters and bone densitometry in young Ukrainian male

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Objectives: To establish the correlations between the body composition, somatotypes and average bone mineral density (BMD) and bone mineral content (BMC) in young (17-18 y.o.) male living in Donbass region (Ukraine).

Materials and methods: Anthropometric and skinfold measurements were carried out. Estimations of the calcaneal BMD (g/cm²) and BMC, (r), estimated on ALOKA-5.0 DXA machine among 156 male were done. Total body fat percentage was calculated by the Mateigka (1921) equation, total body muscular mass by the Kuczmarski R.J, Flegal K.M. equation (2000).The correlation analysis was carried out between the anthropometric measurements and densitometry data.

Results. Densitometry reveals that both BMD and BMC were lower in D than in B persons (BMD 0,96±0,02 g/cm² in D and 1,41±0,01 g/cm² in B; BMC 67,67±2,53 r in D while 91,20±0,04 r – in B). BMD and BMC in D have no correlations with the longitudinal parameters (height, limbs length), but show the negative correlations with the transverse body parameters, such as the intercondylar distances of the elbow, knee and ankle (-0.41 - -0.50). In B the BMD was strongly inversely dependent on the pelvic length, the BMC – with the thigh length. D bear the higher body mass and lean muscular mass (71.80±2.80 kg and 58.20±1.66 kg) than the B (63.44±0.98 kg and 52.09±0.41 kg), but the B have higher body fat content (up to the 3.00% than in D, $p<0.05$). BMC in D directly correlates ($r_{x/y}$ 0.65) with the body fat, and negatively - with the lean muscular mass ($r_{x/y}$ -0.42), when in B both the BMD and BMC were predicted ($r_{x/y}$ 0.27 и 0.51) by the lean muscular mass.

Conclusion. BMC in young dolychomorphes directly correlates with the body fat. In brachymorphes the mineral density higher than in dolychomorphes and directly correlates with the muscular content of the body.

P56

Effect of Puberty on the Muscle-Bone Relationships in Indian Children and Adolescents

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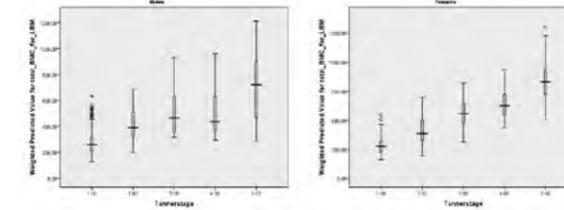
Objective: To describe changes in the muscle-bone unit, assessed as the ratio of bone mineral content (BMC) to lean body mass (LBM) at skeletal sites during puberty in Indian males and females, after adjusting for age and fat.

Methods: Data on arm, leg and total body (Less head) BMC, LBM and fat mass (FM) assessed by DXA for 888 apparently healthy children and adolescents (426 females), 5 to 17 years of age from a cross-sectional study used to generate bone mineral density reference data were used [Bone. 2011;48(4):810-9]. Tanner staging (TS) was performed. Amount of BMC per unit LBM (arms, legs and total body) was computed. Linear regression was performed to examine change in mean BMC/LBM at various TS (adjustment: age, fat).

Results: Mean total BMC/LBM increased significantly (7.7%) at successive TS from 1 (4.76±0.04) to 4 and by 5% from stage 4 to 5 (adjusted for age) in females ($p<0.001$). In males, % increase in total BMC/LBM was small (1.8 to 4%) and insignificant from TS 1 (4.89±0.05) to 4 ($p>0.1$) but increased significantly by 7.4% from 4 to 5 ($p<0.001$). Similar results were seen for total BMC/LBM adjusted for fat. Significant increase of 6.6 to 14.7% in mean arm BMC/LBM (age adjusted) was seen in females; in males increase (7.4%) was significant



only from stage 1 to 2 and 4 to 5 (4.9%) ($p<0.001$). BMC/LBM increased significantly by 18.5% from stage 3 to 4 in males ($p<0.001$); increase in leg BMC/LBM in females was small ($p>0.1$).



Conclusion: BMC for LBM increased with TS, but was more marked at arms and total body in females; this 'extra' BMC may act as a reservoir for later demands of pregnancy and lactation.

P57

Heterozygous mutation in GALNT3 in a case of Hyperphosphataemic Familial Tumoral Calcinosis

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Background: Hyperphosphataemic familial tumoral calcinosis (HFTC) is a rare autosomal recessive condition in which increased renal phosphate reabsorption is associated with elevated serum phosphate, inappropriately normal or raised PTH and extrasosseous calcification. It is caused by mutations in genes related to phosphate metabolism: fibroblast growth factor 23 (FGF23), UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) and Klotho (KL). We present a patient with HFTC and a heterozygous mutation in GALNT3.

Presenting Problem: A 9 year Afro Caribbean old girl presented with a hot, swollen, tender mass of her right hip and buttock associated with several months of fatigue and pain. She was previously well and from a non consanguineous union with no family history of renal or skeletal disorders. Examination revealed a 7-8 cm mass in her right buttock and X-ray and CT imaging demonstrated a large calcified mass in the muscle and subcutaneous fat in the region of the right greater trochanter. Serum phosphate was elevated at 1.83mmol/l, the fractional excretion of phosphate (FEPO₄) was 4.09% with an elevated theoretical tubular maximum of phosphate reabsorption of >2.0 mmol/L (NR 0.8 – 1.35 mmol/L). Serum creatinine and calcium were within the normal range and serum PTH was inappropriately normal (4.5 pmol/L).

Clinical Management: Treatment with sevelamer and acetazolamide resulted in a serum phosphate of 1.6mmol/l, reduction in pain and increased mobility.

DNA analysis showed no mutations in FGF23 or KL. The patient was heterozygous for a novel missense variant, p.Val267Phe (c.799G>T) in exon 3 of GALNT3. Familial studies revealed that the non affected father also had the same mutation in heterozygous form.

Discussion: HFTC has been described to result from homozygous or compound heterozygous mutations in FGF23, GALNT3 and KL. In this case, it is unclear if the heterozygous GALNT3 missense mutation detected is related to HFTC. Her unaffected father also carries this mutation as do 3.4% of individuals from Sub-Saharan Africa and 3.31% of African Americans. HFTC in this child may be as a result of an autosomal recessive compound heterozygous mutation in which the maternal mutation in GALNT3 remains undetected or due to mutations in a gene related to phosphate metabolism which is as yet unidentified.

P58

Puberty is critical for the development of bone mineral density impairment in patients with congenital adrenal hyperplasia

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Objectives. Congenital adrenal hyperplasia (CAH) is a rare condition characterized by the inability of the adrenal gland to produce cortisol. The classical form is due to the deficit of 21-hydroxylase activity (21-OHD) and it accounts for 90–95% of all CAH cases. Treatment of CAH patients consists of life-long glucocorticoid therapy, which must be dosed carefully to avoid excessive or insufficient adrenal suppression. There are data showing low bone mineral density (BMD) in adult patients with CAH, particularly evident in male patients. Few data are available describing the bone mass situation in children. The objective of the current study was to describe bone mineral status in prepubertal CAH patients, and the evolution of their BMD measurements through puberty.

Methods: We enrolled 29 prepubertal children with the classical form of CAH (16 girls and 13 boys), aged 4.8 to 11.5 years at baseline. All patients were receiving cortisone as replacement therapy. We assessed BMD by dual-energy x-ray absorptiometry at the lumbar spine and the whole skeleton at enrollment, and after completion of the pubertal period. We compared BMD values of CAH patients with those of 116 healthy controls of comparable ages.

Results: At baseline lumbar spine BMD values of CAH patients (0.755±0.112 g/cm²) were not different from those of healthy controls. Similarly, whole body BMD measurements (0.902±0.139 g/cm²) did not differ from those of healthy subjects. After puberty we observed significantly lower BMD values of CAH patients both at the lumbar spine (1.162±0.133 g/cm², $P<0.05$), and in the whole skeleton (0.161±0.098, $P<0.05$). When BMD data were expressed as z-scores, we could observe a mean decrement after puberty of -0.2 (1.1) in the lumbar spine, and of -0.7 (1.1) in the whole skeleton. The mean decrement in BMD z-score of male patients was significantly larger than that of female patients in both skeletal sites.

Conclusions: Our data identify puberty as the critical period for the development of bone density impairment in CAH patients. It is therefore important to monitor closely the bone mass acquisition in relation to the replacement therapy during puberty to promote bone health in CAH patients.

P59

The rapid effect of vibration on bone formation and resorption in the growing skeleton

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Background: Mechanical stimulation is thought to be critical for bone anabolic activity. It is unclear how quickly the growing skeleton responds to additional externally-applied mechanical stimuli. We wished to determine the acute effect of a standardised mechanical stimulus to the growing skeleton.

Objectives: To determine the acute time course and magnitude of bone's response to whole body vibration (WBV) in pre-pubertal boys.

Methodology: Healthy boys aged 9-12 years were recruited to stand on either the Juvent 1000 (low magnitude, high frequency) or Galileo Advanced (high magnitude, variable frequency) vibrating platforms for 1, 3 or 5 days for 10 minutes (n=36). A control group (n=15) stood on a non-vibrating platform. Pre- and 10 minutes post vibration blood samples were taken to measure changes in bone formation (PINP, osteocalcin), bone resorption (CTX) and the bone derived factors osteoprotegerin and sclerostin. Samples were collected half hourly for 2 hours in the control group.



Findings: In the immediate pre to post vibration period P1NP decreased by up to 7.9% and CTx by up to 8.8% in the Juvent and Control groups; no change was seen in the Galileo group. There was no difference in the response between groups across the pre- to post-vibration period. At 8 days, in boys exposed to 5 days of WBV on either platform, there was an increase from baseline in P1NP of 25.1% (p=0.005) and in CTx of 10.9% (p=0.009). There was no intergroup difference. There was no change in osteocalcin. Osteoprotegerin increased by 7.2% at day 8 (p=0.08) possibly explaining the smaller increase in resorption. No change was seen in sclerostin.

Conclusion: In the growing skeleton a short period of mechanical stimulation resulted in increased bone activity, with a greater response in the formation marker P1NP than the resorption marker CTx after 5 days WBV. The lack of change in osteocalcin may reflect the "early" nature of the response measured by P1NP in the bone formation process. Increased formation was not due to repression of sclerostin, nor due solely to increased remodelling/resorption, suggesting that other pathways mediate the acute anabolic response seen here to WBV.

KW is funded by Medical Research Council Grant Code U105960371.

P60

Failure of free, public vitamin D supplementation program for Quebec infants - temporal trends and significant predictors

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Over 80% of Quebec woman initiate breastfeeding, and rates of exclusive breastfeeding at 6mo doubled from 2003 (9.7%) to 2009 (19.0%). To prevent deficiency, current recommendations for these infants include 400IU/day of vitamin D. For 20y, Quebec has offered a program of free vitamin D supplements through its public medication insurance plan (RAMQ).

Objective: Program evaluation over the last decade.

Methods: This is a retrospective descriptive analysis of infants born between Jan. 1998 and Dec. 2008. All healthy term infants covered by RAMQ drug plan were eligible; ~30% of pregnant women and infants are covered. Data were extracted from the Quebec Pregnancy Registry linking three databases: RAMQ (diagnoses, medications, SES), MedEcho (hospitalizations), and ISQ (birthweight, SES). Multivariable logistic regression (GEE) examined predictors of participation.

Results: A total of 123,018 infants were eligible. Mean annual prevalence of vitamin D exposure was 17.9%±5.6. Median age at which the first bottle of supplements was obtained was 36 days (range=0-370). The majority (51.0%) obtained only one bottle of fifty doses (median=1, range=1-20). Significant predictors (p<0.05) were prescription by pediatrician (Odds Ratio OR=1.23, with 95% confidence interval CI=1.19-1.27); increasing maternal age (OR=1.018 per year, CI=1.015-1.021); non-synthesizing season for vitamin D (OR=1.04, CI=1.01-1.07); women living alone (OR=0.87, CI=0.83-0.91), less education (OR 0.88, CI=0.84-0.92) and rural residence (OR 0.94, CI=0.91-0.98). There was also a decline in program participation over time (OR 0.89 per year, CI=0.88-0.90).

Conclusions: Program participation was low and decreased with time. Additional risks for rickets included long delay prior to filling the first prescription, failure to renew, younger mothers, mothers with less education, and care by a non-pediatrician. Overall, poor uptake suggests difficulty in administration of supplements, poor understanding of their potential benefits, or low acceptance of health professionals' advice. New preventive strategies need to be developed to increase long-term vitamin D compliance.

Funded by Montreal Children's Hospital-Research Institute and Gray Family Fund.

P61

Elevated FGF23 levels in premature infants without excessive phosphaturia

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Preterm infants develop reduced bone mass and fragility fractures. Nevertheless, normal ranges in preterm infants are poorly defined for concentrations of vitamin D; its isomers (C3a-epimer of 25(OH)D3) and metabolites (24,25(OH)2D3, 1,24(OH)2D); and other mineral-regulating hormones, including FGF23 in both intact (iFRG23) and inactive (C-terminal) forms.

Objective: To clarify normal concentrations of minerals and hormones in healthy, preterm infants (AGA).

Methods: We conducted a longitudinal, pilot, cohort study on infants born at 28-32 weeks gestation, monitoring blood and urine at 1, 3, 5 weeks and at term. Analyses included morning plasma and urine calcium, phosphorus, creatinine (Beckman Coulter, USA), whole blood ionized calcium (Radiometer, Denmark), PTH, cFGF23 (C-terminal & intact; Immunotopics, USA), iFGF23 (Kainos, Japan), 1,25(OH)2D (IDS, UK) and 25(OH)D3 and its C3a-epimer and metabolites by LC-MS/MS (Warnex, Canada). Data are mean±sd.

Results: Eleven infants (mean GA 30.9±1.5 wk, wt 1866±21.6 g, male=5) were recruited. At weeks 1,3, 5, and term, the % achieving 50 nM concentrations of 25(OH)D3 were 40, 60, 100, and 67%, with good intakes of vitamin D. Levels of iFGF23 were mildly elevated (double adult norms) with a marked increase in cFGF23 (10 times adult norms). Nevertheless, tubular resorption of phosphorus (TRP) was normal (88±8%) for those with urine samples. Preterm infants had substantial concentrations of C3a-epimer, much higher than in adults. Calcitriol levels were robust at all ages.

Conclusions: Many infants achieved acceptable 25(OH)D3 concentrations. Despite elevated concentrations of FGF23, there was not excessive phosphaturia.

TABLE: Results of blood biochemistry and hormone concentrations across time in preterm infants

Wk	Ionized calcium mM	Phos mM	i-CGF23 pg/ml	c-FGF23 RU/ml	25(OH)D3 nM	C3a epimer %	PTH pM	1,25(OH)2D pM
1 (n=10)	1.39 ±0.05	2.26 ±0.5	68.2 ±59.9	839 ±494	54.3±21.0	32.4 ±5.7	6.0 ±5.0	213.0*
2 (n=10)	1.42 ±0.05	2.23 ±0.26	71.7 ±31.6	737 ±355	57.1±25.7	43.4 ±6.7	6.0 ±5.2	134.7±60.5*
5 (n=2)	1.41 ±0.02	2.17 ±0.13	95.5 ±3.5	888 ±80	53±3.9	49 ±14.8	5.7 ±1.8	185.0±53.3*
Term (n=2)	1.40 ±0.03	2.35 ±0.20	75.8 ±22.7	1060 ±397	62.2±21.1	47.7 ±13.6	3.2 ±1.9	224.0*

P62

The effect of the ketogenic diet on the developing skeleton

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Objectives: The ketogenic diet (KD) is a medically supervised, high fat, low carbohydrate and restricted protein diet which has been used successfully in patients with refractory epilepsy. Only one published report has explored its effect on the skeleton. We postulated that KD



impairs bone mass accrual and examined skeletal health parameters in this patient group.

Methods: Patients commenced on the KD from 2002-2009 were enrolled in a prospective, longitudinal study; with monitoring of Dual-energy Xray absorptiometry (DXA) derived bone parameter including bone mineral content and density (BMD). Areal BMD was converted to bone mineral apparent density (BMAD) where possible. Biochemical parameters, including Vitamin D, and bone turnover markers, including osteocalcin and urinary deoxypyridinoline, were assessed.

Results: 29 patients were on the KD for a minimum of 6 months (range 0.5-6.5 years, mean 2.1 years). There was a mean reduction in lumbar spine (LS) BMD Z score of 0.37 SD. 20 patients (68%) had a lower BMD at the end of treatment. There was no correlation between change in LS BMD and ambulatory status (R=0.02). Height adjustment was possible for 13 patients, with a mean reduction in BMAD Z score of 0.19 SD. ALP levels were in the normal range but osteocalcin showed a mean 26.5 nmol/L, which was elevated. Only one patient sustained fractures (bilateral femoral fractures). Urinary calcium-creatinine ratios were elevated (mean 0.77) but only 1 patient developed renal calculi.

Conclusion: The KD has a small but significant effect on the developing skeleton, independent of height and ambulatory status. Effects on bone turnover and calcium/creatinine ratios point to abnormal mineral metabolism. Clinicians should be aware of potential skeletal side effects and monitor bone health during KD treatment. Longer term follow up is still required to determine adult/peak bone mass and fracture risk.

P63

Bone status of Indian children and adolescents with type 1 diabetes mellitus

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Objective: Type 1 diabetes mellitus (T1DM) has been shown to adversely affect bone health in children. Hence, objective was to assess bone health status of children with T1DM, and to assess relationship between bone status and disease duration.

Methods: Bone mineral content for total body (less head) (TBBMC) and lumbar spine was measured by DXA in 47 (25 boys) children with T1DM. Z scores for TBBMC for bone area (TBBA), TBBA for height, lean body mass (LBM) for height, TBBMC for LBM and lumbar spine bone mineral apparent density (LSBMAD) were computed reference data [Bone. 2011;48(4):810-9].

Results: Mean age was 10.9±2.5 years, height Z scores were -1.1±1.2 (32% below -2 SD); duration of diabetes was 2.7±3 years, glycosylated hemoglobin was 9.1±2.2%. Mean Z scores for total body (TBBMC for TBBA, TBBA for height, LBM for height, TBBMC for LBM) were within normal range for all; in contrast, mean LSBAMD Z-score was -5.2±0.8. Children were divided according to median for disease duration; children with diabetes for > 1.5 years had significantly lower LSBMAD scores (-5.0±0.7 vs -5.5±0.8, p<0.05, Figure 1). 71% children with diabetes for >1.5 years had LSBMAD Z scores lower than the median values (Z Score= -5.1). Whereas 64% children who had diabetes for < 1.5 years had LSBMAD Z scores above -5.1. Further, boys had significantly lower LSBMAD Z scores (-5.5±0.8 vs -4.9±0.6) whereas the LBM for height Z scores in girls were significantly lower than in boys (-0.2±0.8 vs -0.3±0.9, p<0.05).

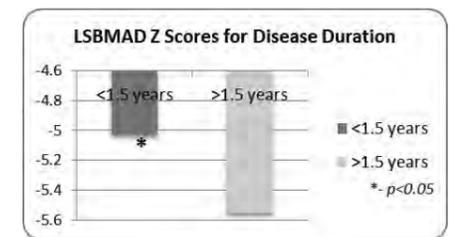


Figure 1

Conclusion: Children with T1DM with longer duration had lower bone mineral content at the lumbar spine possibly due to trabecular bone loss.

P64

The level of vitamin D and calcium in urban pregnant women in Russian Federation

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Background: Optimal intake of calcium and vitamin D of pregnant women is an important component for the normal course of pregnancy, but here are the contradictory opinions about the case of shortage of the consumption, there is no uniform approach to the appointment of them.

Aim: To assess the level of consumption of alimentary calcium, and characteristic of the status of vitamin D and calcium in blood serum of pregnant women.

Materials and Methods: We examined 80 women at the age of 20-35 years (mean age of 27,12±4,36 year) in the third trimester of pregnancy (gestation 31,71±3,37 weeks) residents of Yaroslavl city, Russian Federation. The average consumption of calcium in foodstuff was estimated according to the tables with norms from Russian guidelines for osteoporosis, 2012. The analysis of calcium and vitamin D (25(OH) vitamin D) in blood samples was carried out during the whole year. The concentration of vitamin D in blood serum was determined by the electrochemiluminescence immunoassay («ECLIA»), the concentration of calcium - by the method of optical density on the unit COBAS 400 PLUS.

Results: The average consumption of calcium in foodstuff was 1148,25 ±368,91 mg per day (from 350 to 2064 mg). 29 (36,25 %) of women consumed less than 1000 mg, less than 1200 mg - 46 (57,50%), more than 1500 mg - only 16 (20,00%) women. The total blood calcium was 2,14 ±0,28 mmol/l (from 1,43 to 2,48 mmol/l). Hypocalcaemia was in 40 (50,00%) of the pregnant women. The average level of 25(OH) - vitamin D was 26,61±13,69 ng/ml (from 4,10 to 58,16). The normal content ration (more than 30 ng/ml) was detected in 29 (36,25%) of people, insufficiency (from 21 to 29,9 ng/ml) - at 18 (22,50%), deficit (less than 20 ng/ml) - at 33 (41,25%) women. There was no difference in mean concentration of vitamin D in blood samples from different seasons (from OCT to APR - 26,51±11,69 ng/ml, from MAY to SEP - 26,79±17,32 ng/ml, p>0,05). The clinical manifestations of the vitamin D deficiency and/or hypocalcaemia were noted in 17 (21,25%) women.

Conclusions: Our data demonstrate the wide prevalence of the deficiency of dietary calcium and low level of vitamin D of the urban pregnant women in the third trimester of pregnancy.



P65

Improvement in morphological properties of trabecular bones and longitudinal growth in tibia for growing rats through an impact stimulation

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Mechanical stress and strain generated by physical exercises or the other passive stimulations are well known to have a positive effect on the growing musculoskeletal system. Especially, when the impact stimulation which evokes high magnitude of strain in a second is applied to bone, it improves bone qualities. Thus, to verify the effect of impact stimulation, we conducted longitudinal study on morphological properties of the tibia in growing rats. Free falls from designated heights were implemented to induce high impact stimulation. 6-week-old male Wistar rats were randomly allocated to one of three conditions: free fall from 20-cm-height (W20; n=7), free fall from 40-cm-height (W40; n=7), and control (WC; n=7). The impact stimulations were administered to the free fall groups, 10 times/day, and 5 days/week for 8 weeks. The right tibia was scanned by in-vivo micro-CT at 0, 4, and 8 weeks of experiment and structural parameters and tibia's length were measured to evaluate the variation in morphological characteristics and bone length with maturing. After 4 weeks of the experiment, relative values (value of 4 or 8 weeks / 0 week, thus 1 at 0 week) of BV/TV and Tb.N were significantly higher in W40 group than in WC and W20 groups (all p<0.05), while relative values of Tb.Sp was significantly smaller (p<0.05). However, there were no difference in relative values in Tb.Th and bone length among the groups (all p>0.05). No significant difference in all structural parameters between W20 and WC groups (p>0.05). After 8 weeks of the experiments, relative values in Tb.Th and tibial length were significantly higher in W40 group than in WC and W20 groups (p<0.05). Only relative value of Tb.Sp in W20 group was significantly smaller than that in WC group (p<0.05). Overall, impact stimulation for 8 weeks generated by free falls at a 40-cm-height affected not only trabecular bone homeostasis but also bone growth. Furthermore, since a significant bone growth was appeared after 8 weeks of stimulation, but not after 4 weeks, continuous stimulation may be needed to improve bone growth length in growing rats.

P66

Effect of resistance training on peripheral bone mineral density and muscle strength in adolescents with motor difficulties

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Objectives: Adolescents with motor difficulties may have a higher fracture risk due to limited participation in high impact physical activities that improve bone mineral density (BMD). Equipment constrained resistance training (RT) interventions may be an effective way to improve both muscle strength and BMD in this population. The aims of this study were to investigate the effect of a gym-based RT intervention on peripheral BMD, and to determine the extent of the relationship between BMD and muscle strength, among adolescents with motor difficulties.

Methods: Participants were 21 adolescents (13 intervention and 8 control) with motor difficulties, with a mean age of 14 (1.54) years. The participants were recruited from a larger research project (Adolescent Movement Program; AMPitup) and its wait list. The intervention was a 13-week aerobic and resistance exercise program that participants attended for 90-minutes twice a week. The exercise program included 5 pre-set exercises targeting the forearm and lower leg (Leg-press, push-ups, seated row, calf raises, and up-right rows) to be completed every session. Measures taken pre and post intervention included peripheral

BMD scans (tibia and radius; trabecular and cortical density) using peripheral Quantitative Computer Tomography (pQCT), height, weight, upper (grip strength, chest pass) and lower (IRM leg press, distance and vertical jump) body muscle strength. General linear models, adjusting for physical maturity, and correlations were used to analyse the data.

Results: Improvements in muscle strength, in particular for the upper body (right hand grip strength $p = .01$; chest pass $p = .01$) were observed in the intervention group but not the control group. Changes in BMD measures from pre to post test in the intervention group were less conclusive due to the small sample size and short time frame, however positive trends were apparent. Muscle strength and BMD was related as evidenced by moderate to strong correlations, particularly for the lower leg.

Conclusion: A targeted resistance training program may be effective in improving muscle strength and stimulating bone changes in adolescents with motor difficulties. Further research is needed to clarify the most effective exercises for site specific BMD improvements in this group.

P67

Dual Energy X-Ray Absorptiometry in predicting clinical bone disease in adults with childhood onset end-stage renal disease

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Objectives: Metabolic bone disease is a frequent complication of end-stage renal disease, characterised by a decreased bone mineral density, which can be measured with Dual Energy X-ray Absorptiometry. Its validity as a marker for clinical bone disease and increased fracture risk has never been established in adults with pediatric onset of end-stage renal disease (1-3). Adult survivors of pediatric end stage renal disease have very low bone mineral density and small stature (4). In this cohort study, the association between low bone mineral density measured with Dual Energy X-ray Absorptiometry in adult survivors of pediatric end-stage renal disease in 2000 and manifestations of clinical bone disease between 2000 and 2010 was assessed.

Methods: 113 from all 187 living patients of the so called LERIC-cohort(4) agreed to participate. Data regarding bone mineral density and clinical bone disease were gathered in 2000. Volumetric bone mineral density was calculated. Data on clinical outcome were gathered retrospectively by reviewing medical charts over the period 2000-2010 and by questionnaires.

Results: Mean volumetric bone mineral density Z-scores [95% CI] of the lumbar spine was -1.0 [-1.2 - -0.7] and femoral neck was -3.0 [-3.5 - -2.5]. 27% of the patients suffered from clinical bone disease. 16 patients (14%) had suffered from a fracture in the last 10 years, of which 4 were pathological fractures. The odds ratios [95% CI] of volumetric bone mineral density for clinical bone disease and fractures over 10 years were 1.033 [0.867-1.231] and 0.679 [0.437 - 1.055], respectively.

Conclusion: Adult survivors of pediatric end stage renal disease have low volumetric bone mineral density and the prevalence of clinical bone disease is high. However, we found no significant association between bone mineral density and clinical bone disease. Therefore, the usefulness of Dual Energy X-ray Absorptiometry in predicting clinical bone disease in patients with pediatric end-stage renal disease is still uncertain.



P68

Engineered tridimensional hydroxyapatite scaffold to support bone resorption

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Objective: In many traumatic or pathological conditions, bone turnover is low and osteoclast activity is reduced or abolished. We developed innovative hydroxyapatite (HA) scaffolds carrying RANKL expressing cells with the aim of supporting bone resorption when this is defective. Membrane-bound (m)RANKL is cleaved into soluble (s)RANKL by MMP14. We hypothesized that the osteoclastogenic potential of RANKL-producing cells could be improved if they were seeded on scaffolds engineered by immobilization of MMP14 to rise the shedding of sRANKL.

Methods: Primary osteoblasts (OBs) and stromal bone marrow cells from 10-days old WT CD1 mice were used. MC3T3 cells were stably transfected with sRANKL-vector. MMP14 was immobilized on 3D-HA scaffolds using procedures based on protein-to-substrate binding by glutaraldehyde (10% v/v). For in vivo studies, the scaffolds were entrapped in diffusion chambers (Millipore).

Results: We identified in OBs the best spontaneous sRANKL source, then on these cells we demonstrated a concentration-dependent RANKL shedding ability of the catalytic domain of MMP14. Next, we quantified in about 50% the enzymatic efficiency of MMP-14 functionalized scaffolds versus soluble MMP14, and tested the efficacy of MMP14-engineered 3D-HA scaffolds on OBs noting increased release of sRANKL vs scaffolds not subjected to MMP14 immobilization. Intact scaffolds are also seeded with MC3T3 cells stably overexpressing sRANKL. Moreover, we assembled devices with scaffolds embedded in diffusion chambers and proved the safety of their implants in WT mice. Finally, we tested the efficiency of devices harboring either OBS or sRANKL-MC3T3 implanted in RANKL KO mice. In tibial sections we noted the appearance of TRAcP positive cells in both groups of implanted animals in contrast with sham KO mice, which were TRAcP-negative.

Conclusion: Our results demonstrated the feasibility of a strategy based on engineered bio-device for supporting sRANKL release from osteoblast mRANKL.

P69

sRANKL/OPG in children with Idiopathic Hypercalciuria

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Objectives: To determine any relationship of serum concentrations of osteoprotegerin (OPG), sRANKL and sRANKL/OPG ratio with Idiopathic Hypercalciuria (IH) in children, as there is some evidence of increased bone resorption in these patients.

Methods: In a prospective study, twenty four children of median age 6.5 yrs (range 2.3-16.4) with IH (5 had urolithiasis & 2 nephrocalcinosis) were examined at the time of diagnosis and after 3 months of salt free and adequate Ca diet. Clinically healthy children (n=46) matched for age, sex and season were used as controls (median 8.0 yrs, range 1.8-16.3). sRANKL (total), OPG, 25(OH)D, 1,25(OH)₂D, PTH, Ca, Pi, osteocalcin (N-MID OC), ALP and CTX-Crosslaps were determined in serum and Ca, creatinine, oxalate and citrate in urine. Height was recorded and BMI z-score was assessed.

Results: The BMI z-score was lower in patients than controls (-0.425±0.641 vs +0.106±1.0, p=0.016), but height did not differ. Although urinary Ca excretion (24hCa & UCa/UCr) decreased at 3mo

(24hUCa: 6.29±2.0 vs 5.19±2.3 mg/Kg, p=0.06; UCa/UCr: 0.30±0.19 vs 0.21±0.12 mg/mg, p=0.014) on average it had not reached control values (2.14±1.08, p<0.0001; 0.10±0.06, p=0.0003). Ucitrate/UCr, UCa/UCitrate and 24h oxalate did not differ in patients before and after diet or compared to controls. No significant differences were found for serum Ca, Pi, 25OHD, 1,25(OH)₂D, PTH, osteocalcin, ALP, OPG and sRANKL or for the sRANKL/OPG ratio. Only serum concentrations of CTX-Crosslaps were significantly higher in both patient samples (1.63±0.67, p<0.02; 1.56±0.45, p<0.05 than controls (1.24±0.56 pmol/L).

Conclusion: No evident changes in the serum cytokines OPG and sRANKL were noted in children with IH. However growth might have been affected by increased bone resorption, as indicated by the higher levels of serum CTX-Crosslaps and unaffected formation, since osteocalcin was not found different from controls. Hence, an autocrine role of the above cytokines cannot be excluded.

P70

Isolated bilateral zeugo-autopodal segments agenesis of the lower limb: Unusual malformation case report

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Congenital limb abnormalities represent a prevalence of 0.79/1,000 of live births in Massachusetts¹. A better understanding of their physiopathology could improve the management of the patients. We report on a 23 weeks female fetus affected by an isolated bilateral terminal transverse defect of the lower limbs with nubbins. Both familial history and chromosomal analyses were irrelevant. We performed a deep morphological examination of the fetus in comparison with an age-matched control fetus in order to precise the diagnosis. The estimated skeleton age corresponded to the gestational age. The bones of both legs and feet were completely lacking, whereas no abnormality was observed in the axial and upper limb skeleton as well as in the other systems. The distal femur epiphysis presented an unusual shape and was composed of a cartilage bud including the proximal tibia anlage. Targeted sequencing of exons involved in limb morphogenesis was carried out with skin tissue and showed no significant mutation. Such a rare malformation was never reported in the medical literature. Considering the data available in the literature, we can suggest the genetic cause is the most probable. Only few theories exist to explain the limb and joint development and malformation. This extremely rare malformation comes up against some contradictory theories, like the Progress Zone model² and the Early Specification model³. The minute description of the present case is a real chance to give cues to the correct understanding of limb and joint formation, especially the chronological sequence of the different genes, molecules and targets involved in limb morphogenesis.

P71

The microarchitecture of bone in osteochondromas

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Introduction: Hereditary multiple osteochondromas (HMO) is characterized by the outward growth of cartilage-capped bone tumors. Osteochondromas contain a bone marrow cavity continuous with the normal bone cavity. Because of their off-axis position, osteochondromas are expected to carry less load than normal bone tissue. According to Wolff's law, we therefore hypothesized that osteochondromas would



have a less developed, osteoporotic-like microstructure. To test this hypothesis, we measured the bone morphology of osteochondromas with microCT scanning and compared it to normal bone. To our knowledge this is the first study done with microCT and human osteochondromas.

Materials and Methods: Micro-CT scans were made of thirteen osteochondromas from human subjects to evaluate tissue mineralization and bone structural parameters. Values for normal bone were taken from the literature.

Results: Large differences in tissue mineralization were found, with 3 specimens being hardly mineralized and the others being less mineralized than normal bone. The osteochondromas have less but thicker trabeculae than normal bone. The spacing between the trabeculae is increased. The structure lacks a clear orientation, being more isotropic than normal bone.

Discussion: Osteochondromas resemble an osteoporotic structure in some aspects (increased trabecular spacing, decreased trabecular number) but the osteochondromas have a higher trabecular thickness. This may be due to the influence of the cartilage cap nearby. The osteochondromas also lack a clear trabecular orientation, probably due to the absence of mechanical loading.

Conclusion: osteochondromas have a less developed structure, as witnessed by the lower mineralization and the absence of a clear trabecular orientation, but is not comparable to osteoporotic bone.

Significance: This study is the first to show that the architecture of the osteochondromas is not osteoporotic. It only resembles an osteoporotic structure in some aspects (increased trabecular spacing, decreased trabecular number) but that they have a higher trabecular thickness.

P72

Abstract Withdrawn

P73

Histomorphometric parameters of the alveolar ridges of the mandible in immature rats after thymectomy

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Aim: Study the features changes of histomorphometric parameters of alveolar ridges in immature rats after thymectomy

Methods: The study was conducted on 120 immature male rats were divided into two groups: 1st group – sham-operated animals, 2nd group – rats with removed of the thymus under mask ether anesthesia (thymectomy). Periods of observation were 7, 15, 30, 90 and 180 days. Rats were euthanized under mask ether anesthesia. For histological examination the mandible was cut at the level of the second molar, separated dentoalveolar segments were fixed in 10% neutral formalin, decalcified in 5% formic acid, dehydrated in increasing concentrations of alcohol, embedded in paraffin and stained with hematoxylin-eosin. Morphometry program included the following parameters: the total width of the alveolar ridge at the level of 2nd molar and the width of its layers: the outer cortical plate, inner cortical plate, osteon layer and diameters of osteons and their channels.

Results: In animals of 2nd group the total width of the alveolar ridge and the width of the outer cortical plate decreased, compared with the same parameters in 1st group, from 30 to 180 day of observation respectively by 3.55%, 7.13%, 8.89% and 5.44%, 8.71%, 9.41%, width of osteon layer - by the 90th and the 180th days by 8.83% and 9.28% and the width of the inner cortical plate – by the 180th day by 7.87%. Diameters were lower than those of 1st group by the 90th and 180th days of observation by 4.35% and 4.46%, and the diameter of osteons channels, in contrast, were more by 8.46% and 15.88% respectively.

Conclusions: Thus, under conditions of thymectomy in immature rats there was a disturbance in the structural organization of alveolar ridge of the mandible in the later period of observation (from 30 to 180 per day), which may indicate a generalized periodontitis.

P74

Assessment of bone mass and bone metabolism in children with chronic inflammatory bowel diseases

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Skeletal integrity during childhood may be compromised by diseases interfering with bone metabolism. Chronic inflammatory bowel diseases (CIBD; Crohn's disease, ulcerative colitis) in children is a recognized risk of osteoporosis in adulthood. This study was aimed at assessment of bone mass and bone metabolism in children with CIBD at diagnosis and after one year during therapy. Patient population comprised 64 children (boys 23, girls 42) and adolescents aged 14.6±2.7 years (7-20). Dual x-ray densitometry (lumbar spine) and measurement of 25-OH D and bone markers (osteocalcin, P1CP, CTX, crosslaps urine, osteoprotegerin) by standard methods were performed. No difference between sexes was found. Densitometry z-scores (-0.67±1.28; range -3.95 to 2.47) indicating decreased bone mass (<-2) were found in 5 patients. At time of diagnosis hypovitaminosis D (<50 nmol/L) was found in 69% of patients. Increased bone markers (as compared to adult reference ranges) were observed in 75% for osteocalcin, 95% for P1CP, 98% for CTX and 45% for crosslaps urine. Osteoprotegerin was similar to normal adult values in 92% of patients. After one year follow-up statistically significant increase was found for osteocalcin (0.002) and P1CP (0.001), and decrease for crosslaps urine (0.0005) and osteoprotegerin (0.03). Hypovitaminosis D was still present in 41% of cases. These results indicate that CIBD in children and adolescents did not considerably impair the skeleton. Increased bone markers probably indicate increased bone turnover characteristic of growth and puberty. The observed changes of bone markers during follow-up probably reflects recovery and continuation of growth processes. Normal osteoprotegerin levels suggest that bone resorption at diagnosis and during monitoring was not predominant. Prevalence of hypovitaminosis D is a serious problem of this disorder, its treatment and prevention should be a measure for preventing osteoporosis risk.

