111

Bone: a story of breakthroughs, a promising future

EDITORIAL

" Issues confronting post-menopausal women represent one of the fastest growing areas of biomedical investigation. In the last 50 years basic and clinical research in osteoporosis has been extremely productive, generating new bone modeling concepts, new definitions combining BMD values with clinical evaluation of fracture risk factors, and effective new tools for prevention and treatment, reducing an average patient's lifetime number of fractures by one or two."

Fifty years of discoveries in osteoporosis

by P. J. Meunier, *France*

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The continuous increase in life expectancy during the last five decades for females and males in developed countries—about 12 years—has made osteoporosis a major health issue. The age-related increase in fracture risk has had significant medical and economic consequences. These observations and the pessimistic predictions of the epidemiologists could lead us to consider fragility fractures of spine, hip, humerus, or wrist as an inevitable price to pay for a longer life.

Fortunately, this pessimism is unjustified because the last 50 years have seen major advances in the diagnosis of osteoporosis, now defined in terms of fracture risk factors that can be detected before a fracture occurs. The first fracture is an irreversible event that also heralds subsequent fractures.

Major advances have also been accomplished in understanding normal bone remodeling, the cellular mechanisms of postmenopausal and age-related bone loss, the pathophysiology of most forms of fragile bone disease, and in the discovery of the mechanisms of action of several new drugs offering effective prevention and treatment of fragility fractures.

Bone densitometry provides an accurate and reproducible measure of bone mineral density (BMD) with minimal radiation exposure. It became established in the early 1990s as a tool for diagnosing prefracture osteoporosis. In 1992, a working group of the World Health Organization (WHO) proposed diagnosing osteoporosis in women on the basis of a BMD 2.5 or more standard deviations below the mean values of a young reference population (T-score below 2.5 sd).[1] More recently, in 2010, the WHO Fracture Risk Assessment Tool (FRAX) was shown to be more accurate than BMD alone in predicting 10-year fragility fracture risk: in addition to BMD, the FRAX tool incorporates age, sex, body mass index, long-term steroid use, smoking, alcoholism, any fracture after 50 years, and history of parental hip fracture.[2]

There have been parallel improvements in understanding the cellular events responsible for age-related bone loss, bone disease histopathogenesis, and the long-term effects of new osteotropic agents. These developments include the discovery of the intermediary level of bone organization, new methods of preparing undercalcified bone sections, quantitative and dynamic evaluation of bone formation using in vivo prebiopsy tetracycline double labeling, measurement of microstructure and mineralization parameters on bone biopsy microradiographs, and overall improvement in the range of bone quality assessment parameters.

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In 1964, using rib cortical bone sections, Harold Frost showed that the human adult skeleton is in a dynamic state, being continually broken down and reformed by the coordinated activities of osteoclasts and osteoblasts on trabecular surfaces and the Haversian systems of cortical compact bone.[3,4] This turnover, or remodeling, occurs in focal and discrete packets, or bone multicellular units (BMU), throughout the adult skeleton. The remodeling of each BMU takes a finite period of time estimated as 3 to 4 months, and is topographically and chronologically distinct from the remodeling in neighboring BMUs. The new bone formed by a BMU is known as a bone structure unit (BSU) in trabecular bone and an osteon in cortical bone. BSUs are the end products of osteoblast activity occurring exclusively at sites of recent osteoclast resorption. The last five decades have seen marked progress in quantitative histology or bone histomorphometry thanks to new methods of preparing undercalcified bone sections. Quantitative evaluation of bone formation using tetracycline double labeling provides a direct measure of the mineral apposition rate and mineralizing trabecular surface. Introduction of the time dimension into quantitative histological analysis is crucial to the dynamic evaluation of bone formation and remodeling at the BMU/BSU level. Neither noninvasive BMD measurement nor the specific and sensitive assays for circulating and urinary bone turnover markers developed in the 1980s[5,6] can identify abnormal bone remodeling at the BMU/BSU level because they each reflect events at the whole-skeleton level. Bone turnover markers are clinically useful in following up individual patients receiving antiosteoporosis therapy, but not for diagnosing osteoporosis.[7] The advances accomplished in the histomorphometric approach of bone remodeling have also included the measurement of histological parameters of microstructure reflecting the connectivity or the thickness of the trabeculae, the mean wall thickness of the BSUs, and the number of microcracks.

The late 1990s saw a "historical" step backward in hormone replacement therapy prescribing after large controlled studies showed a significant increase in breast cancer risk in postmenopausal women receiving long-term estrogen or estrogen plus progestin therapy.[8,9] Many menopausal women discontinuing estrogen were transferred to raloxifene, which is effective in preventing postmenopausal bone loss and reducing breast cancer risk. As a selective estrogen receptor modulator (SERM) with mixed estrogen agonist/antagonist activity, raloxifene is an alternative to estrogen replacement therapy.[10,11] Unlike estrogen, it does not stimulate endometrial cells. Its main side effects are hot flashes and muscle cramps. Like estrogen, it is also associated with a two- to threefold increase in the risk of venous thromboembolism.

Considerable pharmacological activity in the last 20 years has also focused on the synthetic P-C-P compounds known as bisphosphonates, pioneered by Herbert Fleisch.[12] These are potent inhibitors of osteoclasts, resorption, and bone remodeling generally. By decreasing bone turnover they decrease bone loss and increase the degree of mineralization of bone.[13,14] Most extensively studied to date have been the aminobisphosphonates (pamidronate, alendronate), followed by clodronate, risedronate, ibandronate, and zoledronate. The first controlled clinical study was performed with the first-generation bisphosphonate etidronate, launched in France in 1981. It showed a favorable effect on spinal BMD and a modest decrease in vertebral fracture rate. In contrast, oral alendronate, daily (10 mg/day) or weekly (70 mg/week), halved spine, hip, and distal radius fracture rates.[12] Oral risedronate (5 mg/day or 35 mg/week) also reduced vertebral and nonvertebral fracture rates in postmenopausal osteoporosis. Alendronate and risedronate products were recently supplemented with vitamin D and calcium due to the high incidence of vitamin D insufficiency among the elderly.[15] An American epidemiological survey in 460 584 women recently estimated that oral bisphosphonates prevented 144 000 fractures from 2001 to 2008 in women aged 45 years or more.[16] The post-launch years have revealed extremely rare side effects from long-term use, including osteonecrosis of the jaw and intertrochanteric fractures of the femur, each presumed due to oversuppression of bone remodeling.

Over the last 50 years, several other antiosteoporotic compounds have been launched and/or withdrawn after controlled studies of fracture rates as the primary end point. Thus, between 1980 and 2011, I was directly involved in histological studies on the effects of parathyroid hormone (PTH) and fluoride salts. In 1980 we reported a 70% increase in iliac cancellous bone volume after injections of PTH at an average dose of 500 U daily for 6 months, with marked extension of trabecular mineralizing surfaces on unstained sections of bone biopsies examined under fluorescence after tetracycline double labeling.[17] It was not until 21 years later that Neer et al reported decreased vertebral and nonvertebral fracture risk in postmenopausal women with prior vertebral fracture at baseline in response to PTH.[18] We now know that daily injections of teriparatide or intact parathormone protect against fracture and are effective in severe symptomatic multifracture osteoporosis.

In 1982, fluoride salts (sodium fluoride or monofluorophosphate) were proposed as curative in vertebral crush fracture syndrome on the grounds that fluoride substantially increased vertebral BMD in toxic fluorosis.[19] However, controlled trials failed to confirm a decrease in vertebral fracture rates. Indeed, fluoride dose-dependently increased stress fractures and, more importantly, hip fractures.[20] In addition, iliac biopsy in fluoride-treated patients showed interstitial mineralization defects compromising bone quality, despite an increase in the bone mass and osteoblast population. For these reasons, fluoride salts were not approved by the Food and Drug Administration (FDA) and they were withdrawn from the European market. The contrasting fates of these two bone-form-

ing agents, PTH and fluoride, remind us that any new antiosteoporotic must not only reduce fracture risk, but also promote bone of good histological quality.

In the last 50 years, several combination products have been launched with osteotropic treatments to combat the low calcium and vitamin D levels often seen in osteoporotic elderly patients. In a French study in women living in nursing homes, supplementation with vitamin D (800 U/day) and calcium (1200 mg/day) for 3 years significantly decreased the incidence of hip fractures compared with placebo.[21]

In the last 5 years, an alternative strategy has been developed for inhibiting bone resorption: blockade of the endogenous promoters of osteoclast recruitment. A key such factor is receptor activator of nuclear factor kappa-B ligand (RANKL), which is essential for osteoclast formation, function, and survival. Denosumab is an anti-RANKL human monoclonal antibody that reversibly inhibits osteoclast-mediated resorption. Subcutaneous denosumab 60 mg every 6 months for 3 years increased BMD and reduced bone turnover and fracture risk in a randomized placebo-controlled trial in 7868 postmenopausal women (FREEDOM [Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months]).[22] It reduced vertebral fractures by 68% and hip fractures by 40%.

In addition, histomorphometry confirmed potent and sustained inhibition of bone turnover, and maintenance of normal bone architecture, with no evidence of impaired mineralization or lamellar bone formation.[23]

Strontium ranelate is a new orally active agent consisting of two atoms of stable strontium and an organic moiety, ranelic acid. It stimulates the formation of new bone tissue and decreases bone resorption, as shown in in-vitro and animal studies. A 2-year placebo-controlled dose-response study in 353 postmenopausal women with osteoporosis showed that oral strontium ranelate 2 g/d significantly reduced the incidence of vertebral fractures during the second year of treatment and simultaneously increased BMD (STRATOS [STtrontium RAnelate for Treatment of Osteoporosis Study]).[24] Two large phase 3 randomized placebo-controlled trials have documented the effects of strontium ranelate on the risks of vertebral and nonvertebral fractures in postmenopausal osteoporotic women. The SOTI study (Spinal Osteoporosis Therapeutic Intervention) in 1649 women with prevalent vertebral fracture showed a risk reduction of 49% in the incidence of new vertebral fractures in the first year of treatment and 41% over the 3-year study period.[25] Strontium ranelate also reduced the incidence of new symptomatic vertebral fractures by 52% in the first year, and benefit was maintained. TROPOS (TReatment Of Peripheral Osteoporosis Study) confirmed significant reduction in hip fracture incidence in 1977 high-risk patients (age >74 years; femoral neck BMD Z score <–3).[26] Histomorphometry and quantitative microradiography of transiliac bone biopsies from patients in these three studies showed that strontium ranelate was exclusively present in bone formed during treatment. Secondary mineralization and bone tissue quality were unimpaired.[27]

Issues confronting postmenopausal women represent one of the fastest growing areas of biomedical investigation. In the last 50 years basic and clinical research in osteoporosis has been extremely productive, generating new bone modeling concepts, new definitions combining BMD values with clinical evaluation of fracture risk factors, and effective new tools for prevention and treatment, reducing an average patient's lifetime number of fractures by one or two. Such fractures, particularly those of the hip, are associated with high mortality and/or seriously impaired quality of life.

However, awareness of these advances remains clearly inadequate at prescriber level, with the result that osteoporosis continues to be underdiagnosed[28] and undertreated despite the best efforts of the International Osteoporosis Foundation at world level, and national societies such as the National Osteoporosis Foundation in the US and the Research and Information Group on Osteoporosis (GRIO) in France. ■

Keywords: *bisphosphonate; denosumab; densitometry; estrogen; osteoporosis; parathyroid hormone; strontium ranelate*

ÉDITORIAL

" *La pathologie des femmes ménopausées est un des domaines de la recherche biomédicale se développant le plus vite. Ces 50 dernières années, les recherches cliniques et fondamentales sur l'ostéoporose ont été à l'origine de nouveaux concepts sur le remodelage osseux et de nouvelles définitions associant la DMO à l'évaluation clinique du risque de fractures. Elles ont également permis la création de nouveaux outils efficaces pour la prévention et le traitement de l'ostéoporose, permettant la prévention d'une ou deux fractures au cours de la vie du patient.* "

Cinquante ans de découvertes dans l'ostéoporose

par P. J. Meunier, *France*

L'augmentation continue de l'espérance de vie ces 50 dernières années pour les hommes et les femmes des pays développés (d'environ 12 ans) a fait de l'ostéoporose un problème majeur de santé. L'augmentation, liée à l'âge, du risque de fracture a eu des conséquences médicales et économiques significatives. Ces observations et les prédictions pessimistes des épidémiologistes pourraient nous conduire à considérer les fractures par fragilité du rachis, de la hanche, de l'humérus ou du poignet comme le prix inévitable à payer pour une vie plus longue.

Heureusement, ce pessimisme n'est pas justifié, car en parallèle, des progrès majeurs ont également eu lieu dans le diagnostic de l'ostéoporose, défini maintenant en termes de facteurs de risque. Le risque de fracture peut maintenant être détecté en amont. La première fracture est un événement irréversible, annonciateur d'autres fractures à venir.

Des progrès importants ont également été faits dans la compréhension du remodelage osseux normal, des mécanismes cellulaires de la perte osseuse post-ménopausique et liée à l'âge et dans la physiopathologie de la plupart des maladies liées à des os fragiles. Les mécanismes d'action de plusieurs nouveaux médicaments permettant une prévention et un traitement efficaces des fractures par fragilité ont également été découverts.

Aujourd'hui, la densitométrie osseuse permet une mesure précise et reproductible de la densité minérale osseuse (DMO) avec une exposition minimale aux radiations. Elle s'est imposée au début des années 1990 comme un outil de diagnostic de l'ostéoporose préfracturaire. En 1992, un groupe de travail de l'Organisation Mondiale de la Santé (OMS) a proposé de diagnostiquer l'ostéoporose chez les femmes à partir d'un écart type de la DMO de 2,5 ou plus, en dessous des valeurs moyennes d'une population jeune de référence (T-score en dessous de 2,5 SD)[1]. Plus récemment, en 2010, l'outil FRAX (Fracture Risk Assessment Tool) de l'OMS s'est montré plus précis que la DMO seule pour prédire le risque de fracture de fragilité à 10 ans : par rapport à la DMO, l'outil FRAX comprend l'âge, le sexe, l'indice de masse corporelle, l'utilisation de glucocorticoïdes à long terme, le tabagisme, l'alcoolisme, les fractures après 50 ans et les antécédents de fracture de hanche chez les parents[2].

Parallèlement à l'amélioration des méthodes densitométriques, des progrès importants ont été accomplis dans la compréhension des événements cellulaires responsables de la perte osseuse liée à l'âge, de l'histopathogenèse des maladies osseuses

Cinquante ans de découvertes dans l'ostéoporose – *Meunier*

et des effets à long terme des nouveaux traitements osseux. Ces progrès comprennent non seulement la découverte de l'organisation osseuse intermédiaire et des nouvelles méthodes de préparation des coupes osseuses, mais également la découverte de méthodes d'évaluation quantitative et dynamique de la formation osseuse grâce à l'utilisation d'un double marquage par la tétracycline préalable à la biopsie osseuse in vivo et de méthodes de mesure des paramètres de microstructure et de minéralisation sur des microradiographies de biopsies osseuses. Une amélioration globale de l'ensemble des paramètres histologiques mesurant la « qualité osseuse » a également été apportée.

En 1964, en utilisant des coupes d'os cortical de côtes, Harold Frost a montré que le squelette adulte humain est dans un état dynamique, en permanence démoli et reformé par l'activité coordonnée des ostéoclastes et des ostéoblastes sur la surface trabéculaire et dans le système haversien de l'os cortical compact[3,4]. Ce renouvellement, ou remodelage, intervient dans tout le squelette adulte, au niveau des paquets focaux produits par des unités osseuses multicellulaires (UOM). Le remodelage de chaque UOM se produit en un temps limité estimé entre 3 et 4 mois, et est topographiquement et chronologiquement indépendant du remodelage des UOM voisines. Le nouvel os formé par une UOM est désigné comme étant une unité de structure osseuse (USO) dans l'os trabéculaire et un ostéon dans l'os cortical. Les USO sont les produits finaux de l'activité ostéoblastique, celle-ci ayant eu lieu exclusivement sur les sites de résorption récents crées par les ostéoclastes. Les 50 dernières années ont vu d'importants progrès en histologie quantitative ou en histomorphométrie osseuse grâce à de nouvelles méthodes de préparation des coupes osseuses non décalcifiées. Une évaluation quantitative de la formation osseuse utilisant le double marquage à la tétracycline fournit une mesure directe du taux d'apposition minérale et de la surface trabéculaire en minéralisation. L'introduction de la dimension « temps » dans l'analyse histologique quantitative est fondamentale pour l'évaluation dynamique de la formation et du remodelage osseux au niveau de l'UOM/USO. Ni les mesures non invasives par DMO, ni l'évaluation des marqueurs circulants et urinaires du remodelage osseux développés dans les années 1980[5,6] ne peuvent identifier un remodelage osseux anormal au niveau UOM/USO parce qu'ils reflètent chacun des événements intervenant au niveau du squelette entier. Ces marqueurs du remodelage osseux sont cliniquement utiles dans le suivi de chaque patient traité par un antiostéoporotique mais pas pour le diagnostic de l'ostéoporose[7]. D'où l'utilité des paramètres microstructuraux plus récents comme la connectivité trabéculaire, l'épaisseur moyenne de la paroi des USO trabéculaires et le nombre de microfractures.

Sur le plan thérapeutique, les années 1990 ont vu un retour en arrière historique dans la prescription du traitement hormonal substitutif. De grandes études contrôlées ont en effet démon-

tré une augmentation significative du risque de cancer du sein chez les femmes ménopausées recevant des estrogènes ou un traitement estrogénoprogestatif à long terme[8,9]. De nombreuses femmes ménopausées ayant arrêté les estrogènes ont reçu à la place du raloxifène, efficace dans la prévention de la perte osseuse post-ménopausique et dans la réduction du risque de cancer du sein. En tant que modulateur du récepteur sélectif de l'estrogène (SERM) et ayant une activité estrogénique mixte agoniste/antagoniste, le raloxifène est une alternative au traitement hormonal substitutif[10,11]. Contrairement aux estrogènes, il ne stimule pas les cellules endométriales. Ses principaux effets indésirables sont des bouffées de chaleur et des crampes musculaires. Comme les estrogènes, il s'associe à une multiplication par 2 ou 3 du risque thromboembolique veineux.

L'activité pharmacologique importante de ces 20 dernières années s'est également concentrée sur des composés synthétiques P-C-P connus sous le nom de bisphosphonates, dont Herbert Fleisch a été le pionnier[12]. Ce sont des inhibiteurs puissants des ostéoclastes, de la résorption et du remodelage osseux en général. En diminuant le renouvellement osseux, ils diminuent la perte osseuse et augmentent la minéralisation[13,14]. Les aminobisphosphonates ont été largement étudiés (pamidronate, alendronate), suivis par le clodronate, le risédronate, l'ibandronate et le zolédronate. La première étude clinique contrôlée a été réalisée avec un bisphosphonate de première génération l'étidronate, lancé en France en 1981, qui avait montré un effet favorable sur la DMO au niveau du rachis et une diminution modeste du taux des fractures vertébrales. Au contraire, l'alendronate par voie orale, pris quotidiennement (10 mg par jour) ou une fois par semaine (70 mg), a montré une diminution de moitié des taux de fracture du radius distal, de la hanche et du rachis[12]. Le risédronate par voie orale (5 mg/jour ou 35 mg/semaine) diminue également les taux de fractures vertébrales et non vertébrales dans l'ostéoporose post-ménopausique. Des suppléments en vitamine D ont récemment été ajoutés à l'alendronate et au risédronate en raison de l'incidence élevée d'insuffisance en vitamine D chez les personnes âgées[15]. Un essai épidémiologique américain chez 460 584 femmes a récemment estimé que les bisphosphonates oraux ont prévenu 144 000 fractures entre 2001 et 2008 chez des femmes de 45 ans et plus[16]. Les années qui ont suivi leur lancement ont révélé quelques bien rares effets secondaires à long terme, comme des ostéonécroses de la mâchoire et des fractures intertrochantériques du fémur, sans doute dues à une suppression excessive du remodelage osseux.