P75

Vitamin D Receptor Gene *FokI* Polymorphism and bone mass accrual in Indian Girls

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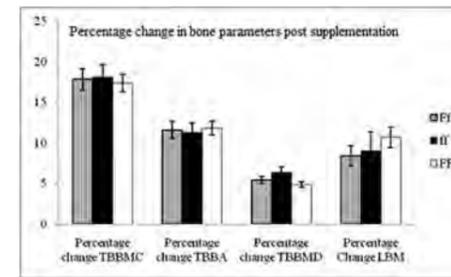
Objective: To study association of VDR gene *FokI* polymorphism locus on bone mass accrual in adolescent girls

Methods: An intervention trial was carried out in 102 girls aged 8-16 years (Pune, India). All girls received 500 mg elemental calcium (daily) and 30,000 IU of vitamin D3 quarterly for one year. Outcome variables were measured at baseline and end of the year. Serum levels of ionised calcium (iCa), inorganic phosphorous, parathyroid hormone (PTH) and 25-hydroxy vitamin-D were measured (25[OH]D). Bone mineral content (BMC), bone area (BA), bone mineral density (BMD) and lean body mass (LBM) were measured at total body by DXA. Percentage increase in BMC, BA and BMD was calculated. Polymorphisms (FF, Ff, ff) of VDR gene at *FokI* locus were detected using SYBR Green quantitative PCR.

Results: Overall prevalence of *FokI* genotypes were 43.1% Ff, 9.8% ff and 47.1% FF. There were no significant differences in percentage change in serum parameters during the intervention period between groups (p>0.05). At baseline, FF genotype had significantly lower BMD as compared to ff & Ff genotype (p<0.05) and significantly lower BA as compared to ff genotype (p<0.05). A significant increase in BMC, BA, BMD and LBM was observed post supplementation in all the 3 *FokI* polymorphism groups (p value < 0.05). However, there were no significant differences in the percentage increase in BMC [Ff (17.9%);



ff (18.1%); FF (17.4%)], BA [Ff (11.6%); ff (11.2%); FF (11.8%)], BMD [Ff (5.4%); ff (6.3%); FF (4.9%)] and LBM [Ff (8.4%); ff (9.2%); FF (10.7%)] (p>0.05)(fig).



Conclusion: Girls with FF genotype had significantly lower bone indices as compared to ff and Ff genotypes. VDR gene polymorphism as defined by *FokI* genotype had no positive influence on bone mass accrual in study girls.

P76

Final height and bone health in young adults, transplanted in childhood

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This study analyses the long-term effect of rhGH on final height (FH) and bone health in renal transplanted patients.

21 young adult patients, aged 17 to 26 yrs, were studied. Group A consisted of 15 pts (12 boys) who received rhGH during 3.0 yrs before transplantation. After transplantation 3 boys needed rhGH again for 3.5 yrs. In group B 6 patients (3 boys) didn't need rhGH before transplantation, 3 girls received rhGH afterwards for 4 yrs. There is no significant difference between the two groups for median age at transplantation, median follow-up period afterward and median GFR at final evaluation. At FH (defined as adult bone age or height velocity of <1 cm/year), clinical evaluation included measurement of height and weight and GFR measurements by Cr EDTA. Lumbar spine and total body mineral content and density and body composition were studied by DXA (Discovery A).

FH was expressed as SDS (Cole 1995). We compared the lumbar spine density, expressed as standardized lumbar bone density L2-L4 (sLBMD), with normative data of Belgian healthy young adults. Osteopenia was defined as a sLBMD T-score of <-1.0, osteoporosis as sLBMD T-score of <-2.5 SDS. Lean body mass and fat mass were expressed as percentage of the total body weight.

Median height SDS at transplantation was better in group A (Sd -0.3) than in group B (Sd -1.7, p = 0.03). Final height SDS was no longer different. For the total group FH SDS ranged from -2.3 to +1.0 SDS. BMI SDS was within the normal range. Fat mass % was lower in group A (16.5) compared to group B (24.5) (p= 0.016).

Lumbar density values were better at the moment of renal transplantation in group A, however there was no difference at final height. In both groups 1 patient has an osteoporotic sLBMD.

In children with chronic renal failure rhGH treatment improves height and results in better bone health at the moment of transplantation. At final height patients with rhGH before transplantation have significantly better lean body mass. Future studies in a larger cohort should confirm these data.

P77

A novel mutation in *CRTAP* gene in a patient with severe Osteogenesis imperfecta type VII

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Background: Osteogenesis imperfecta (OI) is a genetic disorder with low bone mass and bone fragility. Type VII OI is one of the autosomal recessive subtypes and clinically moderate to lethal. It is caused by mutations in the cartilage associated protein (*CRTAP*) gene. Currently less than 20 mutations are known.

Case description: An 11-year-old Iraqi female was referred to our hospital after immigration to Finland. She had suffered numerous peripheral and spinal fractures. At presentation she had severe vertebral and limb deformities and could not stand, her height was 75 cm. The lumbar spine and whole body BMD Z-scores were -5.6 and -3.0, respectively. The clinical and radiographic features were consistent with OI. Her parents and three siblings were healthy; one sibling had died soon after birth due to similar bone disease. The parents were 1st cousins.

All known OI genes were analysed with flanking markers. The genes flanked by homozygous markers were chosen for direct sequencing. A homozygous mutation (c.141dupC) was identified in exon 1 of the *CRTAP* gene. The mutation leads to a premature stop codon. Both parents were heterozygous carriers of the mutation. The mutation has not previously been described.

The patient has received intravenous pamidronate and zoledronic acid treatment for 3 years. Spinal deformity required surgery. Bisphosphonate treatment improved subjective well-being and reduced bone pain and she is able to sit for much longer periods.

Discussion: This family illustrates the extreme severity of type VII OI. Bisphosphonate treatment was of subjective benefit even though it was not started until age 11. The reported novel *CRTAP* mutation expands the genetic defects associated with OI type VII.

P78

Variable relationship of fat mass and bone with pubertal staging in obese and normal weight Indian children and adolescents

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Objective: In children, factors such as age and puberty are confounders when investigating associations of fat or lean with bone mineral content (BMC). Our aim was to assess influence of fat on BMC in overweight/obese (ow/ob) and normal weight (nw) children at different stages of puberty.

Methods: Cross-sectionally, 244 children+adolescents (6-16yrs) (73nw, 177ow/ob) were assessed [tanner staging (TS), body composition (DXA) (Total body BMC, lean mass (LBM) and fat (BF))].

Results: BMC was highly correlated with LBM (r=0.68 in boys; r=0.72 in girls, p<0.0001) more so than with BF (r=0.56 in boys, r=0.67 in girls, p<0.0001). After adjustments (age, LBM), ow/ob boys showed higher values of BMC than controls at TS1&2 (910±74 g vs 1340±41 g; 1598±39 vs 1358±64 g resp) (p<0.05); no difference was observed at TS3,4&5; adjusted BMC values in ow/ob girls were significantly higher than controls at all TS's (p<0.05). Multiple linear regression (entire cohort) was performed at each TS in both genders to assess simultaneous effect of lean, fat on BMC (age adjusted). In boys, at all TS's, lean was significant predictor of BMC (p<0.001); additionally at TS's 2, 3, 5 fat was significant predictor (r² = 0.71, p<0.01). In girls, at TS's 1, 2, 3



($r^2=0.77$) and 5 ($r^2=0.41$) lean was significant predictor ($p<0.05$); at TS3 fat was also predictor ($R^2=0.77$), at stage 4 ($r^2=0.53$) fat was the only predictor.

Conclusion: Differential relationships between lean, fat and bone were observed in both genders at different TS's; lean was the main predictor in all children (nw, ow/ob) at almost all TS's. In boys, at earlier TS's, bone mass was higher in ow/ob boys, but this effect of fat on bone was not maintained at later TS's, suggesting that intervention may be necessary at later TS's to improve bone mass in overweight/obese boys.

P79

High sedentary lifestyle practices has adverse influence on musculoskeletal health in adolescent boys and girls - findings from a population-based study in Malaysia

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The combined effect of lifestyle physical activity (PA) and sedentary behavioral practices on musculoskeletal health in growing populations is still poorly understood.

Objectives: To investigate the combined influence of mechanical loading of weight-bearing PA (WBPA) and screen-based sedentary practice (SSR) on musculoskeletal profiles among 455 adolescents aged 12 to 19y.

Methods: Validated PA and SSR behaviour assessments were used to determine daily physical activities and sedentary practices, respectively. Bone mass of total body (TB), lumbar spine (LS) and proximal femur (PF) was assessed using dual energy X-ray absorptiometry, while muscular strength of the upper and lower extremity were determined using handgrip and isokinetic dynamometers respectively.

Results: Four groups of combined influence of the WBPA and SSR were i) HighWBPA_{LowSSR}, ii) HighWBPA_{HighSSR}, iii) LowWBPA_{LowSSR} and iv) LowWBPA_{HighSSR}. Multivariate analysis model showed that adolescents with high SSR and low WBPA had significantly lower size-adjusted bone mineral content (BMC) of the LS and PF (All, $P<0.01$) and TB bone area (TBBA) ($P<0.001$), muscular strength of the handgrip ($P<0.001$) and hamstring ($P<0.01$) than those at high WBPA and low SSR group, after adjusting for body size, pubertal growth status, socio-demographic and calcium intakes. Furthermore, participants with higher levels of WBPA and SSR practices had significantly higher size-adjusted PF BMC ($P<0.01$) and TBBA ($P<0.01$) compared to those adolescents engaged only in high SSR levels.

Conclusion: These findings suggest that adolescents with higher participation of daily WBPA and low SSR practices exert positive impact on bone mass and muscular strengths than those involved only in high sedentary practice. Effective strategies to promote active lifestyle practices should be emphasised in children and adolescents in order to maximise bone mass accretion and muscular strength during these growing years.

(This study was funded by University Science Malaysia Research University (RU grant: 1001/PPSK/812015))

P80

Osteogenesis imperfecta and short stature: effect of sclerostin antibody treatment in oim/oim mice

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Introduction: Osteogenesis imperfecta (OI) is characterized by low bone mass, skeletal fragility and, frequently, short stature. We previously showed in oim/oim mice that sclerostin inhibition increased bone mass, mineral content and strength. Here, we compared the body length and the sizes of long bones, head and vertebrae between oim/oim and wildtype mice and analyzed the effect of sclerostin antibody (Scl-Ab) on these parameters

Materials and methods: Five-week-old oim/oim and wild-type mice received subcutaneous injections of either Scl-Ab VI (25 mg/kg) or vehicle (PBS) twice a week for 10 weeks. Body length and bone growth were evaluated on DXA scans collected at 5 and 15 weeks of age by measuring the snout-sacrum length. After termination at 15 weeks, the length of humerus and tibia and the craniofacial dimensions of the head were measured on radiographs. The heights of vertebrae L5, L6 and sacrum were measured on sagittal pQCT slices.

Results: All mice had a significant increase in body length during the experiment, but Scl-Ab-treated oim/oim mice remained significantly shorter than the vehicle-treated animals. Humerus and tibia length was similar in all the groups, without influence of Scl-Ab-treatment. Similar results were obtained in the craniofacial dimensions. In the spine, the sacrum (S1-S3) was significantly shorter in vehicle-treated oim/oim than in vehicle-treated controls (-20%). Individual vertebrae of oim/oim mice showed a non-significant increase in their height with Scl-Ab treatment.

Conclusions: In oim/oim mice, short stature could be due to global vertebral shortening. The absence of an effect of sclerostin antibody on stature could be related to its administration after the rapid growth phase. Future studies will investigate earlier phases of growth.

P81

Reference point indentation testing detects age-related changes in tissue mechanical properties in mice

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Objectives: The ability to discriminate bone fractures that result from non-accidental injuries and those that result from underlying bone fragility is limited by the lack of clinical instrumentation to directly measure bone mechanical properties. The BioDent Hfc (Active Life Scientific) is an experimental device that has been validated for measuring cortical bone fracture resistance in adults. We are currently developing protocols to test the feasibility of its use in infants and children. As part of our studies, we are investigating the practical limitations of testing fracture resistance using animal models. In this study we assessed the ability of the instrument to detect age-related differences in bone mechanical properties between young and old mice.

Methods: Femora from C57BL/6 male mice aged 3, 12 and 24 months (n=6 per group) were dissected free of muscles and tendons and stored at -80°C wrapped in gauze wetted in Hanks Balanced Salt Solution. For measurements, bones were thawed at room temperature for 2h and indented at five locations using a reference force of 260g and a test force of 2N over 10 cycles at 2Hz. Indentation parameters were calculated using dedicated software from the manufacturer.



Results: Both the total indentation distance (TID) and indentation distance increment (IDI) were significantly higher in the 24 month old mice: (TID [μm]: 3mo, 30.7 \pm 2.8; 12mo, 38.3 \pm 8.9; 24mo, 48.4 \pm 11.8; $P<0.05$, 3mo v 24mo. IDI [μm]: 3mo, 6.4 \pm 0.8; 12mo, 9.4 \pm 2.8; 24mo, 15.5 \pm 7.0; $P<0.01$, 3mo v 24mo).

Discussion: The increase in both IDI and TID implies a reduction in fracture resistance with aging. With increasing age, the variation in both measurements increased, possibly indicating that genotype exerts less of an effect than environmental factors in the age-related deterioration of the skeleton. The data show that age-related changes in bone mechanical properties can be detected using the reference point indentation technique, and suggest that the instrument will be a useful tool in studying paediatric bone diseases.

P82

The influence of sclerostin serum levels on bone mineral density and body composition in patients with Rett syndrome and healthy adolescent girls

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Objective: Sclerostin, product of the SOST gene, is an important determinant of bone formation and resorption. Rett patients, frequently present marked decreases in bone mineral density (BMD) beyond that expected from disuse atrophy. However, sclerostin has not been yet examined in Rett subjects as a potential mediator of impaired bone metabolism.

Methods: This study aimed to investigate whether there is any associations between sclerostin levels, body composition and BMD in Rett patients and in healthy controls. We studied 32 Rett girls (mean age 11.8 \pm 5.9 yrs) and 25 age-matched controls. Serum calcium, bone alkaline phosphatase, 25-hydroxyvitamin D and sclerostin were measured in both Rett patients and controls. In all subjects bone mineral density at whole body (BMD-WB), BMC-WB and body composition were measured by using a DXA machine (Hologic QDR 4500). QUS parameters were assessed at phalanges by Bone Profiler-IGEA (amplitude dependent speed of sound: AD-SoS and bone transmission time: BTT).

Results: The values of BMD-WB, AD-SoS and BTT were significantly lower in Rett subjects than in controls; as expected Rett patients had lower weight, lean mass and fat mass. The values of sclerostin were not different in Rett girl respect to healthy controls. Sclerostin showed a significant correlations with BMD-WB that remained significant after adjustment for age ($r=0.27$; $p<0.05$). Sclerostin was significantly correlated with both lean mass ($r=-0.41$; $p<0.05$) and fat mass percentage ($r=-0.42$; $p<0.05$). In Rett girls sclerostin serum levels were not predictors of bone status. Instead, we found that in the Rett subjects, weight and age were positively associated with BMD-WB ($p<0.05$).

Conclusion: Our preliminary study shown that sclerostin presents mild, but significant relationship with both BMD-WB and body composition parameters. Further studies are needed to elucidate the role of sclerostin on bone metabolism and body composition in Rett subjects.

P83

Anti-RANKL Nanobody® ALX-0141 Shows Sustained Biomarker Inhibition in a Phase I Study in Healthy Postmenopausal Women

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Objectives: The interaction between RANK and its ligand RANKL is critical for the regulation of osteoclastogenesis and bone resorption. Inhibition of this interaction helps restore the balance between bone resorption and bone formation. ALX-0141, a novel biological agent (Nanobody) that specifically targets RANKL, was studied in a Phase I trial to assess the safety, tolerability, immunogenicity and PK after a single subcutaneous injection.

Methods: Forty-two healthy postmenopausal women (53-77 years, mean age 66 years) were included in this randomised, double-blind, placebo controlled study. Participants received a single SC injection of ALX-0141 (n=31) at 6 dose levels, ranging from 0.003 mg/kg to 1 mg/kg, or placebo (n=11). PK, PD and safety parameters were monitored for 3 months in the lowest dose level and for more than a year in the higher dose levels.

Results: The safety analysis indicated that ALX-0141 was well tolerated. No serious adverse events related to ALX-0141 or dose-limiting toxicity occurred. The frequency of treatment emergent adverse events (TEAE) was similar in placebo-treated subjects (16 events in 7 subjects [64%]) and in subjects treated with ALX-0141 (93 events in 23 subjects [74%]). The most frequent TEAE were musculoskeletal and connective tissue disorders (n=27, reported by 14 subjects) and all TEAE were transient, of mild intensity, and did not result in any study withdrawals. After SC injection, ALX-0141 showed a favourable PK profile, triggering a prolonged PD response. Serum levels of the lead biomarker for bone resorption, cross-linking telopeptide of type 1 collagen (CTX-1), decreased rapidly in all ALX-0141 treated subjects and stayed suppressed (below 70% of the baseline level) up to 390 days after a single administration of 1 mg/kg ALX-0141.

Conclusion: The results from this Phase I trial indicate that ALX-0141 is a potent RANKL inhibitor that is well tolerated over a wide range of doses. Collectively, this data supports the further development of ALX-0141 in bone-resorptive diseases with a reduced bone mineral density and increased fracture risk, such as in cancer-related bone diseases, osteoporosis and other disorders.

P84

Comparison of socioeconomic status on bone mass assessed by Quantitative ultrasound of hand phalanges of boys from 7 to 15 years old

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Several environmental and genetic factors are determinant, also including sex, hormonal, ethnics, nutritional and lifestyle, may interferer on bone mass of children. However, the pediatric studies that approach the socioeconomic status and the quantitative ultrasound parameters (QUS) are scarce. The aim of this study was to compare the socioeconomic status on bone mass assessed by QUS in boys from 7 to 15 years old. In this cross-sectional study, were evaluated 520 Brazilian boys (9.7 \pm 1.5) from different socioeconomic status. The measurements of QUS parameter, amplitude dependent of speed of sound (AD-SoS) were obtained by using the 3th generation of DBM Sonic BP device (IGEA-Carpi, Italy). The socioeconomic status was classified according



with the Criteria of Economic Classification Brazil of Brazilian Association of Research Company (ABEP-Brasil). Regarding to the socioeconomic status, most boys (48.8%) were classified in the lower class. The AD-SoS values were significantly higher ($p < 0.05$) in the high class (1926.8 ± 45.1), when compared to the lower class (1906.6 ± 50.8), as shown at Fig. 1.

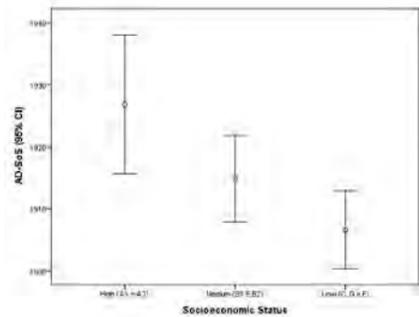


Fig. 1. Comparison of AD-SoS values according to the socioeconomic status. The results of this study shows that the socioeconomic factors may interfere significantly on the bone mass of this sample.

P85
Comparison of socioeconomic status on bone mass assessed by quantitative ultrasound of the phalanges in girls from 7 to 15 years old

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Several environmental and genetic factors may interfere on bone mass in children. However, the pediatric studies that approach the socioeconomic status and quantitative ultrasound (QUS) parameters are scarce. The aim of this study was to compare the bone mass in girls from 7 to 15 years old of different socioeconomic status. The sample consisted of 860 Brazilian girls (8.95 ± 1.32) of different socioeconomic status. The bone mass parameter, Amplitude Dependent Speed Sound (AD-SoS) in meters for seconds (m/s) was assessed for QUS of the proximal phalanges using DBM Sonic BP (IGEA, Carpi, Italy) device. The socioeconomic status was classified according to the Economic Classification Criteria Brazil of Brazilian Association of Research Company (ABEP-Brazil). Regarding to the socioeconomic status, most girls (43.6%) were classified in the lower class. The AD-SoS values were significantly higher ($p < 0.05$) in the lower class (1941.2 ± 53.7) when compared to medium (1927.4 ± 52.1) and high class (1928.1 ± 54.0), as illustrated in Fig. 1.

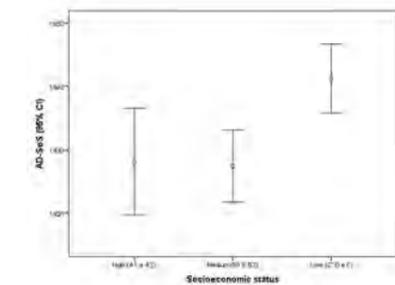


Fig.1. Comparison of AD-SoS values according to the socioeconomic status.

In conclusion, the results presented in this study demonstrates that socioeconomic factors may interfere significantly on the bone mass of the population studied.

P86
Assessing bone quality and fracture resistance in children using microindentation

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Background: At present, clinical assessment of bone strength in children predominantly relies bone mass measurement using absorptiometry (DXA) or QCT densitometric approaches. However, bone strength is not only dependent on mass/density, but also structural and material mechanical properties. Currently no technique measures bone mechanical properties. Recently, a new micro-indentation device, the Reference Point Indentation (RPI) instrument has been validated for the measure of cortical bone fracture resistance in adult subjects.

Aim: To establish safe and effective protocols for using the RPI instrument in infants and children.

Methodology: We planned to perform RPI testing on bone samples from 18 children aged 0-16 undergoing orthopaedic operations where bone was routinely excised. The RPI parameters for total indentation distance (TID) and indentation distance increase (IDI) were measured at 3, 5 and 7N force. The RPI parameters were compared with participants' vitamin D, PTH and calcium levels. Scanning electron microscopy (SEM) of bone samples was carried out to quantify damage cause by RPI testing.

Preliminary Results: Of the 18 samples obtained, only 5 had cortices >2mm and were suitable for testing. In these samples, SEM analysis showed that micro-indentations using the three forces (3, 5 and 7N) did not cause cracks greater than microcracks formed physiologically in cortical bone; crack length was statistically greater for the 7N force ($194.9 \mu\text{m}$ 95%CI 136.0-253.9) compared with either 3N ($124.7 \mu\text{m}$, 95%CI 101.0, 148.3) or 5N force ($116.8 \mu\text{m}$, 95%CI 97.1, 136.5); $p=0.001$ and $p=0.005$ respectively. There was no difference between 3N and 5N. There was no clear correlation of RPI outputs and measures of vitamin D homeostasis within this limited group.

Conclusion: Preliminary results suggest RPI testing using 3N and 5N indentation forces is safe on children's bone with a cortical bone thickness of over 2mm. Further testing will ensure the RPI instrument is safe to use on children of all ages and on children with metabolic bone diseases such as osteogenesis imperfecta, who have fragile bones.

P87
Serum Homocysteine levels in children and adolescents with impaired bone health

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Background: Association between high serum homocysteine (S-Hcy) levels and low bone mineral density (BMD) and increased fracture risk in postmenopausal women has been repeatedly documented. There are scarce data concerning S-Hcy and bone health in children and adolescents.

Patients and Methods: We assessed S-Hcy levels in 37 children and adolescents (22 boys and 15 girls; mean age 13.9 ± 3.5 years) with prevalent low-energy trauma fractures (mean 3.3 ± 2.3 per patient) and/or low spinal L1-L4 BMD (below -2SD Z-score; DXA Lunar GE). We also evaluated S-ALP, serum CrossLaps, osteocalcin (S-OC), body height, weight, body mass index (BMI). At the time of assessment, the children were not taking any drugs known to influence bone metabolism.



The age-dependent parameters (S-Hcy, BMD, S-ALP, S-OC, S-Crosslaps, height, weight and BMI) were expressed as Z-scores \pm SD.

Results: Mean S-Hcy Z-score was significantly higher (1.3 ± 1.5 ; $p < 0.0001$) and mean L1-L4 BMD Z-score was significantly lower (-1.7 ± 1.3 ; $p < 0.0001$), respectively, in comparison with reference values. S-ALP did not differ from reference values ($p=0.88$), while S-CrossLaps and S-OC were higher (1.2 ± 1.8 and 0.4 ± 0.5 respectively; $p=0.001$). Mean body height, body weight, and BMI Z-score was -0.05 ± 1.31 , 0.18 ± 1.62 and 0.48 ± 1.88 , respectively, which did not differ from reference values. The body height, weight and BMI Z-scores positively correlated with L1-L4 BMD Z-score ($r=0.44$, 0.53 and 0.50 , respectively; $p=0.01$). S-Hcy was inversely correlated to L1-L4 BMD ($r=-0.33$; $p=0.05$) and S-ALP ($r=-0.40$; $p=0.02$) and not related to number of prevalent fractures ($r=0.01$), S-osteocalcin ($r=-0.22$) or S-CrossLaps ($r=-0.003$). There were positive correlations between S-ALP and S-OC ($r=0.58$; $p=0.01$), S-ALP and S-Crosslaps ($r=0.39$; $p=0.05$), S-OC and S-Crosslaps ($r=0.36$; $p=0.05$), respectively. These results suggest increased bone turnover and negative influence of elevated S-Hcy on bone formation and BMD in children and adolescents with recurrent fractures.

Conclusion: Our results suggest that elevated S-Hcy should be considered a risk factor of impaired bone health in children and adolescents.

P88
Children and adolescents with cystic fibrosis have normal volumetric BMD and geometry at the radius, but low muscle area at the forearm

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Objectives: While studies in adults with cystic fibrosis (CF) showed increased fracture risk and decreased bone mineral density (BMD), the results of the pediatric studies have been contradictory. Our aims were to assess volumetric BMD, bone geometry and the muscle-bone relation at the forearm in children with CF using peripheral quantitative CT (pQCT), and to correlate these bone parameters to pulmonary function.

Methods: Fifty-three patients with CF (median age 12.9 yrs, range 6.7-18.8, 29 girls) were examined by pQCT at the non-dominant forearm. Results were expressed as Z-scores using published reference data. Median forced expiratory volume in one second (FEV1, % predicted) of the spirometry examinations performed during the last year before densitometry was selected as a surrogate of pulmonary function. The differences from reference data were tested by one-sample T test, Pearson correlation coefficient was used to correlate pQCT-derived bone parameters with FEV1.

Results: Trabecular BMD was normal (mean Z-score -0.2 ± 1.3 , n.s.) in children with CF. Total bone cross-sectional area, cortical bone area and cortical thickness were all normal when adjusted for height (mean Z-scores 0.0 ± 1.1 , 0.0 ± 1.0 and 0.0 ± 0.8 , respectively). Cortical BMD was increased (mean Z-score 1.0 ± 0.9 , $p < 0.001$). As a consequence of decreased muscle area (MA, mean Z-score -1.5 ± 1.5 , $p < 0.001$) the bone mineral content to MA ratio was increased (mean Z-score 1.3 ± 1.0 , $p < 0.001$). Whereas FEV1 was positively correlated to muscle area ($R^2=0.20$, $p < 0.002$) and bone geometry (i.e. cortical thickness ($R^2=0.24$, $p < 0.001$) and cortical bone area ($R^2=0.20$, $p < 0.002$)), the correlation with BMD was weak ($R^2=0.08$, $p=0.035$ and $R^2=0.007$, n.s. for trabecular and cortical BMD, respectively).

Conclusions: Children and adolescents with CF have normal volumetric bone density and geometry at the radius but decreased muscle mass at the forearm. FEV1 seems to be a good predictor of changes in muscle area and bone geometry in children with CF.

GRANT SUPPORT: Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic)

P89
Painful vertebral fractures during pregnancy: Be aware of a potentially underlying genetic cause

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Background: The baby growing in its mother's womb needs calcium for skeletal development. Maternal osteoporosis has been attributed to pregnancy in some cases.

Presenting Problem: A 27-year old woman in the 7th month of her first pregnancy complained of mid-thoracic back pain after lifting a non-heavy object. The pain remained with differing intensity and was attributed to her pregnancy. After the delivery of a healthy child, the back pain prevented her from lifting her baby. Physical therapy had no effect. Her past medical history was uneventful without fractures, except for severely reduced vision of her left eye since birth. She used three dairy products daily and no medication. Family history revealed that her maternal grandmother had osteoporosis at old age. Physical examination: Height 1.58m, weight: 53 kg. No blue sclerae; amblyopic left eye. No hyperlaxity of skin or joints. Laboratory examination including serum calcium, phosphate, alkaline phosphatase, creatinine, bCTX 25-hydroxyvitamin D and TSH was normal. Spinal X-ray showed end-plate compressions of thoracic vertebrae (Th7,9,10 and 12). DXA-scanning showed severe osteoporosis (T-score L2-L4: -5.7 SD, femur neck: -3.9 SD). DNA analyses revealed two mutations in the *LRP5* gene. Family screening with DXA and DNA analyses was performed. Her mother, recently postmenopausal, had osteoporosis and carried the *LRP5* mutation. Her only brother, treated with cabergoline for a microprolactinoma, had osteopenia on DXA and was an *LRP5* mutation carrier.

Clinical Management: The patient was diagnosed with osteoporosis pseudoglioma syndrome and treated with risedronate for 2.5 years. BMD and back pain improved. She stopped the use of bisphosphonate 6 months before planning a second pregnancy.

Discussion: Potentially underlying genetic causes like osteogenesis imperfecta or osteoporosis pseudoglioma syndrome should be excluded in pregnancy-associated osteoporosis. A genetic diagnosis has implications for the patient and relatives. More studies regarding bisphosphonate treatment preceding conception are desirable.

P90
Intravenous pamidronate in the treatment of severe Idiopathic Infantile Hypercalcemia

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Background: Idiopathic infantile hypercalcemia (IIH) is a rare disorder caused by CYP24A1 loss-of-function mutation, resulting in impaired degradation of 1,25-dihydroxyvitamin D₃. Typical signs include muscle hypotonia, dehydration, failure to thrive, psychomotor retardation, constipation, nephrocalcinosis. IIH should be distinguished from other causes of hypercalcemia in infancy. Treatment includes low calcium diet, glucocorticoids, furosemide, calcitonin. Pamidronate, an intravenous bisphosphonate, which is a potent inhibitor of bone resorption, has been so far used only in infants with osteogenesis imperfecta, hypercalcemia due to Williams-Beuren syndrome or neonatal severe primary hyperparathyroidism, and just once in IIH.

Case Report: We present a case of previously healthy 5-month old boy with IIH, where calcemia peaked to 5mmol/L. The boy suffered from poor appetite, dehydration, constipation, and failure to thrive. Treatment



with methylprednisone and furosemide had only minor effect, therefore two intravenous infusions of pamidronate (0.6 mg/kg/dose) were necessary to correct the serum calcium level to 2.95 mmol/L. Furthermore, *CYP24A1* homozygous mutation p.R396W (c.1186c>t) was identified in this patient, confirming the clinical diagnosis of IH. Currently, the boy is five years old, thriving with normal psychomotor status and is being followed on an out-patient basis.

Conclusion: IH has a favourable outcome once properly detected and appropriately treated. Pamidronate has a beneficial effect in those patients with IH where glucocorticoids and furosemide fail to meet the expectations.

P91

Hypovitaminosis D in chronically ill children in Greece and its Management

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Objectives: Study the prevalence of hypovitaminosis D in chronically ill children in Greece, as well the efficacy of a proposed replacement therapy.

Methods: A total number of 213 children (121 males, 92 females), with mean age 10.1 years (1-18), suffering various pathological conditions (cerebral palsy, Duchenne myopathy, epilepsy), were sampled to measure serum 25(OH)D and parameters of bone turnover. 101 children were given replacement therapy according to levels of hypovitaminosis [15-20ng/ml pos ergocalciferol and calcium (4000IU+500mg/day), <15ng/ml intramuscular ergocalciferol (400.000IU) once per month for 2 months combined with pos ergocalciferol and calcium (4000IU+500mg/day)] while 60 children were re-evaluated during the four months follow-up.

Results: The mean serum 25(OH)D was 14.20ng/ml on total (15.5ng/ml for patients<12years old and 12.6ng/ml for patients>12years old), while the highest rate was measured at 44.53ng/ml. Respectively median value for PTH was 48.87pg/ml on total. 34.74% of patients had vitamin D deficiency (10-20ng/ml) while overt vitamin D deficiency (<10ng/ml) was measured in 21.59% of patients of whom 21.73% had less than 4ng/ml. Of the 22 children receiving antiepileptics, 86.34% had vitamin D deficiency. 9% presented secondary hyperparathyroidism. Higher serum PTH levels were observed in the group of 25(OH)D<10ng/ml for both age groups, however the inverse relationship between serum 25(OH)D and PTH was statistically nonsignificant at 25(OH)D levels>10ng/ml. A positive correlation between 25(OH)D and serum phosphorus was also observed (p = 0.003) After replacement therapy, children displayed higher levels of 25(OH)D on total (median 25(OH)D=25.8ng/ml, p<0.0005) and for both age groups(fig1).

P92

Bone mineralization in children with chronic gastroduodenitis

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Children with chronic gastroduodenitis (CGD) have decreased absorption of nutrients, minerals particularly, which can influence on bone strength and linear growth.

Objectives: we evaluated bone mineral density (BMD) in children with CGD and compared with healthy individuals.

Methods: BMD was measured in 97 children aged 5 – 13 years by lumbar spine DXA (DPX MD+, Lunar) with pediatric reference database. BMD was evaluated according official positions of International Society of Clinical Densitometry (2007). Calcium supplementation was estimated by urine calcium/creatinine relation. The

main group consists of 42 CGD children, the control group - 55 healthy children.

Results: BMD and calcium supplementation was related to age, height and weight (r=0.25; r=0.5 r=0.3 relatively, p<0.05). BMD, BMC, BA and calcium/creatinine relation in CGD children were lower (p<0.04) than in healthy controls. Decreased BMD had 36% CGD children and 26% healthy controls when children's BMD was compared with densitometer reference database. After adjustment of BMD with height the number of children with decreased BMD reduced more than 3 times in both groups, so low BMD for chronologic age (<-2SD) in CGD children was detected in 12% compare to healthy controls - 7%, p<0.05). False-positive decreased BMD was detected predominantly in children with growth delay (height was lower 10th percentile for chronological age). Applying ISCD recommendations allowed reduce more than 3 times the overdiagnosis of decreased BMD which prevent them from inappropriate treatment.

Conclusion: Children with CGD had insufficient calcium supplementation. Near 10% of CGD children had growth delay. Clinically significant BMD declining was detected more often in CGD children.

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Long Term Efficacy of Low Dose Pamidronate Treatment in Osteogenesis Imperfecta and Its Effects On Growth

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Aim: To assess long term safety of low dose pamidronate therapy and the effects on pubertal growth spurt.

Methods: A Retrospective study was conducted in 36 girls and 21 boys whose mean age was 4,1±3,6 years at baseline. Intravenous pamidronate 3-4 mg/kg/year once daily therapy with 2-4 cycles/year for 0,5-15 years) was given with physical therapy and orthopedic surgery as appropriate. Mobility score, height, puberty and bone mineral density (BMD) was evaluated through follow up (6,4±4,08 years).

Results: Puberty started before study inclusion in 2 patients and during the study 26 additional patients. 10 of these patients completed puberty during study. Overall lumbar spine BMD z score improved from -3,2±1,8 g/cm2 to -2,1±1,7 g/cm2 (p:0,0). The overall mobility score improved from 2,7±1,6 to 3,36 ±1,1 (p:0,0) (13 of the patients were in infancy so they were not included in the mobilization scores). Mean height SDS was -2,12±2,6 at baseline and -2,0±2,3 at treatment completion (p:0,7). Catch-up linear growth did not occur. Mean height SDS was -2,9±3,4 at the start of puberty and -3,6±4,7 at completion of puberty (p:0,0). Although these patients did not catch up linear growth during puberty, their lumbar BMD z score increased from -2,1±1,5 to -0,9±0,95 during puberty (p:0,3).

Conclusion: Our data suggests that cyclic low dose pamidronate therapy combined with physiotherapy and orthopedic procedures may improve the clinical manifestations as mobilization and bone mineral density. No significant catch up growth occurred during the study period and especially a decrease in height occurred during puberty. This decrease in linear growth can be related to the nature of the disease or to pamidronate therapy.

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Bone anomalies in Noonan syndrome

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Background: Noonan syndrome (NS) is a genetic multisystem disorder characterized by distinctive facial features, learning difficulties, short stature, and cardiac defects. Other important findings include skeletal



anomalies, especially spinal and chest deformities. Skeletal dysplasia was proven to be secondary to a disorder of RAS-mitogen activated protein kinase (MAPK) pathway which is essential for regulation of cell differentiation and growth including bone homeostasis. We describe the pattern and severity of bone deformities and other related clinical characteristics in five patients with Noonan syndrome.

Methods: We reviewed the cases of five children with NS (3 boys, 2 girls, aged between 5 and 13 years), evaluated at the Endocrinology Department between January 2010 and January 2013. Evaluation included clinical and hormonal data as well as radiographic assessment of deformities.

Results: All patients had specific phenotype for Noonan syndrome. Three had also a molecular confirmation (the first with KRAS mutation, the second – PTPN11, and the third – SOS1). All children had short stature, with a mean standard deviation of -3.27 in heights. Hand-wrist radiography showed a moderately delayed bone age in all patients. None of the patients had congenital bone deformities, and the birth weight and length were within normal ranges for gestational age. The most frequent skeletal anomaly was chest deformity – four children had inferior pectus excavatum and two of them associated superior pectus carinatum. One patient, with SOS1 mutation, had scoliosis with double major curve, developed at age two. Another patient also associated cubitus valgus and slightly shortness of the fingers and toes.

Discussions: All five patients with Noonan syndrome had short stature with late bone development and skeletal deformities. Chest deformities are usually correlated with a PTPN11 positive genotype, confirmed in only one case of the four children with this type of skeletal anomaly. Scoliosis and kyphosis occur in about 15% of patients, mainly associated with SOS1 mutations, as it was in our case with severe double curved scoliosis. Since bone anomalies tend to develop early and some are relatively severe, patients require clinical and, if necessary, radiographic assessment with careful follow-up for early detection and treatment.

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Abstract withdrawn

P96

Cross-sectional associations of sex steroids with bone maturation, bone mineral density and bone geometry in boys

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Background: Although both testosterone and estrogens are considered essential for normal bone growth, epiphyseal maturation and bone mass accrual during adolescence, only very few data concerning the changes of estrogens, bone maturation, bone mineral density (BMD) and bone geometry in healthy boys at different pubertal stages have been published.

Objectives: This study aimed to analyze the relationship between sex steroids and more especially estrogens and skeletal maturation, BMD and bone geometry in healthy boys and adolescents.

Methods: Two hundred and five healthy boys (aged 6-18 y; median age 13 y) were included in this cross-sectional study. Pubertal status of the subjects was assessed according to the method established by Tanner. Estradiol (E2) and testosterone (T) levels were determined by liquid chromatography tandem mass spectrometry. Whole body areal bone mineral density (aBMD) and area, lumbar aBMD and area were determined by DXA. Trabecular (4%) and cortical (66%) volumetric BMD and bone geometry (trabecular area, cortical area, periosteal circumference, endosteal circumference and cortical thickness) were assessed at the non-dominant forearm using pQCT. Skeletal age was determined by an X-ray of the left hand and wrist

Results: As expected increasing sex steroids (E2 and T) levels were found with advancing pubertal development (p<0.001). Lumbar and whole body aBMD and area, trabecular and cortical vBMD, trabecular and cortical area, periosteal and endosteal circumference and cortical thickness increased significantly throughout puberty (p<0.001). Regression models including age and BMI showed that estradiol (E2) was positively associated with lumbar (E2: β : 0.35 p<0.001) and whole body aBMD (E2: β : 0.20 p<0.001), trabecular vBMD (E2: β : 0.32 p<0.01), and cortical thickness (E2: β : 0.27 p<0.01) at the radius. Moreover, E2 was positively associated with the degree of bone age advancement (E2: β : 0.26 p<0.01). No associations were found between E2 and cortical vBMD and endosteal circumference. Testosterone was positively associated with lumbar (T: β : 0.36 p<0.001) and whole body area (T: β : 0.23 p<0.001), trabecular (T: β : 0.29 p<0.01) and cortical area (T: β : 0.21 p<0.02), periosteal circumference (T: β : 0.28 p<0.001).

Conclusion: Circulating estradiol is strongly associated with bone maturation and areal and volumetric bone mineral density in healthy boys, whereas testosterone determines mainly bone area (lumbar spine area, whole body area, cortical area and trabecular area, periosteal circumference). These cross-sectional findings need confirmation by a longitudinal study.

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Vitamin D status and bone health in survivors of childhood lymphoblastic leukemia

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Introduction: Lymphoblastic leukemia is the predominant form of childhood malignancies with survival rates of > 80%. Late effects of cancer and treatment can affect endocrine function and may account for acute and chronic impairment of bone health.

Aim and Design: To assess bone health in pediatric patients after therapy for lymphoblastic leukemia we initiated a clinical trial investigating clinical and biochemical parameters of growth, puberty, bone turnover, and vitamin D metabolism, as well as bone densitometry using DXA scans in a subgroup of patients. Additionally a questionnaire was developed assessing life style parameters including calcium and vitamin D intake and levels of physical activity. Patients (n=90) were treated for leukemia according to applicable ALL-BFM protocol (1994 – 2011) and recruited at follow-up visits at the Children's Hospital Essen.

Results: 39 of the 90 patients were female. Mean (range) chronological age (CA) was 11.47 (3.8 – 20.9) y, age at diagnosis was 6.9 (0.8 – 16.9) y, height SDS -0.04 (-3.69 – 2.72), BMI SDS 0.48 (-4.12 – 2.74), SDS for Tanner stage 0.22 (-3.2 – 3.5). Mean serum 25-OH vitamin D levels were 17.34 (1 – 62.6) ng/ml, 1.25 (OH)²vitamin D levels were 60.2 (13 – 158) ng/l, BAP was 119.9 (18.3 – 283)U/l, PTH 46.7 (9.6 – 159.8) pg/ml, N-telopeptide in urine 908.8 (21 – 3000) mmol/mg creatinine and calcium/creatinine ratio in urine was 0.12 (0.01 – 0.4) mg/mg. Mean calcium intake/week was 5.64 g (1.2 – 10.5), vitamin D intake: 15.4 mcg (0.22 – 46.6). Average screentime was 1.9 (0 – 5) hours /day, average daily activity: 5.12 hours/day. Mean age corrected z-score in DXA scans was -0.55 (-3.3 – 2.1, n=30).

11% of the patients experienced fractures after chemotherapy, 3 patients reported frequent fractures. Vitamin D deficiency (25 OH-vitamin D < 20 ng/ml) was observed in 68% and secondary hyperparathyroidism in 17%. 15% reported bone pain after physical activity. Male and female patients did not differ significantly with respect to biochemical or clinical findings. There was a trend to lower height SDS in boys in this cohort. Accordingly mean bone age (BA) was delayed in boys (Delta BA/CA: -1.7 (-5.1 – 0.33)y. Osteopenia (z-score <-2) was detected in



15% of the patients screened. No correlation between z-scores and N-telopeptide levels in urine or total dosage of prednisone, dexamethasone or methotrexate was observed.

Conclusion: Bone health is impaired in some patients after treatment for childhood leukemia. Clinically this is reflected in bone pain and/or fractures. Prediction or identification of children at risk is difficult and requires assessment of additional clinical, biochemical and radiological measures. As previously reported, vitamin D and calcium deficiencies are frequent findings in this group and adequate monitoring and supplementation is recommended.

P98
Effects of denosumab on bone biochemistry and calcium metabolism in a girl with Juvenile Paget's Disease

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Juvenile Paget's disease (JPD) is an extremely rare, yet painful and debilitating bone disease with onset occurring during early childhood. JPD can be caused by loss of function of osteoprotegerin, resulting in subsequent osteoclast stimulation via the activated receptor activator of nuclear factor-kappa B (RANK) pathway. Increased bone turnover and a lack of bone modelling lead to severe deformities, frequent fractures, short stature and loss of hearing.

The treatment of JPD is challenging and has previously been based on either calcitonin or cyclic administration of bisphosphonates. However, with the development of denosumab, a RANK-ligand antibody, a treatment targeting pathophysiology in JPD may be available.

We report clinical and biochemical effects of denosumab treatment on an 8-year-old girl with a severe form of JPD. The patient is the second child of healthy, consanguineous parents of Turkish descent. Genetic analysis revealed a novel homozygous mutation in the osteoprotegerin gene *TNFRSF11B:c.[2T>G]; [2T>G]*, which results in a methionine to arginine exchange at position one of exon 1, presumably resulting in the loss of the start codon and therefore a significant loss of functional osteoprotegerin protein. Osteoprotegerin levels in serum were diminished in the patient (0.7 pmol/L, normal range 1.69 – 3.6 pmol/L) and within the lower normal range in mother and father.

Before starting the denosumab treatment regimen, the patient had been treated for 3.5 years with intravenous pamidronate. However, with an intensified pamidronate treatment bone turnover markers stayed elevated and the girl suffered from frequent bone pain and pathological fractures.

The administration of denosumab resulted in a prompt improvement of disease control. Bone pain ceased on the day of the injection and N-telopeptide and desoxypyridinoline levels in urine decreased the same day. Alkaline phosphatase levels dropped within the normal range and remained at normal levels for 5 months following the final dose of denosumab.

However, concomitantly with the first injection, severe hypocalcemia developed, for which the patient was hospitalized and intravenous calcium substitution was required for a total of 13 days. Calcium demand remained high (900 mg/d) for the six weeks following the injection. A second dose of denosumab was well tolerated and markers of bone turnover stayed within the normal range. With ongoing calcium supplementation a sudden but severe hypercalcemia developed 6 weeks after the second dose of denosumab. At that time denosumab was discontinued despite the clinical improvement and pamidronate treatment was commenced.

In summary, denosumab appears to be significantly effective for osteoclast inhibition for the treatment of JPD. However, severe hypocalcemia and possibly hypercalcemia later on are side effect for which close patient monitoring is required.

P99
Potential association between vitamin D deficiency and hypertension in adolescents: A pilot study

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Objective: Insufficient vitamin D supply defined as serum hydroxyvitamin D (25OHD) less than 20 ng/ml is considered as one of possible cardiovascular risk factors among adults with hypertension. Furthermore, some data also suggest an independent association between vit. D deficits and hypertension and obesity during growth. The aim of the study was to assess vit. D status in children and adolescents with hypertension.

Methods: The cross sectional study was performed on a group of 99 subjects (43 girls, 56 boys) aged 8.4 – 17.6 years (mean: 12.1), stratified according to their values of blood pressure (BP). Blood samples were taken to determine 25OHD; anthropometric traits and BP measurements were carried out using standard methods and equipment. The study was performed after winter time i.e. in March 2012.

Results: Only 8% of all studied children and adolescents demonstrated vitamin D level above 20 ng/ml, whereas predominantly severe deficits were found (92%). Significant correlations were observed between 25OHD and systolic BP ($r = -0.37, p = 0.0003$) and mean BP ($r = -0.3, p = 0.002$). Children and adolescents with normal systolic BP had better supply with vit. D compared with age- and sex-matched hypertensive pairs i.e. subjects with critically increased systolic BP (> 90 percentile). The association ($p = 0.03$) was independent of age, BMI and anthropometric parameters.

Conclusions: Our findings show associations between decreased levels of serum 25OHD and hypertension in adolescents suggesting potential role of vitamin D deficits in the development of this condition. However, these relationships and possible causal links need to be investigated in further prospective studies including larger groups of pediatric patients.

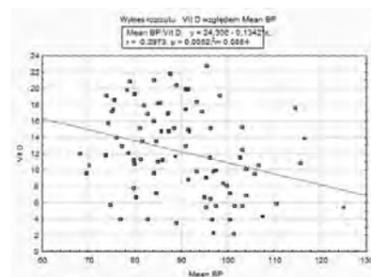


Fig. Correlation between mean blood pressure (Mean BP) and serum concentration of 25(OH)D in subjects aged 8 – 17 years



P100
Preliminary evidence of reduced Volumetric Trabecular Bone Mineral Density in children with Idiopathic Hypercalciuria. A Peripheral Quantitative Computed Tomography (pQCT) study.

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Objective: Idiopathic hypercalciuria (IH) is defined as excessive 24 h urinary calcium excretion (>4 mg/kg/24h), that persists after correction of dietary imbalances in the absence of secondary causes. Recent studies with DXA in children with IH provide evidence of decreased areal BMD. We used peripheral Quantitative Computed Tomography (pQCT) of the tibia, to test the hypothesis that IH results in decreases of volumetric (mg/cm³) BMD of the trabecular and/or cortical compartment of bone.

Patients and Methods: We studied 14 children (8 boys- 6 girls, aged 6–18 years) with newly discovered IH that were admitted to our clinic. Most of them presented with either hematuria or recurrent abdominal or lumbar pain. After establishment of the diagnosis, all children underwent DXA of the lumbar spine. We also performed pQCT of the tibia (Stratec XCT-2000 scanner), 4 slices were obtained at the 4%, 14%, 38% and 66% of tibia length sites. For the 4% slice, we assessed variables of trabecular bone and especially trabecular BMD (TRAB_DEN, mg/cm³). For 14% we assessed parameters of subcortical bone and for the 38% and 66% sites parameters of cortical bone. pQCT data of the children with IH were compared to those of healthy race-, age- and sex-matched children from the published pQCT database of Moyer-Miller et al (J Clin Densitom, 2008) who used the same pQCT device, software, and site measurements that we did.

Results: 7/14 children with IH (50%) were found to have z-scores <-1 SD in the DXA measurements of the lumbar spine. For the pQCT measurements, we report here only the preliminary results of trabecular BMD (ongoing analysis). 8/14 children with IH (57%) had reduced volumetric bone mineral density (TRAB_DEN < 2 SD) when compared with healthy children of the same age, race, sex and height of the Moyer-Miller study.

Conclusion: Our study provides preliminary evidence of reduced trabecular bone mineral density in children with IH as compared to healthy ones.

P101
Rapid Bone Mass Recovery after Parathyroidectomy for Primary Hyperparathyroidism in a 15-year-old Boy

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Primary hyperparathyroidism is extremely rare in childhood and adolescence. Here we report a clinical case of a 15-year-3-month-old boy who began 2 years before with pain in his knees, genu varum, and fatigue. Physical examination showed severe genu valgum with an intermalleolar distance of more than 30 cm. Biochemical tests showed hypercalcemia (12.2 mg/dl), hypophosphatemia (2.3 mg/dl), hypercalciuria (6.4 mg/kg/day), high alkaline phosphatase (2812 IU/L), low 25-hydroxyvitamin D (6 ng/ml), and hyperparathyroidism (PTH: 2653 pg/ml, normal value 12-72). Skull x-rays showed salt-and-pepper appearance, and loss of lamina dura in the mandible, x-rays of the hands showed subperiosteal resorption and severe rickets, and x-rays of the knees showed rickets and osteomalacia. Kidney ultrasonography showed increased echogenicity on both sides. Urine catecholamines were normal. Neck ultrasonography and technetium-99m Sestamibi revealed

parathyroid adenomas. Spine, femora, and whole body bone densitometry disclosed markedly reduced bone mineral density: L2-L4: 0.87 g/cm², Z-score: -2.3, right femur 0.67 g/cm², left femur 0.76 g/cm², and whole body 0.82 g/cm², Z-score: -3.4. Two parathyroid adenomas situated on the upper and lower part of the right lobe of the thyroid gland were successfully removed. Histopathology reported parathyroid adenoma of chief cells. PTH fell to 38 pg/ml 30 minutes after the adenomas were removed. He developed severe hungry bone syndrome 96 hours after surgery requiring up to 7.5 µg of calcitriol and 14.5 g of oral calcium/day and intravenous calcium for 19 days to normalize serum calcium and phosphate. Bone densitometry, repeated after four months, showed a dramatic increase of bone mineral density with normalization in all sites: spine: L2-L4: 1.192 g/cm², Z-score: +0.7; right femur: 1.097 g/cm², left femur: 1.088 g/cm², whole body: 1.065 g/cm², z-score +0.4. In conclusion: Here, we report a case of primary hyperparathyroidism due to parathyroid adenomas in an adolescent boy. After surgery, the patient needed high doses of calcitriol and calcium to normalize calcemia and phosphatemia. Follow-up and outcome were good and four months after surgery he had completely recovered bone mass.

P102
Influence of nutritional status in the bone mass assessed by Quantitative Ultrasonography (QUS) of boys

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The childhood is an important period for bone mass acquisition, and this tissue may be influenced by the nutritional status. However, the relation between the nutritional status and the bone parameters of Quantitative Ultrasound (QUS) remains unclear. The aim of this study was to verify the influence of nutritional status on bone mass assessed by QUS in male children from 7 to 10 years old. The sample consisted of 461 Brazilians pre-pubertal schoolchildren (8.30 ± 1.13 years). The nutritional status was classified according to extended International body mass index cut-offs (Cole and Lobstein, 2012). For the bone mass measurements was used the QUS of proximal hand phalanges, using the DBM Sonic BP (IGEA, Carpi, Italy) device, which provides the parameter AD-SoS (amplitude-dependent speed of sound). Most subjects (64%) presented a normal nutritional status (15.8 kg/m²), 11.3% at thinness (13.7 kg/m²), and 14.8% at overweight (19 kg/m²) and 9.5% at obese (23 kg/m²). The obese subjects (1875.4±43.76 m/s) presented lower values ($p < 0.04$) of AD-SoS when compared to the other groups (1902.2±49.2; 1896.3±48.47; 1900.7±49.5 m/s, thinness, normal e overweight, respectively), as shown at Fig. 1.

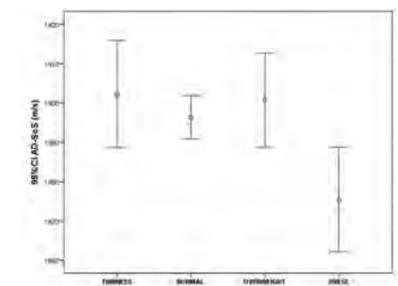


Fig.1: 95% Confidence Interval of AD-SoS values according to nutritional status.

The results presented in this study shows that the nutritional status may interfere negatively at the bone mass parameters, and may implicate the bone health in adulthood. Preventive actions must take place with children to minimize the negative effects of obesity.



P103
Celiac Disease and Bone Disorders: Diagnosis age influence

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Celiac Disease (CD) is a frequent cause of malabsorption in childhood and affects calcium and vitamin D absorption.

Aim: analyze the vitamin D levels and bone mineral density (BMD) at diagnosis in patients affected with Celiac Disease, and the influence of the age of diagnosis in the clinical presentation. Patient: 52 patients (Female: 26) were evaluated. Results (Table 1)

The percentage with Vitamin D deficiency (<20ng/ml) was 37, 7 % (n=20) at the moment of diagnosis and 26,4 % (n: 14) of the patients had BMD LS below 2 SD. In the whole group we found positive correlation between: BMD Z score and Vitamin D levels (p: < 0.01, r : 0.527) and BMD Z Score and Chronological age at the moment of diagnosis (p: < 0, 05 r :-0, 345). Levels of 25(OH)D correlated with and BMD Z score (p<0,005.r:0,486) in the group with more than 6 years old at diagnosis .

Conclusions: We found an important percentage of vitamin D deficiency and low bone mass at the moment of diagnosis in the whole group. The age at the diagnosis influenced bone mineral density and biochemical manifestations. Our results suggest that the early diagnosis and treatment is essential for the bone health of patients affected with CD.

Table 1 (result expressed in mean ± SD)

	Whole group (n: 52)	< 6 years old at diagnosis (n:16)	> 6 años years old at diagnosis (n: 36)
Age at diagnosis (year)	9,11 (± 4,60)	3,44 (± 1,28)	11,6 (± 2,99)
Z score height (SD)	-0,82 (± 1,46)	-0,40 (± 0,79)	-1,01 (± 1,66)
Z score weight (SD)	-0,73 (± 1,36)	-0,33 (± 1,03)	-0,90 (± 1,47)
BMI	18,11 (± 4,14)	18,10 (± 4,09)	17,30 (± 3,74)
BMD Lumbar Spine (SD)	-1,1 (± 1,5)	-0,57 (± 1,3) *	-1,33 (± 1,15) *
BMD Lumbar Spine (g/cm2)	0,642 (± 0,18)	0,48 (± 0,45)	0,687 (± 0,035)
25 (OH) D (ng/ml)	25,58 (± 13,48)	30,26 (± 10,36) **	22,94 (± 11,94) **

* p < 0,02 LS: L2 - L4 Lumbar Spine
 ** p < 0,03

* p < 0,02 LS: L2 - L4 Lumbar Spine

** p < 0,03

P104
Immunohistochemical localization of Bone Morphogenetic Proteins and their receptors in Human Osteochondromas

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Objectives: To define the role of bone morphogenetic proteins (BMP) in human osteochondromas. The expression of bone morphogenetic proteins and their corresponding receptors has not been clarified in osteochondromas. We determined immunohistochemically the localization and distribution of bone morphogenetic proteins 2, 4, 6 and 7, bone morphogenetic protein receptor types 1A, 1B and 2 and the functional effectors phosphorylated Smad proteins 1, 5 and 8 in the cartilaginous cap of human osteochondromas and compared with growth plate and articular cartilage of bovine knee joints.

Methods: The localization of antibodies against human BMP-2/4, BMP-6, BMP-7, BMP receptor type 1A, 1B and 2 and phospho-Smads 1/5/8 on sections of solitary (n=6) and multiple hereditary (n=6) osteochondromas was examined using immunohistochemical localization methods.

Results: The distribution and localization of BMP-2/4, BMP-7 and receptors BMPR-1A, BMPR-1B and BMPR-2 was observed uniformly in chondrocytes in osteochondromas and growth plate. A difference in the distribution of BMP-6 and phospho-Smads 1/5/8 was observed between osteochondroma and growth plate. BMP-6 was localized throughout the cartilaginous cap of osteochondromas while BMP-6 was mainly found in the hypertrophic zone of the growth plate. In osteochondromas phospho-Smads 1/5/8 was observed in hypertrophic chondrocytes undergoing calcification. In contrast, phospho-Smads 1/5/8 was observed throughout the growth plate with greater staining intensity in the resting zone. The expression of BMP-6, BMPR-1A, BMPR-1B and BMPR-2 varied among the cartilaginous cap of solitary and multiple hereditary osteochondromas, the difference is not significant (P > 0.05).

Conclusion: The localization of BMP 2, 4, 6 and 7, BMP receptor types 1A, 1B and 2 and phosphorylated Smad proteins 1, 5 and 8 was analysed in human osteochondromas immunohistologically. Distributions of BMP-6 and phosphorylated Smad proteins 1, 5 and 8 in human osteochondromas were different from normal growth plate. Since bone morphogenetic proteins can stimulate proliferation and differentiation of chondrocytes, these findings suggests that there may be a dysregulation of chondrocyte proliferation and differentiation in the cartilaginous cap of human osteochondromas.

P105
Bisphosphonate Treatment in Non-ambulatory Patients with Spastic Quadriplegic Cerebral Palsy and Other Neuromuscular Disorders: Effectiveness of Pamidronate vs. Zoledronic Acid

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Objectives: To examine the bone mineral density (BMD) response to intravenous pamidronate (Group 1) vs. intravenous zoledronic acid (Group 2) in non-ambulatory children and young adults with severe cerebral palsy or other neuromuscular disorders.

Methods: A total of 50 non-ambulatory children and young adults, (mean age 11.3 years, range 2.1-32) with low BMD and/or history of fractures were retrospectively studied. Thirty-nine patients (30 spastic quadriplegic cerebral palsy, 6 spinal muscular atrophy, 3 myelomeningocele) were treated with IV pamidronate (from 1999-2007). Eleven patients (7 spastic CP, 2 spinal muscular dystrophy, 2 myelomeningocele) were treated with zoledronic acid (from 2006-2011). Intravenous pamidronate, 1 mg/kg/day was administered 3 consecutive days, repeated at 3 month intervals for the first year. IV zoledronic acid, at 0.025 mg/kg/dose, was given at 3 month interval for the first year. Frequency of both treatments was decreased in subsequent years to every 4, 6 or 12 months depending on BMD response. Lumbar BMD (Hologic DEXA), Z score (adjusted for weight and pubertal status) at baseline and 1, 2 and 3 years were analyzed.

Results: Mean lumbar BMD Z score increased significantly from baseline to 1, 2, and 3 years in both groups (p < 0.01): from -3.89 at baseline to -2.68 at 1 year, -1.97 at 2 year, and -1.73 at 3 years in Group 1; from -3.89 at baseline to -2.43 at 1 year, -1.52 at 2 years, and -1.35 at 3 years in Group 2. There was no significant difference in BMD Z score response between groups; treatment was well tolerated with no serious adverse reactions.

Conclusion: Both pamidronate and zoledronic acid treatment are effective in improving BMD and BMD Z score in children and young adults with neuromuscular disorders. The BMD response was comparable between these 2 treatments. Zoledronic acid should be considered as a treatment of choice in this patient population, given the much shorter duration of infusion. Long term follow up study is needed to confirm safety and efficacy in fracture reduction and prevention.



P106
Acquired hypophosphatemic rickets in a 13 year old boy presenting with knee pain and valgus deformity

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Background: Hypophosphatemic rickets commonly presents in early childhood as inherited disorders. Acquired hypophosphatemic rickets or osteomalacia is a rare condition in children caused by paraneoplastic production of phosphaturic factor or FGF23 and is called tumor-induced rickets or osteomalacia. Localization of tumor is important as hypophosphatemic rickets completely resolves after resection of tumor.

Presenting Problem: We present a challenging case of a 13 year old male with acquired hypophosphatemic rickets, the etiology of which has not yet been identified.

Clinical Management: The patient presented to orthopaedic clinic with bilateral knee pain and genu valgum at age 10.5 years. Knee X ray showed widened physes in distal femur and proximal tibia with mild metaphyseal fraying of distal femur, suggesting remote rickets. Additional X ray revealed mild widening and irregularity of distal radius and ulnar physes. Lab showed normal calcium, normal 25-OH vitamin D (28 ng/dL; normal 32-100), normal 1,25 dihydroxy vitamin D (45 pg/mL; normal 15-90). He continued to have worsening knee pain with difficulty walking. He was referred to the Metabolic Bone Clinic at age 13 years, and was noted to have valgus angulation of 24 degree with persistent mildly widened physes on his knee X ray. Biochemical studies showed normal calcium (9.1), low phosphorus (2.8 mg/dL; normal 3.3-5.4), elevated alkaline phosphatase (488 U/L; normal 100-400), normal 25-OH vitamin (28), normal 1,25 dihydroxy vitamin D (51 pg/mL), and normal PTH (51 pg/mL; normal 10-65). Hypophosphatemic rickets was suspected as he also had low tubular resorption of phosphorus. He had normal knee X ray at age 1 year with no signs of rickets. Serum FGF23 was 220 RU/mL (normal < or = 230; Mayo Laboratory). His bone mineral density (BMD) showed normal lumbar BMD Z score at -0.3, while his total body BMD Z score was low at -2.3. Patient was started on calcitriol and phosphate therapy. A month later, he had medial distal femur hemiepiphyseal diaphysis performed to correct his deformity.

Discussion: Hypophosphatemic rickets in the case is acquired as he was well until age 10 years and had normal joint X ray during early childhood. Lab investigation is ongoing.

P107
Bone cross-sectional geometry and volumetric density at the distal radius in female adolescents with anorexia nervosa

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Introduction: Osteopenia is a well-known complication of anorexia nervosa (AN) in older adolescents and adults, especially in those with a long duration of the disease and a severe underweight.

Aim: We investigated whether young premenarchal girls with AN have similar risk factors for a disturbed bone growth and mineralization.

Methods: Twenty-four female premenarchal AN patients as well as 24 age and height matched female controls underwent a peripheral quantitative computed tomography (pQCT) scan at the distal radius of the non-dominant arm.

Results: The age of the AN subjects ranged between 11.8 and 18.5 years. Mean (± SD) weight loss was 22.3 ± 9.3%, which occurred during the preceding 7.8 ± 3.9 months. Mean weight z-score (-2.19 ± 1.40 vs. 0.15 ± 0.90; p<0.0001) as well as mean BMI z-score (-2.28 ± 1.09 vs. 0.07 ± 0.83; p<0.0001) was significantly lower in AN patients, whereas height z-score (-0.36 ± 1.02 vs. 0.23 ± 1.02; p=0.1) was comparable at the moment of examination. AN patients had a significantly lower mean

total bone cross-sectional area (CSA) (294.9 ± 31.5 vs. 334.1 ± 54.4 mm²; p=0.004) and periosteal circumference (60.8 ± 3.2 vs. 64.6 ± 5.0 mm; p=0.003) in comparison with the control subjects. Trabecular bone mineral density (186.1 ± 32.2 vs. 203.5 ± 30.9 mg/cm³; p=0.06) and bone strength index (30.1 ± 9.0 vs. 34.6 ± 10.5 mg/mm⁴; p=0.1) were also lower, but at borderline significance. Total bone CSA z-score correlated with duration of the weight loss (r=0.43; p=0.03), but not with the age and BMI z-score at diagnosis and the severity of the weight loss was found. None of the other bone parameters correlated with any of the disease related factors.

Conclusion: In female premenarchal AN patients the rapidity but not the severity of the weight loss before diagnosis affects the periosteal bone apposition, which is more disturbed than the bone mineralization at the distal bone end of radius.

P108
Antenatal glucocorticoid injections do not aggravate stress-induced bone loss in young adult mice

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Antenatal glucocorticoid (GC) injections are not only used to enhance fetal lung maturation in preterm children but also for the treatment of maternal conditions such as autoimmune diseases or infections. Animal models and clinical studies suggest that the regulation of the hypothalamic-pituitary-adrenal axis is altered in the offspring of GC-treated mothers with increased sensitivity to stress.

Objective: The aim of this study is (1) to define the effects of chronic mild stress (CMS) on the skeleton of young adult mice and the molecular pathways involved; and (2) to determine the effect of antenatal exposure to exogenous GCs on CMS-induced bone loss in young adult mice.

Methods: We used a transgenic (tg) mouse model in which the GC-inactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 2, is overexpressed in osteoblasts and osteocytes, resulting in disruption of pre-receptor GC-signaling in these cells. 8-week old tg mice and their wild type (WT) littermates were exposed to CMS for the duration of 4 weeks, followed by euthanasia and analysis of lumbar spine vertebrae by micro-CT. Stressors included: restraint stress, exposure to hot and cold, tilted cages and overnight illuminations. A subset of mice was exposed to elevated levels of GC at the end of the gestational period when their mothers received dexamethasone injections (0.2 mg/kg) on day 14.5 and 15.5 of pregnancy.

Results: Exposure to CMS resulted in spinal bone loss in WT but not tg mice (BV/TV WT: -17%; p=0.10; tg: -0.3%, p=0.97) when compared to their respective non-stressed controls. This was mainly due to a decrease in trabecular number (WT:-17%; p=0.08; tg: -1%; p=0.84) and a corresponding increase in trabecular separation (WT:+14%; p=0.04; tg: +2%; p=0.84) in WT mice only.

At 12 weeks of age the bone phenotype of mice exposed to antenatal dexamethasone did not differ from non-injected, non-stressed mice. Also, antenatal GC exposure did not aggravate stress-induced bone loss in either WT or tg mice (BV/TV WT: -10%; tg: -2%).

Conclusion: 1. CMS induces vertebral bone loss in mice via osteoblastic GC signaling. 2. Antenatal exposure to GC does not aggravate stress-induced bone loss in young adult mice.



P109

Vertebral morphology assessment in children: can a modified algorithm-based qualitative technique be used reliably?

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Purpose: There is no agreed standardised method for assessing vertebral morphology. The algorithm-based qualitative technique appears to be promising in adults to rule out non-osteoporotic fractures and non-fracture deformities, however it has not been previously trialled in children. We aim to modify a technique for use with both dual energy x-ray absorptiometry and radiographs in children, and to evaluate its reliability and reproducibility.

Methods: 50 patients with a suspected reduction in bone density, having a lateral spine radiograph and lateral dual energy x-ray absorptiometry on the same day, between the ages of 5 to 15 years, were recruited prospectively.

Three observers have independently assessed all images (blinded to corresponding results of radiographs and dual energy x-ray absorptiometry) using the modified algorithm-based qualitative criteria. A consensus read was performed by the observers simultaneously to act as a gold standard for sensitivity and specificity assessment. Inter-observer and modality agreement of fracture detection and characteristics have been assessed.

Interim results using the modified algorithm-based qualitative criteria had limitations. It was useful for fracture detection, severity grading and shape analysis, but not for endplate or position assessment. A survey was sent to 14 British Paediatric and Adolescent Bone Group members to assess the clinical relevance of the criteria; 13 responded. Following consultation simplifications were made.

The images will be re-assessed after an interval of 3 months using the new modified algorithm-based qualitative criteria and the data analysed as before. The reliability of the two techniques will be compared.

Results: Analysis with the original modified algorithm-based qualitative technique showed 26 patients had 1 or more vertebral fractures. The inter-observer agreement for fracture detection, severity grading, shape analysis, end plates affected and position analysis on dual energy x-ray absorptiometry ranged between 68 and 78% and on radiographs between 66 and 77%. Analysis using the second scoring system is in progress.

Conclusion: The modified algorithm-based qualitative assessment had limitations. We have further modified the scoring system to allow standardisation of readers' observations and note only findings of clinical relevance. We are currently evaluating the new system and comparing its reliability to the previous technique.

P110

Replacing conventional spine radiographs with dual energy x-ray absorptiometry (DXA) in children with suspected reduction in bone density

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Purpose: Children have greater lifetime risks of radiation-induced complications compared to adults. In children with osteogenesis imperfecta conventional radiographs are obtained to assess spine morphology, while DXA assesses bone density. In adults DXA is now used for both. We aim to establish whether iDXA can replace spine radiographs in the assessment of paediatric vertebral morphometry.

Methods: An 18 month prospective recruitment of 200 consented children with, and 50 without suspected reduction in bone density, aged 5 to 15 years, will have lateral spine radiographs and lateral DXA scans on the same day. Three observers will independently assess all images (blinded to corresponding results of radiographs and DXA) using a modified ABQ technique. 100 random images will be interpreted twice. Diagnostic accuracy and inter and intra-observer reliability of DXA will be compared to the gold standard of radiography.

Patient/carer experience, radiation dose of DXA compared to radiographs and health economics will be assessed.

Results: Interim results of the first 50 recruited patients showed 26 had 1 or more vertebral fractures. The fracture detection sensitivity for DXA was 62.7% and specificity 87.8%. The overall accuracy for vertebral fracture detection with DXA was 83.5%. The overall agreement between radiographs and DXA was 68.7%.

Conclusion: Using the ABQ technique in children has limitations and a more reliable scoring system is required. Compared to radiography DXA does not appear to be worse for morphometry and given the reduced dose and comparable CEC qualities, DXA should be recommended for morphometry in children after more robust analyses.

P111

Vitamin D status and association to bone health in 781 healthy 8-11 year old Danish school children - preliminary results from the OPUS School Meal Study

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Background: Low vitamin D concentrations among children and adolescents at northern latitudes are frequently observed. Also, inverse associations between 25-hydroxyvitamin D (25(OH)D) and PTH concentrations have been found in children of different ages. More studies on the link between vitamin D status and childhood bone health are needed.

Objective: To evaluate the status of serum 25(OH)D in autumn and the association between 25(OH)D concentrations and bone health in 781 healthy 8-11 year old Danish children (55°N).

Method: A Cross-sectional analysis was performed using baseline data from the OPUS (Optimal well-being, development and health for Danish children through a healthy New Nordic Diet) School Meal Study, including 3rd and 4th graders from nine public schools. In autumn 2011, fasting blood samples were drawn and serum 25(OH)D and intact PTH analysed. Background interviews were conducted and anthropometry, puberty stage, intake of dietary supplements and physical activity was measured. Whole body DXA scans were performed and Total body less head (TBLH) DXA values were used in data analyses.

Results: Serum 25(OH)D ranged from 15.2 to 132 nmol/l, with mean of 60.7 nmol/l±18.7. Twenty-six per cent of the children had concentrations between 25-50 nmol/l, while 2.4% had concentrations <25 nmol/l. Intake of dietary supplements ≥4 days/week (n=305) was associated with higher serum 25(OH)D (p<0.001). Girls had significantly lower 25(OH)D (p<0.001), and significantly higher iPTH (p=0.012) concentrations than boys. Serum 25(OH)D was inversely associated with iPTH without, and with, adjustment for age, gender, pubertal stage, month and ethnicity (p<0.001). No significant associations were found between serum 25(OH)D and Bone Mineral Content (BMC) without, nor with, adjustment for Bone Area (BA), age, height, weight, gender, pubertal stage, ethnicity and physical activity. Likewise, no associations were found between serum 25(OH)D and BA or BMD.



Conclusion: A substantial number of Danish children did not reach the recommended level of 25(OH)D (>50 nmol/l) during autumn. Despite a significant association with iPTH, no overt association between serum 25(OH)D and bone health was established.

The OPUS project (Optimal well-being, development and health for Danish children through a healthy New Nordic Diet) is supported by a grant from the Nordea Foundation.

P112

Abstract withdrawn

P113

Everyday chemicals modulate bone properties during development

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Objectives: In the modern society, all individuals are exposed throughout life to a variety of chemicals, which may contribute significantly to the human disease burden. Most of these chemicals are transferred to the fetus in-utero and to the infant via mother's milk. Until recently, few studies have addressed effects of chemicals on the development of mineralized tissues following exposure during these early stages of life. In this experimental study we analysed consequences of human relevant chemical exposure on developing bone.

Methods: Pregnant Sprague-Dawley rats were exposed to a mixture of polychlorinated biphenyls (PCBs), pesticides and mercury, or to the PCB- and mercury components of the mixture alone, from gestational day 1 to prenatal day 23. Tibias from male and female offspring at postnatal day 21 were analysed using peripheral quantitative computed tomography.

Results: Exposure to the mixture resulted in bone with smaller mid-diaphysis cross-sectional area, but higher cortical thickness. Further, the trabecular bone area was smaller, while the trabecular bone mineral density was higher. The increase in trabecular bone mineral density was larger in the female offspring, while the effects on bone geometry were generally more pronounced in the males. Offspring exposed to PCBs alone showed the same trend of bone alterations as caused by the mixture, although less pronounced and with no increase of cortical thickness. In contrast, exposure to mercury alone resulted in increased cortical thickness, but had no effect on trabecular bone mineral density.

Conclusion: Higher trabecular bone mineral density and increased cortical thickness were the most notable alterations of bone following early life exposure to a mixture of chemicals mimicking the human exposure situation. The functional consequences of these observations should be further investigated in order to identify preventive measures.

P114

Association between parameters of bone mass measured by dual energy X-ray absorptiometry and quantitative ultrasound of proximal phalanges in children and adolescents with congenital adrenal hyperplasia

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The chronic use of glucocorticoids in patients with congenital adrenal hyperplasia (CAH) may result in decreased bone mass. Therefore, using

simple and accurate methods for assessing bone status in these patients could facilitate the treatment of disease. The purpose of this study was to verify the association between parameters of bone mass measured by dual energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) of proximal phalanges in children adolescents with congenital adrenal hyperplasia. We evaluated 26 patients (15 girls and 11 boys) with CAH due 21-hydroxylase deficiency, aged six to 14 years. Bone mineral content (BMC) and bone mineral density (BMD) were assessed with a DXA whole-body scan, model Discovery Wi (Hologic, Bedford, MA). The ultrasound bone parameters, amplitude dependent speed sound (AD-SoS) and bone transmission time (BTT) were assessed for QUS of proximal phalanges using DBM Sonic BP (IGEA, Carpi, Italy) device. No significantly associations were observed between hydrocortisone dose (HC) and bone parameters assessed by DXA and QUS. BTT demonstrated higher correlations with BMC and BMD than AD-SoS (Table 1).

Table 1. Descriptive values and correlations in patients with CAH.

	Mean	SD	Correlations			
			BMD	BTT	AD-SoS	HC
BMC (g)	1153.96	305.83	0.94*	0.78*	0.46*	0.13
BMD (g/cm ³)	0.858	0.094	-	0.65*	0.53*	0.07
BTT (µsec)	0.97	0.17	-	-	0.33	0.32
AD-SoS (m/s)	1897.12	84.97	-	-	-	0.13
HC (mg/m ² /day)	13.42	2.61	-	-	-	-

*p<0.01; **p<0.05.

In our sample with children and adolescents with CAH due 21-hydroxylase deficiency the bone parameters assessed by DXA and QUS of proximal phalanges demonstrated significantly association. More studies are needed to evaluate the usefulness of QUS method in these patients, given that the QUS is a radiation-free method can be advantageous in monitoring of children and adolescents with CAH.