Ces 50 dernières années, plusieurs autres produits antiostéoporotiques ont été lancés et/ou supprimés après des études contrôlées des taux de fracture comme critère primaire. Ainsi, entre 1980 et 2011, j'ai été directement impliqué dans des études sur les effets de l'hormone parathyroïdienne (PTH) et les sels de fluor. En 1980, nous avons rapporté une

augmentation de 70 % du volume d'os spongieux iliaque après injection de PTH à une dose moyenne de 500 U par jour pendant 6 mois, avec une extension manifeste des surfaces de minéralisation trabéculaire sur des coupes non colorées de biopsies osseuses examinées en fluorescence après double marquage à la tétracycline[17]. Ce n'est que 21 ans plus tard que Neer et al ont rapporté une diminution du risque de fractures vertébrales et non vertébrales en réponse à la PTH, chez des femmes ménopausées ayant un antécédent de fracture vertébrale initiale[18]. Nous savons maintenant que des injections quotidiennes de tériparatide ou de parathormone « intacte » protègent contre les fractures et sont efficaces dans l'ostéoporose multifracturaire symptomatique sévère.

En 1982, les sels de fluor (fluorure de sodium ou monofluorophosphate) ont été proposés comme agent curatif dans le syndrome de tassement vertébral, au motif que le fluor augmentait de façon substantielle la DMO vertébrale dans la fluorose toxique[19]. Cependant, les études contrôlées n'ont pas réussi à confirmer de diminution des taux de fractures vertébrales. En effet, le fluor a non seulement augmenté de façon dose dépendante les fractures de fatigue, mais également les fractures de hanche[20]. De plus, des biopsies iliaques de patientes traitées par fluor ont montré des défauts de minéralisation interstitielle, compromettant la qualité osseuse, malgré une augmentation de la population ostéoblastique. Pour ces raisons, les sels de fluor n'ont pas été autorisés par la FDA (Food and Drug Administration) et ont été retirés du marché européen. Les destins contrastés de ces deux agents ostéoformateurs, la PTH et le fluor, nous rappellent que tout nouveau antiostéoporotique doit non seulement réduire le risque de fracture, mais aussi ne pas compromettre la bonne qualité histologique de l'os.

Ces 50 dernières années, plusieurs associations ont été lancées avec des traitements osseux pour compenser des taux de calcium et vitamine D bas apparaissant souvent chez les personnes âgées ostéoporotiques. Dans une étude française effectuée chez des femmes en maison de retraite, l'administration d'un complément de vitamine D (800 U/jour) et de calcium (1 200 mg/jour) pendant 3 ans a diminué de façon significative l'incidence des fractures de hanche par rapport au groupe recevant un placebo[21].

Ces 5 dernières années, une stratégie alternative a été développée pour inhiber la résorption osseuse : le blocage des promoteurs endogènes du recrutement ostéoclastique. Le RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand) est un de ces promoteurs clef. Il est essentiel à la formation, la fonction et la survie des ostéoclastes. Le dénosumab est un anticorps monoclonal humain anti-RANKL qui inhibe de façon réversible la résorption médiée par les ostéoclastes. L'injection sous-cutanée de dénosumab 60 mg tous les 6 mois pendant 3 ans a montré une augmentation de la DMO ainsi qu'une diminution du remodelage osseux et du risque de fracture dans une étude randomisée contrôlée versus placebo chez 7 868 femmes ménopausées (FREEDOM [Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months])[22]. Les fractures vertébrales ont été réduites de 68 % et les fractures de hanches de 40 %. De plus, l'histomorphométrie a confirmé une inhibition puissante et prolongée du renouvellement osseux, ainsi que le maintien de l'architecture osseuse normale, sans preuve d'une altération de la minéralisation ou de la formation d'os lamellaire[23].

Le ranélate de strontium, constitué de 2 atomes de strontium stable et d'une fraction organique, l'acide ranélique, est un nouveau traitement actif par voie orale. Il stimule la formation de nouveau tissu osseux et diminue la résorption osseuse, comme le montrent des études animales et in vitro. Une étude dose-réponse de 2 ans contrôlée contre placebo chez 353 femmes ménopausées ostéoporotiques a montré que 2 g/jour de ranélate de strontium réduit de façon significative l'incidence des fractures vertébrales pendant la deuxième année de traitement tout en augmentant simultanément la DMO (STRATOS [STrontium RAnelate for Treatment of Osteoporosis Study])[24].

Les effets du ranélate de strontium sur les risques de fractures vertébrales et non vertébrales chez des femmes ménopausées ostéoporotiques ont été ensuite décrits dans deux grandes études de phase 3 contrôlées versus placebo. L'étude SOTI (Spinal Osteoporosis Therapeutic Intervention) a montré chez 1 649 femmes ayant une fracture vertébrale prévalente une réduction du risque de 49 % de l'incidence de nouvelles fractures vertébrales dans la première année de traitement et de 41 % au cours des 3 ans de l'étude[25]. Le ranélate de strontium a aussi réduit de 52 % l'incidence des nouvelles fractures vertébrales symptomatiques la première année, et ce bénéfice a été maintenu les années suivantes. L'étude TROPOS (TReatment Of Peripheral Osteoporosis Study) a confirmé une réduction significative de l'incidence de fracture de la hanche chez 1 977 patientes à haut risque (âge > 74 ans ; score Z de DMO au col fémoral <– 3)[26]. L'histomorphométrie et la microradiographie quantitative des biopsies osseuses transiliaques des patientes incluses dans ces 3 études ont montré que le ranélate de strontium était présent exclusivement dans l'os formé pendant le traitement. La minéralisation secondaire et la qualité du tissu osseux n'ont pas été altérées[27].

La pathologie des femmes ménopausées est un des domaines de la recherche biomédicale se développant le plus vite. Ces 50 dernières années, les recherches cliniques et fondamentales sur l'ostéoporose ont été très productives. Elles ont été à l'origine de nouveaux concepts sur le remodelage osseux et de nouvelles définitions associant la DMO à l'évaluation du risque de fractures. Elles ont également permis la création de nouveaux outils efficaces pour la prévention et le traitement de l'ostéoporose, permettant la prévention d'une ou deux frac-

tures au cours de la vie du patient. De telles fractures, en particulier celles de la hanche, sont associées à une forte mortalité et/ou à une détérioration sérieuse de la qualité de vie. Cependant, le prescripteur reste mal informé de ces progrès, aboutissant au sous-diagnostic et au sous-traitement de l'os-

téoporose[28] malgré les efforts soutenus de l'IOF (International Osteoporosis Foundation) *au niveau mondial et des sociétés nationales comme la* National Osteoporosis Foundation *aux USA et le GRIO (Groupe de Recherche et d'Information sur l'Ostéoporose) en France.* ■

References

1. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser.* 1994;843:1-129.
2. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the measurement of fracture probability in men and women from the UK. *Osteoporos Int.* 2008;19(4);385-397.
3. Frost HM. Intermediary organization of the skeleton. Boca Raton, Fla: CRC Press; 1986.
4. Frost HM. Bone remodeling dynamics. Springfield, Ill: Charles C Thomas Company; 1963.
5. Price PA, Parthemore JG, Deftos LJ. New biochemical markers for bone metabolism. Measurement by radioimmunoassay of bone GLA protein in the plasma of normal subjects and patients with bone disease. *J Clin Invest.* 1980; 66(5):878-883.
6. Delmas PD. Biochemical markers of bone turnover in osteoporosis. In Riggs BL, Melton LJ, eds. *Osteoporosis: Etiology, Diagnosis, and Management.* New York, NY: Raven Press; 1988:297-316.
7. Garnero P. Markers of bone metabolism. *Medicographia.* 2004;26:299-307.
8. Schairer C, Lubin J, Troisi, Sturgeon S, Brinton L, Hoover R. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. *JAMA.* 2000;283(4):485-491.
9. Chen CL, Weiss NS, Newcomb P, Barlow W, White E. Hormone replacement therapy in relation to breast cancer. *JAMA.* 2002;287(6):734-741.
10. Ettinger B, Black DM, Mitlak BH, et al; Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. *JAMA.* 1999;282(7):637-645.
11. Lufkin EG, Whitaker MD, Nickelsen T, et al. Treatment of established postmenopausal osteoporosis with raloxifene: a randomized trial. *J Bone Miner Res.* 1998;13(11):1747-1754.
12. Fleisch H. *Bisphosphonates in Bone Disease: From the Laboratory to the Patient.* 4th ed. San Diego, Calif: Academic Press; 2000.
13. Meunier PJ, Boivin G. Bone mineral density reflects bone mass but also the degree of mineralization of bone: therapeutic implications. *Bone.* 1997;21(5):373-377.
14. Boivin G, Meunier PJ. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcif Tissue Int.* 2002: 70(6):593-511.
15. Meunier PJ, Chapuy MC. Vitamin D insufficiency in adults and the elderly. In: *Vitamin D,* 2nd ed. Feldman D, Pike JW, Glorieux FH, eds. San Diego, Calif: Elsevier Academic Press; 2005:1085-1100.
16. Siris ES, Pasquale MK, Wang Y, Watts NB. Estimating bisphosphonate use and fracture reduction among US women aged 45 years and older, 2001-2008. *J Bone Miner Res.* 2011;26(1):3-11.
17. Reeve J, Meunier PJ, Parsons JA, et al. Anabolic effect of human parathyroid hormone fragment on trabecular bone in involutional osteoporosis: a multicentre trial. *BMJ.* 1980;280(6228):1340-1344.
18. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med.* 2001;344(19);1434-1441.
19. Riggs BL, Hodgson SF, O'Fallon WH, et al. Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. *N Engl J Med.* 1990: 322(12);802-809.
20. Meunier PJ, Sebert JL, Reginster JY, et al. Fluoride salts are no better at preventing new vertebral fractures than calcium-vitamin D in postmenopausal osteoporosis: the FAVO Study. *Osteoporosis Int.* 1998;8(1):4-12.
21. Chapuy MC, Arlot ME, Delmas PD, Meunier PJ. Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. *BMJ.* 1994; 308(6936):1081-1082.
22. Cummings SR, San Martin J, McClung MR, et al; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med.* 2009;361(8):756-765.
23. Reid IR, Miller PD, Brown JP, et al; Denosumab Phase 3 Bone Histology Study Group. Effects of denosumab on bone histomorphometry: the FREEDOM and STAND studies. *J Bone Miner Res.* 2010;25(10):2256-2265.
24. Meunier PJ, Slosman DO, Delmas PD, et al. Strontium ranelate: dose-dependent effects in established postmenopausal vertebral osteoporosis—a 2-year randomized placebo controlled trial. *J Clin Endocrinol Metab.* 2002;87(5):2060-2066.
25. Meunier PJ, Roux C, Seeman E, et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med.* 2004;350(5):459-468.
26. Reginster JY, Seeman E, De Vernejoul MC, et al. Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab.* 2005;90(5):2816-2822.
27. Boivin G, Farlay D, Khebbab MT, Jaurand X, Delmas PD, Meunier PJ. In osteoporotic women treated with strontium ranelate, strontium is located in bone formed during treatment with a maintained degree of mineralization. *Osteoporos Int.* 2010;21(4):667-677.
28. Chesnut CH 3rd. Osteoporosis, an underdiagnosed disease. *JAMA.* 2001; 286(22):2865-2866.

> " Microcracks are a normal component of bone and bone intrinsic properties are able to deal with it up to a certain level, avoiding loss of stiffness that leads to fracture. However, this is not enough to keep a bone healthy. Remodeling is the bone's repair mechanism, helping to prevent the propagation of microcracks and their evolution into macrocracks, which can lead to complete fracture… and involves a mechanism that couples bone resorption and formation."

Bone biology: from macrostructure to gene expression

by J. E. Fonseca, *Portugal*

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The overall architecture of bone (cortical thickness, trabecular morphology, and bone geometry) determines the basic functional characteristics of bone. However, this overall structure is influenced by the nanostructural properties of bone, which are directly dependent on the way bone cells, collagen, and calcium crystals interact. Delving deeper into the complex hierarchical structure of bone, at the cellular and molecular level, bone remodeling through coupled resorption and formation plays out again and again in an intricately regulated manner within bone remodeling compartments. Progress in imaging technologies, mechanical tests, and cell biology has facilitated a new understanding of the complexity of bone biology. This review draws the parallel between the advent of innovative technical approaches and the progress of our knowledge in bone biology.

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Bone properties are a result of the complex compromise between stiffness (for efficient protection and locomotion) and ductility (to absorb impacts). Bone's intricate hierarchical structure can be analyzed at different levels. On progressively smaller scales, bone can be viewed at the macrostructural level (cortical and trabecular bone), microstructural level (Haversian system and trabeculae), nanostructural level (collagen and mineral phases), and at cell and molecular levels (cell biology activity).

In this review, we will address how the introduction of new research tools and how successive discoveries in the field of bone biology have reshaped our perception of bone hierarchical structure.

Bone: the macrostructural level

Beyond the classic structure of bone—it's inner trabecular aspect covered by a cortical shield—and the traditional measurement of bone mineral density (BMD) by osteodensitometry, new image processing techniques have facilitated development of innovative ways to look at bone macrostructure. For example, proximal femoral bone strength is not only a function of femoral BMD, but also a function of structural geometric properties such as diameter, area, length, and angle of the femoral neck, which can be calculated automatically by specific software installed in the densitometers. One very simple approach is to measure the section formed by an imaginary plane cutting through femoral bone, at right angles to the axis, in order to measure the cross-sectional area (CSA). In addition, information about bone mass and

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its distribution relating to the squared distance from a neutral axis (center of mass) can be given by the cross-sectional moment of inertia (CSMI). More complex information can be obtained by integrating CSMI, CSA, BMD, patient age, height, and weight into a score called the Femur Strength Index (FSI), which represents the biomechanical properties of the hip and provides an estimate of the risk of fracture resulting from a fall on the hip.[1]

Another way to obtain more information from bone macrostructure is to take advantage of computed tomography (CT). There are algorithms to calculate the minimal rigidity of bone, using serial transaxial CT images to measure both the bone tissue mineral density and cross-sectional geometry. The accuracy of this approach, CT-based rigidity analysis (CTRA), has been validated in a series of ex vivo and in vivo studies.[2-5] This approach provides an image-based "mechanical assay" that characterizes bone tissue material and geometric properties. In fact, CTRA determines axial, bending, and torsional rigidities that correlate with the capacity of the bone to resist axial, bending, and twisting loads, respectively.

SELECTED ABBREVIATIONS AND ACRONYMS

|	
AFM	atomic force microscopy
ASBMR	American Society of Bone and Mineral Research
BMD	bone mineral density
BMDD	bone mineralization density distribution
BMU	bone multicellular unit
BRC	bone remodeling compartment
CSA	cross-sectional area
CSMI	cross-sectional moment of inertia
CT	computed tomography
CTRA	CT-based rigidity analysis
DMP1	dentin matrix protein 1
EFNB2	ephrin-B2
EPHB4	ephrin receptor B4
FGF-23	fibroblast growth factor 23
FSI	Femur Strength Index
IGF-I	insulin-like growth factor-I
IGF-II	insulin-like growth factor-II
LUT	look-up table
M-CSF	macrophage colony-stimulating factor
MicroCT	microcomputed tomography
NFATc1	nuclear factor of activated T-cells, cytoplasmic 1
OPG	osteoprotegerin
qBEI	quantitative backscattered electron imaging
RANKL	receptor activator of nuclear factor κB ligand
S1P	sphingosine-1-phosphate
SEM	scanning electron microscopy
SHG	second-harmonic generation
TEM	transmission electron microscopy
TGF-β	transforming growth factor beta
TPE	two-photon excitation fluorescence

Despite these in vivo approaches to assessing bone mechanical properties, classic bending tests are still the best way to understand macrostructural bone properties. These kinds of tests gave rise to a number of very relevant mechanical concepts, such as the modulus of elasticity, yield stress and yield strain, post-yield stress and post-yield strain, and the total area under the stress-strain curve. The modulus of elasticity shows the stiffness of bone and describes bone behavior before any kind of permanent damage occurs, characterizing the elastic phase. Yield stress and strain determine how much energy can be absorbed before irreversible changes take place at the yield point (microdamage), the moment where bone starts to suffer plastic deformation. Post-yield stress and strain determine mainly how much energy can be absorbed after yield, but before fracture, defining ductility. The total area under the stress-strain curve corresponds to the work done before complete failure of the structure, that is to say, the energy needed for causing a fracture. These bone mechanical properties are intimately related to the microstructural organization of this tissue.

Bone: the microstructural level

The classic microscopic description of cortical bone includes the bone concentric layers around blood vessels, which are designated as Haversian systems, and the transversal channels, known as Volkmann's canals. Trabecular bone has a cellular foam–like structure made of an interconnected network of rods and plates forming the bone trabeculae. The Haversian system layers and the trabeculae in trabecular bone have osteocyte lacunae which connect to each other and to vessels by canaliculi.

The first step toward obtaining more accurate functional information from bone tissue was the advent of bone histomorphometry. This procedure can be defined as the microscopic quantitative and qualitative study of calcified bone samples to obtain data on the microarchitecture and metabolism of bone. Common terminology that fostered the scientific evolution of this technique was established by the American Society of Bone and Mineral Research (ASBMR). Bone, cut into thin slices with a microtome and stained, is evaluated with specific software that analyzes microscopic images, quantifying pixels of 2D images, to determine several histomorphometric parameters, such as bone volume, total volume, trabecular thickness, trabecular separation, trabecular number, and cortical thickness. In addition, the osteoid volume can be measured and the mineralized volume—representing the calcified fraction of trabecular bone volume—calculated *(Figure 1, page 144)*.