P115

Bone mineral density in late adolescence of transsexuals treated with GnRH-analogues in their teens

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Young transsexuals at a minimum age of 12 years are treated with GnRH-analogues (GnRHa) to suspend pubertal development until the addition of hormones of the desired sex is started at a minimum age of 16 years. The effect of this treatment on adult bone mineral density (BMD) is still unknown. We aimed to assess BMD at the age of 22 as this is near its peak in healthy individuals.

In this prospective observational study 19 female to male (FtM) and 16 male to female (MtF) transsexuals were included. In the MtFs mean lumbar and hip BMD were 0.85 (0.66–1.1) and 0.89 (0.68–1.0) g/cm², respectively at start GnRHa (age 15.0 years (12.3–17.9)) and at start estrogen (after 16.5 months (7–45) GnRHa treatment) 0.82 (0.63–0.95) and 0.88 (0.78–0.99) g/cm², respectively. At the age of 22 these were 0.93 (0.75–1.1) and 0.94 (0.74–1.1) g/cm², respectively.

In the FtMs mean lumbar and hip BMD were 0.95 (0.76–1.1) and 0.92 (0.77–1.1) g/cm², respectively at start GnRHa (age 15.2 years (11.6–18.6)) and at start testosterone (after a GnRHa-treatment duration of 18 months (3–62)) 0.92 (0.76–1.1) and 0.92 (0.75–1.0), respectively. At the age of 22 mean lumbar and hip BMD was 1.0 (0.77–1.1) and 0.94 (0.71–1.1) g/cm², respectively.

BMD remained stable during GnRHa-therapy, deterioration was not observed. Upon hormonal administration, BMD increased until the age of 22. These first data appear to support the safety of the treatment protocol. However, study population was heterogeneous regarding pubertal stage and age at start of treatment and duration of GnRHa-



treatment. Further investigation is aimed at continued monitoring of BMD until the age of 30.



P116
Vitamin D dependent rickets type 1 (VDDR-1): two years after discontinuation of treatment

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Background: Vitamin D dependent rickets type 1 (VDDR-1) is an autosomal recessive disorder caused by 1 α hydroxylase enzyme deficiency, that leads to low or undetectable levels of 1,25 dihydroxyvitamin D despite normal levels of 25-hydroxyvitamin D levels. Additional laboratory findings include hypocalcemia and increased levels of parathyroid hormone. Clinically it presents as early onset rickets and severe hypotonia.

Presenting Problem: A 6 year old Caucasian girl was referred to our Clinic after a 6 month history of hypotonia. Rickets was diagnosed at the age of 9 months, based on typical laboratory and radiological findings. Severe hypophosphatemia and generalized aminoaciduria were identified then. The levels of 25-hydroxyvitamin D were normal. She was started on 1- α hydroxyvitamin D with prompt clinical and laboratory improvement. Under medication, the biochemical findings normalized and the child remained asymptomatic. That misled the parents into discontinuing treatment 3,5 years after diagnosis and for the next two years.

Clinical Management: Physical examination on admission revealed genu valgum, short stature and generalized hypotonia. Laboratory workup revealed hypocalcemia, hypophosphatemia, marked elevation of alkaline phosphatase and parathormone, with normal levels of 25-hydroxyvitamin D and low levels of 1,25 dihydroxyvitamin D. Based on these findings and the fact that she had previously responded well to 1- α hydroxyvitamin D, VDDR-1 was diagnosed. Awaiting genetic confirmation she was restarted on 1- α hydroxyvitamin D. Biochemical and radiographic improvement was achieved within 3 and 6 months respectively.

Discussion: Clinical and radiological findings of VDDR-1 are indistinguishable from those of other forms of rickets. Distinction between the two can be based on characteristic biochemical findings. Normal levels of 25-hydroxyvitamin D exclude nutritional rickets, while normal serum calcium combined with elevated PTH excludes hypophosphatemic rickets.

Patients with DDR-1 should receive life long replacement treatment with 1- α hydroxyvitamin D or calcitriol.

P117
Impact of age and pubertal development on bone mass assessed by Quantitative Ultrasound of the Proximal Phalanges in boys and girls aged 10 to 14 years

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The quantitative ultrasound (QUS) of the proximal phalanges has been used for the indirect evaluation of bone status. Furthermore, the relative simplicity and non-exposure to radiation, presents advantages for the use of QUS compared to other methods in children and adolescents. The aim of this study was to determine the influence of age and pubertal development on Amplitude Dependent Speed Sound (AD-SoS) assessed by QUS of proximal phalanges in girls and boys aged 10 to 15 years old. The QUS measurements were performed on 1892 Brazilian white students (1165 girls: weight=43.4 \pm 10.8 kg and height=152.7 \pm 10.1 cm, and 727 boys: weight=43.8 \pm 11.9 kg and height=152.0 \pm 11.8 cm) using the DBM Sonic BP device (IGEA, Carpi, Italy). The pubertal development was evaluated by self-assessment according to breast stage (I-V) for girls and pubic hair (I-V) for boys. Girls demonstrated significantly higher AD-SOS values (range 1808-2190 m/s) compared to boys (range 1976-2140 m/s), in all ages (Fig.1 left panel) and pubertal stages (Fig.1 right panel), as illustrated in Fig1. This result is repeated when the groups were compared in relation to maturational stage, the same stage for girls presented higher values of Ad-SOS. The AD-SoS increased significantly from 11 to 14 years old (p<0,01) for girls and for boys the increase was significant from 13 to 14 years old (p<0,001). As for the pubertal development, a statistical difference were found between the stages II to IV for girls (p<0.001) and between stages III to IV for boys (p<0.001).

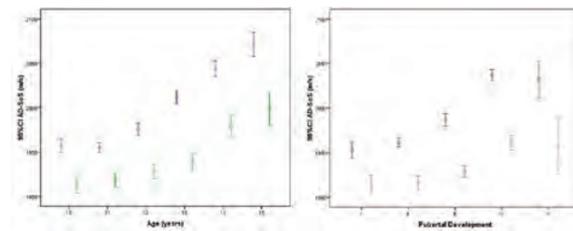


Fig1. AD-SOS values for boys (green) and girls (blue) by age (left panel) and pubertal stage (right panel)

This sample of Brazilian children and adolescents, the values of AD-SoS of girls suffered greater influence of age and pubertal stage. It is suggested that more longitudinal studies are needed to confirm these results.

P118
Variation in response to vitamin D therapy in a series of consecutive children referred to a paediatric bone disease service

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Objectives: Guidelines for treatment of vitamin D deficiency (VDD) vary. We aimed to review the range of treatment regimens for VDD used locally and variation in responses.

Methods: We conducted a retrospective review of the records of consecutive patients referred to a children's bone disease service with a putative diagnosis of VDD over a 14 month period. Data collected includes vitamin D type used, dose and duration of treatment, and pre-



and post serum 25-hydroxy vitamin D (25OHD). Cases were excluded if these data were missing. Weight was used to calculate total dose of vitamin D per kg administered. In the absence of a contemporary weight, subjects were assumed to have mean weight for age. Presence of rickets, radiographic reports, history of fracture and adverse events were collected.

Results: From 66 consecutive subjects, 29 had pre- and post-treatment 25OHD concentrations and a confirmed serum 25OHD <25nmol/l. Demographics - 18/29 male; 12/29 South Asian, 7/29 Black African, 4/29 White British. 13/29 had radiographs looking for signs of rickets. In 2/13 rachitic features were identified. In both cases repeat radiographs confirmed resolution with treatment. 4/29 had ever suffered fractures. Cholecalciferol treatment was variably instituted by primary or secondary care physicians. Daily doses ranged 3000-10000 U (median 6000); duration 4-12 weeks (median 6). 2/29 subjects received two doses of 100000 U each. Estimated total dose/kg ranged 3400-35000 U/kg (mean 13500, SD 7400). The increase in serum 25OHD correlated with estimated total dose/kg (R²=29%, p=0.003). 2/29 remained vitamin D insufficient (34 and 45nmol/l). 4/29 subjects had an increase in 25OHD >200nmol/l. These were all under 18 months of age and received estimated total doses 11000-35000 U/kg over 4-12 weeks. There were no recorded adverse effects.

Conclusion: We found that commonly used regimens for treatment of VDD were effective in raising 25OHD to >50nmol/l with no evidence of harm. This study is limited by its retrospective character, small size, incomplete data and potential bias regarding subject selection. However, there is variability in response to vitamin D treatment and we believe that clinicians should consider the weight-related total dose, particularly in younger children.

P119
Depressive symptoms and bone mineral density in a cohort of Portuguese adolescents: No association

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Objective: Since depressive symptoms, which have been related to low bone quality in adulthood, may also be associated with suboptimal bone mineral accrual, we aim to quantify the association between depressive symptoms and bone mineral density (BMD) throughout adolescence.

Methods: We analysed prospective data from 969 adolescents (56.2% girls) from a population-based cohort of urban adolescents, born in 1990, evaluated during the 2003/2004 and 2007/2008 school years in public and private schools of Porto (EPITeen). In both evaluations, at 13 and 17 years of age, the severity of depressive symptoms in the previous two weeks was evaluated by self-report using the Beck Depression Inventory-II (BDI-II). Bone mineral density (g/cm²) was measured at the non-dominant forearm by dual-energy X-ray absorptiometry (DXA) using a Lunar® Peripheral Instantaneous X-ray Imager (PIXI) device. In order to estimate the cross-sectional and prospective associations between depressive symptoms and BMD during adolescence, linear regression coefficients (β) and 95% confidence intervals crude and adjusted for body mass index (BMI), age at menarche, smoking and sports practice were estimated.

Results: In girls, mean (standard-deviation) forearm BMD was 0.360 (0.057) at 13 years-old and changed 0.024 mg/cm²*year, while in boys was 0.346 g*cm² (0.053) at 13 years of age and changed 0.035 mg/cm²*year. In girls, median (25th; 75th percentiles) BDI-II score remain the same at 13 and at 17 years-old (6.0 (2.0; 10.0) and 6.0 (3.0; 11.0), respectively), while in boys BDI-II scores decreased from 13 to 17 years of age (3.0 (1.0; 6.0) and 2.0 (1.0; 6.0), respectively) and, comparing with girls, were lower in both evaluations. In both sexes, cross-sectional associations between depressive symptoms and BMD at 13 and 17 years-old were not detected even after adjustment for major confounders. Similarly, there were no significant associations between

depressive symptoms during adolescence and the annual BMD change from 13 to 17 years old.

Conclusion: Depressive symptoms were not associated with BMD accrual throughout adolescence. The mechanisms explaining the relationship between depression and BMD are not largely known and explored, but they may not be present at this period of life.

P120
Osteoblasts communicate with their neighbouring cells via extracellular vesicles

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Objectives: Strong coordination between osteoblasts and bone marrow cells is fundamental for the regulation of healthy bone turnover and other (patho)physiological processes. Extracellular vesicles (EVs) mediate communication between cells via horizontal transfer of proteins and nucleic acids. Osteoblasts secrete EVs in the form of matrix vesicles involved in bone mineralization, however, information about a role in intercellular communication is still lacking. In this study, we focus on the characterization of human osteoblast-secreted EVs and study their role as mediators of communication with neighbouring cells in their bone marrow microenvironment.

Methods: We used a human pre-osteoblast-based *in vitro* bone formation model to isolate EVs at various time-points during osteoblast differentiation and mineralization by a series of ultracentrifugation steps. We characterized the EVs by electron and atomic force microscopy and proteomics. Furthermore, we studied their interaction with neighbouring cells by fluorescent labelling and flow cytometric analysis.

Results: Microscopic analyses demonstrated that osteoblast EVs are very heterogenic in size and morphology. Mass spectrometry-based proteomic analyses identified known matrix vesicle proteins (annexins, phosphatases, etc.) and an interesting range of membrane and signalling proteins that may be linked to cell communication. Fluorescently labelled osteoblast EVs were internalized by neighbouring cells such as CD34⁺ hematopoietic stem cells (HSCs) as well as bone-metastasizing prostate cancer cells in a dose-dependent manner. Cultures of CD34⁺ HSCs with osteoblast EVs led to a donor-dependent 2-3 fold increased proliferation of the CD34⁺ expressing cells.

Conclusion: We demonstrated that osteoblasts secrete EVs that are taken up by the cells residing in the surrounding microenvironment. Osteoblast EVs are specifically packaged not only with common matrix vesicle proteins showing their importance in bone metabolism but also with proteins not-primarily linked to mineralization suggestive of a novel mechanism of intercellular communication.

This study has been supported by Erasmus Stem Cell Institute.

P121
Tibial metaphyseal shape varies between children according to history of fracture

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Background: Measurement and interpretation of metaphyseal shape in children is difficult. We aim to develop relevant assessment methods using commonly available spiral computed tomography (CT) scanning.

Methods: We analysed 12 pairs of age and pubertal stage matched subjects from a larger group of children recruited into a non-interventional case control study in which all had suffered trauma; half had sustained a fracture. Each subject underwent anthropometry,



pubertal stage self-assessment, LS DXA and spiral CT of the whole of both tibiae. Total area and volumetric trabecular bone density (TBD) were extracted from each 1.25mm CT slice between the growth plates. 8 nested cylinders were defined within the metaphyseal volume. Metaphyseal extent was defined as being between the growth plate and where TBD reaches zero in the central 1/8 area. Equal-length datasets were created to allow data comparison. We assessed metaphyseal extent, absolute metaphyseal area at 50% metaphyseal length (50%Area) and change in area across the 40-60% metaphyseal length (40-60ΔArea) in relation to tibial length, tibial width at narrowest point and aspect ratio (length/width).

Results: Subjects were aged 5-13 years. Girls: 4/12 controls; 3/12 fractures. Mean tibia length was 29.2±5.1cm. There was no significant difference in height or tibial length between fractures and controls. Aspect ratio varied from 13-19 (median 16).

The proximal metaphysis varied from 11-25% tibial length (median 15%); distal metaphysis from 8-20% (median 13%). There were no significant differences between fracture and control groups for these parameters or for the ratio of 50%Area to shaft width. 40-60ΔArea correlated with age, height, tibial length and height Z score. 40-60ΔArea of the proximal metaphysis was greater in controls compared to fractures (p=0.02). This was not the case for the distal metaphysis. There was no correlation of aspect ratio or metaphyseal data with LS DXA values.

Conclusion: We have described a method for determining the size and variability of the shape of the tibia and its metaphyses. The combination of observed differences suggests less in-waisting of the proximal metaphysis in children with a history of fracture.

P122

Fasting total Ghrelin levels are increased in patients with adolescent idiopathic scoliosis (AIS)

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Objectives: Ghrelin is an orexigenic hormone produced by the stomach that reflects body weight changes and stimulates growth hormone (GH) secretion. Recently, it has been shown to be associated with bone metabolism and eating behaviour. The underlying pathophysiology of adolescent idiopathic scoliosis (AIS) refers to possible abnormal bone development. AIS patients also frequently present with low body mass index (BMI) level.

Eating behavioural disorders, endocrine disorders, abnormal growth pattern and osteopenia have been well documented in AIS. However, the circulating levels of Ghrelin have never been evaluated in patients with AIS.

Methods: a study was designed to investigate circulating Ghrelin levels in adolescent girls with AIS and normal control subjects. 49 AIS girls and 15 controls were included. Anthropometric parameters and fasting circulating total Ghrelin were measured. Curve severity was evaluated in AIS girls. The relationships between Ghrelin and age, body weight, height, body mass index (BMI), BMI Zscore and corrected anthropometric parameters were analyzed in AIS girls and controls.

Results: A significant increase of circulating Ghrelin was found in AIS girls compared with controls. Elevation of Ghrelin levels remained significant when considering corrected BMI or corrected BMI Z-score. Unlike in controls, positive correlations were found between Ghrelin and age in AIS girls. Indeed, a gradual increase of circulating Ghrelin was observed until 13.9 years of age, while remaining stable thereafter. There was no significant difference in body weight, height, BMI or BMI Z-score between AIS and controls.

Conclusion: We observed significantly higher circulating ghrelin levels in AIS as compared to controls with a positive correlation with age. These observations suggest that Ghrelin might play a role in the initiation or development of AIS and consequences on bone status.

P123

Inadequately treated hypophosphataemic rickets (HR) in 14-year-old boy: a case report

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Inadequately treated hypophosphataemic rickets (HR) in 14-year-old boy: a case report

Background: Hypophosphataemic rickets (HR) is a rare cause of short stature associated with limb deformities.

Objective: To report the clinical, laboratory and radiologic features of HR in a 14-year-old boy.

Presenting Problem: We present the case of the 14-year-old boy, with short stature and deformities of extremities, who complained for limb pain and weakness. The boy was diagnosed with vitamin D deficiency four years and was receiving vitamin D. He had a previous surgery for bowleg deformity.

Clinical Management: The clinical examination showed signs of rickets/osteomalacia and biochemical evaluation revealed severe renal loss of phosphate with hypophosphatemia, normal calcium, normal PTH, moderately elevated serum alkaline phosphatase, increased GFG23 and normal 1,25 (OH)2D3. Bone densitometry, PET-CT and abdominal ultrasound were normal. The final diagnosis was hypophosphatemic rickets

Radiographic findings included cupping and fraying of metaphysis, poorly mineralized epiphyseal centers with delayed appearance, irregular widened epiphyseal plates, periosteal reaction, Looser's zones and bowing of long bones. The child received phosphorus along with vitamin D resulting in remission of pain and weakness.

Discussion: Hypophosphatemic rickets occurs rarely, and although it leads to physical development disturbance and to severe deformities of the extremities, it makes a significant medical problem as it may be misdiagnosed or delayed diagnosed. Phosphorus along with vitamin D are administered whereas in case of severe leg deformities, orthopaedic surgical intervention is, additionally, needed.

P124

Adipokines and bone turnover throughout adolescence: an exploratory approach in a cohort of girls

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Objectives: By prospectively evaluating a cohort of girls we aim to identify population patterns linking adipokines and bone turnover during early and late adolescence and to assess the associations of those patterns with forearm bone mineral density (BMD).

Methods: The study was developed within a population-based cohort of urban adolescents born in 1990 and assembled in public and private schools of Porto, Portugal (EPITeen). We analysed prospective data from 300 girls evaluated at 13 and 17 years of age. Anthropometric



assessment included height, weight and body fat percentage. BMD was measured at the distal forearm using dual-energy X-ray absorptiometry. Pubertal development status was estimated through menarche age. Serum concentrations of leptin, adiponectin, receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), collagen type 1 cross-linked C-telopeptide (CTX), and procollagen I N-terminal propeptide (PINP) were determined using commercially available enzyme-linked immunosorbent assays. Exploratory factor analysis was used to identify patterns of associations between serum parameters at each age and to assess their maintenance between 13 and 17 years of age. Associations between factors at each age and BMD were estimated using linear regression coefficients (95% confidence intervals), crude and adjusted for height, weight and menarche age.

Results: We found that the same two factors at 13 and 17 years of age, named "factor leptin CTX RANKL" and "factor PINP OPG", accounted for more than 40% of the variability observed in the selected set of variables. "Leptin CTX RANKL" factors were positively associated with leptin and negatively with CTX and RANKL at 13 and at 17 years of age. There were crude positive associations between these factors and BMD, ($\beta=25.1$ (18.8, 31.4) at 13 and $\beta=9.6$ (3.8, 15.3) at 17 years) that lost significance after adjustment. "Factor PINP OPG" was directly correlated with PINP and inversely with OPG but not associated with BMD at 13 or 17 years of age.

Conclusion: By using an exploratory approach to population data, we identified an important pattern linking fat and bone in adolescent girls, involving systemic mediation by leptin and local mediation by RANKL, reflecting an effect on bone resorption.

P125

Growth plate modifications in lysophosphatidic acid (LPA) LPA1 receptor-invalidated mice

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Objectives: lysophosphatidic acid (LPA) is a potent lipid growth factor which possess several G protein-coupled receptors LPA1-6. We have recently demonstrated that LPA1 receptor-invalidated mice display abnormal bone development and osteoporosis, suggesting abnormal endochondral ossification. We have here further studied the growth plates of LPA1 receptor-invalidated mice.

Methods: we performed a microscopic and immuno-histochemistry analysis of the femoral and tibia bones of 1-4 weeks old LPA1(-/-) mice raised on a C57BL/6 background.

Results: Microscopic analysis demonstrated marked narrowing of the growth plate of the long bones. The proximal tibia was significantly affected with a 27.7% decrease. In the proximal growth plate of the femur, the reserve zone was relatively preserved in LPA1(-/-) mice. In contrast, the columnar proliferative zone was clearly decreased. The hypertrophic zone was not significantly affected. Quantification of the different zones showed a significant decrease in the proliferative zone of LPA1(-/-) mice, especially in the tibia growth plates which were diminished by 37.8%. Other abnormalities were also detectable in the growth plate of LPA1(-/-) mice. The columnar structures were less regular and less organized in comparison to WT. Application of Ki67 antibody to quantify the mitotic index in the proliferative zone, cell proliferation appeared decreased by 31% in the proliferative zone of LPA1(-/-) mice.

Conclusion: These results suggest that LPA1 receptor is involved in the development of growth plate by influencing the proliferating rate of chondrocytes. LPA and LPA1 are new factors potentially involved in patho-physiological situations where endochondral ossification is impaired.

P126

The role of severity of growth hormone deficiency on clinical and instrumental features and response to treatment in children

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Aim: to determine the response to treatment, clinical and instrumental features in children with partial growth hormone deficiency (pGHD) and severe GHD (sGHD).

Methods: We examined retrospectively 30 children with isolated GHD (stage on Tanner 1) in the Endocrinological department of University hospital (Minsk) over 2004-2012 years. Group 1 (G1) – children with pGHD (n=5) mean±SD age 6,3±1,4 yrs.; group 2 (G2) sGHD (n=25), 4,8±0,9 yrs (p = 0,4). Children in both groups were treated with GH more than 3 years. Stimulating GH levels, height on the moment of diagnosis (H); bone X-Ray with the calculation years of delay relative to chronological age on diagnosis (D1), 1 year (D2), 2 years (D3) and 3 years (D4) of treatment; magnetic resonance imaging (MRI) were analyzed. The results were processed using SPSS 17.

Results: The maximum GH levels (IU/l) in clonidine sample G1 was 14,8±0,5, G2 6,2±0,6 (p=0,6), insulin sample 5,7±1,5 and 5,6±0,4 (p=0,8). G1 was treated with GH in the average dose 0,75±0,3 mg per day, G2 0,7±0,3 (p=0,5). H G2 3rd percentile was in 5 (100%) children; G1 <3 percentile was in 13(52%), 3rd 9(36%), 10th 3(12%) children.

6 (24%) children in G2 had been confirmed pituitary pathology by MPI (2 microadenoma, 4 pituitary hypoplasia), G1 microadenoma in 1 patient.

Maturation by X-ray was delayed in both groups, the dynamics G2: D1 3,1±1,7, D2 2,4±1,2, D3 2,1±0,4 (p(D1-D2)=0,2), (p(D2-D3)=0,3), (p(D1-D3)=0,4); G1 – D1 2±1,4, D2 1,5±0,5, D3 1±0,5 (p(D1-D2)=0,1), (p(D2-D3)=0,01), (p(D1-D3)=0,01).

Conclusions: We didn't notice the reliable differences of bone age retardation in children with sGHD at the age of diagnosis and all years of treatment. There were the reliable reducing of bone age retardation in group with pGHD between the beginning of the treatment, 2nd (p=0,01) and 1st years (p=0,01).

P127

Mechanosensitive TRP Channels are Essential for Ca²⁺ Signaling in Osteoclastogenesis

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Objectives: Bone remodeling and maintenance require a fine balance between bone formation of osteoblasts and resorption of osteoclasts. Therefore, various skeletal disorders cause by imbalanced differentiation and activities of these cells. RANKL (receptor activator of NF-κB ligand) induces Ca²⁺ oscillations and activates NFATc1 (nuclear factor of activated T cells 1) during osteoclast differentiation. Although Ca²⁺ oscillations play a key role for osteoclastogenesis, the molecular identification of Ca²⁺ influx via mechanosensitive calcium channels located on the plasma membrane for the generation of Ca²⁺ oscillation are not well known.

Methods: We investigated the expression and functional role of mechanosensitive TRP (transient receptor potential) channels on Ca²⁺ signaling during osteoclastogenesis in RAW264.7 and bone marrow macrophage (BMM) cells using RT-PCR, western blot, Ca²⁺ imaging, TRAP staining, and immunocytochemistry.

Results: Ca²⁺ oscillations and entry were changed by the over-expression of TRPC3 and TRPC6, and the agonist of TRPM7. Activation of these channels had effects on the expression of NFATc1, PLCγ1, and IP₃Rs, and TRAP⁺ cell formations.

Conclusion: These results suggest that mechanosensitive TRP channels play a key role in the Ca²⁺ signaling of osteoclastogenesis. This research



was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-007673).

P128
Bone mineral density in survivors of childhood lymphoma

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Objective: Assessment of bone mineral density in children completing therapy for lymphoma.

Methods: 35 children treated for childhood Hodgkin and non-Hodgkin lymphoma at the Department of Pediatric Hematology and Oncology of Medical University of Białystok in Poland were included. Patients were scanned with DXA at two time points: 1st- up to 5 years and 2nd – above 5 years after the completion of the treatment. Bone mineral density [total (TBMD) and spine (BMDS)] were determined using dual-energy X-ray absorptiometry (DXA). Results were compared to age- and sex-matched references ranges and expressed as a Z-score for bone mineral density according to both the local reference population and manufacture’s database. Statistical analysis were performed using the Wilcoxon Matched-Pairs Ranks test. A p value < 0.05 was considered significant.

Results: Median time interval from the completion of the treatment to the beginning of the study was 3.4 years [1.6, 4.5] for 1st group and 7.1 [6.4,8.9] for 2nd group. We did not find any significant difference between analyzed patients for TBMD and BMDS Z-scores (p=0.702, p=0.093, respectively). Low bone mass (defined as a Z-score ≤ -2) was observed in two children in the 1st group (5.7%) and three children in the 2nd group (8.6%).

Conclusion: In this study, time interval from the completion of the treatment did not influence on bone mineral density. Overall, pediatric lymphoma survivors had negligible bone mineral density deficits.

P129
Radiographic Evidence of Rapid Healing of Vitamin D Deficient Rickets after 2 weeks of Therapy

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Background: Following supplementation with adequate vitamin D and calcium, healing of vitamin D deficient rickets has generally been demonstrated on radiographic films 3-6 months following the initiation of therapy. However, we report a case that demonstrates radiographic evidence of rapid healing of vitamin D deficient rickets in only 2 weeks after starting therapy.

Presenting Problem: An 8 month old African American male presented to the emergency room with a distal left femur fracture near the metaphysis on radiographic film following a fall from height of approximately 2 feet. Orthopedics was consulted and placed a long leg cast. Due to the location and suggested mechanism of his fracture, non-accidental trauma was suspected. A skeletal survey was obtained which revealed irregular cupping and fraying of the metaphysis of the long bones, suggestive of rickets. Vitamin D studies, serum calcium, phosphorus and alkaline phosphorous were obtained which established the diagnosis of severe nutritional vitamin D deficient rickets. He had markedly low 25-OH vitamin D (2.8 ng/mL; normal 32-100), low serum calcium (6.7 mg/dL; normal 8-10.5), low phosphorous (4.1 mg/dL; normal 4.2-6.5), elevated alkaline phosphatase (753 U/L; normal 55-380), markedly elevated PTH (368 pg/mL; normal 10-65). Subsequently, following a thorough investigation, non-accidental trauma as a cause for the injury was excluded.

Clinical Management: This patient was started on calcium and ergocalciferol 2000 IU daily for treatment of nutritional vitamin D deficient rickets. Due to his femur fracture, a repeat radiographic film was obtained approximately 2 weeks following his initial injury. Repeat radiographic film demonstrated remarkable resolution in cupping and fraying of the metaphysis. Repeat vitamin D studies 4 months after starting treatment with ergocalciferol demonstrated adequate vitamin D stores with 25-OH vitamin D level of 59 ng/mL and normalization of serum calcium, phosphorous and PTH.

Discussion: This case demonstrates radiographic evidence of rapid healing of vitamin D deficient rickets following initiation of treatment with calcium and ergocalciferol after only 2 weeks of therapy. This is much more rapid than previously demonstrated. Furthermore, this case also demonstrates that vitamin D deficiency can predispose infants to fractures mimicking those caused by non-accidental trauma.

P130
“Bone in bone” sign in Juvenile Osteoporosis (IJO) in a 13-year-old girl

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Background: We present an interesting case of a young child under bisphosphonates, for the treatment of Juvenile Osteoporosis (IJO), that developed a “bone in bone sign”, in several vertebral bodies, evident both on radiographs (CRX) and on Magnetic Resonance (MR) studies.

Presenting Problem: A 13-year-old girl was admitted in our hospital complaining with thoracolumbar pain. The patient had been diagnosed with IJO two years ago because of a history of a low energy fracture of a thoracic vertebra and a low BMD, measured by DEXA (Z score: - 2,2). The girl was receiving bisphosphonates for one year and six months before her current admission.

She underwent a new BMD and radiographic study, with CRX and MRI of the thoracolumbar spine and the pelvis.

Clinical Management: Bone, new, densitometry values, by DEXA, were normal (Z score: -1,2).

Radiographs of the thoracolumbar spine and pelvis revealed a “bone within a bone” appearance of the vertebrae and iliac bones. MR imaging of the thoracic and lumbar spine, showed a moderately low signal of the vertebral end plates, producing a “frame” like picture, on T1 weighted images after fat-suppression and administration of contrast agent.

In a retrospective study of previous radiographs of the thoracic spine, there was a reduction of the visual height of the vertebral bodies, and the uncalcified end plates were barely visible.

Discussion: The bone within bone” radiographic pattern, has been frequently described in IJO patients and it is either attributed to the disease it’s self or the treatment with bisphosphonates or even the termination of the use of such drugs.

P131
Ghrelin differentiates human osteoblasts via GHS-R1a receptor

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Objectives : Ghrelin is a peptide hormone secreted in the stomach, which stimulates growth hormone release and food intake. It is also known to have an effect on bone metabolism. The ghrelin specific receptor, GHS-R1a, belongs to the G protein-coupled receptors



(GPCRs). Its downstream pathway in osteoblasts remains unclear. We attempted to clarify the way by which ghrelin acts on osteoblast differentiation.

Methods: we studied two human osteosarcoma cell lines, MG63 and SaOs, previously described as preosteoblastic and osteoblastic cell lines, respectively, which were submitted to ghrelin during differentiation.

Results: Ghrelin stimulated alkaline phosphatase activity, mineralization and expression of RUNX2 in differentiated osteoblastic cells: MG63 previously cultured in osteogenic medium, and SaOs cells. Ghrelin decreased the cAMP content of these differentiated cells. The inhibiting effect of ghrelin on osteoblastic cells cAMP level was reversed by GHRP6 [D-Lys], a GHS-R1a inhibitor. Conversely, in undifferentiated MG63 cells, ghrelin slightly increased proliferation. Lastly, ghrelin increased the expression of GHS-R1a. Overall, our data showed that ghrelin contributes to progression in the osteoblastic lineage of already differentiated osteoblasts only, and not of undifferentiated cells. It is therefore suggested that GHS-R1a along the differentiation of osteoblastic cells contributes to their optimal differentiation.

Conclusion: These results emphasize the potential role of ghrelin and GHS-R1a in the regulation of bone formation and maturation of osteoblasts. They may have consequences in pathophysiological conditions like puberty or anorexia, both known to significantly modify ghrelin secretion.

P132
Studies on bone and osteoclasts in patients with Shwachman Diamond Syndrome

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Shwachman Diamond syndrome (SDS; MIM 260400) is a monogenic, autosomal recessive, pancreatic condition often accompanied by low bone mass and fracture. In SDS, as in Cystic Fibrosis, a low bone mass may be secondary to poor nutrition or chronic low grade infection, but it has also been suggested there may be a primary bone phenotype. Paradoxically, recent studies in cell lines and in a mouse knockout for the SBDS gene, have suggested changes in important osteoclast growth factors and impairment of osteoclast formation and migration. Here we evaluated whether there were osteoclast abnormalities in bone biopsies of 7 patients with SDS and assessed bone histology (but not histomorphometry) using trephine biopsies. We also studied whether previously reported abnormalities in signalling through the osteoclast receptor RANK (Receptor Activator of Nuclear factor Kappa B) in the mouse SBDS knockout, was seen in osteoclasts carrying mutant SBDS generated in vitro from patient monocytes (in 5 patients). Bone histology was variable: osteoclast function was evident in all patients and evidence of bone formation, but also osteoid accumulation was seen in some. Osteoclast ultrastructure revealed no gross anatomical abnormalities. In vitro studies did not reveal defects in osteoclast formation and RANK signalling, both in osteoclast precursors and in mature osteoclasts, was not impaired. Our data suggest that in SBDS patients, osteoclast formation is normal and that osteoclast activity may be increased, rather than reduced, in keeping with the increased fracture risk in patients. Our findings suggest that the SBDS mouse knockout model does not reproduce the phenotype seen in patients harbouring mutated SBDS alleles. Further studies using histomorphometry with dynamic bone markers are indicated to fully understand the basis of the increased fracture risk in these patients. Bone mass in SBDS patients should be monitored, including by non-invasive procedures.

P133
Linear growth over 2 years of velaglucerase alfa therapy in children with type 1 Gaucher disease previously treated with imiglucerase

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Objectives: As children with confirmed type 1 Gaucher disease (inherited metabolic disorder) may have linear growth retardation, we evaluated linear growth over 2 years in children enrolled in the interventional study TKT034, in which patients receiving imiglucerase enzyme replacement therapy were switched to velaglucerase alfa.

Methods: Trial TKT034 enrolled patients who were ≥2 years of age with type 1 Gaucher disease and stable clinical parameters on imiglucerase therapy. They were allocated to velaglucerase alfa at the same dose as their previous imiglucerase dose. Eligible patients who had completed TKT034 (1 year) could enrol in an extension study (HGT-GCB-044). The height percentiles and height z-scores of children were determined by gender and age in months using World Health Organization 2007 growth reference data (exploratory analysis).

Results: Nine children, who had previously received imiglucerase for ≥4 years, received velaglucerase alfa in both TKT034 and HGT-GCB-044 for a total of 2 years. One child was at or below the 50th percentile at Baseline of TKT034 (Table). At Baseline and 2 years respectively, the median (range) z-scores were 0.65 (-1.11, 1.31) and 0.79 (-1.33, 1.38), corresponding to percentiles 74.2 and 78.5. Comparing Baseline with 2-year values, no child crossed a major percentile (3, 15, 50, 85, or 97) downward; the z-score change in each child was no greater than ±0.51 (half a standard deviation); and the mean z-score change was not significant (0.06 [95% confidence interval -0.17, 0.30]; P>0.05).

Baseline age, years	Gender	Prior exposure imiglucerase		Tanner stage	Height, centimetres	Percentile		Z score			
		Units/kilogram/2 weeks	Duration, months			2 years	2 years	Baseline	2 years	Baseline	2 years
9	Female	35	69	1	Not done	139.9	153.0	78.2	78.5	0.78	0.79
10	Female	51	65	1	3	147.0	161.5	70.9	81.6	0.55	0.90
12	Female	34	92	3	4	145.0	151.0	13.3	9.2	-1.11	-1.33
13	Female	56	54	5	5	165.6	171.5	82.6	91.1	0.94	1.35
13	Male	44	49	2	4	160.0	175.4	54.8	73.6	0.12	0.63
14	Female	57	95	4	4	170.6	170.2	90.5	86.4	1.31	1.10
14	Female	60	108	4	5	164.0	165.0	65.9	63.3	0.41	0.34
14	Male	47	93	4	5	169.8	175.8	74.2	62.2	0.65	0.31
16	Female	62	124	4	5	170.9	172.2	89.3	91.6	1.24	1.38

¹ at 1 year.

Conclusion: In nine children with type 1 Gaucher disease switched from long-term imiglucerase therapy, height relative to age- and gender-matched reference populations was stable on average over 2 years of velaglucerase alfa therapy.

Funding: TKT034 and HGT-GCB-044 were sponsored by Shire Human Genetic Therapies, Inc.



P134
Cessation of ambulation results in a dramatic loss of trabecular bone density in boys with Duchenne Muscular Dystrophy (DMD)

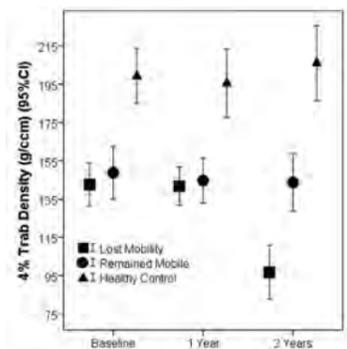
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Steroids are currently used to improve muscle strength and prolong ambulation in boys with DMD although the effect on bone health is still unclear. The aim of this study was to compare bone strength in healthy children and boys with DMD and investigate the interaction between diminished muscle function, loss of ambulation and high dose oral steroids.

Fifty children were studied, 14 healthy boys (HB), 13 boys with DMD who remained ambulant (DMD-RA) and 23 boys with DMD who lost ambulation (DMD-LA). All boys with DMD were taking oral steroids. Peripheral quantitative computed tomography was used to measure bone geometry, density, strength and muscle mass of the non-dominant tibia. Measurements were made at baseline, 12 and 24 months at the distal metaphysis and mid diaphysis sites. Differences between the three groups were evaluated using ANOVA and a repeated measures model.

There were no significant differences in age between the groups, mean age was 9.4(2.7), 8.7(1.9), 8.8(1.8) years for HB, DMD-RA and DMD-LA, respectively. There was no significant difference in steroid exposure between DMD groups. However, boys who lost ambulation had significantly lower muscle function. Healthy boys had significantly greater trabecular bone density (26%) than boys with DMD (p<0.001). However, the rate of change of trabecular bone density was only significant for boys who lost ambulation. By 2 years non-ambulant boys had 51% less trabecular bone than their healthy age matched peers (See Figure).

Previous work has suggested that loss of ambulation can be predicted by assessment of muscle function. Since this study highlights the dramatic loss of bone density with loss of ambulation, it is likely that functional assessment can help identify the point at which medical intervention to strengthen bones should be considered.



P135
Is Vertebral Fracture Assessment by DXA more useful in a high fracture risk paediatric population than in a low risk screening population?

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Vertebral fracture assessment (VFA) by DXA is an accepted tool in adults. However, its use in children has not been validated. The aim of this study was to validate VFA using iDXA against spinal radiographic assessment (RA) for the identification of vertebral fractures in children.

Spine radiographs and VFA (L5-T2) by GE-iDXA were acquired on the same day in 80 children. Forty children were considered high risk for fracture as their metabolic bone specialist had initiated a referral for a radiographic spine assessment. The remaining 40 subjects consisted of children participating in a prospective fracture study and were considered low risk for vertebral fracture. Agreement between RA and VFA was assessed by an expert paediatric radiologist and two metabolic bone specialists. Vertebrae were ranked as normal, mild, moderate or severe if they had <10%, 11-25%, 26-50% and >50% deformity, respectively. Levels of agreement were calculated using the kappa statistic and consistency by the intra-class correlation coefficient (ICC).

Depending on rater, 92.8-94.8% of the vertebrae were analysable by RA. In contrast, 98.5% were analysable by VFA. For the high risk group, moderate agreement was noted between raters for RA [kappa 0.496-0.556], between RA and VFA [kappa 0.510-0.586] and between raters for VFA [kappa 0.630-0.687]. In contrast, for the low risk group, where only mild deformities were observed, poor-to-slight agreement was noted between raters for RA [kappa 0.075-0.2116] and slight-to-fair agreement was noted between RA and VFA [kappa 0.100-0.365] and between raters for VFA [kappa 0.308-0.343]. In the high risk group, agreement improved to substantial if the deformities were dichotomised as normal or mild versus moderate or severe [Kappa 0.810-879]. Subsequently, diagnostic agreement was tested by categorising each subject as having at least one severe or at least one moderate, mild only or no deformity. This approach resulted in consistent levels of moderate agreement between rater and technique [kappa 0.483-0.645].

In conclusion, VFA is accurate and consistent when identifying moderate and severe fractures in chronically sick children. However, since the diagnostic sensitivity of VFA appeared to be comparable to RA for both groups, VFA should prove to be a useful tool in the assessment of bone health in all children.

P136
Time to low bone mass occurrence in children diagnosed with acute lymphoblastic leukemia

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Objective: Assessment of low bone mass in children with acute lymphoblastic leukemia.

Methods: A total of 141 patients (83 boys and 57 girls) treated for acute lymphoblastic leukemia at the Department of Pediatric Hematology and Oncology of Medical University of Bialystok in Poland were assessed for low bone mineral density. Depending on age of diagnosis three clinical groups were analyzed: 1 - up to 5 y.o., 2 - from 6 to 12 y.o., 3 - from 13 to 18 y.o. Survival was measured as the date of birth to the date of low bone mass at DXA scans or most recent follow-up. Statistical analysis was performed using Kaplan-Meier Method, Wilcoxon test and chi² test. A p value < 0.05 was considered significant.

Results: Median age at diagnosis was 5.9 years [3.8, 9.5]. The longest observation period was 20.9, while medium time for a whole group was 17.97 years. There was no statistical difference in time to low bone mass between males and females (p=0.218). However, the number of observed events of low bone mass in boys was lower than expected. Patients diagnosed between 6 and 12 year of age tended to have bone deficits earlier than patients diagnosed before 5 and after 13 year of life (p=0.068). Among all patients a low bone mass (defined as a Z-score ≤ -2) was observed in a total of 17/83 boys and 20/57 girls (p=0.054).

Conclusion: This study suggests that there is no difference in time of occurrence of low bone mass between the analyzed groups. However 20.5% of boys and 35% of girls had a low bone mass during the study. It seems that children diagnosed with acute lymphoblastic leukemia should be screened for bone mass.



P137
Influence of nutritional status in the bone mass assessed by Phalangeal Quantitative Ultrasound of girls aged 7 to 10 years old

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The childhood obesity is a global epidemic. Some studies with pre-pubertal children reported a positive relationship between fat mass and bone. However, there are no available data on the influence of the nutritional status on parameters of quantitative ultrasound (QUS). The aim of this study was to evaluate the influence of nutritional status in bone mass assessed by QUS of proximal phalanges in girls. The bone mass parameter, Amplitude Dependent Speed Sound (AD-SoS) in meters for seconds (m/s) was assessed for QUS of the phalanges using DBM Sonic BP (IGEa, Carpi, Italy) device, in 514 pre-pubertal Brazilian school girls aged 7-10 years old. The nutritional status was classified according to extended International body mass index cut-offs (Cole and Lobstein, 2012). Most girls (69.8%) presented values of body mass index (BMI) considered appropriate for age (BMI: 15.8±1.1 m/kg² and z-score: -0.02±0.54), 14.6% were classified as thinness (BMI: 13.5±0.5 m/kg² and z-score: -1.47±0.40), 11.1% as overweight (BMI: 19.3±1.0 m/kg² and z-score: 1.44±0.28) and 4.5% as obese (BMI: 22.9±1.8 m/kg² and z-score: 2.48±0.31). The AD-SoS values were significantly lower (F=7.54, p<0.05) among overweight (1915.6±46.6 m/s) and obese girls (1896.0±45.6 m/s) in comparison with normal (1935.9±47.8 m/s) and thinness group (1937.6±52.5 m/s) as shown in Fig. 1.

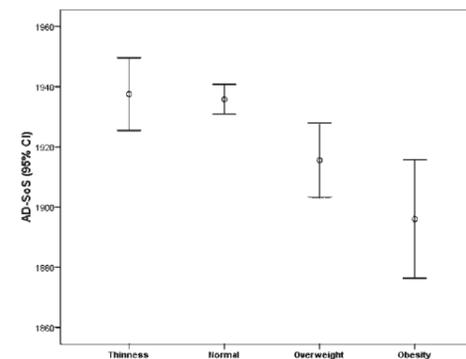


Fig 1. Comparison of AD-SoS values according to the nutritional status.

Our preliminary results demonstrated that the group overweight and/or obese may influence the bone mass parameter measured by QUS at phalanges in girls from 7 to 10 years old. This highlights the need of preventive actions to prevent the excess weight gain in children.

P138
RARE MUTATIONS ASSOCIATED WITH OSTEOCLAST-POOR OSTEOPETROSIS PROVIDE MOLECULAR INSIGHTS INTO RECEPTOR ACTIVATOR OF NF SIGNALLING

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Twelve different mutations in *TNFRSF11A* (encoding the RANK receptor) have been associated with osteoclast-poor autosomal recessive osteopetrosis in patients. Two truncated RANK proteins resulting from

substitution mutations (W434X and G280X), identified in two infants, cause loss of the intracellular oligomerisation motif and in the case of the G280X mutation the TRAF6 binding domain. A third mutation was identified in a 10 year old patient and is a frameshift mutation encoding a protein that is truncated within the extracellular, N-terminal domain (R110Pfs52X) raising the possibility that translation of the C-terminal region of the protein from alternative translation initiation sites may occur. We have been investigating the effects of these mutations on receptor processing and downstream activation of NFκβ to inform on the molecular cause for this condition.

W434X-RANK and G280X-RANK proteins were overexpressed in HeLa cells and immunoprecipitation revealed that, unlike wildtype-RANK, the W434X-RANK and G280X-RANK mutant proteins failed to oligomerise. In addition, whereas wildtype-RANK and W434X-RANK interact with TRAF6, G280X-RANK does not. p65 translocation experiments revealed that RANKL activates NFκβ in cells transfected with wildtype-RANK or W434X-RANK, but not G280X-RANK. These results strongly suggest that, whilst the TRAF6 domain is critical for activation of the RANK signalling pathway, the oligomerisation motif is not essential.

For the R110Pfs52X mutation, in vitro osteoclast cultures surprisingly demonstrated some osteoclast formation in the absence of RANK ligand. We generated seven myc-tagged expression constructs representing the N-terminal and potential C-terminal translation products. p65 translocation experiments demonstrated that the R110Pfs52X product does not support ligand-dependent or ligand-independent activation of NFκβ, whereas the putative C-terminal products of the alternative translation start sites induced only ligand-independent activation of NFκβ which may explain the ligand-independent osteoclast formation observed in osteoclast cultures.

Taken together, these results provide molecular insights into the regulation of RANK signalling underlying the patient phenotypes associated with each mutation.

P139
How to cope with a case of heterotopic ossifications

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Introduction: heterotopic ossification (OH) is a rare condition characterized by the presence of extra-skeletal ossification; in most cases OH is due to the inactivation of the gene of guanine nucleotide-binding protein alpha-stimulating activity polypeptide (GNAS). In some cases they remain confined to skin and subcutaneous tissues (osteoma cutis, Albright hereditary osteodystrophy-AHO-, pseudohypoparathyroidism type 1a and c -PHP1a/c- and pseudopseudohypothyroidism-PPHP), in other they grow also into deep organs (e.g. Progressive Osseous Heteroplasia- POH-).

Case Report: We report the case of a 9-year-old boy, who presented soon after birth several cutaneous lesions, increased over time, above all on legs, which were found to be "cutaneous osteoma" with a skin biopsy. He was -1/M SD for height and M+1 SD for weight, he had a small development delay, but no brachydactyly, nor round face and obesity. The genetic analysis showed GNAS mutation (IVS8+2, T>G+ IVS8+26 STOP). Soon before the visit after an incident to his back, he underwent a MRI, showing an aberrant right paravertebral ossification. It was therefore fundamental to understand if the new finding was a sign of a progressive disease, although usually extra-skeletal ossifications in this kind of disorders are not associated with trauma. Physical examination and laboratory determinations permitted to exclude both AHO and PTH resistance. The most specific way to distinguish POH from PHP1a is the study of the genomic imprinting: maternally-inherited GNAS mutations lead to PHP1a, whereas paternally-inherited ones are linked to POH. In our case RNA analysis clarified that the mutation came from the father, confirming the prior suspect of POH. Our child began periodical visits



to control the progression of the disease and started a therapy with bisphosphonates, obtaining up to now conflicting effects: we observed no progression of the deep lesions by imaging but our patient's pain has not improved.

Conclusion: As there is no specific genotype-phenotype correlations between the more progressive forms of HO and the non-progressive ones it is important to follow the patient with appropriate clinical, laboratory and genetic assessments. Besides, the debate on therapy options and results is still open.

P140 Defects of *SERPINF1* cause progressively deforming recessive Osteogenesis Imperfecta with normal collagen I

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Background: Osteogenesis Imperfecta is commonly due to dominant mutations in type I collagen genes, COL1A1 and COL1A2. Recessive forms, which are rarer, are caused instead by mutations in various genes coding for proteins involved in collagen post-translational modifications, folding and secretion. A novel disease locus, *SERPINF1*, coding for Pigment-Epithelium-Derived-Factor (PEDF), a likely key factor in bone deposition and remodelling, has been found recently.

Methods: We have investigated a group of patients with unresolved genetic diagnoses by means of genomic DNA sequencing and, when possible, by means of further histomorphometric characterization.

Results: In two of them we found homozygous mutations leading to either Nonsense-Mediated-Decay or truncated mutant mRNA. A third subject harbours two different mutations: compound heterozygosity results in a diminished mutant mRNA expression. Another patient, finally, is homozygous for a missense mutation which causes an E→K substitution in a highly conserved domain at the C-term region of the protein.

All patients exhibit a common peculiar phenotype characterized by a progressive worsening of the clinical symptoms, greyish sclerae, normal dentition, severe deformity of the long bones without rhizomelia.

Synthesis and secretion of mutant PEDF are differently affected, accordingly to the type of mutation, but in all cases type I collagen seems to be quantitatively and qualitatively normal.

Clinical, radiographic, histological and histomorphometric findings in these probands are reminiscent of type VI OI, previously described by Glorieux et al in 2002.

P141 Novel splicing mutation in FKBP10 gene in a patient with moderate/severe form of osteogenesis imperfecta

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Background: Osteogenesis imperfecta (OI) is a group of hereditary disorders characterized by bone fragility and osteopaenia, with a broad spectrum of clinical severity. The majority of cases are dominantly inherited and due to mutations in type I collagen genes, whereas recessive forms are less frequent and attributable to mutations in different genes involved in collagen I post translational modifications and folding (prolyl-3-hydroxylase complex, *SERPINH1*, *FKBP10*).

Case Report: We report the case of an Italian 14-years-old boy with an initially mild and then increasingly moderate-severe form of osteogenesis imperfecta. He revealed wild type sequence in COL1A1, COL1A2, CRTAP, LEPRE1 and SERPINH1 genes, respectively. Biochemical analysis, moreover, of dermal fibroblasts type I collagen and procollagen chains showed normal migration and amounts. Subsequent sequencing of FKBP10 gene revealed instead a novel homozygous splicing mutation in intron 8 (c.1399+1G>A), which results in aberrant mRNA processing and consequent lack of FKBP65 chaperone.

The proband's clinical features seem milder than those described in other FKBP10 cases, resembling OI type I at infancy, with slight joint laxity but no arthrogryposis. The histomorphometric analysis confirms an osteomalacic aspect meanwhile a qualitative evaluation shows loss of the normal orientation of the lamellae, with mixed "fish-scale" and "mesh like" patterns.

P142 A case of geleophysic dysplasia

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Background: Geleophysic dysplasia is a rare genetic bone disorder characterized by severe short stature, short hands and feet, characteristic facial features, limited joint mobility, thick skin, progressive cardiac valvular disorders and sometimes upper respiratory stenosis. Diagnosis of this disorder is based on clinical and radiographic criteria. Until now only 60 cases have been reported in the literature.

Case Report: One-month-old male baby was initially referred to our Pediatric Clinic because of short limbs with small hands and feet and a diagnosis of Achondroplasia.

He was born at term to non-consanguineous unaffected parents. At birth his weight and length were both under the 3rd percentile.

Amniocentesis performed at week 16 of gestation revealed a normal male karyotype (46, XY). Ultrasound scan at 28 weeks of pregnancy showed short long bones with a femur length (FL) below the 10th percentile, which worsened below the 3rd percentile in the consecutive checks.

During the first year of life both weight and length remained under the 3rd percentile with a marked growth retardation. At clinical examination the patient had a broad nasal bridge and joint mobility was generally restricted especially at the elbows. The thorax was flared and abdomen was prominent without any apparent hepatosplenomegaly.

Skeletal X-ray examination showed cone shaped and enlarged epiphyses of long bones, hand and feet abnormalities with short tubular bones, widening of the 1st and 5th metacarpals and abnormal shape of the proximal and middle phalanges. The upper respiratory tract and echocardiography findings were normal.

Thus, we diagnosed a case of an intermediate form of geleophysic dysplasia without cardiac and upper respiratory involvement.

Discussion: As a consequence of the clinical variability of this rare genetic bone disorder, a better knowledge of its pathogenesis may probably help to find a therapeutic approach that is actually still lacking; finally only a regular follow up and a multidisciplinary approach could help us to early detect life-threatening signs of the disease and to improve the patient's quality of life.



P143 Fractures in juvenile idiopathic arthritis children: role of disease activity and genetic factors

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Objectives: we evaluated role of disease activity and genetic factors in fractures predisposing among juvenile idiopathic arthritis (JIA) children.

Methods: Bone mineralization parameters were detected by dual-energy X-ray absorptiometry of lumbar spine L₁-L₄ in 197 (81 boys and 116 girls) JIA children. Bone biochemical markers included osteocalcin, C-terminal telopeptides, parathyroid hormone (PTH), Ca, Ca⁺⁺, P, total alkaline phosphatase (TAP) activity. We have detected *Apal-*, *TaqI-*, *BsmI-*, *Cdx2* restriction length polymorphism assay of vitamin D (*VDR*) receptor gene, *Hind III* polymorphism of osteocalcin gene, *TaqI* and *-1997 G/T* polymorphisms of I type collagen Ia chain (*Colla1*), *BclI* polymorphism of glucocorticoid receptor gene (*GCR*). Disease activity was measured by clinical and laboratorial parameters and special indexes.

Results: Differently localized fractures were in 29/197 children (14.7%), 7/81 (8.6%) in boys and 22/116 (19.0%) in girls. In fracture group of JIA children we have revealed higher physician (p=0.004) and parental (p=0.01) overall disease activity by visual-analog scale (VAS), higher Steinbrocker functional class (p=0.01), indexes JADS10 (p=0.027), JADAS27, JADAS71 (p=0.02), lower BMD-Zscore (p=0.027). Girls with fractures had longer disease duration (p=0.04), higher frequency of low BMD (<-2SD, p=0.01), lower BMD-Zscore (p=0.02). Boys with fractures had higher physician (p=0.02) and parental (p=0.02) overall disease activity. Also we have no differences in fracture rate due to glucocorticoids administration in JIA children. Independent predictors of osteoporotic fractures (BMD-Zscore<-2.0 SD) were: physical VAS (OR=4.52, p=0.044), DAS28>5.3 (OR=4.99, p=0.01), Steinbrocker>I (OR=6.17, p=0.01), Steinbrocker>II (OR=18.9, p<0.00001), polyarthritic and systemic course (reference-oligoarthritic course, OR=1.68 and 6.27, respectively, p=0.03), disease duration>5years (OR=25.5, p=0.007), parental VAS>5 (OR=29, p=0.0015), number of active joints>10 (OR=5.5, p=0.007), morning stiffness>120 minutes (OR=4.12, p=0.03), ESR>20 mm/h (OR=3.9, p=0.037), CRP>1.2 mg/dl (OR=5.9, p=0.007). Boys with fractures had significantly frequent H allele of *HindIII* osteocalcin gene polymorphism (p=0.047) and TT genotype (14.3% vs 0%) of *TaqI Colla1* (p=0.004).

Conclusion: We have revealed that fractures in JIA children associated with higher disease activity rather glucocorticoids administration. In JIA boys fractures were associated with presence of H allele of *HindIII* osteocalcin gene and TT genotype of *TaqI Colla1*.

P144 Long term bone sequelae following severe meningococcal septicaemia

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Background: Meningococcal septicaemia in childhood has a high mortality rate in the acute stage, often requiring intensive care support. Survivors are well known to have long term sequelae in the form of neuropathy, renal scarring, loss of limbs and necrotic tissue damage. We describe here a case where a survivor of this disease developed growth plate arrest and consequent severe bowing of both tibias which now require surgical correction. Relevant literature is also reviewed.

Presenting Problem: A 14 month old boy with severe meningococcal septicaemia and wide spread necrotic skin lesions over buttocks and

knees had required ventilation, inotrope support, and renal replacement therapy in the acute stage; but recovered well in 3 weeks with none of the well recognised complications. Three months later he developed progressive bowing of both legs (more noticeable on walking) and a waddling gait but no other clinical or biochemical features of rickets. X-ray of lower limbs revealed abnormal metaphyseal changes at the distal femoral and proximal tibial ends with linear lucencies, sclerosis and irregular widened growth plates. MR scans confirmed growth plate abnormalities secondary to septicemic illness.

Clinical Management: Over the next few months, his bowing became further pronounced. Though he was initially managed conservatively, he will need corrective osteotomies on the medial side. In addition to optimising calcium and vitamin D intake as well as active physiotherapy, he remains under close follow up for growth, limb length and deformity monitoring.

Discussion: Few studies in the literature (Canavese, 2010; Monsell, 2011; Park, 2011) have described similar experiences with larger cohorts of children, and recognised need for monitoring for late bone sequelae, as well as role for corrective osteotomies in this group. Likely aetiologies include ischemia secondary to endotoxin induced microvascular damage to the physes, peripheral growth plate injury due to tethering under areas of skin necrosis, and high metabolic rate with differential perfusion of different areas of the growth plate.

Conclusion: Orthopaedic abnormalities though not very common, can have significant impact on growth and development of children surviving severe meningococcal disease. Increased awareness and prompt recognition as well as early orthopaedic intervention is required to optimise long term growth potential of the long bones and restoration of limb length as well as mechanical axis in this group.

P145 The recurrent IFITM5 c.-14C>T transition which causes osteogenesis imperfecta Type V occurs at a highly methylated CpG dinucleotide. A novel mutational hot-spot?

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Background: Osteogenesis imperfecta (OI) is a heterogeneous group of disorders characterized by bone fragility. The current classification comprises five forms (OI types I-V) with autosomal dominant inheritance and seven rarer forms (OI types VI-XII) with recessive inheritance. OI type V (MIM 610967) has distinguishing radiological features, such as propensity to hyperplastic callus formation, calcification of the forearm interosseous membrane, radial-head dislocation, and a subphyseal metaphyseal radiodense line. The disease gene, coding for IFITM5 (interferon-induced-transmembrane-protein-5) has been identified very recently. A c.-14C>T recurrent mutation in its 5'UTR region has been found in 63 patients so far.

Case Report: The 7-year-old male proband was diagnosed as affected by OI soon after birth because of a forearm fracture and classified as type V after age 5 because of pathognomonic radiological signs. Heterozygosity of the proband for the c.-14C>T transition was revealed by direct sequencing of amplified patient's genomic DNA obtained from a peripheral blood sample.

Methylation Analysis: Total genomic DNA was extracted from cultured skin fibroblasts, leukocytes, sperm and bone marrow, from healthy male and female subjects. Sequence analysis of all samples revealed that c.-14C is 100% methylated in sperm and leukocytes while it appears ≥80% methylated in bone marrow and in fibroblasts.

Discussion: Unlike all other OI types, characterized by high genetic heterogeneity, OI type V appears consistently associated to a unique *de novo* C>T transition within the 5'UTR of IFITM5 gene.



Our data demonstrate that the recurrent *de novo* mutation causing type V OI involves deamination at a CpG dinucleotide which showed the lowest methylation levels, in the fibroblast DNA, in agreement with the connective tissues-specific expression of the gene, while is 100% methylated in male germ cells. We suggest that this recurrent base substitution may well represent a mutational hot spot in the human genome.

P146 A case of familial cherubism

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Background: Cherubism is a rare autosomal dominant bone disease characterized by bilateral painless enlargement of the jaws, that typically first appear at the age of 2 to 7 years. In this condition the affected bone is replaced with fibrous tissue, giving the patient a cherubic appearance. Until now only 300 cases have been reported in the literature.

Case Report: a Caucasian 4-year-old male child came to our Pediatric Clinic complaining pain and bilateral swelling of the face. His parents reported that the swelling started gradually, increasing in size from the age of three years. His past medical history was unremarkable.

Intraoral examination showed bilateral expansion of the mandibular angle extending to the retromolar and molar regions. Panoramic radiography showed generalized multicystic bilateral lesions affecting mandible and maxilla and the absence of several teeth buds. MRI images confirmed the panoramic radiography findings. Mineral metabolism markers are within normal range.

His mother reported that during childhood she suffered from the same problem. At 12 years old she underwent jaw surgery for aesthetic problems. Histopathological examination of the biopsy specimens showed proliferating fibrous connective tissue interspersed by multinucleated giant cells.

Her bone lesions presented a pattern of enlargement until 20 years and then regressed. At the moment of the evaluation of the child, the mother was 35 years old and her condition showed a complete regression.

Thus, we diagnosed a case of familial cherubism. The child is now under clinical and radiological observation. He will undergo biopsy, when he will need surgery.

Discussion: usually, the diagnosis of cherubism is based on a combination of patient age, skeletal distribution of lesions, radiographic findings and histopathological features. A positive family history for cherubism could confirm the diagnosis, but biopsy and gene testing are often recommended. It is important to improve our current knowledge of pathophysiology of this disorder to evaluate future medical treatment.

P147 A case of Gorham-Stout syndrome with Chylothorax

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Background: Gorham Stout syndrome, also called disseminated lymphangiomatosis, is a rare disease of unknown etiology and pathogenesis. This syndrome is characterized by an abnormal proliferation of thin walled capillaries and small lymphatic vessels that results in the massive osteolysis of adjacent bone. Surrounding soft tissues such as muscle, connective tissue, and viscera may also be affected. Chylothorax occurs secondary to direct involvement of the pleural cavity or the thoracic duct and this complication is almost always fatal.

Until now only about 35 cases of Gorham's syndrome complicated by chylothorax have been reported in the literature.

Case Report: A 11-month-old boy presented to our Hospital with respiratory distress and tachypnea. His medical history was significant for a mild growth retard and feeding difficulties with recurrent vomiting since the 6th month.

A chest radiograph showed left pleural effusion. MRI of thorax and abdomen demonstrated multiple cystic lesions containing multiple septa; the major was in the mediastinum and extended from the neck base to diaphragm involving the aorta. Other cystic lesions were described in the spleen, ribs, omerus and dorsal and lumbar vertebrae. Thoracentesis revealed chylothorax and chest tubes were placed bilaterally with continuous and large volume of chylous drainage.

Infection, malignancy and immunodeficiency were ruled out. Several surgical interventions were performed during the following months: excision of the bigger lymphatic mass, ligation of multiple oozing sites, pleurodesis and finally thoracic duct ligation. At 17 months the patient started therapy with alpha-2b interferon. 10 months later MRI showed no recurrence of pleural effusions. but a progressive enlargement of skeletal lesions was described. Thus, after an informed consent, we decided to start therapy with bisphosphonates.

Discussion: we described a case of Gorham syndrome, complicated by chylothorax, that presented a good response to aggressive surgical management. Due to the rarity and varied presentations of Gorham's disease, there is no standard treatment for this disorder but new and focused therapeutic interventions are needed. Only a regular follow up could help us to know if bisphosphonates are helpful in this disorder.

P148 Low bone mineral density in a group of girls with Rett syndrome

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Objective:

In girls with Rett Syndrome:

Describe bone mineral density (BMD) and contributing factors in a cross-section of subjects

Examine serial DXA measures

Examine effect of pamidronate on BMD over time

Methods: We reviewed the clinical course, medications, level of ambulation, 25-OH-Vit D levels, fracture history and DXA results in 13 girls with Rett syndrome. Eight subjects had more than one DXA study, including two treated with Pamidronate. Body sites scanned included whole body when possible (WB), lumbar spine (LS), and lateral distal femur (LDF). Age and gender-matched Z-scores were calculated using manufacturer-provided normative values for LS and WB, and published norms for the LDF.

Results: 13 girls with a mean age of 9.6 years (range 6.6 to 18.2) at the time of initial DXA were included in this study. Eight were partially or fully ambulatory.

We were able to obtain LDF and LS DXAs on all subjects. Mean (and range) LS BMD Z-scores were -1.9 (-0.1 to -4.4); for the LDF: R1 -4.5 (-1.9 to -8.5); R2: -3.4 (-1.3 to -7.8) R3: -3.5 (0.1 to -11.2)

Only 6 WB scans could be acquired, with a mean Z-score of -2.6 (-1.3 to -4.4). The inability to lie still and positioning limitations precluded WB DXA measurement.

25-OH-Vit D levels ranged from 6.0 to 50.8 ng/dl at the time of initial DXA, with a median level of 26.0.

Serial DXA Studies:

Length of observation in the 6 untreated subjects ranged from 3.1 to 6.1 years after the initial study, and 2.8 to 8.2 years in the two treated girls. The 2 girls treated with Pamidronate, of whom one was ambulatory, had



a history of multiple fractures prior to treatment, and the lowest baseline LS and LDF DXA Z-scores. As expected, their Z-scores increased dramatically with Pamidronate. In contrast, there was no clear pattern in BMD Z-scores in the 6 untreated girls; slight increases and decreases were observed over time, seemingly unrelated to ambulatory status and vitamin D levels.

Conclusions: Girls with Rett Syndrome have below normal BMD, particularly of the lower extremities. The two who suffered multiple fractures and then received bisphosphonate, have had no fractures since treatment began. As demonstrated in other disabled populations, the LDF proved to be an easily-obtained, useful alternative DXA site in Rett syndrome.

P149 Bone Mineral Content in healthy Danish Children assessed by DXA-scanning and by computerised determination from hand radiographs.

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Background: The mineral content of the skeleton in children may be estimated by a number of methods including DXA-scanning, ultrasound, pQCT-scanning and from plain radiographs. Recently, a new method offering an estimating of the bone mineral content in children based on computerised assessment from hand radiographs has been introduced. The new measure is named bone health index (BHI) and expresses the mineral content in the metacarpal bones divided by the volume of the same bones. However, it is not clear to what degree BHI correlates to bone mineral content measured by DXA.

Aim: The aim of the present study was to compare bone mineral content in healthy Danish children measured with DXA and BHI.

Methods: Eighty five healthy Danish children were included in the study. The children were all part of the CHAMPS-study-DK. As a part of CHAMPS-study-DK, the children were DXA-scanned on a Lunar Prodigy (GE Medical Systems, Madison, WI), equipped with ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI). The total body less head (TBLH) BMC, BMD and BA were measured. On the same day, radiographs of the participants' left hand were taken. The BHI was determined by the BoneXpert® software (Visiana, Holte, Denmark).

BMC, BMD and BA in each child were correlated to BHI by simple linear regression analyses (SPSS, version 19).

Results: BHI correlated well to BMD with R²= 0.56 (p<0.001). BHI also correlated highly significantly (p<0.001) to BMC and BA, although the R² were lower (0.46 and 0.38, respectively).

Conclusion: BHI may offer a new method to estimate the mineral content of the bones in children.

The present data will be supplied with additional data on the whole study population, which are 700 children. Furthermore, the methods will be compared by Bland-Altman plot, and the change in BHI over a two year period will be compared to the change in BMD and BMC during the same period. These data will be available at the time of the conference.

P150 Markers of bone turnover in obese children - relationship to the nutritional status and oxidative stress level

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Background: Recent data showed that some bone related markers (osteocalcin, 25OHD3) correlate with body mass index (BMI) in the pediatric population. From the other side, obesity in childhood can increase the risk of cardiovascular morbidity and mortality in adulthood. Increased oxidative stress can be one from the causative mechanisms involved in the pathophysiology of almost every complication in obesity. The aim of this study was to determine the relationship between bone turnover markers, nutritional status and oxidative stress markers in obese children comparing to the lean control group.

Material and methods: Bone turnover markers (osteocalcin (OC), N-terminal telopeptide of type I collagen (NTx), sRANKL), oxidative stress markers (TAC – total antioxidant capacity, glutathione peroxidase, oxy-LDL) and leptin were determined in 50 obese children and 79 healthy controls. Nutritional status assessed by BMI calculation and body composition parameters as: fat mass (FAT), fat-free mass (FMM), predicted muscle mass (PMM) and total body water (TBW) were evaluated using bioelectrical impedance analyzer in all children.

Results: OC was significantly lower in obese children and correlated significantly (negatively p<0.01) with BMI in the lean group. There was also significant positive correlation between OC and TAC in obese children. NTx correlated significantly with oxy-LDL (positively) in either, obese and lean group (p<0.05 and p<0.01 respectively). In the lean group only, there were significant relations between NTx vs. leptin and body composition parameters (r = 0.245 vs. leptin, r = 0.245 vs. FAT%, r = -0.252 vs. PMM%, and r = -0.245 v. FFM% respectively). There was no significant correlation between RANKL and every other parameter assessed in both studied groups.

Conclusions: 1. Bone turnover seems to be disturbed in the obese children and pathophysiological factor with can be involved in that mechanism may be an increase oxidative stress level. 2. Even in lean children nutritional status is inversely and directly related with osteocalcin and NTx respectively.

P151 Severity of spine deformity in children and adolescents with idiopathic scoliosis (IS) is associated with nutritional status and body composition

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Background: Body composition changes during the developmental period and differs in children with idiopathic scoliosis (IS). No large-scale study has been performed to reveal the link between scoliotic deformity and body composition assessed by bioimpedance method (BIA). The study objective was to correlate the extent of scoliotic-curve severity with nutritional status of patients with IS based on standard anthropometrical analysis and BIA.

Material and methods: 279 patients (224 girls/ 55 boys) in mean age of 14.21 ± 2.75 years, with IS were qualified into the study. Scoliotic curve was assessed by Cobb angle and angle vertebra rotation (AVR). Curve severity was categorized into a mild (10-19 °), a moderate (20-39°) and a severe group (≥40°) based on Cobb's angle. Height, weight, waist and hip circumferences were measured and body mass index (BMI), BMI Z-score, waist/height ratio (WHtR) and waist/hip ratio (WHR) were calculated in the entire group. Body composition parameters as: fat mass (FAT), fat-free mass (FMM), predicted muscle



mass (PMM) and total body water (TBW) were evaluated using bioelectrical impedance analyzer.

Results: Mean Cobb angle of the mild, moderate and severe groups were $13.69 \pm 2.95^\circ$, $26.74 \pm 5.67^\circ$ and $52.36 \pm 12.43^\circ$ respectively. Cobb's angle and AVR were positively correlated ($p < 0.01$) with FAT% and BMI, but inversely ($p < 0.01$) with FFM%, TBW% and PMM% in the study group. Subgroups analysis revealed the same relationship only in the group of severe spinal deformity, also for BMI Z-score and WHtR. Body fatness expressed as FAT% was significantly higher ($p < 0.05$) in the severe vs. mild group, but FFM%, TBW% and PMM% were significantly ($p < 0.05$) lower.

Conclusions: 1. Body composition parameters assessed by BIA are associated with scoliotic curve severity, mainly in severe spinal deformity patients. 2. Fatness degree (FAT% and BMI Z-score) and fat tissue distribution (WHtR) seems to have significant relation with clinical grade of IS.

P152

Zinc supplementation improves bone density in young adults with thalassemia

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Poor bone mineralization remains a major health problem in patients with Thal and has been linked to functional zinc deficiency despite adequate dietary intake. The global etiology of poor bone mineralization includes inadequate dietary intake of calcium and vitamin D, endocrinopathies leading to disturbed calcium homeostasis, dysregulation of the GH-IGF-1 axis, and delayed puberty, all resulting in limited growth, decreased bone formation and increased bone resorption. These effects are further aggravated by chronic blood transfusions and chelation therapy, which are indispensable for survival. The aim of this study was to ascertain whether zinc supplementation leads to improved bone mineralization in adolescents and adults with Thal. Forty-two subjects (21 Females, 10-30 y) with Thal and low bone mass were randomly assigned to receive 25 g zinc/day or placebo; 33 completed the study (16.9±5.1 y, Mean ± standard deviation), 81% were transfusion dependent. Bone mineral content (BMC) and bone mineral areal density (aBMD) were assessed by dual energy x-ray absorptiometry. Dietary intake was assessed by food frequency questionnaire at 0, 12 and 18 months. Fasting blood was assessed for plasma zinc and copper at 0, 3, 6, 12 and 18 months. Plasma zinc was depressed ($\leq 70 \mu\text{g/dL}$) in 11 subjects (26%) at baseline and increased significantly with zinc supplementation ($p=0.014$). Zinc intake at baseline averaged 123±66% of U.S. dietary recommendations. Using intention to treat analysis, and mixed effects linear modeling controlling for baseline, the zinc group ($n=24$) had significantly greater relative increases in whole body BMC (3.2%, $p=0.027$) and whole body aBMD (2.3%, $p=0.046$) compared to placebo after 18 months. Zinc-supplemented adults ($n=15$) had significantly greater relative increase in spine BMC ($p=0.03$), spine aBMD ($p=0.039$), whole body BMC ($p=0.046$) and whole body aBMD ($p=0.019$) compared to placebo. No significant change was observed in any of the bone variables in the adolescent subjects taking zinc, likely due to the increased variability and smaller sample size. These results suggest that Thal patients suffer a functional zinc deficiency which limits bone mineralization. This nutritional supplement was well-tolerated and merits further investigation in larger trials across a broader range of age and disease severity.

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Low urinary citrate: A risk factor for fragility fractures in children and adolescents

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Objectives. Idiopathic hypercalciuria may infer not only an increased risk of nephrolithiasis but may also be associated with reduced bone mineral density (BMD) in adults. However, little is known about relationships between hypercalciuria, oxaluria, urolithiasis, citruria and fracture risk in children. The aim of this cross-sectional study was to evaluate associations between hypercalciuria, urinary oxalate and citrate, BMD and fractures in hypercalciuric children.

Methods. Medical records were studied to evaluate history of fractures, which were documented by X-ray examination. Dual energy X-ray absorptiometry (DXA) was used to assess body composition, bone mineral content (BMC) and BMD in total body and lumbar spine L1-L4 in 40 children and adolescents (26 boys, 14 girls) aged 3.5-18 years (mean±SD: 14.9±3.3) with hypercalciuria and/or urinary calcium oxalate stones (87.5% of the sample) diagnosed using high resolution ultrasonography. Urinary calcium, phosphate, uric acid excretion, oxaluria, citruria were investigated in the 24-hour urine collections.

Results. Mean Z-score for spine BMD was -1.08 ± 1.09 . Decreased BMD (Z-score below -2.0) was found in 9 subjects (22.5% of the sample). Of all studied children, 13 (32.5%; boys/girls: 10/3) sustained 20 low-energy fractures in the peripheral skeleton (forearm, wrist, tibia, ankle). Subjects with fractures had significantly lower citruria rate ($467 \pm 296 \text{ mg/g creatinine/24h}$) compared with those fracture-free ($484 \pm 266 \text{ mg/g creatinine/24h}$) ($p=0.02$). Urinary citrate excretion was positively correlated with BMD (adjusted for age and lean mass) only in the fractured children ($r=0.76$ $p=0.04$), whereas no such correlation was observed in children without fractures. No associations were found between calciuria, oxaluria, uricosuria, phosphaturia and bone mass or fractures.

Conclusions. Deficits in BMD among hypercalciuric children and adolescents are common although not associated with calciuria or oxaluria. Small sample size in this study, however, limits inferences that could be drawn. Our findings suggest that prolonged hypocitruria may be, at least partly, an independent risk factor of both reduced peak bone mass and an increased fragility during growth. Whether the life-long risk of osteoporotic fractures attributable to low citrates will be sustained in these patients is unclear.

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Smad4 Regulates Growth Plate Chondrocyte Proliferation, Columnar Organization and Proteoglycan Synthesis

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Objective: The physis, or growth plate, is comprised of precisely organized chondrocytes that confer longitudinal growth of the bone. Multiple signaling pathways cooperate to regulate growth through their control of chondrocyte shape, polarity, proliferation, and differentiation.[1,2] Disruption of these cellular events result in physal defects, skeletal deformities, and abnormal limb growth. Loss of function mutations in Smad4, a common intracellular effector of all transforming growth factor β (TGF β) family members, is responsible for human Myhre syndrome characterized by short stature, brachydactyly, and joint stiffness.[3] In the physis of Smad4 deficient mice, the columnar organization is replaced with a disorganized mass of misshapen chondrocytes.[4] Although many studies affirm the key role for Smad4/TGF β family signaling in chondrocytes, the mechanisms



by which Smad4 maintains normal growth plate organization are not defined.