That bone metabolism and mineralization over time can be studied remains one of the best advantages of this technique. This approach is based on the use of tetracycline, which is integrated by bone and can act as an in vivo marker of bone growth.[6] With the exception of the information on bone me-
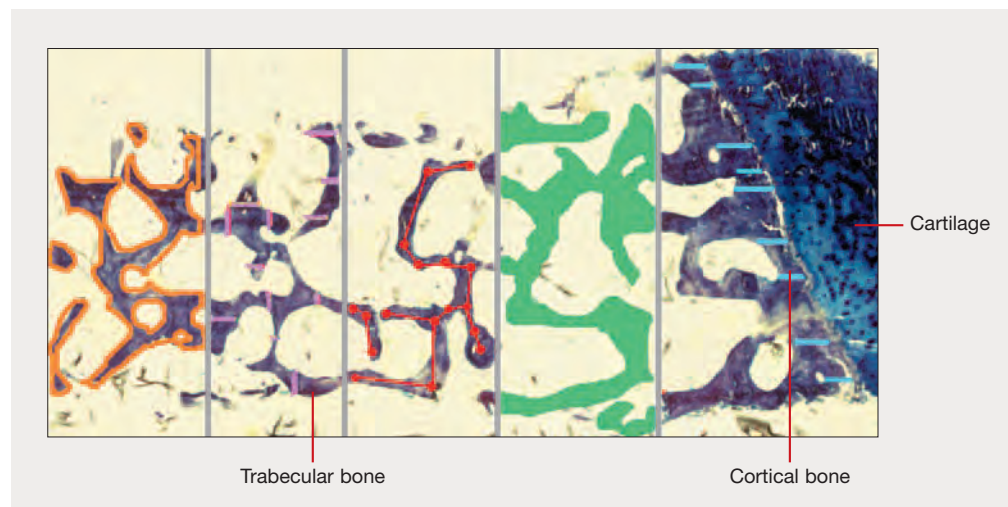
Figure 1. *Example of the main variables that can be obtained from bone histomorphometry analysis.*
● *Perimeter, allows the determination of other parameters such as bone surface, mineralized surface, and eroded surface;* ● *Trabecular thickness, average trabecular thickness, as a function of bone section size;* ● *Trabecular connectivity, supplies information about the organization of the different trabeculae in a bone section, reproducing the trabecular distribution in space (trabecular network);* ● *Trabecular volume, space occupied by mineralized and unmineralized bone, as a function of bone size;* ● *Cortical width, distance between periosteal and endocortical surfaces. (Courtesy of Bruno Vidal).*

Cartilage

Trabecular bone

Cortical bone

Figure 2. *Bone remodeling model results.*
(A) Initial solution. (B) Biological criterion simulation results (13% bone loss). (C) Mechanical criterion simulation results (13% bone loss). (Courtesy of Luis Santos).
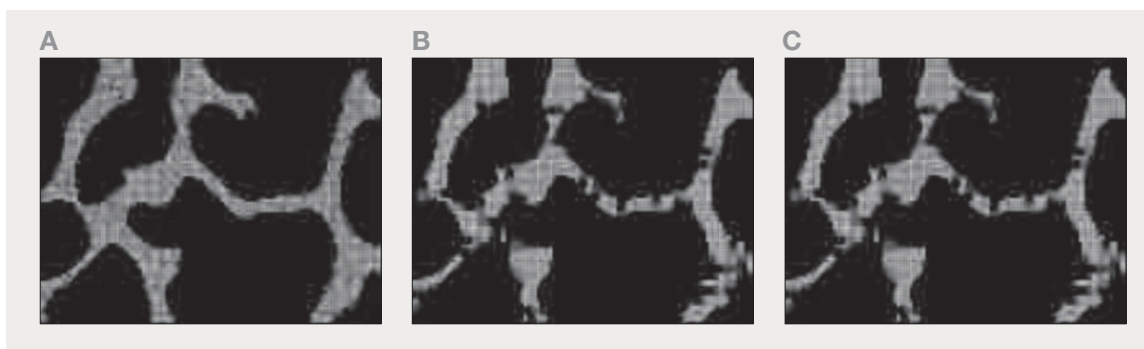
tabolism, classic histomorphometry has been surpassed by bone microcomputed tomography (microCT), which allows a quantitative assessment of the three-dimensional (3D) trabecular bone structural characteristics. The microCT procedure involves three main steps: acquisition, reconstruction, and image analysis. During the acquisition step, scans are performed to obtain approximately 500 images for each sample. In the reconstruction phase, binarization is carried out, which involves the separation of bone from regions without bone tissue. To reconstruct the images, 3D reconstruction software assembles virtual cross-section slices of the bone piece. The image data is then analyzed in order to quantify the parameters that characterize the trabecular bone structure, based on the histomorphometric nomenclature proposed by the ASBMR.[6] The use of mathematical modeling that helps to predict the evolution of a given structure, changing selected variables *(Figure 2),*[7] would be a step forward for analysis of densitometry and microCT data.
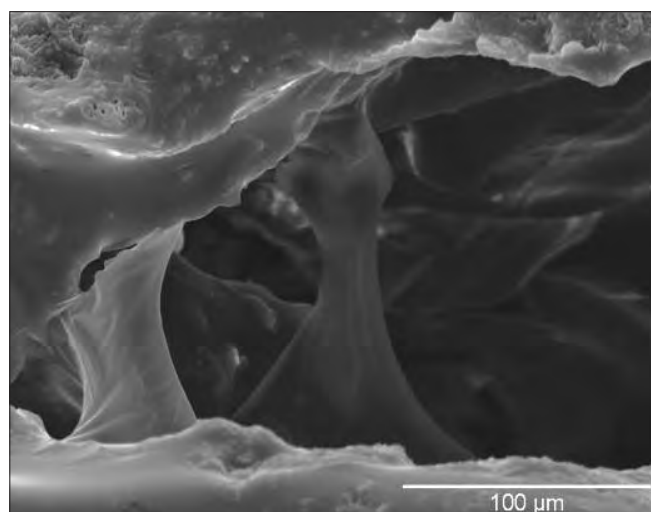
Additional information can be obtained by applying scanning electron microscopy (SEM) to the study of bone. It uses an electron beam that hits the surface of bone and induces secondary low-energy electrons, producing very high–resolution images with a large depth of field *(Figure 3)*. However, histomorphometry, microCT, and SEM are unable to inform on the way bone constituents are organized. This critical information for our current perception of bone is provided by the techniques that will be described below.

Figure 3. *Scanning electron microscopy (SEM) of bone trabeculae.*
Scanning electron microscopy (SEM) uses an electron beam to scan the surface of a sample. Different interactions can be detected when the electron beam hits the material, including emission of secondary electrons, which have low energy as they result from inelastic collisions between the incident beam and the sample, but produce very high–resolution images with a large depth of field. Bone trabeculae can be clearly observed in this image. (Courtesy of Joana Caetano-Lopes).

Bone: the nanostructural level

Transmission electron microscopy (TEM) has been pivotal for understanding the basic nanostructure of bone. TEM uses an electron beam that is transmitted through extremely thin bone samples, generating an image from the beam-sample interactions. It helped characterize bone collagen fibrils and apatite crystals.[8] In fact, TEM established the concept of bone as a heterogeneous and anisotropic material that structurally comprises two phases. The mineral phase is essentially composed of 50%-74% (dry weight) inorganic particles of carbonate-substituted hydroxyapatite embedded in an organic matrix. The organic matrix, which makes up the organic phase, forms the remaining 30%-40% (dry weight) of the bone tissue and is mainly composed of collagen type I (85%-90%).[9] The apatite crystals in bone are mixed structures, with a general formula of $Ca_{10}(PO_4)_6 X_2$.[10] The crystals of bone apatite are plate shaped, with a thickness that ranges from 2 nm (for mineralized tendon) to 7 nm (for some mature bone types), with an average length of 15 nm to 200 nm and a width from 10 nm to 80 nm.[11] The organic phase in bone is mainly made of type I collagen, which is the most abundant fibrillar collagen in the body. The basic unit of collagen type I is the tropocollagen molecule. Each molecule is composed of two identical α_1 and one α_2 polypeptide chains. The three chains are bound together in a tight triple helix, coiled in a right-handed manner, and cross-linked in the extracellular space to form the collagen fibril. In the extracellular space, the tropocollagen molecules are assembled in an axial direction.[12] Collagen molecules are staggered, but there is a gap zone between them in the order of 35 nm, where nucleation of calcium crystals occur. Thus, crystal size and orientation are influenced by collagen organization. Usually, this crystal deposit on the collagen array is in the form of flat plates, parallel to each other and to the axis of collagen fibrils, at regular intervals along the fibrils, at distances corresponding to the distances between collagen molecules. This arrangement is responsible for the anisotropic properties of bone, giving rise to higher values of stiffness and strength in that direction. In addition, the distribution of crystals is not uniform due to bone remodeling, giving rise to different degrees of mineralization, with implications for crack initiation and propagation. Bone mechanical responses are influenced by crystal size, shape, arrangement, and volume fraction, and by collagen spacing, orientation, length, and the strength of intermolecular interactions.[8]

As can be inferred from the bone ultrastructure, herein discussed bone nanostructural properties are independent of the overall bone architecture (cortical thickness, trabecular morphology and bone geometry) and directly dependent on the way bone cells, collagen, and calcium crystals interact. The efficacy of bone as a structural element of our body depends on the balance between the mineral component and the nonmineral component of bone. In fact, bone can be viewed as a collagen-mineral composite made of components with extremely different mechanical properties. Changes in the min-

eralization process or in the interaction between components can have profound effects on bone properties. Further detail can be obtained with atomic force microscopy (AFM) and nanoindentation, which characterize the arrangement of bone components and evaluate its mechanical properties. Complementary information is provided by quantitative backscattered electron imaging (qBEI) that determines bone mineralization density distribution (BMDD), distinguishing differences in the degree of mineralization.

AFM is based on a piezo mechanism, with which the deflection of an arm, due to van der Waals forces between the atom at the tip and the atom at the surface, is measured. Through this mechanism, a structural image can be obtained, detailing crystal and collagen interactions. Besides structural assessment, AFM can be used for nanoindentation, which is performed in three phases: i) a loading phase, during which the tip is pressed into the material up to a maximum force; ii) a holding period, during which the tip penetrates into the material, leaving indentation marks; and iii) an unloading step. Nanoindentation measures the elastic modulus or the stiffness, as well as the hardness of the bone tissue, allowing the measure of intrinsic mechanical properties of bone, such as tissue modulus of trabecular bone and cortical tissue, or the mechanical properties of the osteon for cortical bone and the trabecular wall for trabecular bone. The combination of nanoindentation with AFM offers a surface topography of constant contact force and a force-displacement.[8]

SEM technology is used for detection of backscattered electrons in qBEI. The intensity of backscattered electrons increases with the atomic number. Calcium has the highest atomic number, thus dominating the intensity of the backscattered beam. In this imaging technique, different gray levels correlate to calcium content, producing a BMDD. qBEI clearly shows that mineralization is not uniform, and this is due to the fact that this process evolves over time, starting with a rapid phase of mineralization over a few days combined with a slower phase that takes years. Thus, less mineralized zones are generally found in fresh bone. However, old bone is continuously remodeling, mitigating this effect.[8]

Another fascinating approach to the study of bone nanostructure is multiphoton nonlinear microscopy that uses intrinsic two-photon excitation fluorescence (TPE) combined with second-harmonic generation (SHG) to visualize collagen fiber orientation *(Figure 4, page 146).* This technique produces images with the resolution and detail of standard histology without the use of exogenous dyes. Due to the nonsymmetrical arrangement of collagen when an electric field is applied, an oscillating field is produced at twice the frequency (SHG). The multiphoton microscope acquires the signal by two opposing detectors: i) a detector associated with a green look-up table (LUT) and referred to as backward-SHG, detecting the backscattered SHG signal, and ii) a detector associated with the
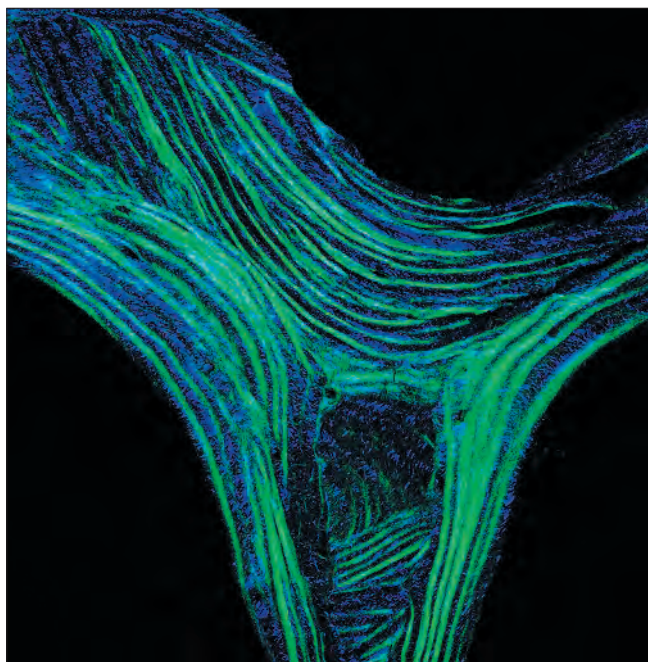
Figure 4. *Collagen fiber orientation.*
Multiphoton nonlinear microscopy uses intrinsic two-photon excitation fluorescence (TPE) combined with second-harmonic generation (SHG) to visualize collagen fiber orientation. The green backscattered-SHG image represents a less dense and immature collagen network, while the blue image coming from the forward-SHG channel captures a more dense and polymerized collagen matrix. (Courtesy of Joana Caetano-Lopes).

blue LUT and referred to as forward-SHG, detecting the forwarded signal. The green backscattered-SHG image represents a less dense and immature collagen network, while the blue image coming from the forward-SHG channel captures a more dense and polymerized collagen matrix. Image analysis software is used to determine the amount of mature and immature collagen in a given area, and the proportions of these 2 variables have been shown to be affected by bone diseases.[13,14]

Understanding the balanced properties of the mineral and organic phases of bone is crucial. In fact, the mineral phase confers strength and stiffness to the bone tissue, but after a certain degree of mineralization, bone becomes brittle, reducing the energy required for fracture. On the other hand, the organic phase is more ductile and defines how much energy can be absorbed after the first microcracks, but before failure (fracture), therefore determining post-yield properties and the toughness of the overall bone. However, the overall picture is more complex and the dis-

crepancies in the degree of mineralization between different bone areas are pivotal in the pro-cess of microcrack formation and accumulation, which is a process that increases bone compliance so that it can sustain larger deformations, thus contributing to bone toughness. It is now clear that the overall bone mechanical properties are dependent on nanostructural aspects, including the amount and distribution of mineral in the tissue; collagen content and orientation of the collagen fibers; crystal shape, size, and arrangement; and accumulation of microcracks.[12]

Microcracks are a normal component of bone and bone intrinsic properties are able to deal with it up to a certain level, avoiding loss of stiffness that leads to fracture. However, this is not enough to keep a bone healthy. Remodeling is the bone's repair mechanism, helping to prevent the propagation of microcracks and their evolution into macrocracks, which can lead to complete fracture. In the next section, we will see how the bone cell molecular machinery ensures that remodeling meets bone repair needs.

Bone: the cell and molecular level

Animal models, cell culture, gene expression studies, and protein analysis have been pivotal in outlining the fine control of the bone remodeling process, which involves a mechanism that couples bone resorption and formation *(Figure 5)*.

Resorption is the first event that occurs in response to a mechanical stress signal and is much faster than formation: an area of bone can be resorbed in 2-3 weeks, but it takes at least 3 months to rebuild it.[15] The working concept of this process is based on the bone multicellular unit (BMU),[16] con-
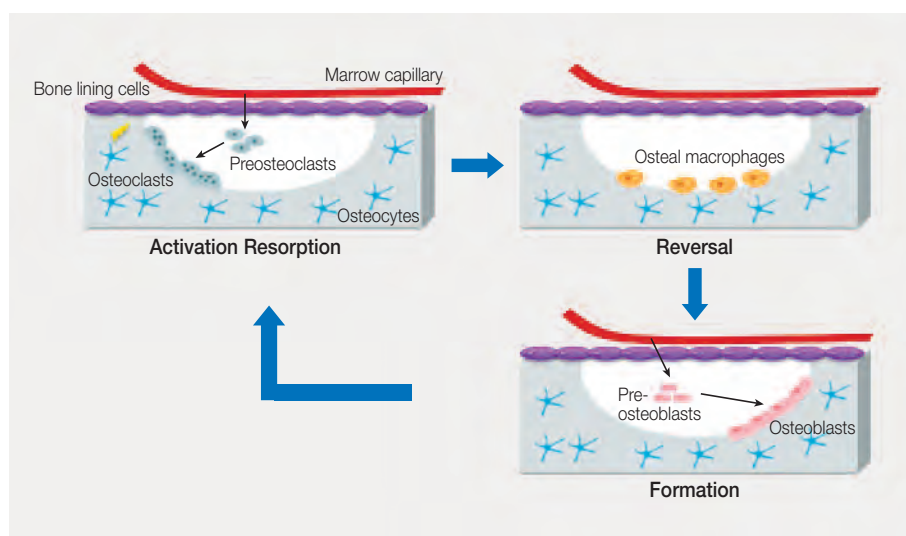
Figure 5. *The remodeling process.*
The remodeling process takes place inside the bone remodeling compartment. Bone remodeling is hypothesized to be initiated by osteocyte apoptosis (activation phase), which sends a signal for the recruitment of osteoclast precursors, which then differentiate and resorb bone in this closed microenvironment (resorption phase). Following osteoclast-mediated resorption, the lacunae remain covered with undigested demineralized collagen matrix and are cleaned by osteal macrophages (reversal phase). At last, osteoblasts arrive at the bone remodeling compartment where they deposit new bone (formation phase). (Courtesy of Joana Caetano-Lopes).

Bone biology: from macrostructure to gene expression – *Fonseca*

stituted by groups of interplaying osteoclasts, osteoblasts, and osteocytes.[17] Inside the BMU, osteoclasts are always at the front of the advancing BMU and osteoblasts are in the back. At any given time, several million BMUs carry out turnover at multiple skeletal sites in an activation-reversal-formation cycle that in a healthy adult human lasts 6-9 months.[18] Osteoblasts and their regulators determine osteoclastogenesis, while osteoclastogenesis and the products of the degraded matrix regulate osteoblastogenesis. In addition, both processes may be regulated by osteocytes and their products. Bone remodeling occurs at the BMU level and involves several sequential steps beginning with osteoclast formation, progressing to osteoclast-mediated bone resorption, a reversal period in which the matrix is prepared for the next phase, a long period of osteoblast-mediated bone matrix formation, and mineralization of the matrix.[19,20] This process occurs in the bone remodeling compartment (BRC), which is covered by a canopy of cells that display the classic osteoblast lineage markers and, therefore, are most probably lining cells.[21] Capillaries infiltrate the BRC, bringing both osteoclast and osteoblast precursor cells.[22] The BRC is the structure that translates microdamage sensed by the osteocyte network into osteoclast and osteoblast activity. Signals sensed by osteocytes are transmitted to the layer of lining cells,[23] which express osteoprotegerin (OPG) and receptor activator of nuclear factor κB ligand (RANKL)[24] and trigger osteoclast precursor recruitment.

Studies of mechanical loading in human bone, ex vivo, and of rat bone, in vivo, determined that osteocyte apoptosis is increased by loading and was accompanied by increased remodeling.[25] Under normal conditions, osteocytes secrete transforming growth factor beta (TGF-β), inhibiting osteoclastogenesis. When these cells undergo apoptosis, TGF-β levels diminish, removing the osteoclastogenesis inhibitory signal and allowing osteoclast formation.[26] Moreover, osteocytes are able to secrete OPG and macrophage colony-stimulating factor (M-CSF) and express RANKL, which enable them to control osteoclastogenesis.[27]

The transition from bone resorption to bone formation might be partially dependent on molecules stored within bone matrix, such as insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), or TGF-β,[28] but other coupling mechanisms are also relevant, such as sphingosine-1-phosphate (S1P) and the bidirectional signaling mediated by ephrin receptor B4/ephrin-B2 (EPHB4/EFNB2). S1P is secreted by osteoclasts, induces osteoblast precursor recruitment, and promotes mature cell survival.[29] EPHB4 receptors are expressed by osteoblasts, and EFNB2 is located in osteoclasts. Forward signaling through EPHB4 into osteoblasts enhances osteogenesis, and reverse signaling through EFNB2 into osteoclast precursors suppresses osteoclast differentiation by inhibiting the osteoclastogenic c-Fos-NFATc1 (nuclear factor of activated T-cells, cytoplasmic 1) cascade.[30] In addition, mechanical stimulation can induce bone formation signals through osteocytes by inhibiting SOST expression and thus removing the inhibition of WNT/β-catenin signaling, allowing bone formation to occur.[31]

Once bone formation is complete and the bone surface is covered with lining cells, the matrix continues to be mineralized in a process dependent on the signaling of osteocytes, particularly on the expression of dentin matrix protein 1 (DMP1), X-linked phosphate regulating endopeptidase homolog (PHEX), and fibroblast growth factor 23 (FGF-23).[32] The lost SOST expression returns toward the end of the remodeling cycle. Following mineralization, mature osteoblasts undergo apoptosis, revert to a lining-cell phenotype, or differentiate into osteocytes, and the remodeling cycle is completed.