Methods: Tibias of Col2a1-Cre;Smad4^{fl/fl} mice were isolated at e18.5 and frozen sections were used for immunofluorescence and immunohistochemistry. Primary chondrocytes were isolated from Smad4^{fl/fl} mice, underwent adenoviral-cre infection and cultured in a pellet for 21 days.

Results: Our data indicate Smad4 is responsible for maintaining normal proliferation, columnar organization and proteoglycan synthesis *in vivo* and *in vitro* with consistent results in the physis and primary murine chondrocyte pellet cultures. Using immunofluorescence, we determined several markers of cell polarity, including cell shape and direction of cell elongation, are aberrant in Smad4-deficient chondrocytes. The number of cells in a proliferative column and markers of proliferation are reduced. Proteoglycan content, through Safranin-O staining and immunohistochemistry, is also reduced.

Conclusions: Smad4 is involved in cytoskeleton reorganization and proteoglycan synthesis in the physis and could alter the extracellular signaling of many critical pathways, including the planar cell polarity (PCP) pathway. This study enriches our knowledge in the development and regulation of the growth plate, cartilage biology, tissue engineering, joint formation, articular cartilage development, and osteoarthritis.

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P155

Assessment of Bone Density in MPS IV (Morquio Disease)

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Objectives: 1) Describe bone mineral density (BMD) of children with MPS IV (Morquio disease), a rare genetic disorder which produces skeletal deformity, small stature and results in physical limitations such as the ability to walk.

2) Examine fracture history and factors affecting bone health in Morquio

3) Describe technical issues encountered in assessing BMD by DXA in Morquio

Methods: In this prospective cross-sectional study, BMD of the whole body (WB), lumbar spine (LS) and lateral distal femur (LDF) were acquired by DXA on a group of children with MPS IV(A) or MPS IV(B). Functional abilities, medical history, Tanner score, and laboratory results were reviewed. Radiologic images of the lateral spine, including x-rays and IVA by DXA were used to aid in correct region of interest placement on the LS DXA and in interpretation. Age and gender-matched norms were used to calculate Z-scores.

Results: Ten children (8 female) with a mean age of 11.8 years (range 3.3 to 18 years) participated in the study. Both subtypes of Morquio (A and B) were represented. While every subject was weightbearing, half were full-time ambulators. Whole body could be obtained on only 4 subjects due to respiratory compromise caused by the position, presence of hardware or positioning difficulties. Mean WB Z-score was -2.2 (range -0.6 to -4). Mean LS BMD Z-score was -3.5 (range -0.8 to -6.9) with 7 subjects exhibiting low BMD. Technical issues encountered with this metabolic condition included kyphosis at the thoracolumbar junction and wedge configuration of the vertebrae. Lateral views of the spine were needed for correct identification of vertebrae. LDF BMD Z-scores

at all regions were low, with mean Z-scores of -2.6 , -2.5 , and -2.8 at regions 1, 2, and 3, respectively. Only one fracture was reported and was due to trauma of the hand.

Conclusion: Children with Morquio exhibited low BMD at all sites measured. Despite the low BMD and skeletal dysplasia, fractures were not reported. WB DXA was not well tolerated or feasible. Anatomical abnormalities of the spine and technical limitations of DXA make assessment of the LS challenging.

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Reflection analysis of infant scans results may improve infant DXA bone density and body composition result that contain motion

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Objectives: Special dual-energy X-ray absorptiometry (DXA) protocols permit quantification of bone mineralization, fat mass, and fat distribution in infants. Our objective was to evaluate the accuracy and precision of a multiscan acquisition protocol designed to allow for reflection and imputation analysis for regions with movement.

Methods: The IMPAACT P1084s Study assesses bone and kidney safety of antiretrovirals used for PMTCT. Newborns received a spine and whole body DXA scan with up to three attempts to acquire a motion free scan. A novel 6-region whole body analysis was used to isolate movement artifacts. Precision was estimated as a root mean square error (RMSE) or percent coefficient of variation (%CV) for scans with multiple valid results. Results from whole body scans without movement were compared to estimated whole body results from either single-scan reflection of arms and legs, or from multiscan imputation using the Student's T test.

Results: Of 229 sequentially recruited infants (100 male), 41 and 132 infants had repeat scans for spine and whole body, respectively. Spine precision for duplicate scans was 5.0% (BMD) and 4.4% BMC ($n=38$). Whole body bone precision was 9.9% for BMC and 6.7% for BMD ($n=3$), and 0.9%, 4.9%, 4.0%, and 1.1% for lean, fat, % fat, and total mass respectively. Omitting one of the four vertebrae did not significantly impact the total BMD no matter which vertebrae was left out. One-vertebra imputation resulted in less than a 1.5% difference in total BMD. The standard error of total body measures using reflected appendages was in all cases less than the precision for any single region, and smaller than for imputed values in most cases.

Conclusion: Motion artifacts can be effectively removed from infant DXA scans using either omission, reflection, or imputation techniques. The reflection method provides the best agreement to whole body results without movement.

P157

Growth and Bone Health after Hematopoietic Stem Cell Transplantation or Tyrosine Kinase Inhibitors in Children with Chronic Myeloid Leukemia: A Single Institution Experience

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Objective: To examine the impact on growth and bone mineral density (BMD) of tyrosine kinase inhibitors (TKI) versus hematopoietic cell transplant (HCT) for treatment of chronic myelogenous leukemia (CML) in patients less than 18 years of age.



Methods: We performed a retrospective review of children with CML in chronic phase treated between 1992 and 2011 at a single institution and evaluated available growth and BMD data.

Results: Twenty-five patients were included in the analysis. Ten patients were treated by HCT without TKI (group 1), 9 with TKI followed by HSCT (group 2) and 6 with TKI alone (group 3). Overall survival at 3 years for HCT +/- TKI (group 1 and 2) was 68%. All 6 patients in group 3 remained in complete hematological remission on TKI for a median of 10.5 months (range, 5-72 months) at time of study but one has since gone on to HCT. Among 4 patients in group 1 with evaluable long-term growth data, only one patient was definitely prepubertal at diagnosis (defined as age <11y girls and <12y boys). Group 2 had 4/6 prepubertal and group 3 had 1/6. Mean change in height Z score diagnosis to 1 year was +0.09 for group 1 (n=2), -0.4 for group 2 (n=3), and -0.03 for group 3 (n=5). Combining all treatment groups, change in height Z score at 1 yr was 0.01 for pubertal versus -0.3 for prepubertal children. For subjects evaluable at 3-5 years from diagnosis, height Z scores tend to be most negatively affected in group 1, while group 3 shows consistently negative Z scores over time but only 1 data point each at 3 and 5 years. BMD Z score by DEXA (using the lowest value between lumbar or whole body less head as available) was positive for the single group 3 patient with a scan at 1 yr, but trends for group 2 (n=4) appeared more negative at 3-5 years than for group 1 (n=2).

Conclusion: Our study looked at patients treated with CML at our institution in the last two decades. In the short term (1 year), growth retardation seems to be most affected by prepubertal status, but in the longer term both HCT and TKI are likely to affect growth. Limited data suggest the BMD appears to be negatively affected by TKI +/- HSCT. Long-term monitoring of growth and BMD in pediatric patients treated with TKI is needed.

P158 Exploring Vertebral Abnormalities in Patients with Thalassemia and Sickle Cell Disease

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Low bone mass is common in thalassemia (Thal) and sickle cell disease (SCD). Bone pain is also reported, though its relationship to low bone mass has not been explored. The aims of this study were to determine the prevalence of vertebral height abnormalities (VHA) and evaluate the relationship between VHA, low bone mass and patient assessed pain in Thal and SCD. Data were collected from the Thalassemia Clinical Research Network Pain Survey study and CHRCO Clinical Bone Density database. The Pain study used a validated pain survey to collect data on patients from CHRCO at multiple time points. This was then analyzed with clinical bone mineral density (aBMD) scans if ± 6 months. Full lateral spine scans conducted at the time of aBMD scan were re-analyzed by one observer using the Vertebral Fracture Analysis (VFA) software (Hologic v12.6.1), and scored according to Genant for VHA (Grades 1 to 3). 232 VFA scans were re-analyzed from 91 patients with Thal (21.5 ± 10.7 y, 55% F) and 46 SCD (38.1 ± 12.7 y, 61% F). Of the patients with Thal who had VFA scans, 26.9% had at least one VHA, compared to 34.8% of those with SCD (p=NS). A similar percentage of Thal patients had substantial VHA deficits (Grade 2 or 3; 15.1%) compared to SCD (13.3%). The average number of vertebrae with abnormalities within a patient was lower in Thal (1.6 ± 0.2) compared to SCD (2.8 ± 0.4 ; p=0.003). Thal had wedge-type whereas SCD had biconcavity-type deformed vertebrae. The presence of a VHA was related to age in Thal (p=0.029) with a trend towards a relationship to low bone mass (p=0.12). In the patients for which both VFA scans and pain studies were performed (n=32), bodily pain was not related to VHA. A high percentage of both SCD and Thal patients had significant VHA. The etiology of the abnormal morphology is unclear but appears to be more developmental in SCD, but apparently a consequence of inherent

bone fragility in Thal. Further research should focus on the clinical relevance of VHA and possible temporal relationship to bone pain in these at risk patient populations.

P159 Novel SLC34A3 mutation causing mild Hypophosphataemia, Hypercalciuria & Nephrolithiasis but no clinical or radiological evidence of Rickets

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Background: Genetic disorders of mineral metabolism causing nephrolithiasis and bone abnormalities are uncommon and have a varied clinical spectrum. Hypophosphataemic rickets with hypercalciuria (HHRH) is a rare autosomal-recessive condition, typically presenting with severe rickets and hypophosphataemia. Milder forms can present with hypercalciuria and nephrolithiasis without bone disease. The underlying pathophysiology is due to mutations in the SLC34A3 gene, which encodes the sodium-phosphate transporter NaPi-IIc in the proximal renal tubules.

We describe HHRH in two siblings; the elder presented with hypercalciuria and nephrolithiasis, the younger subsequently diagnosed on biochemical screening; neither with clinical or radiological evidence of rickets or osteomalacia.

Presenting Problem: A 13-year old Caucasian girl from a non-consanguineous family presented with intermittent loin pain, macroscopic haematuria and passage of a kidney stone. Ultrasound showed a right-sided renal calculus and bilateral nephrocalcinosis. Recurrent episodes of calculi followed. Urine biochemistry demonstrated hypercalciuria [0.16mmol/kg/day (normal <0.1)] and hyperphosphaturia [TmP/GFR 0.59mmol/L (0.93-1.71)]. Plasma analysis revealed hypophosphataemia 0.78 mmol/L (0.95-1.5), suppressed serum parathyroid hormone [9pg/ml (15-65)], elevated serum 1,25-dihydroxyvitamin D 109pg/ml (20-50) and inappropriately low FGF23 30RU/ml considering the hypophosphataemia.

Clinical Management: Genetic studies found a heterozygous missense mutation c.413C>T (a rare but known SNP) and a homozygous inframe deletion c.1576_1578delCTC. Parental DNA analysis found her mother heterozygous for both the missense mutation c.413C>T and the inframe deletion c.1576_1578delCTC, but no evidence of any mutation in her father. Screening of her 10-year old brother revealed hypercalciuria (urine calcium:creatinine ratio 0.87 mol/mol), mild nephrocalcinosis on ultrasound, normal serum phosphate [1.25 mmol/L (1.0-1.8)], raised 1,25-dihydroxyvitamin D [252.0 pmol/L (48-120)] and FGF23 84 RU/ml. DNA analysis is awaited. Maternal biochemistry is also awaited.

Treatment of the index patient with oral phosphate supplements (introduced in incremental doses to prevent side-effects) has reduced urinary calcium excretion to 0.096 mmol/kg/day, with no further episodes of renal calculi. Her brother will also be treated with oral phosphate.

Discussion: Only a few sporadic cases of HHRH without rickets are described worldwide. It is likely to be underdiagnosed and requires treatment with oral phosphate supplementation alone, without Vitamin D supplementation, which will worsen the condition. Treatment with oral phosphate supplements may help to prevent renal calculi and worsening of renal function.



P160 A familial case of Osteogenesis Imperfecta: study of genotype-phenotype correlation.

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Osteogenesis imperfecta is a clinically heterogeneous heritable connective tissue disorder. Most OI cases are due to mutations in type I collagen genes, *COL1A1* and *COL1A2* encoding the pro-alpha1(I) and pro-alpha2(I) chains, respectively. However, genotype-phenotype correlation has not been completely elucidated yet. In this study we evaluated a familial case including a mother and a daughter, classified as OI type I. The daughter had more severe clinical features compared to the mother. Both were carrying a 4005+1G>T mutation in *COL1A1* gene, which leads to loss of a splicing site with retention of intron 49 and insertion of a stop codon in the mRNA. Accordingly, both patients had lower levels of *COL1A1* transcripts compared to control subjects. Due to the retention of intron 49, mRNA derived from the mutated allele should be longer than that derived from the wild type allele. Differential expression analysis of the two alleles was performed on mRNA derived from dermal fibroblasts using RT-PCR. A low amount of transcripts derived from the mutated allele was present only in the daughter. Semi-quantitative determination of allele expression was evaluated by Real-time PCR with primers and probe specific for the mutated and the wild type mRNA. In the daughter, the levels of the mutated transcripts were 17 times higher than in the mother. In contrast, wild type mRNA levels were similar in the two patients. Based on these results, it is tempting to speculate that the more severe clinical characteristics of the daughter might be due to the concomitant presence of a quantitative and a qualitative defect. Furthermore, these findings highlight the importance of a detailed molecular characterization of each genetic variant to explain the different phenotypic consequences of the same mutation.

P161 Autoimmune hepatitis and bone density in children

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Autoimmune hepatitis (AIH) is an immune-mediated chronic inflammatory disease of the liver of unknown origin, that suddenly appears in previously healthy, normally growing children. Standard therapy is long-term prednisone, aimed at avoiding progression to cirrhosis. Considering the inflammatory origin of the disease and the long-term steroid therapy, negative consequences for bone health can be expected, but no data on this complication have been published until now.

We measured lumbar spine bone mineral density in 17 children (8 F, 9 M) affected by AIH and treated with steroids. Two DXA scans were performed, at the time of diagnosis (liver biopsy) and after 12 months of steroid therapy. The bone mineral apparent density (BMAD) Z-score was -1.8 ± 1.3 (calculated with respect to a healthy age- and sex-matched Italian population). In particular, 12 children had a lower-than-normal Z-score (-1.2 to -3.8), and the lower values were observed in pubertal (Z-score -1.9 ± 1.7) rather than in pre-pubertal children (Z-score -1 ± 1.2).

After one year of steroids, even with adequate calcium intake and vitamin D supplementation, BMAD showed a further decrease (Z-score -2.5 ± 1.4): 13 children (76.4%) showed a mean Z-score reduction of -0.95 ± 0.5 . Three children sustained a fragility fracture in the first months of steroid treatment (vertebral fractures in two cases).

The BMAD Z-score values were correlated to age at onset of AIH (r = 0.31, p < 0.02) and cumulative steroid dose (r = -0.30, p < 0.02).

In conclusion, these data call attention upon a problem that has been neglected until now. In AIH, bone mineral density may be reduced at a very early time, even before starting steroid therapy, particularly in pubertal children. The risk of vertebral fractures strongly recommends

an evaluation of bone density with DXA as soon as possible after a diagnosis of AIH. The required immunosuppressive therapy must be carefully and individually tailored to minimize further negative effects on bone.

P162 Low bone mass and fractures in young patients with chronic diseases

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We performed a prospective study on 440 young patients (aged 3-20 years), affected by various chronic diseases (cystic fibrosis; juvenile idiopathic arthritis; nephrotic syndrome; systemic lupus erythematosus; Duchenne muscular dystrophy; autoimmune hepatitis; transplants; etc.). with periodical bone mineral density (BMD) evaluations with DXA, for 3 to 14 years (7.8 ± 6.2).

266 patients were on long-term treatment with glucocorticoids (GCs); among them, 140 stopped GC treatment and were followed for 3 to 5 years after GC withdrawal. BMD was measured at lumbar spine (LS) and on total body (TB); LS BMAD (g/cm^3) was calculated. Z-scores were calculated with respect to a sex- and age-matched healthy Italian population.

Patients never treated with GCs had a higher BMD (TB +2.6%; LS +5.9%) than those (of same sex, age, pubertal status, and disease) who received GCs.

GCs mainly affected trabecular bone, independently of age and disease. GCs influenced bone mass differently in relation to disease and age at onset. The BMD reduction was related to GC cumulative dose (<10 g prednisone equivalent = -23%; 10-30 g = -40%; >30 g = -68%). There was a significant correlation between BMAD Z-score vs. cumulative GCs dose (r = -0.84, p < 0.001). LS bone loss was higher during the first year on GCs (-16%), and was different in different diseases.

Regarding fragility fractures, 83 (31%) of the 266 patients who received GCs sustained at least one fracture, and 33 more than one. In total, 161 fractures (39 vertebral) were recorded. 21 (15%) of the 140 patients followed after GC withdrawal sustained new fractures (all peripheral). 33 (18.9%) of the 174 patients never treated with GCs sustained fractures (all peripheral), only 6 of them having more than 1 fracture (p < 0.01).

This prospective study demonstrates that GCs cause bone loss and fractures also in young patients, affecting normal bone accrual. The fracture rate (at any site) significantly increases during GCs treatment. Vertebral fractures were observed only in patients receiving GC treatment.

P163 Phenotype-genotype correlation and role of ancillary investigations in atypical and rare forms of Osteogenesis Imperfecta

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Background: Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders of bone formation, resulting in low bone mass and an increased propensity to fracture. It is a variable condition with a range of clinical severity. About 90% of patients with a clinical diagnosis of OI have a mutation in the *COL1A1* or *COL1A2* genes, which shows an autosomal dominant pattern (AD) of inheritance. Other genes are associated with the autosomal recessive (AR) forms of OI. Mutations in *IFITM5* have recently been described in Type V OI. Other, rare phenotypes have also been described.



For the purposes of this study, we considered patients with OI who had phenotypic similarities to Russell-Silver syndrome (RSS), OI syndromes (not commonly fitting the Sillence classification) such as Cole-Carpenter and Bruck syndrome and patients with a clinical diagnosis of Type V OI. All these conditions were collectively referred to as Atypical OI.

Aims: To identify and investigate individuals with atypical forms of OI, with a view to proposing sub-classifications and identify genotype-phenotype correlations.

Methods: Patients who fulfilled the inclusion criteria were recruited from the Sheffield Clinical Genetics and OI Services. One patient was excluded as the family did not consent to participation. Detailed phenotyping, skin biopsy for histology, including electron microscopy (EM) and collagen species analysis (CSA), urine N-terminal telopeptide (Urine NTx), skeletal survey, and sequencing of OI genes and aCGH were performed.

Results: Recruited patients (n=14) were phenotypically divided into three sub-groups: Group 1) predominant features of RSS (n=5); Group 2) OI with additional features (n=6); and Group 3) Type V OI (n=3). Common clinical features included poor growth, feeding difficulties, facial dysmorphism +/- fractures. Pathogenic single gene mutations were identified in seven patients (four in Group 1 and 2; three in Group 3); chromosomal imbalances were identified in three patients. Skeletal surveys and skin biopsies (histology and EM) have revealed some common findings. CSA has shown good correlation with molecular findings.

Conclusions: This study has enabled us to start to sub-classify patients with atypical OI. It has also established the need to consider aCGH, skin histology, EM and CSA, routinely in the investigation of such patients.

Reference: Parker MJ, Balasubramanian M, *et al.* Type 1 collagenopathy presenting with a Russell-Silver phenotype. *Am J Med Genet A* 2011; **155**(6): 1414-18

P164 Vitamin D deficiency in Moscow children and adolescents

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Objectives: to determine the prevalence and intensity of D-deficiency in children and adolescents in the metropolitan area with subtotal deficiency of ultraviolet B (55° N).

Methods: from May 2008 to May 2010 in a random sample of 163 Muscovites 0-18 years of old (9.9±0.4; girls/boys = 81/82) serum 25-hydroxyvitamin D content was determined by chemiluminescent analysis [DiaSorin Inc., USA; n=56 and Roche Diagnostics, Switzerland; n=107]. The results were evaluated according to the criteria McKenna M.J. and Freaney R. (1998).

Results: the 2-year overall frequency of subnormal vitamin values (<40 ng/ml) was 77%. Mild decrease (20-<40) is set at 32% of young Muscovites, moderate (10-<20) - 30%, severe (<10) - 15%. A mild deficiency prevailed from May to August (43±7% of n=54 vs. 27±4% of n=109 in the remaining months, RR=1.6 with 95% CI [1.03-2.5]; p<0.04), and more severe (<20 ng/ml) - from September to April (53±5%, n=109 vs. 28±6%, n=54 for others, RR=1.9 [1.2-3.1]; p=0.002). Gender differences were not found. Between chronological age and separate degrees of D-deficiency was found the weak correlation (r=+0.23-0.32; p=0.0008-0.004). The sole category of children with the best vitamin status were at the age of 0-3 years: they had normal levels three times more common (">40 ng/ml": 57±11%, n=23 vs. 18±3%, n=140 in patients 4-18 years, RR=3.2 [1.9-5.2], p=0.0001) and three times more rare were cases with moderate to severe D-deficiency ("<20" 17±8% vs. 49±4%, RR=0.35 [0.14-0.87], p<0.009).

Conclusion: in Russian largest metropolis with geographical and social disadvantage (due to low insolation and absence of mass prophylaxis)

most children older than 3 years and adolescents have vitamin D insufficiency; this unfavorable feature revealed regardless of sex, often manifests with moderate to severe degree, for the most part of calendar year.

P165 Morquio disease in two sisters: Clinical case

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Background: Morquio disease was described by the Uruguayan pediatrician Luis Morquio. It's a congenital disease caused by a deficiency of the N ACETILGALACTOSAMINE 6 SULFATASE (MPS IV A) or B GALACTOSIDASE (MPS IV B) and his frequency is 1/100.000 live births. Accumulation of mucopolysaccharides in tissues results in short stature, skeletal anomalies (vertebral column deformities), loss of hearing, visual anomalies (corneal opacities), cardiac, hepatic and respiratory problems with a life expectancy of 40 years.

Presenting Problems: Two sister with 18 month and 4 years of age, consulting for short stature (>3 SD lower for age), bone deformities at 18 months, dolichocephaly, serrated teeth, thoracic kyphosis, pectum carinatum, globular abdomen, joint thickening and genu valgus and corneal opacities in one. With spinal cord compromise toxic and atlanto occipital subluxacion with fixing atlanto occipital. Leukocytes enzymatic activity of galactose 6 sulfate with decreased (Morquio type IVA).

Clinical Management: Patient with short stature and skeletal deformities showed be suspected disease Morquio type IVA in this same family, currently in multidisciplinary follow up. Since other tissue involvement appear later on, a cardiology and ophthalmology evaluation should be done including a bone marrow analysis.

Discussion: There is promising results with new enzymatic replacement therapy and management multidisciplinary

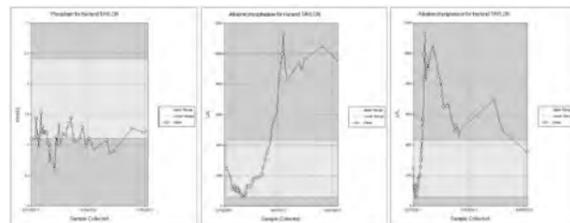
P166 Severe metabolic bone disease of prematurity following continuous veno-veno haemofiltration (CVVH)

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A first twin born at 28 weeks gestation weighing 630g underwent an end-to-end anastomosis for colonic stricture on day 92 of life. He collapsed with severe E. Coli sepsis post-operatively and became anuric. Veno-veno haemofiltration was instituted as a life-preserving measure, continuing for 3 days.

On day 119, osteopaenia and rachitic changes were noted on a chest x-ray. Review of his prior biochemistry showed a precipitous fall in serum phosphate, as well as alkaline phosphatase, followed by a rapid rise in serum alkaline phosphatase activity dating to the period of haemofiltration. The filtrate solution contained no phosphate; phosphate supplementation (1 mmol/kg/d) had been discontinued prior to his end-to-end anastomosis and not recommenced.



His skeletal survey showed multiple fractures including both femurs, both lower legs, the small bones of the feet, left distal ulna and left distal humerus and vertebral crush fractures. The fractures had not been suspected clinically. X-rays prior to his operation showed thin cortices but no rickets or fractures.

Fractures have been previously reported in the context of metabolic bone disease of prematurity (preterm MBD) in association with prolonged intravenous feeding, physiotherapy, diuretic and steroid use and with conjugated hyperbilirubinaemia. The profound loss of phosphate during hemofiltration likely resulted in severe bone disease with substantial increase in bone fragility. Loss of alkaline phosphatase activity may have allowed a transient increase in mineralisation inhibitors, contributing to the problem. To our knowledge vertebral crush fractures have not been reported previously in preterm MBD. Care should be taken to ensure adequate provision of mineral substrate in infants undergoing even brief periods of CVVH.

P167 Recurrent fractures and low bone mass in a patient with new mutation of LRP5 gene

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In recent years, the important role in bone remodelling Wnt/b-catenin pathway is highlighted. Key receptor of this pathway is LRP5 (low-density lipoprotein receptor-related protein 5). It was demonstrated in adults that polymorphism in LRP5 gene was associated with bone mineral density and fracture risk. So far no such studies were conducted in children.

Aim: The aim of the study was the analysis of LRP5 and COL1A1 genes in a patient with recurrent fractures and low bone mass of unknown aetiology.

Patient and Method: 14-year-old boy was admitted to the hospital because of recurrent low-trauma fractures. The patient sustained 5 fractures: the first took place at the age of 4, the last at 14 years of age; it was fracture of forearm (three times), humerus, metatarsal bone. There was no fracture history in the family. The father of the patient suffered from pain in the extremities and in the spine; he was diagnosed with scoliosis. There were no abnormalities in the physical examination of the patient with exception of a slight scoliosis. We diagnosed low bone mass by DXA method: Z-score index in the AP Spine was -2.2. Hormonal disorders, malabsorption syndrome, chronic inflammation, kidney and liver diseases were excluded. An analysis of the LRP5 and COL1A1 gene by direct sequencing was performed. LRP5 analysis was also conducted in the parents and siblings of the patient.

Results: We identified a new, not yet described in the literature, R1146C heterozygous mutation (c.3436 C> T, CGC> TGC) in the LRP5 gene sequences. The same mutation was found in the patient's father, but it was not present in the mother and siblings. We did not identify COL1A1 gene mutation.

Conclusions: • LRP5 gene mutation may be the cause of recurrent fractures and low bone mass in the examined patient.

• It seems likely that the scoliosis, and bone pain in the patient's father are also related to LRP5 mutation.

Acknowledgements. The study was financed as a grant NN407 060 938.

P168 Osteogenesis imperfecta - diagnostic difficulties due to clinical symptoms diversity - the results of own studies

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Osteogenesis imperfecta (OI) is a genetic bone dysplasia characterized by recurrent fractures and reduced bone mineral density. The severity of its symptoms varied from very mild to severe, which strongly affect the quality of life and cause premature death.

Aim: The aim of the study is to compare the clinical symptoms of different types of osteogenesis imperfecta and to present diagnostic difficulties based on the analysis of our patients.

Patients and Methods: The study included 83 patients with a diagnosis of osteogenesis imperfecta (type I - 34 children, Type III - 30, Type IV - 19), at the age from 1 week to 18 years. A survey on the appearing ailments and the previously used therapy, paediatric and anthropometric examination were conducted. Bone densitometry using dual energy X-ray absorptiometry was performed. In 20 patients COL1A1 gene by direct sequencing was analyzed.

Results: We revealed a statistically significantly lower bone mineral density in patients with type III OI, the best bone mineral density was in patients with OI type I (p <0.05). The total number of fractures ranged from 0 to 40, but there was no significant difference in the average fracture number between different types of OI. Skeletal deformities were present in 50/83 subjects and were the most common in patients with type III (27/30). The blue sclerae was present in the 68/83 subjects, mostly in type I and III. Dentinogenesis imperfecta was diagnosed only in 18/83 patients, with a similar frequency in all types of OI. There were no significant differences in the number of fractures and bone mineral density, or in other phenotypic manifestations of OI between patients with and without identified mutation in COL1A1.

Conclusions: • OI is a heterogeneous group of skeletal disorders associated with increased fracture risk, characterized by different genetic background and variable clinical course.

• Symptoms subject to variable phenotypic expression in different patients with the same type of OI, which often make it difficult to determine the correct diagnosis and prognosis.

Acknowledgements. The study was financed as a grant NN407 060 938.

P169 Milk, childhood and postmenopausal osteoporosis

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Objective: To determine if milk is always good for preventing osteoporosis in children anyway.

Material and method: Worldwide bibliography review on the problem and our experience.

Results: Studies performed in children and adolescents relate to the subject of the long-term relative effects on bone health of the protein content of the diet compared with that of the diet's net load of acid in the body. Long-term acid loading in humans causes an increase in renal acid excretion. People taking high doses of PPIs are more likely to break a hip. PPIs interfere with the continuous breakdown and rebuilding of bone. Consumption of animal protein, grain, and high amounts of milk increases the acidity of the body. Metabolic acidosis in humans results in a significant decrease in serum IGF-1 concentration without a demonstrable effect on IGF binding protein 3, and is related to a resistance to the hepatocellular action of growth hormone. Scant evidence supports nutrition guidelines focused specifically on increasing



milk or other dairy product intake for promoting child and adolescent bone mineralization. The catabolic effect on bone related to the magnitude of the diet's net acid load can offset the anabolic effect of higher dietary protein intakes. Sodium chloride, elevates urinary calcium excretion. Consumption of fruits and vegetables has been implicated in lowering net acid excretion. The association between fruit and vegetable consumption and indexes of bone health was first identified within the older population by the alkalizing effect of fruit and vegetable consumption.

Conclusions: Increased dietary intake of calcium during childhood, usually as calcium in milk, is associated with increased bone mass in adulthood. Whether the increase is due to the calcium content of milk, however, is not certain. Humans take milk as nourishment at any age but not the other animals.

P170

Achondroplasia: Medical and orthopedic management in a pediatric patient

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Background: Achondroplasia is a genetic disorder which affects bone growth leading to short stature. It occurs in 1 of every 25000 live births and it is characterized by short extremities, hyperlordosis, small hands and macrocephaly, with high forehead and saddle nose. Neurologic complications are due to narrowing of the vertebral foramen. It is transmitted as autosomal dominant and it is due to mutations of the FGFR3 gene which codifies the fibroblastic receptor of growth hormone expressed in provisional cartilage.

Presenting Problem: Female with 15 years old with achondroplasia, hypothyroidism, short stature, dolichocephaly, dental anomalies, shortening of the inferior segment, bilateral genu varus, forearm varus, shorter sign of both hands.

Clinical Management: Patient was treated with sodium levotiroxine and growth hormone at 0.85 mg/kg/week from age 4 until age fourteen. Height increased by 54.5 cm, from 83 cm to 137.5 cm with treatment. An elongation of lower extremities with ILISAROF was performed and height increased by 21.5 cm in two years.

Discussion: Bone elongation combined with growth hormone therapy produced better result in height than growth hormone alone.

P171

Vitamin D levels and signs of metabolic bone disease in adolescents with idiopathic scoliosis

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Objective: Biochemical assessment of teenage patients with Adolescent Idiopathic Scoliosis (AIS)

Methods: Blood sampling from 120 patients with AIS aged 12 – 17 years. The patients all had significant scoliosis and blood samples were taken a few days or weeks before they underwent scoliosis surgery. Serum 25-hydroxy-vitamin D (25-OHD), calcium, phosphate, alkaline phosphatase and parathyroid hormone (PTH) were measured. Patients with a 25-OHD level below 25 nmol/L were classified as deficient in line with current UK guidance.

Results: The 25-OHD levels ranged between 8 and 150 nmol/L. The median level was 45 nmol/L. The mean level was 51 nmol/L (SD = 28). 21 patients (17%) were vitamin D deficient, 47 patients (39%) were vitamin D insufficient meaning that 56% of the AIS patients had inadequate vitamin D levels.

PTH was significantly higher for individuals with vitamin D below 50 nmol/L when compared with individuals with vitamin D above 50 nmol/L (one-way ANOVA p<0.001).

Conclusion: Vitamin D deficiency is common among patients with AIS. Both Vitamin D deficiency and insufficiency were associated with higher PTH levels in this population indicating that metabolic bone disease may play a role in the aetiology of this scoliosis. Patients should be assessed and treated for Vitamin D Deficiency before scoliosis surgery.

P172

Longitudinal Assessment of Spinal Bone Mineral Density (BMD) in Children with Neurofibromatosis type 1 (NF1) using Dual Energy Absorptiometry (DXA) and Quantitative computed tomography (QCT)

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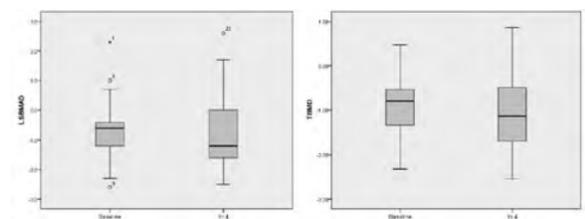
Aim: Scoliosis is a common skeletal problem affecting 10-30% of patients with NF1. NF1 patients have been shown to have reduced BMD which may play a role in the pathogenesis or progression of scoliosis. Our centre is one of four international centres currently evaluating the efficacy of various spinal imaging techniques and BMD as predictors for scoliosis in NF1. In our cohort we measured the lumbar spine (LS) BMD both by DXA and QCT.

Methods: Clinical examination, spinal x-ray and bone densitometry was undertaken in 22 children with NF1 aged 6-9 years (12 female). This was repeated at year 4. BMD of L1-L4 was measured by DXA; data was expressed as bone mineral apparent density (BMAD; g/cm³) and values transformed to Z scores using previously published normative data (ADC:2007;92(1):53-9). Volumetric trabecular BMD (TBMD; mg/cm³) of L1-L3 was also measured using QCT; values transformed to Z scores using the Mindways software (Austin, Texas). The mean difference between years 1 and 4 was calculated using a paired T test.

Results: Year 1 mean Z score LSBMAD (-0.62±1.1; p=0.01) and TBMD (-0.86±0.7; p<0.001) were less than zero. Initial data for year 4 mean Z score LSBMAD (-0.75±1.3; p=0.01) and TBMD (-1.07±0.94; p<0.001) were also less than zero. Mean difference in LSBMAD between year 1 and 4 is (-0.07±0.64; p=0.58) and for TBMD (-0.21±0.5; p=0.07).

Conclusions: Children with NF1 had reduced LS BMD which was more marked in the trabecular compartment. Furthermore this persisted during the period of follow-up.

KW is funded by Medical Research Council Grant Code U105960371.



P173

Vitamin D Prescription: A review of British National Formulary for Children recommendations, and a proposal

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Aims: To review changes in the advice regarding Vitamin D deficiency in the United Kingdom and compare these with other national guidance.

Methods: All Vitamin D guidance in the Royal College of Paediatrics and Child Health 2003 guide (Medicines for Children) and in the 8 editions since the British National Formulary for Children (BNFc) was first published in 2005 was reviewed.

Results: Dosage and Indications of Prevalence are shown in the Table.

Year	Maintenance			Treatment			Indications of Prevalence
	1m+ (units)	6m-12y (units)	12-18y (units)	1m+ (units)	6m-12y (units)	12-18y (units)	
2003 Medicines for Children	190	400	400	3000	6000	10000	
BNFc 2005	400-600 (neonatal 200-400)	400-600	400-600	3000	6000	10000	"Nutritional deficiency uncommon" "Certain ethnic groups"
BNFc 2006	[As above]						
BNFc 2007	400-600 (neonatal 200-400)	400-600	400-600	3000	6000	10000	As above
BNFc 2008	400-600 (neonatal 200-400)	400-600	400-600	3000	6000	10000	As above
BNFc 2009	400-600 (Neonatal 400)	400-600	400-600	3000	6000	10000	"Symptomatic deficiency uncommon" "Certain ethnic groups"
BNFc 2010	[As above]						
BNFc 2011	400-600 (Neonatal 400)	400-600	400-600	3000	6000	10000	"Asymptomatic deficiency common" "Certain ethnic groups"
BNFc 2012	[As above]						

Doses are higher than recommended by the UK Chief Medical Officers in 2005 and 2012. Emphasis in the remarks about prevalence has shifted from "deficiency is uncommon" to "symptoms are uncommon". Each of the 8 editions has stated that children on treatment doses should have a blood test weekly (or twice weekly) to check calcium levels.

Conclusions: The BNFc portrayal of D Deficiency as an uncommon problem has gradually changed through the editions so that since 2011 the high prevalence of asymptomatic deficiency has been acknowledged. However the recommendation that children should have regular blood tests, which is not based on evidence, and is against the consensus of national experts, has remained through the editions. We propose a new clearer national dosage guide in line with the recommendations of the Chief Medical Officer and withdrawal of the recommendation to monitor serum calcium in well children with asymptomatic D Deficiency.

P174

Elite child athlete is our future: bone lumbar spine adaptation in Egyptian children monofin athletes

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Objectives: Over the last several years, the Monofin has appeared with increasing regularity at swim practices throughout the world. Physical activity during childhood is advocated as one strategy for enhancing peak bone mass as a means to reduce osteoporosis. Clinical studies have found that non-impact sport like swimming are associated with normal to low bone densities. Little is known about the influence of monofin swimming during childhood on lumbar spine mass. This is a novel descriptive study examining bone mass indices in prepubertal monofin

children, and to quantify structural bone lumbar spine adaptation of monofin – as a non-weight-bearing sport, and compare the results with non active healthy age-matched children, and the potential determine the effects of monofin training on bone health.

Methods: Monofin children athletes (n=14) who had been training for a minimum of 3 years with a volume of 10/h/aged(12.7±2.6 yr). were compare to age-matched control non-active healthy children (n=14), aged(13.04 yr). All groups underwent Dual energy X-ray absorptiometry (DXA) to determine bone density (BMDg/cm²), bone mineral content (BMC g), and bone Area (cm²) of lumbar spine (L1-L4) were assessed. Anthropometric parameter (weight-height, chest G - Leg length), vertical jump was measured. Statically methods spss, mean ± SD, paired t test were used.

Results: BMD, BMC, and Area of lumbar spine of monofin children was significantly greater than control (p=0.01), BMD (1.07±0.08 vs. 0.74±0.05 g/cm²), BMC (44.8±2.6 vs. 26.9±2.7(g)), Area (67.7±2.03 vs. 49.4±2.31cm²). Anthropometrical and muscular power (vertical jump) of monofin was significantly (p=0.01) greater than controls, chest girth (cm) was (83.1±3.2 vs. 59.7±3.6), leg length (cm) (78.2±3.7 vs. 66.5±1.7), vertical jump (cm) was (39.6±2.5 vs. 23±1.6).

Conclusion: Participation in monofin training may enhance BMD, BMC, and Area of lumbar spine in prepubertal children, and demonstrate superior morphological shape and fitness, and improved body composition. These positive physiological, anthropometrical, and bone health effects suggest the brisk monofin training can be considered as a useful activity for osteogenic stimulus among prepubertal children.

P175

Correlation analysis of bone vibration frequency and its mass-volume ratio

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Background: Vibration analysis is a well-established technique in industry to analyse materials physical properties. The application to bone's physical properties is unclear. This study investigated the relationship between bone vibration frequency and mass/volume ratio (ρ).

Methods: We used 8 turkey bones (tibia tursus). Following soft tissue removal, a 12 cm diaphyseal section was isolated, marrow removed using a water jet and the bones dried at 25 °C for one week. Bone volume was determined by water displacement, and mass by weighing.

Bones were held in a vice at one end in a consistent manner and vibrated either using a miniature vibration motor (continuous vibration approach) or a miniature electronic hammer (impulse vibration approach). Vibration signals were recorded using CM-01B sensor. For the impulse approach, the highest peak in the magnitude frequency spectrum of the vibration signal (F) was used to determine the bone vibration frequency. For the continuous vibration approach, the difference (F_D) between the motor vibration frequency and the bone vibration frequency (obtained from the highest peak in the magnitude frequency spectrum) was used.

Results:

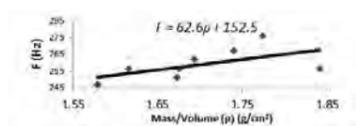


Fig.1 Vibration frequency versus ρ (impulse vibration approach)

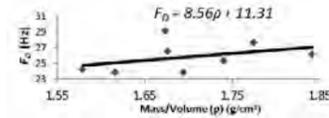


Fig.2 Vibration frequency difference versus ρ

The impulse approach correlated more strongly with ρ than did the continuous approach (correlation of F with ρ 0.57 vs 0.38 respectively).

Summary: This study suggests that vibration analysis may be a valuable technique in assessing bone mass/volume properties. This was a preliminary study and we are currently conducting a larger study to explore the findings further.

P176

One case of pseudohypoparathyroidism, clinical characterisation, follow up and treatment

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Background: Pseudohypoparathyroidism is characterized by a resistance to parathormone, with variable phenotypical and biochemical manifestations. With genetic disorder caused by heterozygous inactivating mutations in GNAS1, the gene encoding the alpha-chain of G(s), and is associated with short stature, obesity, brachydactyly, and sc ossifications. AHO patients with GNAS1 mutations on maternally inherited alleles also manifest resistance to multiple hormones (e.g. PTH, TSH, LH, FSH).

Presenting Problem: The first patient consult at 7 years old with ataxia with serious episodes of spasticity and tapeto retinal degeneration, neuropediatric evaluation shown in tets PTH of 500 pg/ml and low ionized calcium of 1.04 mmol/L and total calcium of 7.5 mg/dl without metabolic acidosis and normal lactato piruvate test and phosphate levels, for this reason the concept was made to us. Moderate deficiency of vitamin D was treated without response in low levels of calcium and whit hypocalciuric persistence. Sestamibi test was normal also renal function. DEXA Osteodensitometer shown low bone mineral density of L1 to L4. Calcitriol was begun with initial phosphate of 6,8 mg/dl, PTH of 581,9 pg/ml and hypocalciuria.

Clinical Management: Two months later with high calcium diet and calcium citrate 1500 mg BID, calcitriol 0,25 ugm BID. PTH antibodies was negative. Baclofen pump was initiated to this patient and clinical espasticity decrease.

Discussion: We comment some points of this clinical association and our experience with this difficult case

P177

The bone mineral density of children with CF

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Objectives: Low bone mineral density (BMD) is reported frequently in adult cystic fibrosis (CF) patients but the data is less consistent for children and adolescents. The aim of our study is to describe bone mineral density (BMD) in a group of children over a period of 10 years and to determine if BMD is related to vitamin D level, calcium intake, lung function, height and age.

Methods: A retrospective review of 123 dxa scans conducted in 50 children with CF was evaluated (Lunar, DPXL/ PED, Winconsin, USA). To adjust for body size we calculated apparent BMD (BMAD) of dxa reports using the Dutch reference database¹ as recommended by the International society for clinical densitometry (ISCD)². Subsequent dxa scans were repeated at 1, 2 or 3 years depending on severity. Nutritional

status (BMI) pulmonary status (FEV1%), vitamin D, calcium intake (5 day food diary) and age were evaluated as potential correlates of BMAD. Minitab statistical package (version 14) was used to analyse the data.

Results: Fifty children (24 girls) with mean age 12.8± 2.1(SD) years at time of first dxa scan were included. BMAD was normal (dxa z score of >-1) in 64% of children while 30% had dxa Z score between -1 to -2 and 6% had low BMD with dxa Z score of < -2. Follow-up dxa scans at 1, 2 and 3 year intervals revealed deterioration rates of 2%, 43% and 75% respectively. The main determinant of BMAD was FEV1, BMI and height. Calcium intake or vitamin D level did not influence BMAD.

Conclusion: Low BMAD was present in a significant proportion of children and adolescents with CF independent of age or sex. BMAD depended on pulmonary function, height and BMI. This study highlights the importance of earlier and more regular monitoring of BMD in children with CF to help prevent deterioration of their bones.

P178

Hypomagnesaemia with hypercalciuria secondary to mutations in the claudin gene: A single centre experience

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Introduction: Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis is a rare autosomal recessive renal tubular disease caused by mutations in Claudin 16 and 19 genes, often complicated by progressive renal failure. We describe the clinical and genetic features and management of this condition in 3 patients at our Centre.

Case 1: A 3 year old south Asian boy with consanguinity presented with hypocalcemic seizures. Investigations revealed hypomagnesaemia and hypercalciuria with extensive nephrocalcinosis. Genetic studies confirmed homozygous inactivating mutation in the Claudin 16 gene. Over last 2.5 years he had stable renal function and normal blood pressure, and he remained on oral citrate and magnesium supplements, and hydrochlorothiazide. He has now developed hyperphosphatemia with secondary hyperparathyroidism and chronic kidney disease stage 2 (GFR 69, normal being >80), and needs 1-alfa hydroxycholecalciferol with dietetic management for phosphate control.

Case 2: A 3 year old white Caucasian girl with consanguinity presented with recurrent urinary tract infections, hematuria, and a large bladder stone requiring lithotripsy. Her investigations confirmed hypomagnesaemia with hypercalciuria and extensive nephrocalcinosis. Genetic studies revealed homozygous missense mutation in the Claudin 16 gene. Over last 4 years, she has remained well on oral magnesium and citrate supplements, and hydrochlorothiazide with normal blood pressure and renal function.

Case 3: A nine month old white Caucasian girl presented with 2 episodes of febrile convulsions. Investigations revealed profound hypomagnesaemia with normal other biochemical parameters. Urine calcium and magnesium levels were initially immeasurable. Once serum magnesium was normalised on oral magnesium supplements, she developed progressive hypercalciuria. Genetic studies show a heterozygous mutation in the Claudin 19 gene. Over last 9 months she has remained well with normal growth and development, and no vision problems. Her renal ultrasonography is currently normal; with normal blood pressure and renal function.

Conclusion: Mutations in the Claudin genes are known to cause hypomagnesaemia, hypercalciuria and progressive nephrocalcinosis due to paracellular absorption defects in the renal tubules. Long term prognosis is poor with eventual renal failure requiring renal replacement therapy. Our experience suggests that children with this condition can have widely variable clinical presentation and progress. While Claudin 19 mutation is also associated with profound mental retardation and visual problems, the milder course in Case number 3 may be due to a heterozygous mutation that influenced the severity of the disease.



P179

The Impact of Selective Serotonin Reuptake Inhibitors on Bone Mineral Density in the Pediatric and Young Adult Population

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Objective: Serotonin is a neurotransmitter with multiple functions in the gastrointestinal tract and CNS. Recent animal studies indicate that serotonin regulates bone mass and remodelling. In humans, a handful of studies have shown decreased bone mineral density (BMD) in adults treated with selective serotonin reuptake inhibitors (SSRI) for depressive symptoms. Although SSRI's are prescribed in pediatric practice, there are few studies examining the effect of SSRIs on bone mass in children and young adults. The objective of this study was to examine if SSRI's are related to BMD in pediatric subjects.

Methods: Cross sectional epidemiologic study utilizing data obtained from the 2005-2010 National Health and Nutrition Examination Survey (NHANES). We compared DEXA scan data from subjects taking a single SSRI aged 8-20 years. DEXA information is only available for subjects aged 8 years and older. We then used multivariate regression analysis adjusting for age, gender, height, weight and ethnicity.

Results: Out of 8,838 subjects, we found 92 subjects with valid DEXA data. The mean age of the subjects in the study group was 15 years. SSRI use was revealed to be a negative independent factor on femur BMD ($R^2 = 0.49$, correlation coefficient = -0.054 , $p \leq 0.00$) and whole spine BMD ($R^2 = 0.64$, correlation coefficient = -0.037 , $p \leq 0.011$).

Conclusion: Our findings suggest that SSRI use has a negative impact on BMD in the pediatric and young adult population. Further prospective studies are needed to characterize this effect.

P180

Beyond Brittle Bones: A Preliminary Report from the Osteogenesis Imperfecta Adult Natural History Initiative

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Background: Osteogenesis imperfecta (OI) is a heterogeneous, rare disorder most commonly affecting Type I collagen. The OI Adult Natural History Initiative (OI ANHI) was established following a 2010 Osteogenesis Imperfecta Foundation (OIF) national meeting at which patient participants noted that (1) there is little information about the natural history and progression of OI beyond childhood, and (2) most of the data available are focused on musculoskeletal issues. Adults with OI face the possibility of complications in nearly every organ system in the body, and yet there is little information about the extent of these complications.

Presenting Problem: Although the cardinal manifestation of OI is bone fragility, the clinical picture includes impaired dentition, joint laxity, hearing loss, cardiopulmonary problems, etc. While the literature offers a range of case studies, the low prevalence of OI means that few papers offer a full picture of these complications. The OIF and members of the medical community, in collaboration with adults with OI, created the OI ANHI to address this gap.

Clinical Management: The OI ANHI established a web-based portal within the existing OIF website to create a cost-effective outreach and data management clearinghouse to survey the OI community and capture a first-ever "snapshot" of the health status, needs, and priorities of adults with OI. The survey included questions on general health concerns and

health behaviors plus a review of systems, with scale-based rankings of respondents' priorities, concerns, and impacts of common impairments and conditions within each medical system.

Conclusions: More than 950 individuals completed the ANHI survey. Over 93% of respondents completed the survey on the web. The OI ANHI survey identified numerous opportunities for future research. As our next step, we plan to pilot a system-specific health module to determine whether a patient reported outcomes tool can measure discernible differences in pulmonary function in individuals with OI. The marked heterogeneity of OI also demands a clearer methodology for stratifying patients; therefore we plan to use survey measures to explore the development of a new classification/stratification system for OI. It will be essential to recruit more men and minorities in future surveys.

P181

Long-term imiglucerase/algucerase treatment in Latin American children with type 1 Gaucher disease: Lessons from the International Collaborative Gaucher Group (ICGG) Gaucher Registry

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Objective: Evaluate the clinical characteristics of all Latin American pediatric patients with Gaucher disease type 1 (GD1) enrolled in the ICGG Gaucher Registry at baseline and investigate long-term outcomes and clinical benefit of prolonged imiglucerase/algucerase therapy in patients with manifestations of GD1 at baseline.

Methods: All Latin American patients with GD1 in the ICGG Gaucher Registry (NCT00358943) who were <18 years of age at the start of therapy and received treatment with imiglucerase (Cerezyme®, Genzyme) or algucerase (Ceredase®, Genzyme) were identified. Of the eligible patients, subsets were classified based on clinical diagnosis of anemia, thrombocytopenia, hepatomegaly, splenomegaly, bone disease and/or growth retardation at baseline and who had 1 or more follow-up assessments. Data were analyzed using nonlinear mixed models. An alpha-level of 0.05 designated statistical significance.

Results: As of October 2011, 443 Latin American patients with GD1 met the inclusion criteria. At baseline, 174 patients reported anemia (mean hemoglobin 9.2±1.29 g/dL); 184 reported thrombocytopenia (mean platelet counts 80.6±1.37 ×10³/mm³); 19 reported hepatomegaly (mean 2.1±0.65 MN); and 33 reported splenomegaly (mean spleen volume 21.4±9.98 MN). Mean height Z-scores were -1.3±2.93 (225 observations); 74 reported bone pain and 24 reported bone crises. After 8 years of imiglucerase/algucerase treatment, patients showed significant ($p < 0.05$) improvements in mean hemoglobin levels, platelet count, liver and spleen volumes, bone pain, bone crises and growth.

Conclusion: Among ICGG Gaucher Registry GD1 pediatric patients from Latin America, continuous and long-term imiglucerase therapy improves hematological, visceral and skeletal symptoms and reverses most of the clinical and biochemical manifestations of GD1.



P182

Reference curves for Bone Health Index for Han children from five large cities in China, and a comparison to Asian-American children

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Objective: The Bone Health Index (BHI) has previously been shown to be 1.5% lower in Asian children than in Caucasian children, both living in USA. The aim of this study is to present reference curves for BHI of Chinese Han children and to compare to Asian children living in USA.

Method: BHI is derived from the cortical thickness in the three middle metacarpals. It is determined with the BoneXpert medical device, which automatically analyses a standard bone age hand radiograph. The measurement result is independent of the sharpness of the image. The data were from 6200 children from five major cities in China recorded in 2005. For comparison we use the Asian-American reference curves based on 280 children from Los Angeles recorded 1993-2006.

Results: The Chinese reference curves cover the bone age range 3 to 17 years for boys and 3-15 years for girls and show a steeper rise in puberty for boys than for girls as previously seen in other populations. An average BHI level is defined by the average over the bone age range 7-14 years for boys and 6-14 years for girls. This BHI level is found to be consistent with being the same in Chinese and Asian-American children.

Conclusion: BHI was designed to exhibit the minimum variance for children of the same bone age and gender in a given population. This lead to a scaling of the cortical bone thickens with a certain power of the bone width and the bone length, and this can be understood to "take out" the "uninteresting" dependence on the highly variable size and proportion of the children. The Asian children in USA and China compared in his study could have different adult heights, but were found to have the same BHI. This suggests that BHI could be a good candidate for a universal measure of bone health and that the amount of cortical bone could have reached a similar optimum in these two populations. The broad availability of bone age hand X-rays across the world, as well as over the long history of radiology, makes BHI a useful tool for auxological studies of children's bone health.

P183

Juvenile Idiopathic Osteoporosis - a case study

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Background: This case illustrates the longitudinal improvements quantified by serial bone mineral density scans in an adolescent female with juvenile idiopathic osteoporosis treated with Pamidronate.

Presenting Problem: The girl in question presented at 11 years and 10 months with a 5 month history of increasing back pain. A MRI scan prior to referral had indicated vertebral collapse and osteopenia. She had no medical, social or developmental history of note. There was no family history of bone problems. Her diet was varied with no restrictions. Her exercise had been restricted by the back pain. Her height was 138cm [$<10^{\text{th}}$ centile] for a predicted mid parental height of 160 cm [25th centile] while her weight had increased recently and was 47.3 kg [75-90th centile].

Clinical Management: Once all secondary causes of osteoporosis were excluded and a clinical diagnosis of juvenile idiopathic osteoporosis was reached, then intravenous Pamidronate treatment was commenced. She received IV Pamidronate 1mg/kg for 3 doses every 3 months for three years then six monthly for a fourth year. After the first six months she became asymptomatic for bone pain and serial DXA scans showed improvement in lumbar spine bone mineral density from 0.381g/cm² at diagnosis [Z-score -4.3] to a peak of 0.884 g/cm² at cessation of

bisphosphonate therapy and this has been maintained at 0.855 g/cm² [Z-score -1.4, 3 years post bisphosphonate therapy]. DXA spinal images demonstrate thickening of superior and inferior endplates, reminiscent of a rigger-jersey spine. Resolution of pain resulted in an early return to exercise and normalisation of her weight to the 25th centile over the subsequent three years.

Discussion: Juvenile osteoporosis is a condition mostly affecting female adolescents that can result in spontaneous resolution. It is a condition of exclusion. Treatment with bisphosphonates has been proposed but the effect can be difficult to quantify in view of the natural improvement of the condition. This case underlines the rapid resolution of symptoms and serial improvements of bone mineral density with Pamidronate therapy, but with residual endplate thickening.

P184

High FGF23 measurements in a child with Vitamin D dependent rickets type I. Cause or consequence?

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Background: Defects in 1- α -hydroxylase enzyme activity result in reduced activity of 1, 25(OH)₂D causing Vitamin D dependent rickets. Physiologically FGF23 levels are stimulated by a rise in 1, 25(OH)₂D which in turn suppresses 1- α -hydroxylase expression to complete the feedback loop.

Presenting Problem: A 15 month old Irish Caucasian girl was referred by her GP for failure to weight bear. She was born at term via elective section, and was bottle fed with no dietary or absorption issues. She had prominent swelling of her wrists, femora and tibiae but no genu valgum or varum, talipes or spinal anomalies. X-rays demonstrated metaphyseal flaring of all long bones suggestive of rickets. Skeletal biochemistry confirmed this with raised alkaline phosphatase, low-normal calcium and low phosphorus. Serum 25OHD was raised while 1, 25(OH)₂D was inappropriately low. Subsequent genetic testing identified a heterozygous mutation in the 1- α -hydroxylase gene considered to be pathogenic because it creates a frameshift mutation changing the amino acid sequence from position 387 with a premature truncation. The mutation is paternally inherited.

Clinical Management: The girl was commenced on calcium supplementation with One Alpha replacement resulting in improvements in serum PTH, alkaline phosphatase, calcium and phosphorus over subsequent months. FGF23 levels were measured at initial assessment and during recovery and are persistently raised [Range 127-190 RU/ml, reference range <100] despite persistent low phosphorus levels thought to be secondary to the raised PTH level. Clinically, her demeanour has improved from initial treatment and after six months she began weight bearing without difficulty and showing interest in walking.

Discussion: The phenotype of this girl reflects the diagnosis of a 1- α -hydroxylase mutation albeit with the identification of only one affected allele. Blunted activity of 1,25(OH)₂D and hypophosphataemia should both result in the lowering of FGF23 levels yet counter intuitively the levels remain high. Is this a cause or consequence of therapy?

LB1

Influence of age and gender on Spine Bone Density and TBS microarchitectural texture parameters in infants

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Children bone knowledge is relatively sparse. This is especially true for infant and for bone microarchitecture data. We have investigated, in this



study, the age-related modifications of spine microarchitectural texture, as assessed by TBS, on male and female infants during their two first years of life.

The study group was composed of 143 and 109 healthy female and male infants aged between 0 and 2 years. Height and weight Z-scores were significantly lower than zero (-0.61 and -0.65 for female; -0.62 and -0.77 for male), showing that infants were shorter and underweight relative to The WHO Child Growth Standards. The areal BMD (aBMD) was assessed at spine L1-L4 using a prodigy densitometer (GE-LUNAR, USA). TBS was evaluated using TBS iNsight v2.0 (Medimaps, France). The LMS statistical method proposed by Cole and Green (Stat Med, 1992) was used to construct aBMD and TBS age-related curves for each gender using R software (v2.15.3).

Female and male infant shave the same mean age, height and weight Z-score and TBS ($p>0.3$) whereas female infants have higher aBMD than male ($p=0.01$). Before and after 12 months of age, TBS and aBMD correlations in both female and male infants were low ($r^2<0.20$). We have observed a first TBS decrease from birth to 12-15 months (for female and male infants respectively) followed by a TBS increasing without reaching the mean birth TBS value. In parallel, the aBMD always increases (see figure 1).

To date, our study is the first which has evaluated the modification of spine microarchitectural texture occurring in infants. Results obtained showed similar aBMD and TBS evolution shapes for both male and female infants. Surprisingly, TBS evolution exhibits a decreasing/increasing pattern. We can hypothesis that this pattern correspond to the infants bed-rest phase (no weight loads on the spine-> trabecular structure adaptation using minimum energy adaptation rule-> TBS decreasing) and the stand/walking phase (weight loads on spine->mechanical stress increasing -> trabecular structure positive adaptation -> TBS increasing). Further studies have to be conduct to confirm these first findings.

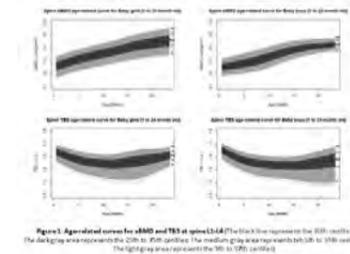


Figure 1. Age-related curves for aBMD and TBS at spine L1-L4. The black line represents the 25th centile. The background area represents the 5th to 95th centiles. The median gray area represents the 5th to 95th centiles. The light gray area represents the 95th to 99th centiles.

LB2

WNT1 Mutations in Early-onset Osteoporosis and Osteogenesis Imperfecta Identify a Key WNT Ligand Regulating Bone Mass

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The role of the WNT pathway in skeletal maintenance has been extensively studied since the identification of mutations in key signaling WNT mediators (LRP5 and sclerostin) in high and low bone mass phenotypes. However, the identity of the key WNT ligand that signals via LRP5/6 has remained unknown. We aimed to identify genes with a major effect on the skeleton by studying individuals and families with early-onset osteoporosis or osteogenesis imperfecta (OI).

We recruited a Finnish family with severe early-onset and dominantly inherited osteoporosis, characterized by low BMD and vertebral fractures, in 10 individuals. Histomorphometry showed severe low-turnover osteoporosis with low bone formation in two adults and reduced bone remodeling in a 14-year-old boy. A genome-wide microsatellite scan, fine-mapping and targeted next-generation sequencing of the linkage region identified a single novel variant in *WNT1* (p.C218G) segregating with the phenotype. We also ascertained a Laotian Hmong family with two severely affected daughters suffering from recessive OI. After exclusion of mutations in the known OI genes we performed whole-exome sequencing and identified a homozygous nonsense mutation in *WNT1* (p.Ser295*) in both affected children.

The mutant *WNT1*^{C218G} and *WNT1*^{S295*} proteins were stable and exhibited similar cellular distribution to the wild type (wt) *WNT1*. In contrast with wt-*WNT1*, *WNT1*^{C218G} and *WNT1*^{S295*} did not induce significant accumulation of active β -catenin in the nucleus. Accordingly, *WNT1*^{C218G} and *WNT1*^{S295*} showed significantly reduced capacity to induce canonical WNT signaling in a TOPFLASH reporter assay. They also had impaired capacity to induce the WNT target gene expression and osteoblast differentiation in vitro. In expression profiling by RT-PCR we detected *Wnt1* expression in mouse brain, femur and spleen but not in calvarial osteoblasts, osteoclasts or human mesenchymal stromal cells. *Wnt1* was clearly expressed in bone marrow, especially in B cell lineage and hematopoietic progenitors. Using a *Wnt1*-Cre crossed with a reporter mice *Wnt1* expression was also detected in a subset of osteocytes.

Our results suggest altered cross-talk of WNT signaling between hematopoietic and osteoblast lineage cells as the pathogenetic mechanism. These findings indicate that loss-of-function heterozygous or bi-allelic mutations in *WNT1* result in early-onset osteoporosis or OI and identify *WNT1* as a key WNT ligand in the regulation of bone mass.



P185

An unusual presentation of progressive osseous heteroplasia in a 7-year-old female child

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Background: Progressive osseous heteroplasia (POH) (OMIM 166350) is a rare autosomal dominant condition, characterized by heterotopic ossification of the skin, subcutaneous fat and deep connective tissue. This condition is distinct from Albright's hereditary osteodystrophy or McCune Albright syndrome (AHO) (OMIM 103580) and fibrodysplasia ossificans progressiva (FOP) (OMIM 135100).

Presenting Problem: We present an unusual presentation of POH in a seven-year-old female child. The clinical features included a painful swelling on the left heel, with mechanical complaints. There was no congenital hallux valgus. Family anamnesis was positive in the father. There were subcutaneous ossifications of his left upper arm, right-sided thorax and lateral side of the right ankle. The father did not allow any radiographs or further examinations. Radiographic examination of the patient revealed ossified subcutaneous plaques on the left heel, thoracic spine and both scapulae. Additional blood samples were analyzed, revealing no pseudohypoparathyroidism. Sequence analysis of the gene associated with POH, the GNAS1 gene, revealed the heterozygote mutation c.565_588del, previously found in AHO. Histopathological examination of the subcutaneous ossification showed presence of chondrocyte clusters, a feature usually found in FOP.

Discussion: The combination of the clinical features, the absence of pseudohypoparathyroidism, histology revealing chondrocyte clusters and the specific GNAS mutation in this patient, makes this a truly unusual presentation of POH. The findings in the described case might denote subdivisions of POH. The condition is associated with progressive superficial to deep ossification, progressive restriction of range of motion and recurrence if excised. We hope to inform pediatricians and orthopedic surgeons in order to create more awareness of this disorder so that unnecessary treatments can be avoided, and proper counseling offered.

P186

Bone mass, Bone microarchitecture (TBS) and anthropometric measurements during childhood growth in Spanish girls

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The aim of the present study was to evaluate bone mass and bone microarchitectural texture as assessed by TBS (Trabecular Bone Score) modification at spine during childhood growth in girls.

The study group was composed of 415 healthy girls aged between 2 and 17 years old. Height, weight and BMI Z-scores were evaluated and compared to The WHO Child Growth Standards. Pubertal stage was evaluated using Tanner score. The areal BMD (aBMD) was assessed at spine L1-L4 using a prodigy densitometer (GE-LUNAR, USA). Pseudo 3D BMD (vBMD) was calculated based on cylindrical model proposed by Kroeger et al. (Bone Mineral, 1992). TBS was evaluated using TBS iNsite v2.0 (Medimaps, France). The LMS statistical method proposed by Cole and Green (Stat Med, 1992) was used to construct aBMD, vBMD and TBS age-related curves using R software (v2.15.3).

Mean age, Weight, Height and BMI Z-scores were respectively 10.9±4.4 years, -0.22±1.3, -0.44±1.3 and 0.02±1.2 SD respectively. Positive significant correlations (p<.05) exists between TBS and age, BMI, aBMD and vBMD (r= 0.39, 0.27, 0.47 and 0.43 respectively). Height,

weight and BMI followed normal pattern with age (data not show). aBMD increases with the growth with an acceleration at the puberty (as presented figure 1 a). This finding is consistent with the data of Kalkwarf et al (JCEM 2007). When normalized by the 3D volume, effect of puberty on vBMD is more visible (see figure 1b). Before the puberty, vBMD trend seems to be flat. Concerning TBS, we observed first a decreasing phase until the puberty follow by an increasing phase until 17 (fig 1c).

DXA can be used to assess trabecular bone microarchitectural texture, as assessed by TBS, in children with a high degree of reliability. Age-related TBS curve can be useful, in complement to the BMD curve, to help clinician to identify children with bone microarchitectural modifications induced by chronic diseases or drug therapies.

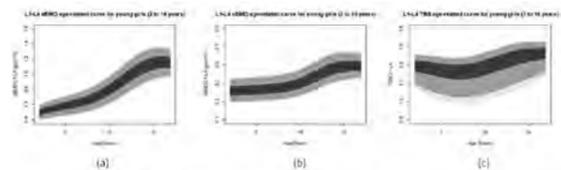


Figure 1: Age-related curves for aBMD and TBS at spine L1-L4 (The black line represents the 50th centile. The dark gray area represents the 25th to 75th centiles; The medium gray area represents the 5th to 95th centiles; The light gray area represents the 3th to 97th centiles)

P187

Juvenile Idiopathic Osteoporosis responsive to intravenous Alendronate

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Background: Osteoporosis in otherwise healthy children demands thorough study as it may be the first manifestation of an occult illness. When other diseases can be ruled out, Juvenile Idiopathic Osteoporosis (JIO) is the purported diagnosis.

Presentation: We report the case of an 8-year old boy with no personal or family history of chronic disease who presented with sudden ankle pain unrelated to trauma and not responsive to rest and ibuprofen treatment. Physical examination revealed no abnormalities other than ankle tenderness. X-ray showed a distal tibial fracture after which DEXA was performed and a lumbar bone mineral density (BMD) of 0.541 g/cm² (Z-score -1.7) was found. Basic haematological, biochemical and endocrine determinations were within normal ranges except for vitamin D. The patient was discharged with supplementation and a 6 month follow-up was scheduled, at the time of which he was unable to walk or stand because of leg pain. He was admitted and new tests performed included renal function, rheumatological and bone turnover markers, with no significant alterations. X-rays found an asymptomatic fracture of the wrist, platyspondilia of lumbar vertebrae and a "salt-and-pepper" image of the skull. Lumbar MRI confirmed lumbar deformities but showed no signs of radicular lesion. New DEXA showed a BMD of 0.448g/cm² (Z-score -2.8, minus 17.8% from previous). Bone SPECT found no new bone lesions suggesting tumoral activity. Bone marrow aspirate revealed normal cellularity, bone biopsy showed diminished number and thickness of bone trabeculae. Eventually the diagnosis of JIO was established and he received a 3-day treatment with IV pamidronate prior to discharge. 4 and 8 months later, BMD Z-scores were -2.4 and -1.5 with no further treatment required. The patient regained full mobility as pain subsided and he had a moderate linear catch-up growth during the following year.

Conclusions: JIO is a rare disease of unknown cause presenting in prepubertal children, often self-limited and with spontaneous resolution, treatment is focused mainly on preventing bone deformation, particularly scoliosis. Biphosphonate treatment may be attempted, as in this case, to hasten recovery.



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Abstract withdrawn

P189

Clinical features of temporary brittle bone disease

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Temporary brittle bone disease has been a controversial explanation of multiple unexplained fractures in early childhood. Evidence for its existence is growing. We report the clinical and laboratory features of 104 patients investigated personally. These patients had in aggregate 984 fractures or fracture-like lesions.

Our patients included disproportionate numbers of infants born preterm or as a result of multiple pregnancy. The fractures were mainly identified in the first six months of life and entirely within the first year of life. Most fractures were asymptomatic, particularly the many rib fractures and metaphyseal lesions. 140 fractures were diaphyseal including 81 transverse fractures. Few patients had evidence of bruising at presentation; none had clinical evidence of inflicted injury commensurate with the fractures found.

In several cases fractures took place while the children were in hospital. Unexplained bruising and sub-conjunctival haemorrhages also occurred in hospital, suggesting a collagen defect. Hernias were recorded; in most these resolved spontaneously, again suggesting a transient collagen defect. Among the unexplained symptoms of the patients was a history of vomiting, often projectile vomiting. Some patients had unusually blue or grey sclerae for the child's age. Many patients had abnormally large anterior fontanelles. Laboratory findings included anaemia, neutropenia and an exceptionally high serum alkaline phosphatase.

Our findings reinforce the view that children with temporary brittle bone disease have a distinctive and identifiable disorder and that they are not the victims of non-accidental injury. The potential causes of this disorder including rickets, scurvy and copper deficiency will be discussed. While the causes remain unclear, its characteristic features should be recognised.

P190

Vitamin D deficiency rickets in neonates

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This paper reviews clinical reports of vitamin D deficiency rickets in neonates from 1930 onwards. In 24 reports there was good evidence of maternal deficiency. In some the diagnosis of the rickets led to the identification of symptomatic osteomalacia in the mothers; several had been severe and longstanding. Of the 15 mothers who had assays for serum 25-hydroxyvitamin D (25OHD) 13 had values < 25 nmol/L (10 ng/mL) and 10 had values < 12.5 nmol/L (5 ng/mL).

Presentations in the infants included craniotabes, wide skull sutures, rachitic rosaries, enlargement of the wrists and ankles, tetany and hypocalcemic convulsions. In three cases rickets had been suspected from antenatal X-rays. In four cases fractures were found at the time of initial presentation. Of the 19 infants with serum calcium assays 12 had values lower than 2.0 mmol/L (8.0 mg/dL). A variety of methods had been used for serum alkaline phosphatase assays but of 18 infants at least 15 had abnormally high levels for a child. Of the 11 infants in whom serum 25OHD was measured before treatment, all had values < 25 nmol/L (10 ng/mL). Eight had values < 12.5 nmol/L (5 ng/mL). Seven infants had assays for serum parathyroid hormone; all had substantially raised values.

These reports provide strong support for the view that maternal deficiency leads to overt bone disease from before birth. Larger surveys have shown that maternal deficiency leads to long-term impairment of

bone quality in postnatal life. The importance of ensuring adequate vitamin D nutrition in pregnancy is emphasised.

P191

Long-Term Follow-Up in Stuve-Wiedemann Syndrome: A Case Report

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Background: Stuve-Wiedemann syndrome (SWS, OMIM 601559) is a severe autosomal recessive condition characterized by bowing of the lower limbs with cortical thickening, wide metaphyses, abnormal trabecular pattern and camptodactyly. Additional features include dysautonomia symptoms with temperature instability, respiratory distress and sucking/swallowing difficulties in the first months of life.

Most SWS cases do not survive beyond the first year of age, unexplained hyperthermia and respiratory distress being a frequent cause of death. The number of survivors is small, and it is not known at present what proportion of infants with SWS may survive.

Therefore, the clinical phenotype with age has not yet been clearly characterized.

Presenting Problem: Here we report on the natural history of a girl with SWS, born from two consanguineous Romanian parents. She presented osteodysplastic signs and autonomic nervous system symptoms: lack of corneal reflex and neuropathic keratitis, absence of fungiform papillae, paradoxical sweating at low temperature, patellar hyporeflexia, and progressive scoliosis.

The diagnosis was molecularly confirmed in Paris by the detection of a homozygous mutation in exon 7 of LIFR gene: c.756_757insT.

At the age of 9 years old she developed significant joint swelling of both knee with subsequent inability to walk. Doppler-US detected synovial hypertrophy and significant effusion.

Clinical Management: Coagulation, metabolic, endocrine, and immunological studies were all normal with the exception of elevated C-reactive protein (3, normal value <0,5 mg/dl) and Serum alkaline phosphatase levels (1,439 U/L; normal values 145-420 U/L).

Radiographically, our patients have a pattern of bowing, metaphyseal undertubulation, rarefaction, and striation that has been reported before in long-term survivors.

In order to avoid the progressive decrease in knee mobility described in survival patients that lead to became wheelchair dependent, ultrasonography was used for guidance of synovial aspiration and steroid injection. Synovial fluid cell count showed a white cell count of about 1,000 with prevalence of monocyte cells. The increased synovial fluid appears to originate from dilated capillaries adjacent to altered joint structures, suggesting a primary role for mechanical factors.

Discussion: Follow up beyond adulthood will permit to better define the range of the clinical manifestations, and the natural history in adulthood.

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The assesement of the vitamin D supply in the population of Polish children at the age of 9-12 years - multicentre research- preliminary report

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Introduction: The importance of vitamin D in metabolism, bone growth and functioning of many organs and systems (the pleiotropic effect) has been broadly discussed in the literature recently. The systemic deficiency of vitamin D connected with the lower sunlight exposure and the decreased diet supply favours to bone mineral density lowering and the bone structure disorganization. More and more often the common vitamin deficiency in children and adolescents has been observed. It is universally known that even temporary shortage of vitamin D during the rapid growth may hamper the correct peak bone mass acquisition.

Aim: The aim of the study was to determine the vitamin D supply in schoolchildren from the different regions of Poland.

Patients and methods: The study comprised research centers from the 6 cities in Poland: Łódź (coordinator), Białystok, Katowice, Szczecin, Lublin, Poznań. The healthy schoolchildren at the age of 9-11,99 were examined. In every child the liver metabolite of vitamin D was detected twice (first after the winter season, secondly after the summer). The serum was analysed in the laboratory with the international certificate in the Department of Biochemistry and Experimental Medicine in The Children's Memorial Health Institute in Warsaw. The serum concentration of vitamin D (25OHD) was detected with the immunochemiluminescence method with the DEQAS international control system. The sufficient 25 OHD serum concentration was recognized at range of 20-100 ng/ml. The concentration at the range of 10-20 ng/ml was recognized as vitamin D insufficiency and below 10 ng/ml as vitamin D deficiency.

Results: The 715 of children were examined. The greatest vitamin D shortages were observed in Szczecin and Białystok – in 95% and in 90% of children (insufficiency in 64% and 67% and deficiency in 31% and 23%). In Katowice and Lublin the lower vitamin D concentration was detected in 89% and 88% of children (insufficiency in 73% and 68% and deficiency in 16% and 20%). The lower shortages were revealed in children from Łódź and Poznań- in 77% and 74% (insufficiency in 57% and 59% and deficiency in 20% and 15%). The results of the vitamin D in the same children proved considerable improvement after the summer. The greatest shortages were obtained in Poznań and Szczecin – in 52,9% and 42,1% (insufficiency in 52,2% and 42,1% and deficiency in 0,7% and 0%). On the other hand in Łódź the decreased 25OHD concentration was observed in 41,5% of children and it was the insufficiency only. The lowest shortages were revealed in Lublin, Białystok and Katowice- in 28%, 26,3% and 26,3% of children and again it was the insufficiency only.

Conclusions: The lower concentration of liver metabolite of vitamin D in as many of examined children indicates on adverse diet and climatic conditions which may influence on bone mineralization in children.

The results of this study confirm the necessity of the prophylaxis of vitamin D deficiency in schoolchildren in Poland.