Conclusion

The perception of bone as a complex living tissue that combines hierarchical structures and properties was the product of the introduction of complementary technologies over the years. Currently, it is clear that a comprehensive study of bone biological phenomena, including the testing of new drugs for osteoporosis, requires use of the full panoply of techniques herein discussed. ■

References
1. Faulkner KG, Wacker WK, Barden HS, et al. Femur strength index predicts hip fracture independent of bone density and hip axis length. *Osteoporos Int.* 2006;17:593-599.
2. Snyder B, Hecht AC, Tedrow JR, Hauser DL. Structural rigidity measured by CT accurately predicts fracture in children with benign tumors of the appendicular skeleton. 45th Annual Meeting of the Orthopedic Research Society. Orlando, FL. 2002:25;243.
3. Aaron M, Snyder BD, Wilson S, et al. Noninvasive prediction of fracture risk in patients with metastatic breast cancer to the spine. 47th Annual Meeting of the Orthopedic Research Society. Dallas, TX. 2002.
4. Entezari V, Basto PA, Vartanians V, Zurakowski D, Snyder BD, Nazarian A. Non-invasive assessment of failure torque in rat bones with simulated lytic lesions using computed tomography based structural rigidity analysis. *J Biomech.* 2011;44:552-556.
5. Nazarian A, Pezzella L, Tseng A, et al. Application of structural rigidity analysis to assess fidelity of healed fractures in rat femurs with critical defects. *Calcif Tissue Int.* 86(5):397-403.
6. Parfitt AM, Drezner MK, Glorieux FH, et al. Bone histomorphometry: standard-

ization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res.* 1987;2:595-610.
7. Santos L, Romeu JC, Canhão H, Fonseca JE, Fernandes PR. A quantitative comparison of a bone remodeling model with dual-energy X-ray absorptiometry and analysis of the inter-individual biological variability of femoral neck T-score. *J Biomech.* 2010;43:3150-3155.
8. Vaz MF, Canhao H, Fonseca JE. Bone: a composite natural material. *Composite Materials. InTech.* In press.
9. Currey JD. The design of mineralised hard tissues for their mechanical functions. *J Exp Biol.* 1999; 202:3285-3294.
10. Posner AS. The Mineral of Bone. *Clin Orthop Relat Res.* 1985;200:87-99.
11. Fratzl P, Gupta HS, Paschalis EP, Roschger P. Structure and mechanical quality of the collagen–mineral nano-composite in bone. *J Mater Chem.* 2004;14: 2115-2123.
12. Abdulghani S, Caetano-Lopes J, Canhão H, Fonseca JE. Biomechanical effects of inflammatory diseases on bone-rheumatoid arthritis as a paradigm. *Autoimmun Rev.* 2009;8:668-671.
13. Caetano-Lopes J, Nery AM, Canhão H, et al. Chronic arthritis leads to distur-

bances in the bone collagen network. *Arthritis Res Ther.* 2010;12(1):R9.

14. Caetano-Lopes J, Nery AM, Henriques R, et al. Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. *Clin Exp Rheumatol.* 2009;27:475-482.

15. Bilezikian JP, Raisz LG, Rodan GA. *Principles of bone biology.* San Diego, CA: Academic Press; 2002.

16. Hattner R, Epker BN, Frost HM. Suggested sequential mode of control of changes in cell behaviour in adult bone remodelling. *Nature.* 1965;206:489-490.

17. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord.* 2010;11(4):219-227.

18. Manolagas SC, Parfitt AM. What old means to bone. *Trends Endocrinol Metab.* 2010;21:369-374.

19. Sims NA, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Semin Cell Dev Biol.* 2008;19:444-451.

20. Proff P, Romer P. The molecular mechanism behind bone remodelling: a review. *Clin Oral Investig.* 2009;13:355-362.

21. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res.* 2001;16:1575-1582.

22. Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. Circulating osteoblast-lineage cells in humans. *N Engl J Med.* 2005;352:1959-1966.

23. Civitelli R. Cell-cell communication in the osteoblast/osteocyte lineage. *Arch Biochem Biophys.* 2008;473:188-192.

24. Silvestrini G, Ballanti P, Patacchioli F, et al. Detection of osteoprotegerin (OPG) and its ligand (RANKL) mRNA and protein in femur and tibia of the rat. *J Mol Histol.* 2005;36:59-67.

25. Mann V, Huber C, Kogianni G, Jones D, Noble B. The influence of mechanical stimulation on osteocyte apoptosis and bone viability in human trabecular bone. *J Musculoskelet Neuronal Interact.* 2006;6:408-417.

26. Heino TJ, Hentunen TA, Vaananen HK. Osteocytes inhibit osteoclastic bone resorption through transforming growth factor-beta: enhancement by estrogen. *J Cell Biochem.* 2002;85:185-197.

27. Heino TJ, Kurata K, Higaki H, Vaananen HK. Evidence for the role of osteocytes in the initiation of targeted remodeling. *Technol Health Care.* 2009;17:49-56.

28. Henriksen K, Neutzsky-Wulff AV, Bonewald LF, Karsdal MA. Local communication on and within bone controls bone remodeling. *Bone.* 2009;44:1026-1033.

29. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc Natl Acad Sci USA.* 2008;105:20764-20769.

30. Zhao C, Irie N, Takada Y, et al. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab.* 2006;4:111-121.

31. Robling AG, Niziolek PJ, Baldridge LA, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J Biol Chem.* 2008;283:5866-5875.

32. Yuan B, Takaiwa M, Clemens TL, et al. Aberrant Phex function in osteoblasts and osteocytes alone underlies murine X-linked hypophosphatemia. *J Clin Invest.* 2008;118:722-734.

BIOLOGIE OSSEUSE : DE LA MACROSTRUCTURE À L'EXPRESSION DU GÈNE

L'architecture globale de l'os (épaisseur corticale, morphologie trabéculaire et géométrie osseuse) détermine ses caractéristiques fonctionnelles de base. Cependant, ses propriétés nanostructurelles influent sur la structure globale et sont directement dépendantes des interactions entre les cellules osseuses, le collagène et les cristaux de calcium. Aux niveaux cellulaire et moléculaire de la structure hiérarchique complexe de l'os se produit un remodelage osseux constant et minutieusement régulé, par l'action conjointe de la résorption et de la formation au sein des différents compartiments de remodelage. Une nouvelle compréhension de la complexité de la biologie osseuse a été possible grâce au progrès des technologies d'imagerie, des tests mécaniques et de la biologie cellulaire. Cet article dresse un parallèle entre l'avènement des approches techniques innovantes et les progrès de notre connaissance de la biologie osseuse.

Bone remodeling: a social network of cells

by P. J. Marie, *France*

> Bone remodeling is a dynamic process that requires coordinated activity between osteoclasts, osteoblasts, and osteocytes to maintain bone homeostasis. Remodeling is tightly controlled by a social network in which these cells communicate with each other by signals that stimulate or inhibit bone formation or resorption. The network plays a key role in maintaining skeletal integrity… An important challenge for the future is to identify the bone cell interaction that is affected during bone remodeling in diseases such as osteoporosis."

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*B*one remodeling is a dynamic process that requires coordinated cellular activities between osteoclasts, osteoblasts, and osteocytes to maintain bone homeostasis. Bone cells differing in origin and function communicate with each other in a social network to stimulate or inhibit bone formation or resorption through signaling processes. The network plays a key role in controlling bone cell activity and maintaining skeletal integrity. The communication mechanisms involved include soluble factors such as cytokines, receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin, produced locally by osteoblasts or osteocytes for the regulation of osteoclastogenesis. Conversely, factors released by osteoclasts during bone resorption or by osteocytes control osteoblast genesis and function. Cell-cell connecting molecules such as cadherins and connexins in osteoblasts and osteocytes are essential to bone cell function, while bidirectional communication molecules (ephrins) control bone remodeling by linking osteoblasts to osteoclasts. This review summarizes current knowledge of the mechanisms that bone cells use to communicate with each other in the control of bone remodeling and outlines future challenges.

The skeleton is composed of a mineralized collagenous bone matrix dynamically maintained throughout life by bone remodeling, a complex process that renews a fraction of cortical or trabecular bone in order to preserve skeletal integrity and properties.[1,2] Remodeling begins with the recruitment and differentiation of bone-resorbing osteoclasts. The resorbed bone is then replaced by bone-forming osteoblasts. A number of these osteoblasts remain embedded within the bone matrix where they give rise to osteocytes, the third bone cell type involved in remodeling.

Bone remodeling is regulated by a variety of systemic hormones as well as by molecules released by the central nervous system.[3] Local factors are also involved, such as cytokines, growth factors,[4] and cell-matrix interactions.[5] Up to recently, bone cells were considered separate entities originating from distinct lineages and having no or little connection. There is now ample evidence that they communicate with each other to control bone homeostasis.[6] Recent data support the concept of a social network of communicating cells that differ in origin and function. Several direct and indirect links between bone cells have been identified. Cells interact by releasing soluble regulatory molecules, while transmembrane molecules create cell-cell

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connections.[7] These network interactions play a major role in the control of bone remodeling and have a significant impact on bone homeostasis. This review summarizes our knowledge of the mechanisms that bone cells use to communicate with each other in the control of bone remodeling.

Bone remodeling: a process controlled by bone cell interaction

Bone remodeling is an important process that continuously renews the cortical and trabecular envelopes throughout life. It repairs microdamage, maintains mineral homeostasis, and ensures mechanical competence by modifying the microarchitecture.[1,2] Remodeling takes place within bone multicellular units (BMUs), beginning with the activation and fusion of preosteoclasts derived from the monocyte-macrophage lineage. These then differentiate to become active bone-resorbing osteoclasts located along the bone surface they are resorbing. Osteoclast activity is highly dependent on cell attachment to the bone matrix and the release of protons and proteolytic enzymes into the subosteoclast compartment. Following bone resorption (which lasts about 1 month in humans), osteoclasts detach and die, leaving time and space for an intermediate reversal phase in which preosteoblasts are recruited from mesenchymal stromal cells in the bone marrow. These differentiate into mature osteoblasts that form a new bone matrix on the resorption site over approximately 3 months. At the end of this period, about 1 osteoblast in 10 embeds within the newly formed bone matrix to become an osteocyte. Osteoblasts and osteocytes keep connected through cytoplasmic extensions within a canalicular network.[8] Other osteoblasts either die by apoptosis or line the newly formed matrix. Usually the amount of bone removed by osteoclasts is equal to the amount of bone formed by osteoblasts and a stable bone mass is maintained. However, this is not the case during aging when significant bone loss results from imbalance between bone resorption and bone formation.

SELECTED ABBREVIATIONS AND ACRONYMS

BMU	bone multicellular unit
Cx	connexin
Eph	ephrin
IGF-I	insulin-like growth factor-I
IL	interleukin
LRP	low density lipoprotein receptor-related protein
MCSF	macrophage colony-stimulating factor
MSC	mesenchymal stromal cell
NFAT	nuclear factor of activated T cells
OPG	osteoprotegerin
PTH	parathyroid hormone
RANK	receptor activator of nuclear factor κB
RANKL	receptor activator of nuclear factor κB ligand
TGF	transforming growth factor
TNF	tumor necrosis factor

At first sight, bone resorption and formation resemble distinct processes occurring at different times and involving different cell types. In fact, however, bone remodeling is tightly controlled to ensure that the right event occurs at the right time and in the right space. It was initially believed this was mainly the work of circulating hormones such as parathyroid hormone (PTH), sex hormones, glucocorticoids, and vitamin D. We now know that bone remodeling largely depends on cell communication between osteoblasts and osteoclasts.[6] In addition, correct coupling of bone resorption to bone formation is essential for maintaining skeletal integrity and bone homeostasis. Coupling is ensured by local factors and cell-cell communication molecules. Impaired communication between bone cells uncouples resorption and formation, resulting in impaired remodeling and bone pathology.

How osteoblasts control osteoclasts

◆ Cytokines and growth factors

Mesenchymal stromal cells (MSCs), preosteoblasts, and mature osteoblasts produce a number of soluble factors such as cytokines that act locally on preosteoclasts and osteoclasts in the bone marrow. Osteoblast cytokines include interleukins (IL) -1 and -6 and tumor necrosis factor (TNF)-α. These and other cytokines control osteoclast differentiation by activating specific receptors on osteoclast precursor cells. Evidence for the importance of these cytokines is that estrogen deficiency increases the secretion of IL-1 and TNF-α, thereby activating osteoclast differentiation from precursor cells.[9] This stimulates bone remodeling with an imbalance in resorption over formation, resulting in net bone loss. Although other locally secreted molecules increase bone resorption by osteoclasts in estrogen deficiency, strategies targeting bone-resorbing cytokines have prevented bone loss in ovariectomized mice.[10] Some growth factors produced by osteoblasts control osteoclast function. For example, osteoblasts produce transforming growth factor-β (TGF-β) which binds to a macromolecule in the bone matrix. On release from the matrix during bone resorption, TGF-β acts locally to protect osteoclasts from apoptosis[11] and promotes osteoclast differentiation and bone resorption.[12] This emphasizes the importance of factors derived from cells of the osteoblast lineage in the control of bone resorption and bone mass (Figure 1).

◆ RANKL and OPG

Besides cytokines and growth factors, cells of the osteoblast lineage produce other soluble molecules that control osteoclast differentiation. Osteoblast precursors and osteoblasts express receptor activator of nuclear factor κB ligand (RANKL), a molecule that binds to the receptor activator of nuclear factor κB (RANK) on osteoclast precursor cells and thereby activates intracellular signaling, resulting in osteoclast differentiation. Osteoblast precursors and osteoblasts also release osteoprotegerin (OPG), a decoy protein that blocks RANK interaction with its ligand, thereby inhibiting osteoclast differentiation.[13] The RANKL/OPG ratio controls bone remodeling
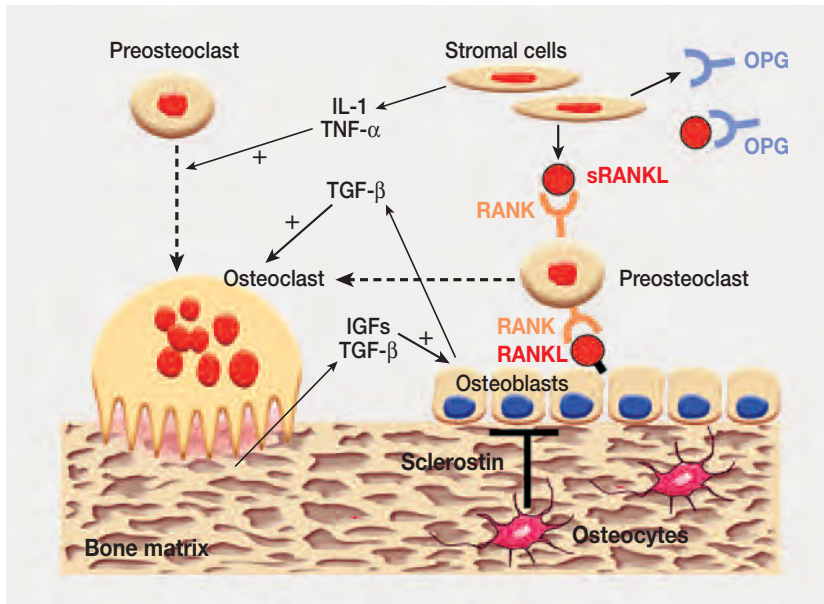
◆ *Ephrins*

Ephrins are important molecules that mediate osteoclast-osteoblast interaction by simultaneous signal transduction in both cell types. Ephrin receptors (Eph) and their ligands (ephrin) are expressed by osteoclasts and osteoblasts, and help to control bone remodeling.[20,21] Cell-cell interaction mediated by the osteoclast-expressed ephrin-B2 ligand and its osteoblast-expressed EphB4 receptor generates bidirectional anti-osteoclastogenic and pro-osteobla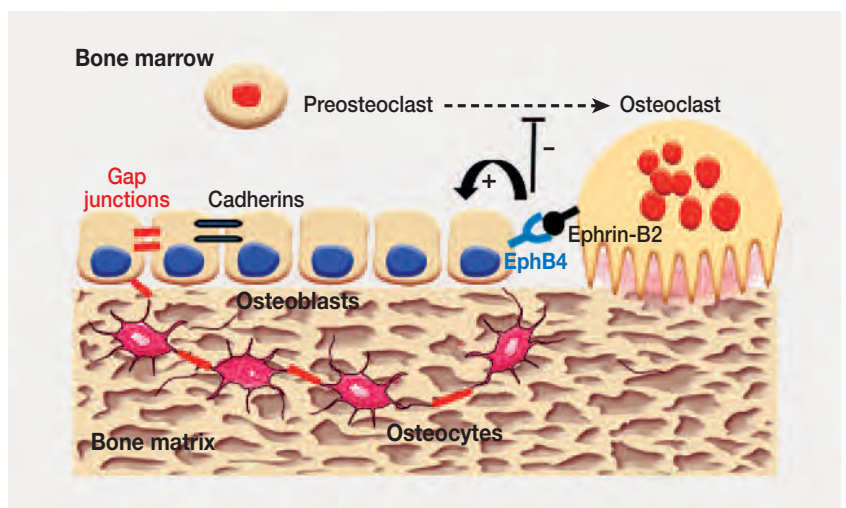stogenic signals. Reverse signaling through ephrin-B2 into osteoclast precursors suppresses osteoclast differentiation by inhibiting the osteoclastogenic c-Fos-nuclear factor of activated T cells (NFAT) c1 cascade. In contrast, forward signaling through EphB4 into osteoblasts enhances osteogenic differentiation, and overexpression of EphB4 in osteoblasts increases bone mass in transgenic mice.[22,23] Furthermore, ephrin-A2/EphA2 interaction facilitates the initiation phase of bone remodeling by enhancing osteoclast differentiation and suppressing osteoblast differentiation.[24] Blocking ephrin-B2/ EphB4 receptor interaction inhibits osteoblast differentiation, which may help to control bone formation at remodeling sites.[25] These data indicate that ephrin-Eph bidirectional signaling links osteoclast and osteoblast differentiation, which may facilitate the transition from bone resorption to bone formation during bone remodeling *(Figure 2).*[26] This concept is supported by the finding that PTH increases ephrin-B2 expression in osteoblasts, which may account for the hormone's anabolic action on bone.[27]

through its key role in osteoclast differentiation.[14] This discovery has important implications since both molecules were found to mediate the increased bone resorption induced by estrogen deficiency and other states characterized by high bone remodeling.[14] Targeting RANKL using specific antibodies has proved a promising therapeutic strategy to reduce osteoclast differentiation, osteoclast number, and bone resorption in several diseases, including postmenopausal osteoporosis.[15] This important finding emphasizes the part played by osteoblasts in the control of osteoclasts *(Figure 1).*

How osteoclasts control osteoblasts
◆ *Coupling factors*
Bone resorption by osteoclasts induces the release of certain growth factors that are included within the bone matrix and can act as coupling factors between resorption and formation.[16] One such factor, TGF-β, is released during bone resorption and acts locally to recruit osteoblast precursor cells, increase osteoblast replication, and stimulate osteoblast production of collagen type 1. Another growth factor released during bone resorption is insulin-like growth factor-I (IGF-I), a potent activator of bone formation.[4,17] This led to the concept that growth factors released during bone resorption may couple osteoclasts to osteoblasts during the remodeling cycle.[18,19] The mechanism linking bone resorption to bone formation is likely to be important in the control of remodeling by osteoclast-osteoblast communication *(Figure 1).*

Figure 2. Adherens junctions and gap junctions in cell-cell communication during bone remodeling.
Abbreviation: EphB4, ephrin B4.

How osteoblasts control osteoblasts
◆ *Adherens junctions*
The coordination of bone remodeling depends on intercellular mechanisms involved in cell-cell communication to generate and synchronize bone cell activity. These mechanisms include adherens junctions (cadherins) and communicating junctions (gap junctions) formed by connexins *(Figure 2).*[28] Cell-cell interaction mediated by cadherins is essential for the function of bone-forming cells during osteogenesis.[29,30] Osteoblasts express a limited number of cadherins, including the classic N-cadherin, which controls the expression of osteoblast differentiation and survival. Cadherins interact with β-catenin and Wnt coreceptors LRP5/6 and thereby control Wnt signaling.[31,32] Thus, altered N-cadherin expression in osteoblasts leads to aberrant Wnt signaling, inhibition of osteoblast differentiation, and delayed osteogenesis.[31-34] In addition, N-cadherins link osteoblasts and osteoclasts. A dominant negative N-cadherin that affects Wnt signaling reduces osteoclastogenesis by altering heterotypic interaction with osteoclast precursors and reducing RANKL expression, which helps to reduce osteoclast formation.[35] N-cadherin thus plays a major role in osteoblast function and osteoblast-osteoclast communication, and hence in the control of bone cells and bone mass.

◆ *Gap junctions*
Osteoblasts are interconnected by gap junctions formed by transmembrane channels (connexin43 [Cx43] and Cx45) that allow efficient cell-cell communication *(Figure 2).* Gap junctions play a critical role in coordinating osteoblast activity. Cx43 is required for normal osteoblast differentiation and function, as its deletion results in osteoblast dysfunction and low bone mass in mice.[36] Transmission of soluble molecules via connexin gap junctions allows propagation of specific signals and response to anabolic agents such as PTH, which then translate into gene expression.[37-39] A communicating intercellular network involving gap junctions connecting osteocytes and osteoblasts is also involved in the skeletal response to mechanical forces.[40] This network mediated by adherens junctions formed by cadherins and communicative gap junctions formed by connexins is therefore an important mechanism controlling cell-cell communication and maintaining skeletal integrity.

How osteocytes control osteoclasts
Osteocytes are the most common cell type in bone and form an extensive connecting network via cytoplasmic processes present in canaliculi. They communicate with osteoblasts and other osteocytes through gap junctions and other mechanisms to transmit information and influence bone formation and resorption.[8,41] There is extensive evidence for interaction between osteocytes and bone remodeling activity. Osteocytes

secrete prostaglandins, nitric oxide, and TGF-β. On release into the bone marrow compartment these molecules may affect the recruitment and function of osteoblasts. However, osteocytes also secrete RANKL and macrophage colony-stimulating factor (MCSF), and when these reach the marrow they can modulate the recruitment and function of osteoclasts.[8,41] Microdamage increases in aging, causing rupture of osteocyte canaliculae and cell death.[42] Dying osteocytes release signaling molecules into the bone marrow, which could in turn recruit osteoclasts to a new remodeling cycle for replacing the damaged bone matrix.[42]

How osteocytes control osteoblasts
Soluble molecules released by osteocytes play a role in the control of osteoblasts. One such important factor is sclerostin *(Figure 1),* a protein that negatively interacts with Wnt signaling and thereby inhibits osteoblast function and bone formation.[43] The finding that osteocyte sclerostin expression increases in response to PTH treatment[44,45] and mechanical stimulation[46] emphasizes the protein's role in the control of bone formation. In summary, all the available data indicate that osteocytes are key cells in the control of bone resorption and formation through the release of soluble mediators targeted at other bone cell types.

Conclusion and future challenges
Bone remodeling is a dynamic process that requires coordinated activity between osteoclasts, osteoblasts, and osteocytes to maintain bone homeostasis. Remodeling is tightly controlled by a social network in which these cells communicate with each other by signals that stimulate or inhibit bone formation or resorption. The network plays a key role in maintaining skeletal integrity. Because impaired bone cell communication leads to bone pathology, we need to explore all the ramifications of this network if we are to improve our understanding of bone remodeling in normal and pathological conditions.

An important challenge for the future is to identify the bone cell interaction that is affected during bone remodeling in diseases such as osteoporosis. Such knowledge would improve our understanding of the pathophysiology at tissue level and generate novel targeted strategies. For example, we already know that we can target RANKL (linking preosteoblasts and preosteoclasts) and sclerostin (linking osteocytes and osteoblasts) to reduce bone resorption or increase bone formation, respectively, in osteoporosis. Identifying novel molecules and other intercellular mechanisms will give us a better understanding of how bone remodeling is controlled and should facilitate the development of new approaches to the control of bone remodeling and bone mass in a variety of pathological conditions. ■

References

1. Riggs BL, Parfitt AM. Drugs used to treat osteoporosis: the critical need for a uniform nomenclature based on their action on bone remodeling. *J Bone Miner Res.* 2005;20(2):177-184.
2. Marie PJ. The molecular genetics of bone formation: implications for therapeutic interventions in bone disorders. *Am J Pharmacogenomics.* 2001;1(3):175-187.
3. Karsenty G, Oury F. The central regulation of bone mass, the first link between bone remodeling and energy metabolism. *J Clin Endocrinol Metab.* 2010;95 (11):4795-4801.
4. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev.* 2008;29(5):535-559.
5. Marie PJ. Bone cell-matrix protein interactions. *Osteoporos Int.* 2009;20(6): 1037-1042.
6. Rodan GA, Martin TJ. Role of osteoblasts in hormonal control of bone resorption—a hypothesis. *Calcif Tissue Int.* 1982;34(3):311.
7. Sims NA, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Semin Cell Dev Biol.* 2008;19(5): 444-451.
8. Bonewald LF. The amazing osteocyte. *J Bone Miner Res.* 2011;26(2):229-238.
9. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. *J Bone Miner Res.* 1996;11(8):1043-1051.
10. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest.* 2006;116(5):1186-1194.
11. Janssens K, ten Dijke P, Janssens S, Van Hul W. Transforming growth factor-beta1 to the bone. *Endocr Rev.* 2005;26(6):743-774.
12. Mohammad KS, Chen CG, Balooch G, et al. Pharmacologic inhibition of the TGF-beta type I receptor kinase has anabolic and anti-catabolic effects on bone. *PLoS One.* 2009;4(4):e5275.
13. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature.* 2003;423(6937):337-342.
14. Hofbauer LC, Kühne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. *J Musculoskelet Neuronal Interact.* 2004;4(3):268-275.
15. Baron R, Ferrari S, Russell RG. Denosumab and bisphosphonates: different mechanisms of action and effects. *Bone.* 2011;48(4):677-692.
16. Howard GA, Bottemiller BL, Turner RT, Rader JI, Baylink DJ. Parathyroid hormone stimulates bone formation and resorption in organ culture: evidence for a coupling mechanism. *Proc Natl Acad Sci U S A.* 1981;78(5):3204-3208.
17. Hayden JM, Mohan S, Baylink DJ. The insulin-like growth factor system and the coupling of formation to resorption. *Bone.* 1995;17(suppl 2):93S-98S.
18. Henriksen K, Neutszky-Wulff AV, Bonewald LF, Karsdal MA. Local communication on and within bone controls bone remodeling. *Bone.* 2009;44(6):1026-1033.
19. Martin TJ, Sims NA. Osteoclast-derived activity in the coupling of bone formation to resorption. *Trends Mol Med.* 2005;11(2):76-81.
20. Edwards CM, Mundy GR. Eph receptors and ephrin signaling pathways: a role in bone homeostasis. *Int J Med Sci.* 2008;5(5):263-272.
21. Martin TJ, Allan EH, Ho PW, et al. Communication between ephrinB2 and EphB4 within the osteoblast lineage. *Adv Exp Med Biol.* 2010;658:51-60.
22. Zhao C, Irie N, Takada Y, et al. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab.* 2006;4(2):111-121.
23. Matsuo K, Irie N. Osteoclast-osteoblast communication. *Arch Biochem Biophys.* 2008;473(2):201-209.
24. Irie N, Takada Y, Watanabe Y, et al. Bidirectional signaling through ephrinA2-EphA2 enhances osteoclastogenesis and suppresses osteoblastogenesis. *J Biol Chem.* 2009;284(21):14637-14644.
25. Allan EH, Häusler KD, Wei T, et al. EphrinB2 regulation by PTH and PTHrP revealed by molecular profiling in differentiating osteoblasts. *J Bone Miner Res.* 2008;23(8):1170-1181.
26. Matsuo K. Eph and ephrin interactions in bone. *Adv Exp Med Biol.* 2010;658: 95-103.
27. Civitelli R. Cell-cell communication in the osteoblast/osteocyte lineage. *Arch Biochem Biophys.* 2008;473(2):188-192.
28. Marie PJ. Role of N-cadherin in bone formation. *J Cell Physiol.* 2002;190(3): 297-305.
29. Stains JP, Civitelli R. Cell-to-cell interactions in bone. *Biochem Biophys Res Commun.* 2005;328(3):721-727.
30. Mbalaviele G, Shin CS, Civitelli R. Cell-cell adhesion and signaling through cadherins: connecting bone cells in their microenvironment. *J Bone Miner Res.* 2006;21(12):1821-1827.
31. Cheng SL, Shin CS, Towler DA, Civitelli R. A dominant negative cadherin inhibits osteoblast differentiation. *J Bone Miner Res.* 2000;15(12):2362-2370.
32. Haÿ E, Laplantine E, Geoffroy V, et al. N-cadherin interacts with axin and LRP5 to negatively regulate Wnt/beta-catenin signaling, osteoblast function, and bone formation. *Mol Cell Biol.* 2009;29(4):953-964.
33. Haÿ E, Nouraud A, Marie PJ. N-cadherin negatively regulates osteoblast proliferation and survival by antagonizing Wnt, ERK and PI3K/Akt signalling. *PLoS One.* 2009;4(12):e8284.
34. Castro CH, Shin CS, Stains JP, et al. Targeted expression of a dominant-negative N-cadherin in vivo delays peak bone mass and increases adipogenesis. *J Cell Sci.* 2004;117(Pt 13):2853-2864.
35. Shin CS, Her SJ, Kim JA, et al. Dominant negative N-cadherin inhibits osteoclast differentiation by interfering with beta-catenin regulation of RANKL, independent of cell-cell adhesion. *J Bone Miner Res.* 2005;20(12):2200-2212.
36. Lecanda F, Warlow PM, Sheikh S, Furlan F, Steinberg TH, Civitelli R. Connexin43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. *J Cell Biol.* 2000;151(4):931-944.
37. Lecanda F, Towler DA, Ziambaras K, et al. Gap junctional communication modulates gene expression in osteoblastic cells. *Mol Biol Cell.* 1998;9(8):2249-2258.
38. Chung DJ, Castro CH, Watkins M, et al. Low peak bone mass and attenuated anabolic response to parathyroid hormone in mice with an osteoblast-specific deletion of connexin43. *J Cell Sci.* 2006;119(Pt 20):4187-4198.
39. Stains JP, Civitelli R. Gap junctions regulate extracellular signal-regulated kinase signaling to affect gene transcription. *Mol Biol Cell.* 2005;16(1):64-72.
40. Grimston SK, Screen J, Haskell JH, et al. Role of connexin43 in osteoblast response to physical load. *Ann N Y Acad Sci.* 2006;1068:214-224.
41. Burger EH, Klein-Nulend J. Mechanotransduction in bone--role of the lacunocanalicular network. *FASEB J.* 1999;13 Suppl:S101-S112.
42. Noble BS. The osteocyte lineage. *Arch Biochem Biophys.* 2008;473(2):106-111.
43. van Bezooijen RL, ten Dijke P, Papapoulos SE, Löwik CW. SOST/sclerostin, an osteocyte-derived negative regulator of bone formation. *Cytokine Growth Factor Rev.* 2005;16(3):319-327.
44. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone.* 2005;37 (2):148-158.
45. O'Brien CA, Plotkin LI, Galli C, et al. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PLoS One.* 2008;3(8):e2942.
46. Robling AG, Niziolek PJ, Baldridge LA, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J Biol Chem.* 2008;283 (9):5866-5875.

Le remodelage osseux : un réseau social de cellules

Le remodelage osseux est un processus dynamique qui nécessite une activité cellulaire coordonnée entre les ostéoclastes, les ostéoblastes et les ostéocytes pour maintenir l'homéostasie osseuse. Des cellules osseuses d'origine et de fonction différentes communiquent entre elles au sein d'un réseau social afin de stimuler ou d'inhiber la formation ou la résorption osseuses par l'intermédiaire de processus de signalisation. Ce réseau joue un rôle clé dans le contrôle de l'activité cellulaire osseuse et dans le maintien de l'intégrité du squelette. Les mécanismes de communication impliqués comprennent des facteurs solubles comme les cytokines, le RANKL (Receptor Activator of Nuclear factor κB Ligand) et l'ostéoprotégérine, produits localement par les ostéoblastes ou les ostéocytes pour la régulation de l'ostéoclastogenèse. Inversement, les facteurs libérés par les ostéocytes ou par les ostéoclastes pendant la résorption osseuse contrôlent la formation et la fonction des ostéoblastes. Les molécules de connexion cellules-cellules comme les cadhérines et les connexines dans les ostéoblastes et les ostéocytes sont essentielles à la fonction cellulaire osseuse, alors que les molécules de communication bidirectionnelles (éphrines) contrôlent le remodelage osseux en liant les ostéoblastes aux ostéoclastes. Cet article résume la connaissance actuelle des mécanismes utilisés par les cellules osseuses pour communiquer entre elles pour le contrôle du remodelage osseux et donne un aperçu des futurs défis dans ce domaine.

Bone remodeling: a social network of cells – *Marie*

"Bone material heterogeneity, especially at the bone surface, can be a source of crack initiation. Local small changes in the mechanical properties of composite material are unlikely to affect the overall stiffness or modulus of bone material (considered as an average of local properties), but may have a profound impact on material strength, which depends essentially on the strength of its weakest component (in the same way as the weakest link determines the strength of a chain)."

The complexity and heterogeneity of bone material

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*B*one has a complex hierarchical structure which provides outstanding mechanical performance at minimal requisite mass. The geometry and inner architecture of trabecular and compact bone represent the macro- and mesoscopic structural levels. At the lowest hierarchical level, bone material is a composite of two components with divergent mechanical characteristics: soft type I collagen fibrils and stiff calcium phosphate particles a few nanometers thick. To this basic heterogeneity of the composite at nanoscale is added microscale heterogeneity in the form of continuous remodeling of the bone matrix. This process generates bone packets with different matrix mineralization and lamellar orientations coexisting within the bone material. Nondestructive techniques with high spatial resolution are therefore required to characterize material structure-function relationships in normal and diseased bone. The present article focuses on the characteristics of the material levels in bone, in particular on the two components collagen and mineral, the mineralized collagen fibril, the lamellar arrangement of fibrils, and bone packets in normal and diseased bone. Technical advances in recent years have yielded specific insights into the structural hierarchy of bone and a better appreciation of the impact of disease on bone material and its mechanical properties. This in turn should deepen understanding of the underlying pathophysiology, enhance prediction of fracture risk, and inform therapeutic decision-making.
*Medicographia. 2012;34:155-162 (see French abstract on page 162)*

*B*one is an outstanding material in that it adapts to changes in mechanical demand and is self-healing. Normal function requires proper interplay between all sizes of its structural components.[1] Bone is a lightweight structure providing maximal mechanical strength at minimal requisite mass thanks to a complex hierarchical organization extending from the nano , through the micro and meso, to the macroscopic level *(Figure 1, page 156).* The outer geometry and inner architecture of cancellous and cortical bone are clearly evident at the macro- and mesoscopic levels. However, the increase in fracture risk associated with aging or disease depends not only on the amount of bone, but also on its material properties.

This article focuses on the lower hierarchical levels comprising the intrinsic bone material. Technical advances have thrown fresh light on the structure-function relationships of the different components down to the nano level, thereby advancing our understanding of this material's remarkable mechanical competence. Bone material is made up of four hierarchical levels of structural organization: the lowest

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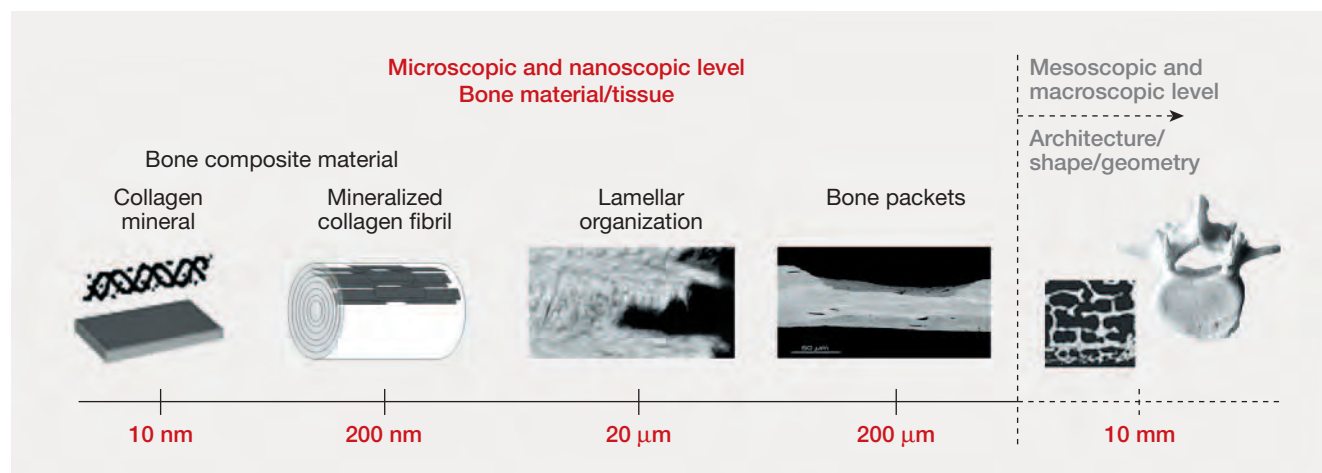
Figure 1. *The hierarchical structure of bone.*
The 4-level structural hierarchy of bone material, beginning at the nanometer level with collagen and mineral, the basic components of the nanocomposite material organized into mineral fibrils, which are then arranged in lamellae and finally in bone packets (the basic structural unit of bone material formed by osteoblasts during a remodeling cycle).

(nano) level features collagen and mineral as the main components of the nanocomposite forming the mineralized fibrils. These fibrils are arranged in lamellae and bone structural units (BSUs, also known as bone packets or osteons).