The considerable improvement of the 25OHD serum concentration in the majority of children after the summer may provide favourable influence of the sun exposure.

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The Effect of Levothyroxine Therapy on Vitamin D and Bone Mineral Density

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Introduction: Treatment in thyroid function disorders, which lead to clinical hypothyroidism, is replacement with levothyroxine. We aimed to investigate the effect of long-term levothyroxine therapy on vitamin D metabolism and bone mineral density in children.

Materials and Methods: Twenty-seven children with hypothyroidism receiving levothyroxine therapy (M/F:13/14, mean age, 12.1 ± 0.7 years) and 21 healthy controls (M/F:13/8, mean age, 11.8 ± 0.5) were enrolled. Calcium, phosphorus, alkaline phosphatase, parathormone and 25-hydroxy vitamin D levels were measured from serum specimens collected from the study and control groups. DEXA scans were performed and Z-scores determined.

Results: Mean duration of levothyroxine treatment was 7.7 ± 0.5 months. Serum vitamin D levels were significantly lower in the study group (17.1 ± 7.6 ng/mL) compared to the control group (23.6 ± 7.8 ng/mL) (p=0.006), but short-term treatment did not appear to have an effect on BMD Z-scores (p>0.05).

Conclusion: Long-term levothyroxine therapy can affect vitamin D levels and impair bone health. Accordingly, bone mineral density analysis would seem to be useful in long-term.

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Celiac disease underlying rickets in an adolescent

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Background: Bone health is negatively affected in children with celiac disease. Alterations in calcium and vitamin D metabolism are frequently encountered in children with celiac disease but rickets is rarely a presenting complaint.

Presenting Problem: The patient was first admitted at the age of 13 due to waddling gait and weight loss for three years.

Clinical Management: Detailed history revealed that loss of appetite and intermittent diarrhea were apparent since 3-4 years of age. His past medical history was otherwise unremarkable. Physical examination revealed a listless child with a height of 130.7 cm (SD score, -3.6), weight 16.5 kg (SD score, -5.6), body mass index 9.7 (SD score, -11.6), genu valgum, rachitic rosary, and pubertal stage Tanner I. Laboratory studies showed iron deficiency anemia, mildly elevated AST and ALT, hypocalcemia, hypophosphatemia, low vitamin D, and elevated levels of alkaline phosphatase and parathormone. Left hand X-ray revealed metaphyseal widening and fraying. Rickets due to malabsorption was considered. Positive celiac antibodies resulted in endoscopic and pathological evaluation, which confirmed the diagnosis. Gluten-free diet was introduced and intramuscular vitamin D (a single dose of 600.000 IU) along with oral calcium (75 mg/kg/d for one week) was given. Evaluation after three months revealed a contented and alert child with a weight gain of 13 kg (29.5 kg, SD score -3.0; BMI 17.1, SD score -0.78) and normal levels of calcium, phosphorus, alkaline phosphatase, 25-OH vitamin D, and parathormone.

Discussion: Undiagnosed celiac disease may result not only in vitamin D and calcium deficiency but also in rickets. Intramuscular administration of high dose of vitamin D is effective along with gluten-free diet and oral calcium.



P195

A case of progressive generalised osteolysis – a fibrous dysplasia or something else?

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Background: In pathological conditions the osteolysis is defined as a process of dissolution or degeneration of bone tissue due to abnormal bone resorption. It is rare in childhood and the diagnosis and the management is always a challenge.

Presenting Problem: We present a 10-year old boy with multiple osteolytic lesions discovered initially when he was 2 years of age because of leg length discrepancy and waddling gait. The lesions are located predominantly in the diaphyseal regions of the long bones, the pelvis and the basis of the skull. They have well-defined borders, with no periosteal reaction and show slow, but progressive evolution. The child experienced two pathological fractures in the affected zone of his distal right femur. A moderate congenital deformity of the head comprised of a hypoplastic mandibulae, small mouth and crowded teeth is also present and multiple orthodontic interventions have been done till now. The patient suffers from frequent ENT infections, difficult breathing and recurrent nose bleeding with unclear etiology. Bone histology shows fibrous changes, but on bone scintigraphy scans there are no pathological accumulations. Interestingly, whole-body densitometric measurements are in the referent limits. There are no physical signs of any skin, hematologic, endocrine or other systemic involvement, too. In 2013, because of an increasing neck pain and weakness in the left limbs, several crushed cervical vertebrae (C3-C6) with narrowing of the spinal canal were found.

Clinical Management: Two neurosurgical interventions were performed including vertebroplasty and occipito-spinodesis. A bisphosphonate therapy has been commenced, too.

Discussion: The patient presents with multiple polyosteoitic osteolytic lesions with aggressive evolution. In the differential diagnosis except *Fibrous dysplasia (McCune-Albright syndrome)* as a leading working diagnosis, *Neurofibromatosis type 1 (von Recklinghausen's disease)*, *Gorham-Stout Syndrome (Generalized lymphangiomatosis)* and many others were discussed. For most of them, the genetic background and the underlying molecular pathoetiologic mechanisms are already well-known but treatment options still remain quite limited.

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Bigger But Not Stronger? Growth Hormone Treatment in Turner Syndrome May Confer No Benefit to HR-pQCT Determined Bone Micro-Architecture

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Turner Syndrome (TS) is known to be associated with increased risk of osteoporosis and fracture. Childhood treatment with growth hormone (GH) has been considered standard of care for treatment of growth failure in TS, while the influence of GH on bone health has been poorly understood. The purpose of this study is to assess the influence of GH on bone microarchitecture on a cohort of TS subjects.

TS subjects aged 16-45 were included. Bone mineral density (BMD) was assessed at the lumbar spine, hip and radius using dual-energy x-ray absorptiometry (DXA). High resolution peripheral quantitative computed tomography (HR-pQCT) scans of the radius and tibia were completed. Bone microarchitecture analysis included total volumetric BMD (Tt.BMD), cortical BMD (Ct.BMD), trabecular BMD (Tb.BMD), total area (Tt.Ar) and cortical thickness (Ct.Th). Simulated bone strength was determined using finite element (FE) analysis. Group means were compared using independent T-tests and two-way analysis of variance.

Sixteen TS subjects were recruited, 6 GH-treated and 10 non-GH-treated. Both groups were similar in regards to age, estrogen exposure and bone health related lifestyle parameters. GH-treated subjects were significantly taller than the untreated group (152.0 +/- 3.3 cm vs 143.9 +/- 6.5 cm respectively, p=0.005). DXA BMD of hip, spine and radius was similar between treatment groups. At the radius, Tt.Ar was greater among GH-treated subjects (+17.3%, p<0.03), while Tt.BMD, Ct.BMD, Tb.BMD, and Ct.Th were similar in both groups. Similarly, at the tibia, Tt.Ar was greater among the GH-treated subjects (+21.8%, p<0.005) while the remaining parameters were not significantly different. FE determined bone strength trended higher in the GH-treated group (radius +2.5 %, p=0.9; tibia +6.3%, p=0.5), but results were not statistically different.

Despite GH treatment in TS resulting in increased height and larger bones, no significant difference in DXA derived BMD, HR-pQCT micro-architectural parameters, or FE simulated bone strength were detected. While these early findings may be due to insufficient statistical power, the significant difference in final height and bone area suggest a substantial GH effect on other aspects of bone growth. Further study seeks to understand the effects of GH on bone health in this unique patient population.

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Parathyroid Hormone administered by Continuous Subcutaneous Infusion is more effective than when given by intermittent injection

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Background: Activating mutations in the calcium sensing receptor can result in severe hypoparathyroidism with symptomatic hypocalcaemia. Complications of treatment with calcitriol or alfacalcidol include hypercalciuria, nephrocalcinosis and renal failure. The use of synthetic parathyroid hormone (PTH 1-34, teriparatide) provides a more physiological treatment option and reduces the risk of hypercalciuria.

We report our experience with such a patient who had increasing requirements of PTH on multiple daily injections over five years. The large PTH requirements significantly reduced when multiple subcutaneous injections were changed to a Continuous Subcutaneous Infusion of PTH (CSIP).

Presenting Problem: A one year old girl presented with hypocalcaemic convulsions and was found to have hypoparathyroidism due to an activating mutation of the calcium sensing receptor (CaSR) (c.2528C>A p.A843E) which prevents secretion of PTH under any circumstances. She was initially treated with alfacalcidol but continued to have episodes of symptomatic hypocalcaemia, particularly associated with intercurrent infections. After lengthy informed discussion with the parents, she was treated with daily injections of teriparatide. Normocalcaemia was initially maintained with twice daily injections but, after three years of treatment, her dose increased to 75mcg daily in three divided injections, almost twice the recommended adult dose. In order to try to reduce the total dose of PTH and the need for multiple daily injections, she was changed to CSIP using an insulin infusion pump to deliver the PTH at a constant rate throughout the day and night.

Clinical Management: Plasma calcium was 2.2 mmol/L prior to starting treatment. PTH 1-34 was infused initially at a rate of 72 mcg/d (equivalent to the dose being received by injection) but she rapidly became hypercalcaemic. 72 hours after starting the infusion, her total dose had been reduced by 50% and her calcium stabilised.

Discussion: Shortly after starting treatment with a continuous infusion of PTH, the total dose was able to be reduced significantly indicating that the PTH was much more effective when given in this manner. She no longer required multiple injections and only needed to have her giving set changed every three days. We recommend that this is the treatment of choice in such circumstances.



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Severe hypercalcemia in an infant with idiopathic infantile hypercalcemia caused by mutation in CYP24A1 gene

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Background: Idiopathic infantile hypercalcemia (IIH) is a rare cause of infantile hypercalcemia characterized by failure to thrive, vomiting, dehydration, and nephrocalcinosis. This condition has recently been associated with mutations in the CYP24A1 gene, which encodes 25-hydroxyvitamin D3 24-hydroxylase, the key enzyme of 1,25-dihydroxyvitamin D3 degradation. Until now, only 13 cases genetically tested for IIH have been reported in the literature.

Case Report: We reported a case of a 10-months-old male infant, who was referred to our Pediatric Clinic because of failure to thrive. His weight was 6560 gr (between -3 SD and -2 SD below the mean) and length was 69 cm (between -1 SD and the mean). Laboratory studies revealed severe hypercalcemia (4,58 mmol/L), hypercalciuria (3,05 u ca / u cr), suppressed parathyroid hormone (0,106 pmol/L) and elevated plasma levels of 25-hydroxyvitamin D3 (169,48 nmol/L). His renal ultrasonography scan revealed marked medullary nephrocalcinosis.

We started a conservative therapy with intravenous rehydration, diuretics and intravenous neridronate (1 mg/kg), that normalized plasma calcium levels (2,7 mmol /L).

Karyotype excluded Wiliams syndrome. Additional tests excluded malignancy, chronic renal disease, hypoadrenocorticism, granulomatous disorders and osteolysis.

Molecular testing of the CYP24A1 gene revealed a homozygous deletion (E143del). Both parents were heterozygous for this mutation.

At subsequent checks, we observed an improvement of his growth velocity and a good weight gain.

In spite of bisphosphonate therapy, the patient maintained elevated plasma levels of osteocalcin (10,4 nmol/L), bone alkaline phosphatase (92 ug/L) and the C-Terminal telopeptide of type I collagen (1210 ng/L).

: We report the case of an infant affected by IHH, caused by a homozygous CYP24A1 gene mutation, who normalized plasma calcium levels and growth velocity with conservative therapy.

The prognosis of these patients is not still clearly known, but, considered the nephrocalcinosis and the high bone turnover markers, we suggest that a closely nephrologic and endocrinological follow up is strictly necessary.

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Levels of 25(OH)vitamin D in Children and Adolescents with Type 1 Diabetes Mellitus and in Healthy Controls in Bulgarian Population

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Objectives: The aim is to examine the serum levels of 25(OH)vitamin D in children and adolescents with type 1 diabetes mellitus and in healthy controls and to determine whether patients with diabetes have higher prevalence of vitamin D deficiency/insufficiency and whether it is correlated to its metabolic control.

Methods: A cross-sectional study of 73 patients (35 males) aged 11,84±4,44 years and 27 healthy controls (15 males), aged 7,36 ± 4,71 years. The participants are divided in 2 subgroups according to the month the sample was taken- May-September and October-April. Patients are divided in 2 subgroups according to their metabolic control:

good (HbA1c ≤ 7,5%) and poor (HbA1c > 7,5%). Levels of 25(OH)vitamin D were determined by electrochemiluminescence detection technology. The statistical methods used are Mann-Whitney and Kruskal-Wallis tests.

Results: There is no statistically significant difference between 25(OH)vitamin D levels in diabetic patients and in controls (p=0,783). The mean level of 25(OH)vitamin D in patients is slightly higher- 25,39 ±8,14 ng/ml than in controls- 24,44±11,88 ng/ml. The mean levels of 25(OH)vitamin D are higher in the period with more outdoor sunlight. In May-September period – 29,01± 9,2 ng/ml in patients (n=28) and 29,85± 8,42 ng/ml (n=9) in controls, for October-April period 25(OH)vitaminD levels are 23,14±6,55 ng/ml vs 21,74±12,63 ng/ml for patients (n=45) and controls (n=18) respectively. We use US Endocrine Society guideline to define vitamin D deficiency and insufficiency as 25(OH)vitamin D level ≤20ng/ml and 21 - 29 ng/ml respectively. 36% of all patients (n=26) and 33% of controls (n=9) have vitamin D deficiency. Vitamin D insufficiency is observed in 37% of patients (n=27) and 37% of controls (n=10).

Mean level of HbA1c in good control group (n=16) is 7,05±0,33%, in poor control group(n=39)- 9,18±1,36%. No correlation between metabolic control and vitamin D levels is found. Level of 25(OH)vitamin D are 25,86±7,29ng/ml and 25,56±8,23ng/ml respectively (p=0,476).

Conclusions: Presence of diabetes mellitus type 1 does not influence vitamin D metabolism. Our results show no significant differences between 25(OH)vitamin D levels in diabetic patients and in healthy controls. Vitamin D deficiency is slightly but not significantly prevalent in diabetic patients and is almost equal to that observed in healthy Bulgarian adults.

Supported by Grant from Medical University Sofia, Bulgaria.

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Low 25-hydroxyvitamin D levels in Scheuermann's disease: a preliminary study of nineteen patients

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Objectives: Scheuermann's disease is a common cause of pediatric kyphotic deformity, and its etiology remains poorly understood. A hypothetical relationship has been described between osteoporosis and the development of the disorder. Also, a recent study revealed that young pigs developed kyphosis when fed low-vitamin D diets. Due to these factors, we designed the present investigation to measure the prevalence of vitamin D insufficiency and deficiency in a group of patients with Scheuermann's disease.

Methods: This is a case series of nineteen patients. In this study, ICD-9 codes for kyphosis, juvenile osteochondrosis of the spine, and low back pain were used to identify patients with the diagnosis of Scheuermann's kyphosis and disc disease from the clinics of the senior author at a tertiary-care children's hospital over a six-month period. Patients with radiographic evidence of Scheuermann's disease (N=20) were evaluated by the authoring surgeon personally, and the diagnosis was confirmed. The study population included eleven males and nine females. High-performance liquid chromatography/mass spectrometry assay was used to measure serum 25-hydroxyvitamin D in all these patients (age ranges 10.6 to 18.3 at testing) during the course of their regular clinic follow-up, and one value is still pending. Vitamin D adequacy was defined by a value within our laboratory's normal range (32-100 ng/mL). Inadequacy was subdivided into insufficiency (20-32 ng/mL) and deficiency (<20 ng/mL).

Results: Of the nineteen currently returned vitamin D levels (for eleven males and eight females), fifteen patients (79%) had inadequate vitamin D levels (range 14-31 ng/mL), including eleven (58%) who were insufficient and four (21%) who were deficient. Only four values (21%) were within normal limits (range 32-43 ng/mL). Mean and median values were 25.7 +/- 7.4 and 24 ng/mL, respectively.



Conclusions: A majority of these Scheuermann's disease patients assayed for vitamin D were found to be insufficient, with a substantial number qualifying as deficient. These observations, coupled with the newly discovered link in vitamin-deficient piglets, demonstrate a need for a more detailed, prospective evaluation of our Scheuermann's disease patients, with an appropriate control group, and this will be the subject of a future longitudinal study, preferably at multiple centers.

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Guided Growth with Hinge Plates for Lack of Extension and Fixed Flexion of the Knee

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Lack of extension of the knee and fixed flexion of the knee may occur in patients with arthrogryposis, rheumatoid arthritis, achondroplasia, osteogenesis imperfecta, cerebral palsy and other conditions. They develop a crouch gait and this is an energy non-efficient condition that causes a compensatory flexion deformity of the hip and lumbar lordosis. Recommended treatments have included bracing, physical therapy, posterior release, distal femoral osteotomy or progressive distraction with external fixation; but these treatments often fails. We studied 11 patients (12 knees) with: arthrogryposis (7), achondroplasia (1), osteogenesis imperfecta (1), postaxial hypoplasia and short femur (1), rheumatoid arthritis (2) and 1 knee with cerebral palsy and hemiplegic pattern. The average age was 6 years (3-13) and the average lack of extension was 40° (20°-60°). Clinical assessment included measurement of knee range motion, gait evaluation, and screening for concomitant deformities. We found three patterns: Type 1: fixed flexion with limitation of flexion and extension; Type 2: lack of extension but normal flexion; and Type 3, pro-curved deformity of the distal femur with lack of extension. One patient had previous treatment with staples that failed because of forward migration and 5 patients had been treated previously with custom plates (without hinges), in these cases we observed that the correction stops when the screws impinge the plate and lock. These 6 patients were treated changing the staple or the plate to a Hinge Plate. 82% of the knees had a full correction in 14 month, the rest are progressing. We do not find loosening of the screws or migration. We observed in some cases that after the screws impinged the plate the hinge started to move. We observed that guided growth is effective with the Hinge Plates.





Judith Adams

Judith Adams is Consultant Radiologist, Manchester Royal Infirmary and Honorary Professor of Diagnostic Radiology, Imaging Science & Biomedical Engineering (ISBE) at the University of Manchester, UK. She is a musculo-skeletal radiologist with a particular interest in metabolic bone disease (especially osteoporosis) and quantitative assessment of the skeleton. Her publications include 155 scientific papers, 20 reviews and 23 chapters and she has collaborated in over £3M research grants. Professor Adams has served as Dean (Vice President) of the Royal College Radiologists, Chairman of the Osteoporosis Group of the European Society of Skeletal Radiology (ESSR) and of the National Osteoporosis Society (NOS) Bone Densitometry Forum.

Paul Baldock

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Maria Luisa Bianchi

Dr Bianchi has been engaged in clinical activity and scientific research on bone metabolic diseases since the early 1980s. Dr Bianchi is currently working at the Istituto Auxologico Italiano IRCCS, a hospital and research institute in Milan, Italy. She has special interests in pathophysiology, problems of diagnosis and treatment of

primary and secondary osteoporosis in children and adolescents; evaluation of adherence to treatment and quality of life in osteoporosis. Dr Bianchi has been a member of the ICCBH International Scientific Committee since 1997 and is on the Board of Directors of the International Bone and Mineral Society and the European Calcified Tissue Society (ECTS) and the Editorial Boards of Bone and Calcified Tissue International. She is author or co-author of over 200 scientific articles and book chapters.

Nick Bishop

The UK's only Professor of Paediatric Bone Disease, Nick Bishop trained in Manchester (clinical), Cambridge (MRC and Wellcome Fellowships) and Montreal (visiting Professor at McGill). He was appointed to Chair in Sheffield in 1998. In 2002 Nick was appointed Head of the Academic Unit of Child Health and in 2008 Director of the Children's Clinical Research Facility. He is also Director for Undergraduate Medical Education at the Sheffield Children's NHS Foundation Trust. His clinical research group focuses on the treatment of childhood osteoporosis and his basic science group on pathophysiology of childhood bone diseases. Professor Bishop has been a member of the ICCBH International Scientific Committee since 1997 and organised the 2002 and 2009 ICCBH meetings in Sheffield and Cambridge.

Annemieke Boot

Annemieke M Boot is paediatric endocrinologist at University Medical Center Groningen - Beatrix Children's Hospital in Groningen, the Netherlands. After her study she worked as general medical doctor in Blantyre and Mangochi in Malawi from 1989 to 1992, Africa. Her training of paediatrics and paediatric-endocrinology (head Prof dr SLS Drop) was in Sophia Children's Hospital - Erasmus MC in Rotterdam, the Netherlands. Her research is focussed on bone mineral density and bone metabolism in children. She was awarded her PhD in 1997. She is a member of The European Society of Paediatric Endocrinology (ESPE) and is active in the ESPE Bone and Growth Plate working group.

Emma Clark

I am a Consultant Senior Lecturer in Rheumatology at the University of Bristol, UK. My research area is Musculoskeletal Epidemiology, with a particular interest in the role of bone mass in determining fracture risk in children and adolescents. Other areas of interest are the epidemiology of hypermobility and scoliosis in adolescents, and identification of osteoporotic vertebral fractures in older adults. I am on the Editorial Boards of



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Dr Paul Dimitri studied Medicine at the University of St Andrew's in Scotland and the University of Manchester where he received a medal in pathology and a distinction in Paediatrics. In 2010 he was awarded a PhD in Medicine and the Michael Blacow Award from the Royal College of Paediatrics and Child Health for his work on the relationship of fat and bone in children. Paul currently works as a Consultant in Paediatric Endocrinology at Sheffield Children's Hospital. He was appointed as the Director of Research and Innovation and the Deputy Director for the Medicines for Children Research Network (East of England) in 2012. Paul's research interests include the effect of obesity on skeletal growth and the development of novel imaging of bone in children and adults.

Rachel Gafni

Rachel I Gafni received her BA from Barnard College and her MD from Temple University. She completed a pediatric residency at the Children's Hospital of Philadelphia followed by a pediatric endocrinology fellowship at the National Institutes of Health (NIH), serving as an officer in the Public Health Service from 1996-2002. She subsequently served as an Assistant

Professor at the University of Maryland. Dr Gafni returned to NIH in 2007 as a staff clinician in the National Institute of Dental and Craniofacial Research. She is also faculty in the NIH Pediatric Endocrinology Training Program. She is an investigator on several protocols studying and treating patients with endocrine disorders including hypoparathyroidism, McCune-Albright Syndrome, hypophosphatemic rickets, tumoral calcinosis, and other metabolic bone diseases.

Francis Glorieux

Dr Francis H Glorieux received his MD from the University of Louvain and his PhD from McGill University. It is there that he developed his interest in heritable pediatric bone diseases. His doctoral thesis focused on hypophosphatemic rickets and the demonstration that calcitriol and phosphate allowed for control of the bone disease, a regimen still used worldwide in such patients. Since 1992 he has documented the beneficial effects of bisphosphonate in severe forms of Osteogenesis Imperfecta. Programs based on the Montreal protocols are now used all over the world. For 40 years, he has been the Head of the Genetics Unit at the Montreal Shriners Hospital for Children and a Professor at McGill University. He is the recipient of numerous Awards, and in 2004 was made an Officer of the Order of Canada, the country's highest honor for lifetime achievement.

Catherine Gordon

Catherine M Gordon, MD, MSc is a Professor of Pediatrics at the Alpert Medical School of Brown University and is Director of the Division of Adolescent Medicine at Hasbro Children's Hospital. She is board-certified in adolescent medicine and pediatric endocrinology. She is on the Board of Directors for the International Society for Clinical Densitometry, and directs the Student Research Program co-sponsored by the American Pediatric Society and Society for Pediatric Research. Her clinical interests include bone loss in pediatric chronic disease, pediatric densitometry, disorders of vitamin D and calcium metabolism, and eating disorders. Her research focuses on the effect of malnutrition on bone loss including the early osteoporosis seen in adolescents with anorexia nervosa, cystic fibrosis, and inflammatory bowel disease.

Wolfgang Högler

Wolfgang Högler is a Consultant Paediatric Endocrinologist, heading the Department of Endocrinology & Diabetes at Birmingham Children's Hospital, Birmingham, UK. Dr Högler is also Honorary Senior Lecturer at the School of Medical and Dental Sciences at the University of Birmingham, UK and current



Chair of the ESPE Working Group on Bone and Growth Plate.

Dr Högler completed his paediatric training at the Department of Paediatrics at Innsbruck University Hospital in Innsbruck, Austria. Following a clinical and research fellowship at the Institute of Endocrinology and Metabolism, Children's Hospital at Westmead, in Sydney, Australia, he worked as an Associate Professor in Paediatrics at the Medical University in Innsbruck, Austria before moving to the United Kingdom. Dr Högler's current research focuses on novel measures of mobility, bone strength and density and growth disorders. His group is currently investigating the role of whole body vibration on mobility and bone strength in children with osteogenesis imperfecta, as well as the complications of the rare growth disorder ALS deficiency on glucose metabolism and bone strength, including novel treatment options. His commitment to postgraduate education has led him to chair the endocrine branch of the IPOKRATES Foundation. He organizes paediatric endocrine specialist seminars across the globe.

JM Kassim Javaid

After completing medical training at Charing Cross and Westminster Medical School, I specialized in adult rheumatology at the Wessex Deanery. During that time, I completed a PhD examining the maternal determinants of intra-uterine bone growth as part of an ARC Clinical Fellowship at the University of Southampton. In my last year of clinical training, I was fortunate to be awarded an ARC travelling fellowship and worked with the OA group in UCSF to study the role of vitamin D and bone in lower limb OA, a fantastic opportunity. Since my return to the UK, I have been appointed as Senior Research Fellow in Metabolic Bone Disease/Honorary Consultant Rheumatologist at Oxford and further extended my research into the role of vitamin D status in musculoskeletal disease, improving outcomes after fragility fracture as well as continuing work looking into the bone phenotypes in osteoarthritis. Balancing clinical and teaching, my direction of research is evermore linking the basic science with the key clinical issues in OA and OP.

Harald Jüppner

Dr Jüppner is a Professor of Pediatrics at Harvard Medical School, chief of pediatric nephrology at Massachusetts General Hospital for Children, and a senior member of the Endocrine Unit at Massachusetts General Hospital. His research focuses on the regulation of mineral ion homeostasis and bone metabolism, with a primary interest in the PTH/PTHrP receptor and understanding its

role in bone, kidney and cartilage biology. He is furthermore interested in parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), has developed assays to measure these hormones, and helped assess their role in patients with phosphate-wasting disorders and chronic kidney disease. For more than a decade, molecular genetic studies have been the main focus of his research. His laboratory identified the molecular defect of several inherited disorders, including pseudohypoparathyroidism type Ib, Jansen metaphyseal chondrodysplasia, infantile cortical hyperostosis, and several hypophosphatemic disorders. To explore the molecular basis of these and other inherited human disorders, he has collaborated with a large numbers of investigators and clinicians.

Salman Kirmani

Dr Kirmani is an Assistant Professor in Medical Genetics and Pediatrics at Mayo Clinic, Rochester, MN. He received his medical degree from Dow Medical College, University of Karachi, Pakistan. He went to Mayo Clinic to pursue a residency in Pediatric & Adolescent Medicine, and stayed there for a fellowship in Pediatric Endocrinology. He went on to pursue further training in Medical Genetics and has been on staff at Mayo Clinic since 2009. His clinical and research interests include pubertal bone mass accrual, hereditary metabolic bone disease and connective tissue dysplasias.

Craig B Langman

Craig B Langman MD is the Isaac A Abt, MD Professor of Kidney Diseases and Tenured Professor of Pediatrics at the Feinberg School of Medicine, Northwestern University and Head, Kidney Diseases at the Ann and Robert H Lurie Children's Hospital of Chicago. He has had a career-long interest in the understanding of genetic or acquired rare and ultra-orphan diseases of kidney and bone, including nephrolithiasis. He participates in creation of evidence-based medicine guidelines, and his research is funded through the National Institutes of Health in the areas of genetic stone disease, chronic kidney disease, and cystinosis. Dr Langman served as the Chair of the ICCBH meeting held in Montreal CA in 2007.

Mary Leonard

Dr Mary Leonard, MD, MSCE is a Professor of Paediatrics and Epidemiology at the Perlmans School of Medicine at the University of Pennsylvania, and the Director of the Office of Clinical and Translational Research at the Children's Hospital of Philadelphia. Her multidisciplinary research program is focused on the assessment of bone health in children, and the detrimental effects of



glucocorticoid therapy, chronic kidney disease, muscle deficits, vitamin D deficiency and inflammation on bone development in chronic pediatric disease. Her research uses quantitative computed tomography and novel micro-MRI imaging techniques. Her research program is supported by multiple NIH investigator-initiated grants.

Agnès Linglart

Dr Agnès Linglart is a Paediatric Endocrinologist working at the Hôpital St Vincent de Paul in Paris, France. She has a special interest in rare diseases.

Craig Munns

Associate Professor Munns is a Senior Staff Specialist in Bone and Mineral Medicine and Endocrinology at the Children's Hospital at Westmead and Conjoint Associate Professor in the Sydney Medical School at the University of Sydney, Australia. Following the completion of his Paediatric and Endocrinology training at The Royal Children's Hospital, Brisbane, Australia, Associate Professor Munns was Clinical Associate in Genetic and Metabolic Bone Disorders at the Shriners Hospital for Children, Montreal, Canada. He was awarded his PhD through the University of Queensland in 2004. Associate Professor Munns' major clinical and research focus is the diagnosis and management of primary and secondary bone disorders in children.

Coen Netelenbos

J Coen Netelenbos is Emeritus Professor of Endocrinology in the Department of Internal Medicine at the VU University Medical Center (VUMC), Amsterdam, Netherlands. He is Author/Co-author of more than 200 scientific publications and Reviewer/member editorial advisory board of several international journals and reviewer of national/international grant applications for MRC's. Professor Netelenbos holds several positions on national and international societies, including President and Chair of the Scientific Committee of the Dutch Osteoporosis Foundation. He organised the First International Symposium on Children's Bone Health in Maastricht, The Netherlands (4-7 May 1999), and has remained on the organising committee since.

Gerard Pals

Gerard Pals is Director of the Centre for Connective Tissue Research at the VU University Medical Center (VUmc) in Amsterdam, Netherlands. Following completion of his MSc in Biochemistry in Utrecht he completed his PhD training in Human Genetics in Amsterdam. In 1987 he held a position as Visiting Scientist/Professor at Wayne State University in Detroit,

USA, and then as Research Scientist from 1989 to 1997 in the Department of Clinical Genetics at the VUmc. He directed the VUmc Molecular Diagnostic Laboratory from 1997 to 2007 when he took up his current position. He is active in many national and international societies, particularly those relating to Human Genetics.

Farzana Perwad

Dr Farzana Perwad is an Assistant Professor at the Department of Pediatrics, University of California San Francisco. Dr Perwad's research focuses on the regulation of vitamin D and phosphorus homeostasis in health and disease. Her research projects include investigating the pathophysiology of X-Linked Hypophosphatemia in mouse models of the human disease, and to study the molecular mechanisms of action of fibroblast growth factor-23 in the kidney.

Frank Rauch

Frank Rauch trained as a pediatrician at the Children's Hospital of Cologne University, Germany, where he started working on pediatric bone disorders in Dr Schoenau's laboratory. He then performed a research fellowship on metabolic bone disorders at the Shriners Hospital for Children, Montreal, Canada. Since 2001 he has been a clinician scientist at the Shriners Hospital and he is an Associate Professor at the Department of Pediatrics of McGill University. Dr Rauch has published 140 peer-reviewed publications and since 2009 has been the Editor of the Journal of Musculoskeletal and Neuronal Interactions. His main research areas are muscle-bone interaction and heritable bone disorders in children.

Susanne Schmidt

Susanne Schmidt graduated with a medical degree from the University of Rostock (Germany) in 1996 and received her pediatric training in Germany, Norway and Sweden. Already during this time she became interested in pediatric inflammatory bowel disease and bone mineral density. After residency, she worked as a pediatric gastroenterologist in the region of Gothenburg (Sweden) and intermittently also at the tertiary centre of pediatric gastroenterology of Sahlgrenska University Hospital. At the Institute of Clinical Science at Sahlgrenska Academy she conducted clinical studies as part of her PhD work, and in 2010 she defended her PhD thesis with the title "Bone mineral density in pediatric inflammatory bowel disease". Susanne moved very recently to New Jersey (USA), but remains involved in an ongoing project in Gothenburg focusing on the development of bone mineral density in patients with inflammatory bowel disease during the transition from adolescence into adulthood.



Marry van den Heuvel-Eibrink

Marry van den Heuvel-Eibrink is Associate Professor of Pediatric Oncology at the Erasmus MC/Sophia Children's Hospital, Rotterdam, Netherlands. She began her medical career with an MD from the University of Utrecht and following a number of years of clinical work in the pediatric oncology field completed her PhD in Rotterdam in 2001. Since 2009 Dr van den Heuvel-Eibrink has been Head of the "late effects after childhood cancer treatment" outpatient clinic. She is a member of the SIOP Renal Tumour Study Group (RTSG) Steering Committee, and Chair of the EWOG-MDS Study Group Protocol Committee of the DCOG. Her research is dedicated to translational research in pediatric oncology/hematology and the genetic variation of toxicity and late effects of childhood cancer, with a special interest in endocrine sequelae.

Katherine Wesseling-Perry

Kate Wesseling-Perry, MD, is an Assistant Professor in Pediatric Nephrology at UCLA. Her research is focused on understanding the regulation of skeletal mineralization in patients with all stages of chronic kidney disease. Her research interest is identifying the abnormalities in bone that lead to the early development of renal bone disease.

Carmen Wilson

Dr Carmen Wilson received a PhD in Epidemiology from the University of New South Wales, Australia, in 2008 for research focusing on the late complications of anti-cancer therapies among individuals diagnosed with childhood cancer. She then worked for a short time as study coordinator for the New South Wales Childhood Cancer Survivor Study before coming to work in the Department of Epidemiology and Cancer Control at St Jude Children's Research Hospital, Memphis, Tennessee in 2010. Dr Wilson's studies have focused on examining genetic and treatment-related factors which modify the risk of long-term complications among cancer survivors. Dr Wilson is the principal investigator of several genome-wide association studies evaluating genetic variation associated with cardiac dysfunction, obesity, bone health and neurosensory impairments in childhood cancer survivors. Her work has been funded by the Rally Foundation.



Registration desk opening times

Saturday	12:00-19:30
Sunday	08:00-19:00
Monday	08:00-19:00
Tuesday	08:00-13:00

Cloakroom

There is a free cloakroom in the entrance foyer. Opening times as follows:

Saturday	12:00-19:30
Sunday	08:00-21:30
Monday	08:00-19:15
Tuesday	08:00-12:45

Attendance Certificates

Attendance certificates will be available through the registration desk from Monday onwards.

Food and beverage

The following are included in the conference fee:

All coffee breaks

Lunch on Sunday and Monday

Welcome Reception on Saturday

Conference Dinner on Monday

In addition, all delegates are invited to attend the Alexion educational symposium on Sunday evening, which is followed by dinner in the Arcadia Zaal.

Learning Objectives and Continuing Medical Education (CME)

On completion of the meeting programme, the participant will have an enhanced understanding of a contemporary approach to clinical and basic paediatric bone science as related to:

- Inherited bone disorders
- Acquired bone disorders
- Bone therapeutics
- Bone diagnostics
- Fracture prevention

The conference has been approved for 18 CME credits by the European Accreditation Council for Continuing Medical Education (EACCME). EACCME credits are recognised by the American Medical Association.

Local information

Nearby ATM: Central Station

Nearest bank: Central Station

Nearest pharmacy: Medsen Apotheek, Westblaak 34

Name badges

Please wear your name badge at all times when you are in the Conference Centre.

Photography/Recording

You are respectfully requested not to take photos or recordings during sessions.

Smoking

Smoking is not permitted in any area within the Conference Centre.

Valuables

You are reminded that you are responsible for your valuables and you should take care not to leave them unattended at any time.

WiFi

There is free WiFi throughout the Conference Centre.

EMERGENCIES

Emergency phone number

Police, Ambulance, Fire: 112

Hospital

Erasmus Medical Center
Doctor Molewaterplein 50-60
Tel: + 31 10 704 0704

First Aid

In case First Aid is required please contact the Registration Desk.

Emergencies and evacuation procedures

Please follow the instructions given to you by the Conference Centre staff



LOCATIONS

Lectures	Willem Burger Zaal (3rd floor)
Exhibition	Willem Burger Foyer (3rd floor)
Coffee/lunch breaks	Willem Burger Foyer (3rd floor) After lunch on Sunday and Monday coffee will be served in the poster area on the 4th floor
Posters	Van Weelde Zaal/Mees Zaal (4th floor) Odd numbers attended 13:30-14:30 Sunday 23 June Even numbers attended 13:30-14:30 Monday 24 June
Welcome Reception	Saturday 22 June, 18:00-19:00 Willem Burger Foyer (3rd floor)
Alexion Dinner	Sunday 23 June, 20:00-21:00 Arcadis Zaal (1st floor)
Conference Dinner	Monday 24 June, 19:30-23:00 Paddlesteamer De Majesteit Embarkation Boomjeskade <i>Buses leave Conference Centre immediately at the end of Danone Satellite</i> <i>Bus returns to Conference Centre via local hotels from 22:30</i>





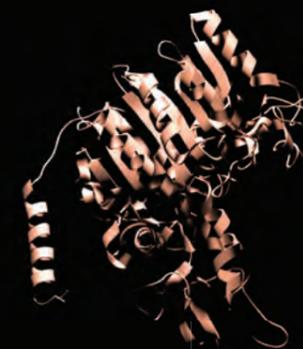
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▶ **To learn more about HPP, visit the Alexion booth.**

References: 1. Whyte MP. Hypophosphatasia: nature's window on alkaline phosphatase function in humans. In: Bilezikian JP, Raisz LG, Martin TJ, eds. *Principles of Bone Biology*. Vol 1. 3rd ed. San Diego, CA: Academic Press; 2008:1573-1598. 2. Barvencik F, Beil FT, Gebauer M, et al. Skeletal mineralization defects in adult hypophosphatasia—a clinical and histological analysis. *Osteoporos Int*. 2011;22(10):2667-2675. 3. Whyte MP. Physiological role of alkaline phosphatase explored in hypophosphatasia. *Ann NY Acad Sci*. 2010;1192:190-200.

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ALEXION

WCO-IOF-ESCEO SEVILLE 2014

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MUSCULOSKELETAL DISEASES



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