Collagen and mineral

◆ Collagen structure and cross-linking

The organic matrix of bone consists mainly of collagen type I, a triple helix of two α_1 and one α_2 collagen chains. It is synthesized by osteoblasts, assembled extracellularly into fibrils, and stabilized by cross-links.[2] The matrix not only serves as a scaffold for the mineral in the composite material, but itself plays a decisive role in the biomechanical competence of the collagen-mineral composite described later. For instance, we know that the decrease in mechanical competence of the organic matrix with increasing age is partly responsible for bone

SELECTED ABBREVIATIONS AND ACRONYMS	
AGE	advanced glycation end product
BMDD	bone mineralization density distribution
BSU	basic structural unit
EDX	energy-dispersive x-ray spectroscopy
ESRF	European Synchrotron Radiation Facility
FTIR	Fourier transform infrared
OI	osteogenesis imperfecta
oim	osteogenesis imperfecta mouse
qBEI	quantitative backscattered electron imaging
SAXS	small angle x-ray scattering
SEM	scanning electron microscopy
SR	synchrotron radiation
SR μCT	synchrotron radiation microcomputed tomography
SR μXRF	synchrotron radiation induced micro x-ray fluorescence
TEM	transmission electron microscopy
XRD	x-ray diffraction

fragility.[3] Many characteristics of organic matrix, such as amount produced by the cell, fibril structure, and the character, number, and distribution of the fibril cross-links, are genetically determined. Consequently, mutations not only in the collagen encoding genes, but also in the proteins involved in synthesis can have an impact on the resulting material properties. This is exemplified in osteogenesis imperfecta (OI), which is caused by a variety of genetic mutations.[4-8] The mechanical consequences of abnormal collagen molecules have been demonstrated in the OI mouse (oim), an animal model for human OI. Affected animals lose 50% of their tendon collagen strength compared with wild-type littermates.[9] Indeed, the properties of collagen are also essential for the plastic (post-yield) behavior of bone during tension.[10]

The properties of collagen/organic matrix and specific noncollagenous proteins can be chemically analyzed with high spatial resolution by spectroscopic techniques such as Fourier transform infrared (FTIR) imaging[11] and Raman microspectroscopy.[12] FTIR imaging measures the absorption of infrared radiation at specific wavelengths. This is dependent on the molecular bonds and their modes of vibration. The absorbance spectra reveal the characteristics of collagen (amide bands and cross-links) at a typical spatial resolution of a few micrometers.[11] In Raman microspectroscopy, the bone sample is irradiated by monochromatic laser light and the inelastically scattered light from the specimen is measured. The resulting vibrational bands are generally very sharp, enabling even small band shifts to be detected. High spatial resolution in the 1-μm range permits the analysis of selective small regions, such as newly formed bone between fluorescence-labeled bands.[12]

These techniques have shown newly formed bone material to possess more immature divalent cross-links destined to mature into trivalent cross-links. Deviations from the normal ratio of trivalent over divalent cross-links (collagen cross-link ratio)

are associated with bone fragility.[11] Additionally, the formation of advanced glycation end products (AGEs) has come into focus during recent years. Increased AGEs are thought to predispose to bone fragility in postmenopausal osteoporosis and diabetes.[13]

◆ *Bone mineral*
The inorganic component of bone material is the mineral consisting essentially of nanosized platelets (particles, crystals) made of carbonated hydroxyapatite ($Ca_5(PO_4)_3OH$). However, the chemical composition of these platelets is not constant, but can change during mineralization and maturation. In particular, substitution of calcium and phosphate ions is frequent.[14] The platelets are about 60 nm long[15] and a few nanometers thick,[16,17] and are embedded in collagen matrix. Their microscopic size is essential for the mechanical properties of the resulting nanocomposite and, moreover, makes platelet strength insensitive to flaws.[18]

Mineral characteristics such as maturity/crystallinity can be obtained from the intensity ratios of vibrational bands for phosphate, carbonate, and other constituents measured by FTIR imaging or Raman microspectroscopy. Other mineral characteristics, such as size, shape, and alignment, can be as-

Scattered x-ray intensities under wider angles (scanning x-ray diffraction [XRD]) give information about the crystal/lattice structure of the mineral particles. A useful elemental analysis technique is SR induced micro x-ray fluorescence (SR-μXRF). It measures the elemental distribution of trace elements, such as strontium and lead, with high sensitivity down to the ppm range.[19] In contrast, electron-induced μXRF, as used in the scanning electron microscope (SEM)/energy-dispersive x-ray spectroscopy (EDX), is much less sensitive (limited to about 0.5 weight % elemental concentration). SAXS has revealed that average mineral platelet thickness increases rapidly in the first four years of life, then slows.[20] It has also shown that sodium fluoride (NaF) administration significantly alters the size distribution of mineral particles in osteoporotic patients.[21] Fluoride is incorporated into the mineral crystals and changes their chemical composition, size, and crystallinity *(Figure 2)*. The resulting abnormalities in bone material may explain the absence of any increase in mechanical competence despite higher bone volume after NaF treatment.[22] Strontium and lead are two other bone-seeking chemical elements. Strontium gets incorporated into newly formed bone packets during strontium ranelate therapy. Depending on the patient's serum levels, strontium exchanges for up to 5% of calcium ions.[23,24] The incorporated element does not modify the lo-
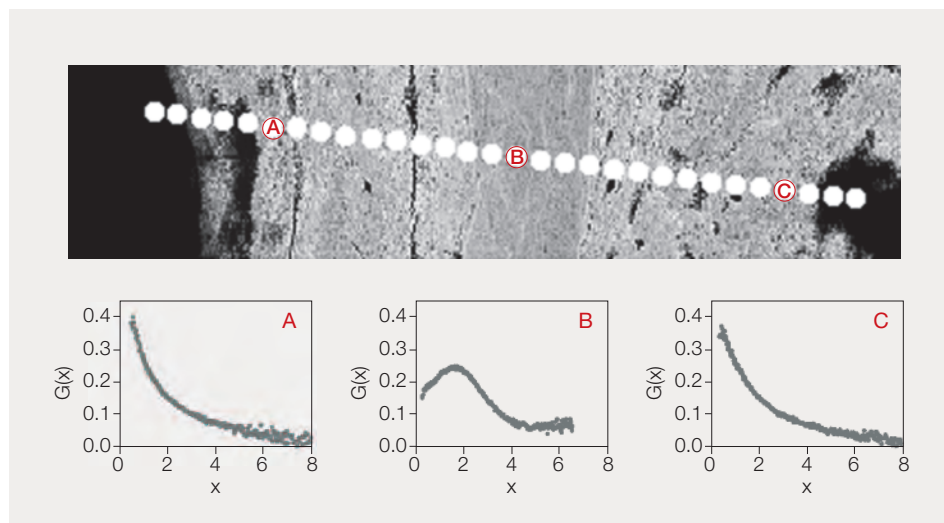
Figure 2. *The effect of sodium fluoride treatment on trabecular bone structure.*
Top: Backscattered electron image of part of a bone trabeculum from a patient treated with sodium fluoride. White circles: areas for synchrotron small angle x-ray scattering measurement. Bone formed during treatment (circles A and C) reveals altered structure compared with bone present before treatment (circle B).
Bottom: Corresponding G(x)-curves for the areas A, B, and C. G(x) was obtained from small angle x-ray scatter measurements and gives information on the shape and size of the mineral particles. G(x) differs qualitatively at positions A and C compared with position B.
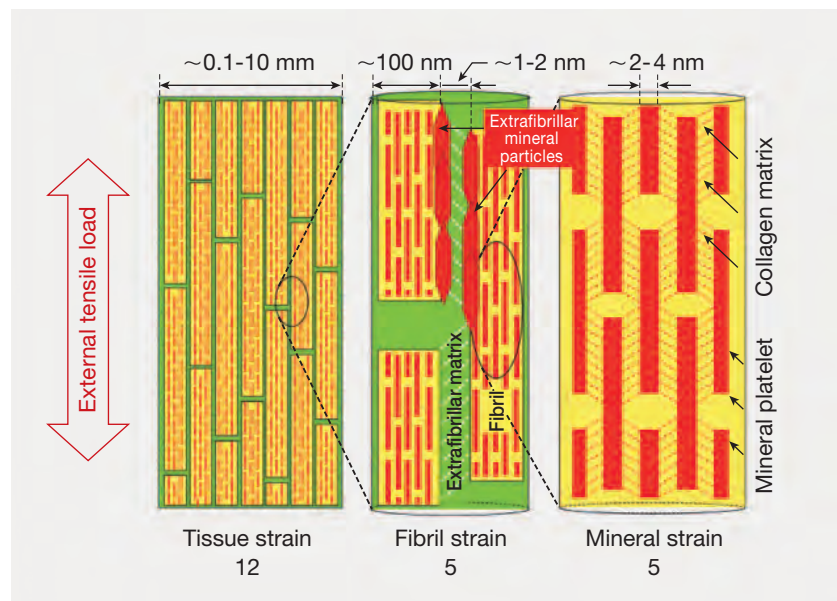*Unpublished material related to reference 21.*

sessed from conventional histological bone sections by x-ray scattering down to micrometer resolutions using synchrotron radiation (SR). Small angle x-ray scattering (SAXS) measures the scattered x-ray intensities within angles no larger than 1° with respect to the direction of the incident beam. The intensities reflect objects between 1 and 50 nm thick within the sample. Information is given on the thickness, shape, and alignment of the mineral platelets.[16] Transmission electron microscopy (TEM) can also be used to characterize platelet size and shape,[15] but it is not applicable in routine as it needs sophisticated preparation, is rather time-consuming, and provides information only on single objects and not on an average of millions of particles, as does SAXS.

cal mechanical properties (nanoindentation) or collagen cross-linking.[23] As for lead, normal environmental exposure results in storage in bone mineral, specifically (up to 13 fold) in the tide mark of the transition zone between bone and articular cartilage, and within cement lines.[19]

Mineralized collagen fibrils
The main building blocks of bone are mineralized collagen fibrils. These form the composite material consisting of the soft yet tough protein stiffened by the hard and brittle mineral platelets. The fibrils are about 100 nm[15] in diameter and consist of collagen molecules staggered in parallel, but displaced by 67 nm, producing a structure with overlap and hole zones[25] in

~0.1-10 mm   ~100 nm   ~1-2 nm   ~2-4 nm

External tensile load

Extrafibrillar mineral particles

Extrafibrillar matrix

Fibril

Collagen matrix

Mineral platelet

Tissue strain
12

Fibril strain
5

Mineral strain
5

Figure 3. *Schematic model for bone deformation in response to external tensile load at three levels in the structural hierarchy.*
In response to an external load (elastic region of deformation), the bone material does not deform homogeneously, but the larger structures take up more strain than the small, stiff elements. This results in characteristic strain contributions of 12:5:2 for whole tissue, fibril, and mineral, respectively. **Modified from reference 30:** *Gupta et al.* Proc Natl Acad Sci U S A. *2006;103(47):17741-17746. © 2006, The National Academy of Sciences.*
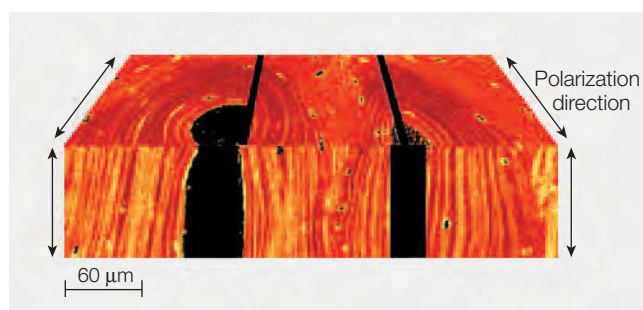
Bone lamellae

In mature bone, the mineralized collagen fibrils are assembled into lamellae. Within each lamella the fibrils are in a predominant orientation that changes from lamella to lamella. During bone formation, collagen is deposited onto the actual bone surface in a way that orients the lamellae parallel to this bone surface. This is mirrored by the orientation of the long axis of the mineral platelets, which follows the direction of the trabeculae.[20] Because of the birefringent properties of collagen fibrils, lamellar orientation can be clearly visualized under polarized light microscopy. Raman microspectroscopy has revealed the lamellar organization of the bone matrix by showing how the scattering intensities of certain collagen and phosphate bands strongly depend on the angle between fibril orientation, laser light polarization, and beam axis direction *(Figure 4).*[32] Scanning SAXS combined with XRD has confirmed the rotated plywood arrangement of the fibrils consistent with the observed lamellar structures.[28]

Mechanical testing has shown that deformation is also not homogeneous at the lamellar level, but distributed between tensile deformation of the fibrils and shearing in the interfibrillar matrix (the so-called "glue").[31] Fibril anisotropy within the lamellae is essentially responsible for the high anisotropy in mechanical behavior found in bone material, in terms, for instance, of the nanoelasticity and nanohardness data obtained

which the mineral particles are thought to nucleate and grow. Not much is yet known about the nucleation of the mineral particles and their growth to final size. It has been suggested that both collagen itself[26] and noncollagenous proteins[27] significantly influence these processes. Interestingly, the amount of mineral within the organic matrix is abnormally high, but similar in different forms of OI with altered or normal collagen structure,[7] suggesting that the structure of the collagen itself is not the only regulator of the amount of mineral deposited.

Insight into the mechanical properties of mineralized collagen fibrils is essential for understanding whole-tissue mechanics. In general, the mineral platelets are oriented with their long axis (c axis) parallel to the long axis of the collagen fibril.[15,28] Mechanical models have shown that the organic matrix in between the mineral platelets transfers the shear forces acting on bone material. The main determinant of the elasticity and hardness of the material is assumed to be the amount of mineral. However, platelet shape and large aspect ratio (length or width over thickness) are also important for stiffening the collagen fibrils.[29] Sophisticated techniques using in-situ mechanical testing with high spatial resolution structural analysis at the European Synchrotron Radiation Facility (Grenoble, France [ESRF]) were recently introduced for studying the interface between collagen and mineral platelets, since the platelets are thought to be important for the deformation behavior of mineralized collagen fibrils. These tensile loading experiments revealed that bone material does not deform homogeneously in response to the external load, but the larger elements take up more strain than the small stiff elements, with characteristic strain contributions of 12:5:2 for whole tissue, fibril, and mineral, respectively, in the elastic deformation region.[30] The results favor a staggered arrangement of mineral platelets within each fibril, and a staggered arrangement of fibrils to fibers and tissue within the extrafibrillar matrix *(Figure 3).*[31]

Polarization direction

60 µm

Figure 4. *Raman images of cortical bone with two Haversian canals in front of the imaged region.*
The arrows indicate the polarization orientation of the laser beam. Note that dependent on the direction of polarization, the lamellae change their contrast in the images (those appearing dark in longitudinal orientation appear bright in transverse laser polarization and vice versa).
Modified from reference 32: *Kazanci et al.* Bone. *2007;41(3):456-461. © 2007, Elsevier B.V.*

A Bone mineralization density distribution
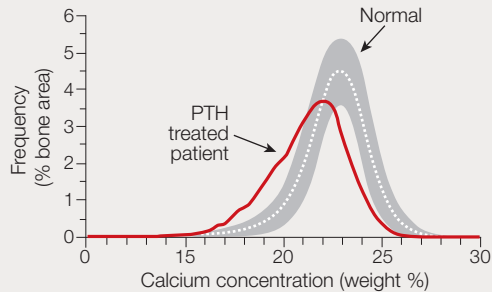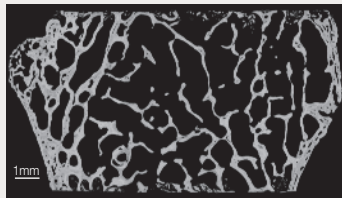
B Bone mineralization profile
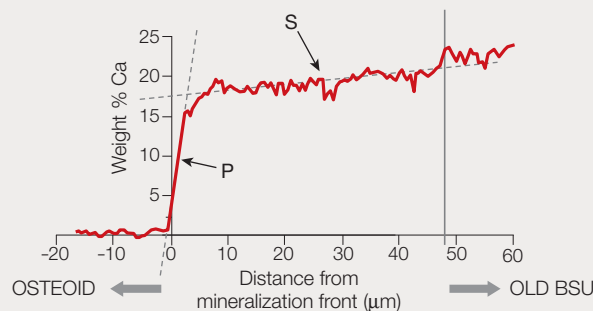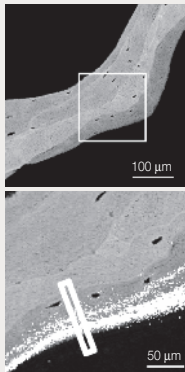
Figure 5. *Formation of bone mineralization density distribution.*
A. *Backscattered electron images of trabecular area (left) in a parathyroid hormone-treated osteoporotic male patient and the corresponding bone mineralization density distribution (BMDD) represented by the solid red line (right). The dotted white line and the gray area in the background show the mean and 95% confidence interval of the normal reference BMDD, respectively.*
B. *Mineralization density profile perpendicular to the mineralization front from the enlarged trabeculum (left). In the first few micrometers from the mineralization front, the calcium content of the matrix increases sharply, and then flattens out further from the mineralization front. Using the tissue age data from fluorescence labeling of the bone sample, this profile can be assigned to the time sequence of mineralization (primary or secondary).*
Abbreviations: BSU, basic structural unit; Ca, calcium; P, primary mineralization phase; PTH, parathyroid hormone; S, secondary mineralization phase.
Modified from reference 38: Roschger et al. Bone. 2008;42(3):456-466. © 2008, Elsevier B.V.

by nanoindentation.[33] In this technique, the tip of an atomic force microscope is used as a nanosized indenter to measure the local elastic response of the sample in the submicrometer range. Evaluation of the elastic modulus and hardness is based on the measurement of load, indentation depth, and projected indentation area (contact area). The results greatly depend on the local orientation of lamellae and fibrils with respect to the indentation axis.[34] Moreover, deformation experiments have clearly shown that lamellar organization is essential in controlling crack propagation:[35] the energy required to propagate a crack is about two orders higher if the crack is perpendicular rather than parallel to the lamellar plane. In consequence, the impaired lamellar structure found in patients with pycnodysostosis[36] is probably responsible for the bone fragility observed in this genetic disease.

Basic structural units

As bone is remodeled throughout life, it is not a homogeneous material, but consists of tissue volumina of differing ages. The mean lifespan of bone material varies from a few to about 20 years, depending on the site considered.[37] In consequence, bone material consists of younger and older bone volumina in BSUs, or bone packets for trabecular bone and osteons for compact bone. Each represents the amount of bone material formed by osteoblasts within one remodeling cycle. Consequently, these BSUs differ in lamellar orientation and mineral content. The differences in degree of mineralization are caused by the characteristic time courses involved. Newly formed unmineralized osteoid starts to mineralize after about 14 days of mineralization lag time. In a first rapid phase of primary mineralization, about 70% of the final mineral content is deposited within a few days *(Figure 5)*.[38] This is followed by much slower phases of secondary mineralization which last months to years before achieving the final mineral content,[38-41] typically in interstitial bone.[38] In pathological cases, however, final mineral content at BSU level can achieve values that are higher (hypermineralization) or lower (hypomineralization) than in normal interstitial bone, due to altered organic matrix, disordered mineralization kinetics, and/or changes in mineral particle size.[38]

In general, the amount of mineral and its distribution within the collagen matrix are important determinants of stiffness and strength.[42] This is reflected in the positive correlation found between mineral content and nanoindentation outcomes.[43] However, there is remarkable scatter in indentation outcomes at a given calcium content.[44] This is because the measurement of mineral may be independent of orientation, but local elasticity and nanohardness greatly depend on the actual orientation of the mineralized collagen fibrils.[34,44] Moreover, BSU heterogeneity in mineral content and lamellar orientation (including cement lines) may be essential in reducing and/or preventing crack propagation.[43,45,46] However, bone material heterogeneity, especially at the bone surface, can also be a source of crack initiation. Local small changes in the mechanical properties of

composite material are unlikely to affect the overall stiffness or modulus of bone material (considered as an average of local properties), but may have a profound impact on material strength, which depends essentially on the strength of its weakest component (in the same way as the weakest link determines the strength of a chain). Thus, it is difficult to predict the extent to which the heterogeneity introduced by BSUs benefits or impairs mechanical performance.

Microradiography,[41,47] SR microcomputed tomography (SR μCT)[48] and quantitative backscattered electron imaging (qBEI)[6,38] are the techniques used to measure the intrinsic bone mineralization pattern. Microradiography has been used for this purpose for some decades. However, it requires bone sections about 100 μm thick, leading to partial volume effects (mimicking lower mineral content) that impair its accuracy. SR μCT is by comparison a rather novel technique analogous to x-ray tomography, but with a specific beam energy (monochromatic beam) and high spatial resolution (a few micrometers in beam diameter) that confer the bonus of full 3D information. However, its disadvantage is that it is time-consuming and requires the synchrotron facility, thereby excluding conventional applications. Attempts to harness laboratory μCT devices (usually used to measure structural indices of bone microarchitecture) to measure matrix mineralization have to be viewed with caution because of the beam-hardening effects introduced by the non-monochromatic x-ray beam used by such devices. Yet another technique for analyzing mineralization density is qBEI, a 2D method that gives information from the bone surface layer <1.5 μm in thickness, but provides high spatial resolution and sensitivity in the detection of different degrees of mineralization. In bone, the backscattered electron intensity signal is dominated by its calcium content. Thus, the different gray levels in qBEI images reflect different local calcium concentrations and can be further analyzed in histograms revealing the percentage of bone areas with a specific calcium concentration (bone mineralization density distribution [BMDD]).

Comprehensive studies on samples from healthy adult individuals have shown minor variations in cancellous BMDD with gender, ethnicity, and skeletal site.[47,49] These amazingly small variations may indicate that BMDD is an evolutionary optimum in terms of biology and mechanics. Thus, even small deviations from the normal BMDD appear of biological and/or clinical relevance in that they reflect altered mineralization kinetics and/or bone turnover rates (Figure 5). Metabolic bone diseases and treatments that increase bone turnover push BMDD values towards lower mineralization and/or into a wider range (ie, more heterogeneous matrix mineralization),[38,47] as in postmenopausal osteoporosis.[38] Antiresorptive treatments that reduce bone turnover (alendronate, risedronate, zoledronic acid, etc), on the other hand, shift BMDD values from lower to normal calcium concentrations and transiently narrow the range (more homogeneous matrix mineralization).[38] Higher than normal degrees of mineralization have been found in patients with different types of OI, making their bone harder and more brittle than normal.[6-8] Interestingly, this shift of BMDD to higher calcium concentrations occurs despite an increased bone turnover, indicating that mineralization must be accelerated in this genetic disease, leading to a hypermineralized matrix. Mathematical modeling of BMDD has greatly contributed to the understanding and interpretation of experimentally measured BMDD in health and disease.[50]

Conclusion

Bone material is heterogeneous and anisotropic based on the hierarchical organization of its principal components (collagen and mineral) into several structural levels starting from the nanometer scale. Metabolic and genetic disease associated with bone fragility usually affects one or more of these structural levels, indicating that the normal structure of healthy bone material is optimized for mechanical performance. Any deviation from normal is likely to compromise mechanical competence. Yet it still remains difficult to predict mechanical strength from changes in single structural components due to the complex interplay of all structural levels and the sensitivity of material strength to local defects, according to the principle of the weakest link in a chain. ∎

Complexity and heterogeneity of bone – *Roschger and others*

References

1. Fratzl P, Gupta HS, Paschalis EP, Roschger P. Structure and mechanical quality of the collagen-mineral nano-composite in bone. *J Mater Chem.* 2004;14: 2115-2123.
2. Hulmes DJ. Building collagen molecules, fibrils, and suprafibrillar structures. *J Struct Biol.* 2002;137(1-2):2-10.
3. Wang X, Shen X, Li X, Agrawal CM. Age-related changes in the collagen network and toughness of bone. *Bone.* 2002;31(1):1-7.
4. Rauch F, Glorieux FH. Osteogenesis imperfecta. *Lancet.* 2004;363(9418): 1377-1385.
5. Marini JC, Cabral WA, Barnes AM. Null mutations in LEPRE1 and CRTAP cause severe recessive osteogenesis imperfecta. *Cell Tissue Res.* 2010;339(1):59-70.
6. Boyde A, Travers R, Glorieux FH, Jones SJ. The mineralization density of iliac crest bone from children with osteogenesis imperfecta. *Calcif Tissue Int.* 1999; 64(3):185-190.
7. Roschger P, Fratzl-Zelman N, Misof BM, Glorieux FH, Klaushofer K, Rauch F. Evidence that abnormal high bone mineralization in growing children with osteogenesis imperfecta is not associated with specific collagen mutations. *Calcif Tissue Int.* 2008;82(4):263-270.
8. Fratzl-Zelman N, Morello R, Lee B, et al. CRTAP deficiency leads to abnormally high bone matrix mineralization in a murine model and in children with osteogenesis imperfecta type VII. *Bone.* 2010;46(3):820-826.
9. Misof K, Landis WJ, Klaushofer K, Fratzl P. Collagen from the osteogenesis imperfecta mouse model (oim) shows reduced resistance against tensile stress. *J Clin Invest.* 1997;100(1):40-45.
10. Jepsen KJ, Goldstein SA, Kuhn JL, Schaffler MB, Bonadio J. Type-I collagen mutation compromises the post-yield behavior of Mov13 long bone. *J Orthop Res.* 1996;14(3):493-499.
11. Paschalis EP, Mendelsohn R, Boskey AL. Infrared assessment of bone quality: a review. *Clin Orthop Relat Res.* 2011;469(8):2170-2178.
12. Gamsjaeger S, Buchinger B, Zwettler E, et al. Bone material properties in actively bone-forming trabeculae in postmenopausal women with osteoporosis after three years of treatment with once-yearly zoledronic acid. *J Bone Miner Res.* 2011;26(1):12-18.
13. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int.* 2010;21(2):195-214.
14. Posner AS. The structure of bone apatite surfaces. *J Biomed Mater Res.* 1985; 19(3):241-250.
15. Rubin MA, Jasiuk I, Taylor J, Rubin J, Ganey T, Apkarian RP. TEM analysis of the nanostructure of normal and osteoporotic human trabecular bone. *Bone.* 2003;33(3):270-282.
16. Fratzl P, Groschner M, Vogl G, et al. Mineral crystals in calcified tissues: a comparative study by SAXS. *J Bone Miner Res.* 1992;7(3):329-334.
17. Weiner S, Wagner HD. The material bone: Structure-mechanical function relations. *Annu Rev Mater Sci.* 1998;28:271-298.
18. Gao H, Ji B, Jager IL, Arzt E, Fratzl P. Materials become insensitive to flaws at nanoscale: lessons from nature. *Proc Natl Acad Sci U S A.* 2003;100(10):5597-5600.
19. Zoeger N, Roschger P, Hoefstaetter JG, et al. Lead accumulation in the tidemark of articular cartilage. *Osteoarthritis Cartilage.* 2006;14(9):906-913.
20. Roschger P, Grabner BM, Rinnerthaler S, et al. Structural development of the mineralized tissue in the human L4 vertebral body. *J Struct Biol.* 2001;136(2): 126-136.
21. Gourrier A, Li C, Seigel S, et al. Scanning small-angle X-ray scattering analysis of the size and organization of the mineral nanoparticles in fluorotic bone using a stack of cards model. *J Appl Cryst.* 2010;43(6):1385-1392.
22. Fratzl P, Schreiber S, Roschger P, Lafage MH, Rodan G, Klaushofer K. Effects of sodium fluoride and alendronate on the bone mineral in minipigs: a small-angle X-ray scattering and backscattered electron imaging study. *J Bone Miner Res.* 1996;11(2):248-253.
23. Roschger P, Manjubala I, Zoeger N, et al. Bone material quality in transiliac bone biopsies of postmenopausal osteoporotic women after 3 years of strontium ranelate treatment. *J Bone Miner Res.* 2010;25(4):891-900.
24. Li C, Paris O, Siegel S, et al. Strontium is incorporated into mineral crystals only in newly formed bone during strontium ranelate treatment. *J Bone Miner Res.* 2010;25(5):968-975.
25. Hodge AJ, Petruska JA. Recent studies with the electron microscope on ordered aggregates of the tropocollagen molecule. In: Ramachandran GN, ed. *Aspects of protein structure.* New York, NY: Academic Press; 1963;289-300.
26. Landis WJ, Silver FH. Mineral deposition in the extracellular matrices of vertebrate tissues: identification of possible apatite nucleation sites on type I collagen. *Cells Tissues Organs.* 2009;189(1-4):20-24.
27. Boskey AL. Matrix proteins and mineralization: an overview. *Connect Tissue Res.* 1996;35(1-4):357-363.
28. Wagermaier W, Gupta HS, Gourrier A, et al. Scanning texture analysis of lamellar bone using microbeam synchrotron X-ray radiation. *J Appl Cryst.* 2007;40(1): 115-120.
29. Jager I, Fratzl P. Mineralized collagen fibrils: a mechanical model with a staggered arrangement of mineral particles. *Biophys J.* 2000;79(4):1737-1746.
30. Gupta HS, Seto J, Wagermaier W, Zaslansky P, Boesecke P, Fratzl P. Cooperative deformation of mineral and collagen in bone at the nanoscale. *Proc Natl Acad Sci U S A.* 2006;103(47):17741-17746.
31. Gupta HS, Wagermaier W, Zickler GA, et al. Nanoscale deformation mechanisms in bone. *Nano Lett.* 2005;5(10):2108-2111.
32. Kazanci M, Wagner HD, Manjubala NI, et al. Raman imaging of two orthogonal planes within cortical bone. *Bone.* 2007;41(3):456-461.
33. Zysset PK. Indentation of bone tissue: a short review. *Osteoporos Int.* 2009;20 (6):1049-1055.
34. Rho JY, Tsui TY, Pharr GM. Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation. *Biomaterials.* 1997;18(20): 1325-1330.
35. Peterlik H, Roschger P, Klaushofer K, Fratzl P. From brittle to ductile fracture of bone. *Nat Mater.* 2006;5(1):52-55.
36. Fratzl-Zelman N, Valenta A, Roschger P, et al. Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis. *J Clin Endocrinol Metab.* 2004;89(4):1538-1547.
37. Parfitt AM. Misconceptions (2): turnover is always higher in cancellous than in cortical bone. *Bone.* 2002;30(6):807-809.
38. Roschger P, Paschalis EP, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. *Bone.* 2008;42(3):456-466.
39. Akkus O, Polyakova-Akkus A, Adar F, Schaffler MB. Aging of microstructural compartments in human compact bone. *J Bone Miner Res.* 2003;18(6):1012-1019.
40. Fuchs RK, Allen MR, Ruppel ME, et al. In situ examination of the time-course for secondary mineralization of Haversian bone using synchrotron Fourier transform infrared microspectroscopy. *Matrix Biol.* 2008;27(1):34-41.
41. Bala Y, Farlay D, Delmas PD, Meunier PJ, Boivin G. Time sequence of secondary mineralization and microhardness in cortical and cancellous bone from ewes. *Bone.* 2010;46(4):1204-1212.
42. Currey JD. The mechanical consequences of variation in the mineral content of bone. *J Biomech.* 1969;2(1):1-11.
43. Gupta HS, Schratter S, Tesch W, et al. Two different correlations between nanoindentation modulus and mineral content in the bone-cartilage interface. *J Struct Biol.* 2005;149(2):138-148.
44. Oyen ML, Ferguson VL, Bembey AK, Bushby AJ, Boyde A. Composite bounds on the elastic modulus of bone. *J Biomech.* 2008;41(11):2585-2588.
45. Fratzl P. Bone fracture: When the cracks begin to show. *Nat Mater.* 2008;7(8): 610-612.
46. Zioupos P, Gresle M, Winwood K. Fatigue strength of human cortical bone: age, physical, and material heterogeneity effects. *J Biomed Mater Res A.* 2008; 86(3):627-636.
47. Boivin G, Meunier PJ. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcif Tissue Int.* 2002; 70(6):503-511.
48. Nuzzo S, Lafage-Proust MH, Martin-Badosa E, et al. Synchrotron radiation microtomography allows the analysis of three-dimensional microarchitecture and degree of mineralization of human iliac crest biopsy specimens: Effect of etidronate treatment. *J Bone Miner Res.* 2002;17(8):1372-1382.
49. Roschger P, Gupta HS, Berzlanovich A, et al. Constant mineralization density distribution in cancellous human bone. *Bone.* 2003;32(3):316-323.
50. Ruffoni D, Fratzl P, Roschger P, Phipps R, Klaushofer K, Weinkamer R. The effect of temporal changes in bone turnover on the bone mineralization density distribution: A computer simulation study. *J Bone Miner Res.* 2008;23(12): 1905-1914.

COMPLEXITÉ ET HÉTÉROGÉNÉITÉ DU MATÉRIEL OSSEUX

La structure hiérarchique de l'os est complexe et sa performance mécanique pour une masse requise minimale est remarquable. La géométrie et l'architecture intérieure de l'os compact et trabéculaire représentent les niveaux structuraux macro- et mésoscopiques. Au plus bas niveau hiérarchique, le matériel osseux est un composite de deux composés aux caractéristiques mécaniques divergentes : les fibrilles souples de collagène de type I et les particules rigides de phosphate de calcium d'une épaisseur de quelques nanomètres. À cette base hétérogène du composite à l'échelle nanométrique s'ajoute une hétérogénéité micrométrique sous la forme d'un remodelage continu de la matrice osseuse. Ce processus génère des paquets osseux avec différentes orientations lamellaires et de minéralisation matricielle coexistant au sein du matériel osseux. Des techniques non invasives à haute résolution spatiale sont donc nécessaires pour caractériser les relations structure-fonction du matériel osseux dans l'os normal et dans l'os pathologique. Cet article s'intéresse aux caractéristiques du matériel osseux en particulier aux deux composants collagène et minéral, les fibrilles de collagène minéralisées, les fibrilles lamellaires et les paquets osseux dans l'os normal et dans l'os pathologique. Les avancées techniques de ces dernières années ont permis une meilleure compréhension de la hiérarchie structurale de l'os et de l'impact de la maladie sur le matériel osseux et ses propriétés mécaniques. Ceci permet en retour de mieux comprendre la physiopathologie sous-jacente, de mieux prévoir le risque fracturaire et de contribuer à la prise de décisions thérapeutiques.

"Bone continually adapts its size, shape, and/or matrix properties to optimize resistance to changes in mechanical load (compression or tension). Adaptation depends on the three-dimensional physical arrangement of trabecular and cortical bone, their relative proportion, and their capacity for self-renewal and repair. Such adaptation is readily observed in tennis players where bone in the dominant arm is larger than in the supporting arm."

The fragile beauty of bone architecture

by N. Dion and L. G. Ste-Marie, *Canada*

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In order to resist biomechanical loading and torsion while allowing movement, bone needs to be stiff, flexible, and light. These properties are determined by a complex set of interdependent factors, including bone mass, geometry, and tissue material composition, that define bone quality and maintain structural integrity and strength. Throughout life, the material and structural properties of bone are modulated to better respond to stresses such as growth, menopause, and aging. Inability to adapt its macro and microarchitecture to such stresses makes bone fragile and may initiate fracture. This review focuses on the components that define the macro and microarchitecture of bone. It describes their structural differences, relationships to skeletal sites, and respective roles. It discusses the influence of major life stresses on structural bone adaptation, and addresses structural bone fragility in terms of the abnormalities in bone material composition observed in some bone diseases. Better understanding of the biomechanical properties of bone is needed in order to maintain bone health and prevent or treat bone disease. This requires methods that evaluate overall bone quality in terms of the correlations between material composition and three-dimensional structure.

The structure of bone must be strong enough to support body weight and, in some cases, such as the skull and ribs, to protect vital organs. However, it must also be light enough to make movement possible.

Structural differences per skeletal site (geometry/macroarchitecture)

Bone achieves its mechanical performance by virtue of its geometric properties and biomaterial composition. These two parameters determine bone strength, which is a balance between stiffness/flexibility and lightness/mass. During impact loading, bone must be stiff enough to resist deformation. It must also be elastic or flexible enough to avoid fracture by absorbing energy. Like a spring, bone must be able to change its shape to absorb compression energy and light enough to allow rapid movement.

In other words, depending on its location and specific role in the body, bone adopts different shapes allowing a balance between load resistance, bone mass, and structural flexibility. These specifications produce five types of macroarchitecture: long bone, flat bone, irregular bone, short bone, and sesamoid bone, the first three of which we characterize briefly below.

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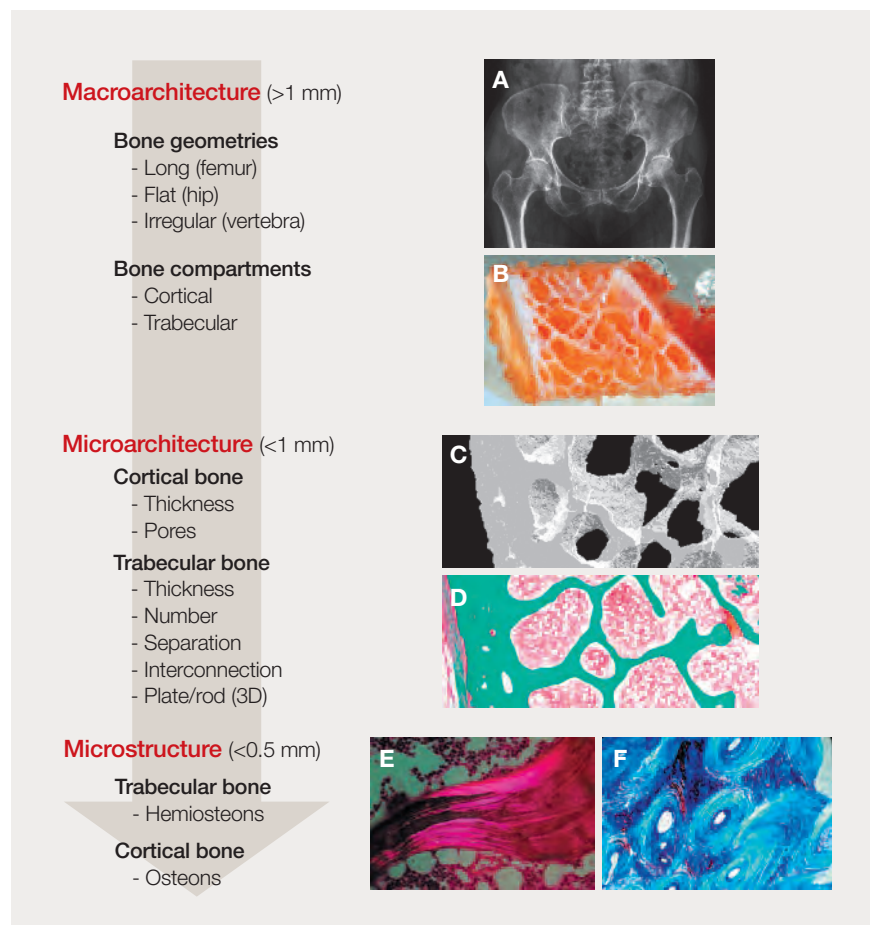
Figure 1. *Architectural organization of bone (inspired by Chappard et al[3]).*
A. *Pelvic x-ray showing a variety of bone geometry.*
B. *Undecalcified transiliac bone biopsy showing external cortical bone and internal trabecular bone within the medullary cavity.*
C. *Microarchitectural analysis using 3D micro-computed tomography.*
D. *Microarchitectural analysis using 2D histomorphometry (Goldner's trichrome stain).*
E. *Basic structural unit (hemiosteon) of trabecular bone (hematoxylin phloxine saffron stain under polarized light).*
F. *Basic structural unit (osteon) of cortical bone (toluidine blue stain under polarized light).*

ton depends on interaction between the genetic contribution and mechanical loading and modeling during growth and development. It also reflects nutrition, intercurrent illness, and other factors encountered during growth and development.[1]

Roles of cortical and trabecular bone tissue

At the macrostructural level, bone can be classified as cancellous (trabecular) and compact (cortical). However, each type is best distinguished by its specific microarchitecture, structural organization, and role in bone strength. Their relative proportions vary considerably between sites. The trabecular:cortical ratio is about 75:25 in vertebrae, 50:50 in the femoral head, and 95:5 in the shaft (diaphysis) of the radius.[2]

Long bones, such as the femur, tibia, or humerus, are levers allowing load and movement, achieved mostly by a structural design that emphasizes rigidity over flexibility. One characteristic is that they are longer than they are wide, having a growth plate (epiphysis) at either end with a hard compact outer surface (cortical bone) and a spongy cancellous marrow-containing interior (trabecular bone). Hyaline cartilage covers each end for protection and shock absorption.

Flat bones, such as the pelvis, protect vital organs and anchor muscles. The anterior and posterior surfaces are basically formed of compact bone to provide good mechanical strength while the center consists of marrow-containing cancellous bone. In adults, red blood cells are formed mostly in the marrow of flat bones.

Irregular bones, such as vertebrae, have a spring action and consist primarily of cancellous bone under a thin outer layer of compact bone.

The macroarchitecture of bone refers to the length, size, and shape of the intact adult skeleton, which is mostly genetically determined. In addition, bones have special angulations and curvatures enabling them to resist compression, tension, and torsion. However, the final shape and mass of the adult skele-

Cortical bone constitutes approximately 80% of the skeleton and provides strength. It consists of mineralized matrix layers stacked tightly to form a solid organized structure (compact bone). The structural and functional unit of compact bone (bone structural unit [BSU]) is the osteon, which contains a central Haversian canal parallel to the long axis of the bone, according to the direction of maximal stress. The canal provides a passage for blood vessels and sympathetic nerve fibers through the hard bone matrix. In addition, the presence of transverse Volkmann's canals ensures communication be-

SELECTED ABBREVIATIONS AND ACRONYMS

BMU	bone multicellular unit
BSU	bone structural unit
3D μCT	three-dimensional microcomputed tomography
GH	growth hormone
IGF	insulin-like growth factor
IOP	idiopathic osteoporosis
OI	osteogenesis imperfecta

tween the Haversian canals and circulation between the outer and inner spaces (periosteum and medulla). Under the periosteum, the osteon layer is lined by a number of parallel lamellae constituting the periosteal bone. On the medullary side, the osteons are also covered by lamellae forming the endosteum (Figure 1).[3]

Osteons are complex composite structures whose lamellae (aligned collagen-mineral fibers) are organized for optimal resistance to the type of load they are required to bear. Three types have been defined: transverse, alternate, and longitudinal (Figure 1F).[4,5] Recent numerical compression experiments studying the finite elements of transverse osteons suggest that the composite microstructure has specific biomechanical functions depending on its position (internal or external) in the Haversian canal.[6] This new type of experiment helps us to better understand how bone microstructure behaves.

Cortical bone is found mainly in long bones, such as the femur, tibia, and radius, and the outer surfaces of flat bones, such as the skull, mandible, and scapula. A key feature of cortical microarchitecture is the number of pores (porosity). The cortical width and the inner and outer diameters of the main shaft of long bones are also evaluated.

Trabecular bone constitutes approximately 20% of the skeleton and comprises an interconnected network of irregularly arranged (highly anisotropic) trabeculae, allowing maximal strength, allied to a porous lightweight bone structure. The trabecular network is basically composed of plates parallel to the stress lines and connected laterally by transverse rods or pillars that ensure cohesion of the entire organization.[7] The trabecular BSU is the arch-like hemiosteon, resembling an incomplete osteon (Figure 1E). Remnants of partially eroded hemiosteons persist between newly laid down BSUs and constitute interstitial trabecular bone. Trabecular bone is normally found within either end of long bones, such as the femur and tibia, and within flat or irregular bones (Figure 1).

The microarchitecture of bone tissue refers mainly to the morphology of the trabecular compartment. Its structural features include the volume, shape, number, thickness, and connectivity of trabeculae present, plus the marrow in the medullary cavity. Turnover is higher in trabecular bone because it has more surface per unit of volume than cortical bone. This also explains why microarchitecture reforms more rapidly in the trabecular compartment.

Structural influence of mechanical, hormonal and nutritional stress

◆ Mechanical stress
Bone continually adapts its size, shape, and/or matrix properties to optimize resistance to changes in mechanical load (compression or tension). Adaptation depends on the three-dimensional (3D) physical arrangement of trabecular and cor-

tical bone, their relative proportion, and their capacity for self-renewal and repair. Such adaptation is readily observed in tennis players where bone in the dominant arm is larger than in the supporting arm.[8] Similarly, recent comparison between elite female soccer players and swimmers highlighted the relative benefit of high-impact sport (soccer) on hip geometry and strength.[9]

Greater body weight and height account for the larger bone size in men over women at all ages. Hence their greater resistance to loads on the skeleton during regular activity, and their overall pattern of more favorable geometric adaptation.[10]

Biomechanical behavior influences overall bone size and shape. Even a small increase in the external diameter of a long bone can markedly improve its resistance to mechanical stress since resistance to bending increases to the fourth power of the distance from the neutral axis.[11] However, the relationship between bone size and resistance to stress is also heavily dependent on cortical thickness, porosity, and degree of bone matrix mineralization. A power law with mineralization and porosity as explanatory variables accounts for over 80% of the variation in cortical stiffness and strength.[12]

Immobilization and microgravity influence bone mass and architecture, as has been documented in astronauts. The abnormalities in astronaut bone during spaceflight result from reduced skeletal loading, reminding us that everyday gravitational loading is important in maintaining normal bone mass and architecture.[13] Trabecular bone has a higher remodeling level (balance between formation and resorption) than cortical bone. This maximizes adaptability by orienting its principal axes along the most common loading directions, making the trabecular bone network better able to resist mechanical forces.[14] The growth phase exemplifies trabecular adaptability. Whereas in childhood trabecular bone mostly consists of a dense network of plates in a frequently isotropic 3D distribution (ie, uniform in all directions), in adults the plates gradually change their preferential orientation along the direction of the primary stress exerted on the bone.[15]

◆ Hormonal stress
◆ Gender differences
The sexual dimorphism of bone becomes apparent during puberty, with men achieving higher peak bone mass, greater bone size, and ultimately a stronger skeleton than women.[16] These structural modifications cater for the greater biomechanical load of male weight and height.[17] Histomorphometry shows greater bone volume and thicker trabeculae in male vertebrae.[18]

◆ Puberty
During puberty, bones in men become wider, but not denser, as bone mineral acquisition in long bones occurs in proportion to bone volume. Boys develop a larger periosteal perime-

ter than girls from midpuberty onward. This is generally attributed to the contrasting effects of sex steroids on bone structure in men and women. As a result, cortical bone in male long bones is further from the neutral axis, which confers more resistance to bending. During aging, initial changes in trabecular bone are similar in both sexes and characterized by decreases in bone volume and trabecular thickness. However, in women, bone fragility becomes more common due to menopause. Estrogen withdrawal accelerates bone loss and produces structural deterioration, due to a combination of rapid remodeling and greater imbalance (reduced osteoblast lifespan with increased osteoclast lifespan) in the bone multicellular unit (BMU).[19]

◆ *Growth*
In addition to sex hormones, growth hormone (GH) and insulin-like growth factor I (IGF-I) are probably the most important determinants of structural gender differences characterized by wider but not thicker bones. Although it is now well accepted that sex hormones interact with the GH/IGF-I axis to regulate peak cortical bone size, the relative contributions of each have yet to be precisely determined.[16,20] Growth is associated with age-related changes in bone geometry in an attempt to preserve whole-bone strength. In the appendicular skeleton, these changes involve the redistribution of cortical and trabecular bone, specifically endosteal resorption and periosteal apposition, resulting in an increase in long bone diameter (ensuring resistance to bending and torsion[21-23]) and a decrease in cortical thickness. Cross-sectional data have also shown that the bone of the axial skeleton can increase in size with aging.[24]

◆ *Aging stress*
Although bone stability appears to follow the completion of growth, aging corresponds to a period of bone loss with structural deterioration. During early adulthood, bone is lost in men and women, probably due to a negative BMU balance characterized by early decrease in bone formation within each individual BMU with no change in bone resorption. Thus, aging not only decreases bone mass, it also gradually affects bone microarchitecture by reducing trabecular thickness and connectivity.[23] In addition, it affects cortical thickness by increasing endocortical bone resorption and reducing periosteal apposition, thereby altering the overall distribution of the remaining bone.[25]

Throughout life, mechanical loading produces fatigue damage in the bone matrix. Accumulation of such microdamage or microcracks can initiate fracture, although continuous reparative remodeling is designed to prevent this eventuality. It has been suggested that, in aging bone, accumulated microdamage results from interaction between altered 3D microarchitecture, including changes in trabecular shape and connectivity, and mechanical loading. A study of the relationship between 3D microstructure and the accumulated microdamage induced by compression loading in cancellous bone cores from adult human tibial plateaus of varying ages showed that the bone volume fraction (bone volume/trabecular volume) and changes in microarchitecture predispose trabecular bone to accumulated microdamage.[26] Thus, in a less dense trabecular network, rod-like trabeculae may have a greater role in the accumulation of microdamage than impaired removal due to the suppression of bone turnover. This study supports the assumption that 3D microarchitecture has a direct impact on bone fragility.

◆ *Nutritional stress*
An appropriately balanced diet is essential for developing and maintaining a bone structure capable of withstanding daily mechanical loading. Calcium, vitamin D, and protein are the main nutrients required to maintain bone health and prevent diseases such as osteoporosis. Calcium along with phosphorus forms hydroxyapatite crystals, the mineral component of bone, providing the requisite rigidity for weight-bearing. The skeleton contains 99% of the body's calcium, the remaining 1% being found in blood, extracellular fluid, and soft tissue. In addition to its structural role, calcium has metabolic functions so important that its extracellular concentrations are maintained under fine control. Bone is involved in the regulation of blood calcium levels via bone remodeling, while calcium intake has a reciprocal impact on remodeling: calcium deficiency increases parathormone secretion, which stimulates bone resorption, leading to a decrease in bone density and a gradually weakened bone structure.[27]

Sunlight and diet are the main sources of the vitamin D that helps to maintain blood calcium levels by promoting calcium absorption in the gut. Even moderate vitamin D deficiency causes secondary hyperparathyroidism, impacting bone density and structure. Severe vitamin D deficiency markedly impairs mineralization, rigidity, and structural integrity, producing osteomalacia in adults and rickets in children.[27] As well as indirectly affecting bone health, vitamin D has a direct impact on bone cell activity, notably by determining osteoclast differentiation and function, with osteoclasts actually metabolizing vitamin D in an autocrine manner.[28] The vitamin D metabolite, $1\alpha,25$-dihydroxyvitamin D3, also regulates osteoblast gene transcription, proliferation, and mineralization.[29]

Selective deficiencies in dietary protein markedly lower bone mass and undermine microarchitecture. Low protein intake is commonly associated with hip fracture in the elderly, while protein supplementation attenuates post-fracture bone loss and increases muscle strength, possibly via an increase in IGF-I, thereby markedly reducing medical complications and hospital stay. [30]

Structural impairment in bone disease
Bone structure correlates with biomaterial composition and the manner in which this material is fashioned into a 3D structure endowed with stress-resistant geometric properties. Meta-
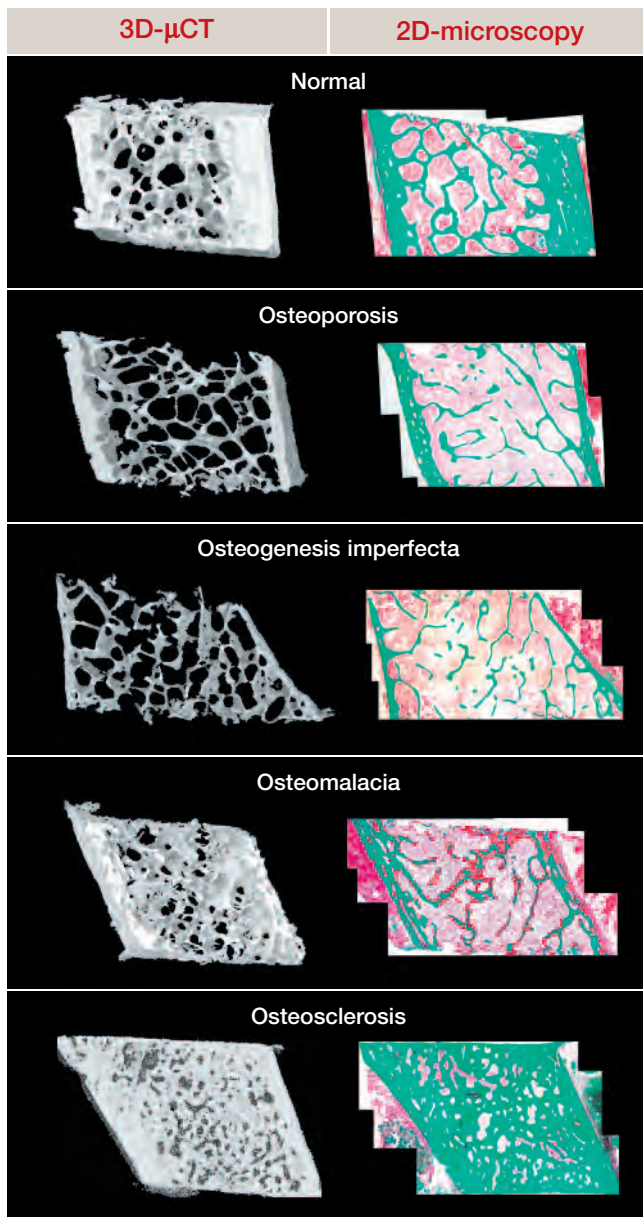
Figure 2. *Structural impairment in bone disease.*
Undecalcified bone biopsies (3D microcomputed tomography or 2D micro-scopic histology [Goldner's stain]). Green: mineralized bone. Red (pink to red gradient): osteoid (nonmineralized bone) and bone marrow cells.
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bolic bone disease that distorts biomaterial composition and/or macro/microarchitecture therefore results in bone fragility. The cellular mechanism by which one component attempts to compensate for abnormalities in another has not been elucidated. There are also few clinically validated methods for assessing and monitoring the microarchitectural response to bone disease and its treatment. However, study of the collagen disorder osteogenesis imperfecta (OI), the mineralization disorder osteomalacia, and postmenopausal osteoporosis (low bone mass plus altered microarchitecture) has highlighted the contribution of each of these components to bone quality and strength *(Figure 2).*[31]

◆ *Osteogenesis imperfecta: a collagen disorder*
OI is a genetic disorder of collagen synthesis characterized by fragile bones with recurrent fractures resulting in skeletal deformity. The phenotype ranges from cases that are lethal in the perinatal period to mild cases diagnosed in adulthood.[32] The abnormal quantity of collagen and its poor quality interfere with mineral crystal size, accounting for low bone mass and bone fragility. The microarchitecture is also affected due to fewer and thinner trabeculae and decreased bone formation at cellular level. Taken together, these abnormalities severely impair resistance to load and torsion stress.

◆ *Osteomalacia: impaired mineralization*
Osteomalacia and rickets are characterized by a defect of primary mineralization due to calcium and/or phosphate deficiency. Osteomalacic bone comprises a very small amount of mineralized tissue with an accumulation of osteoid (nonmineralized newly formed bone), due to delay between bone matrix deposition and mineralization onset. In addition, bone resorption is usually increased, and trabecular microarchitecture impaired, in cases of secondary hyperparathyroidism due to calcium malabsorption. Biomechanical properties are thus markedly impaired, with weakened bones at risk of fracture from minimal trauma.[31]

◆ *Osteoporosis: low bone mass and altered microarchitecture*
The World Health Organization and International Osteoporosis Foundation define osteoporosis as "a systemic skeletal disease characterized by low-bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk."[33] Estrogen deficiency is the most important factor in the pathogenesis of postmenopausal osteoporosis. Its main effects are to increase bone resorption and remodeling. These may be transient, but in combination they accelerate trabecular microarchitectural damage, such as increased spacing (loss of interconnectivity), reduced thickness, induced perforation, and ultimately transformation of the 3D structure from plates to rods.[31] Estrogen withdrawal compounds the effect of aging on cortical bone by slowing periosteal apposition while maintaining vigorous endocortical resorption, resulting in a gradually thinner cortex and net bone loss. In more severe cases, erosion of endocortical bone leads to the trabecularization of cortical bone by producing irregularly shaped giant canals and allowing adjacent resorptive cavities to coalesce, thereby blurring the distinction between cortical and trabecular bone. The combination of such cortical changes with structural deterioration of the trabecular network accounts for postmenopausal bone fragility.[25]

A prospective study using 2D histomorphometry and 3D microcomputed tomography (μCT) found significant microarchitectural abnormalities in bone biopsies from premenopausal women with idiopathic osteoporosis (IOP) compared with normal women. Cortices were thinner and trabeculae fewer, thin-

ner, more widely separated, and heterogeneously distributed.[34] Although these architectural changes resemble those in postmenopausal women, high bone turnover does not seem to be involved. Some of the affected women showed osteoblast dysfunction, with repercussions on bone formation and microstructure, but there was no evidence of an association with IGF-I, in contrast to men with IOP, where low serum IGF-I directly induces osteoblast dysfunction, resulting in low bone mineral density. Further studies are needed to determine the factors involved in the pathogenesis of IOP in premenopausal women.

Bone diseases involving very high bone formation rates (fibrous dysplasia, metastatic bone in bone metastases, Paget's disease) synthesize bone matrix in an anarchic pattern that lays down collagen fibers in random directions. The biomechanical properties of the resulting woven or nonlamellar bone are poorer than those of lamellar bone, despite its greater mineralization.[35,36]

◆ *Effects of osteoporosis treatments on bone architecture*

Although it is the main aim of osteoporosis treatments, an increase in bone mass only partly accounts for the resulting decrease in fracture incidence. Studies using quantitative techniques are now seeking to identify the effects of these therapies on bone microarchitecture.

In postmenopausal women, a high proportion of nonvertebral fractures occur at sites comprising between 70% and 80% of cortical bone. Recent reports have paid particular attention to the Haversian canals that traverse the cortex carrying blood vessels and sympathetic nerve fibers. They provide an appropriate surface for bone remodeling. 3D μCT quantification has shown that cortical fragility in osteoporotic women is partly due to bone loss associated with increased intracortical porosity. The same study found that antiresorptive agents not only improved trabecular bone microarchitecture, but also lowered the number and size of cortical pores, which could explain the observed reduction in nonvertebral fracture risk.[37]

Anabolic agents such as parathyroid hormone/recombinant teriparatide improve trabecular and cortical microarchitecture by inducing bone formation at quiescent surfaces and increasing bone turnover with greater stimulation of formation than re-

sorption. Histomorphometry of transiliac bone biopsies from postmenopausal women receiving teriparatide for 12 to 24 months showed significantly higher trabecular and endosteal hemiosteon mean wall thickness than in placebo controls.[38]

Strontium ranelate is an antiosteoporotic treatment with a dual mode of action. Rizzoli et al recently showed that treatment for 2 years significantly improved bone microarchitecture as well as bone resistance. Improvement was significant not only versus baseline, but also versus bisphosphonate.[39]

Several agents with different modes of action are available to treat osteoporosis. Yet few studies have investigated the impact of switching therapies on bone cells and microstructure. Jobke et al recently reported that the bone of osteoporotic patients switched from an antiresorptive agent (bisphosphonate) to strontium ranelate responds by trabecular reorganization. Bone biopsy histomorphometry and μCT just one year after the switch to strontium ranelate revealed a substantial increase in bone volume fraction and enhanced indices of connectivity density, structure model index, and trabecular bone pattern factors, indicating that it was the architectural transformation from trabecular rods to plates that was responsible for the bone volume increase, rather than changes in trabecular thickness and number.[40]

Conclusion

The bone fragility observed in systemic skeletal disease is characterized by low bone mass and poor bone quality in terms of both biomaterial and microarchitecture. Although bone mineral mass is readily accessible and routinely measured, the investigation of bone geometry/structure represents a real challenge. Hence the burgeoning number of studies incorporating 3D iliac crest biopsy data in addition to conventional 2D histomorphometry. However, the technique is invasive, with limitations that include low sample size, sampling variation, and bone site differences (hip versus spine). A number of computerized methods have emerged, such as high-resolution magnetic resonance imaging and peripheral quantitative computed tomography, to improve the monitoring of longitudinal changes in bone quality during skeletal disease, recovery, and treatment.[3] However, the difficulty of developing a single method for the comprehensive investigation of bone quality reflects the complexity of interdependent factors accounting for both the strength of bone and its fragility. ■

References

1. Recker RR, Armas L. The effect of antiresorptives on bone quality. *Clin Orthop Relat Res.* 2011;469(8):2207-2214.
2. Brandi ML. Microarchitecture, the key to bone quality. *Rheumatology.* 2009;48 (suppl 4):iv3-iv8.
3. Chappard D, Baslé MF, Legrand E, Audran M. New laboratory tools in the assessment of bone quality. *Osteoporos Int.* 2011;22(8):2225-2240.
4. Ascenzi A, Bonucci E, Bocciarelli DS. An electron microscope study on primary periosteal bone. *J Ultrastruct Res.* 1967;18(5):605-618.
5. Martin RB, Ishida J. The relative effects of collagen fiber orientation, porosity, density, and mineralization on bone strength. *J Biomech.* 1989;22(5):419-426.
6. De Micheli PO, Witzel U. Microstructural mechanical study of transverse osteon under compressive loading: the role of fiber reinforcement and explanation of some geometrical and mechanical microscopic properties. *J Biomech.* 2011;44(8):1588-1592.
7. Parfitt AM, Mathews CH, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. *J Clin Invest.* 1983;72:1396-1409.
8. Jones HH, Priest JD, Hayes WC, Tichenor CC, Nagel DA. Humeral hypertrophy in response to exercise. *J Bone Joint Surg Am.* 1977;59:204-208.

9. Ferry B, Duclos M, Burt L, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. *J Bone Miner Metab.* 2011;29(3):342-351.

10. Bouxsein ML, Karasik D. Bone geometry and skeletal fragility. *Curr Osteoporos Rep.* 2006;4(2):49-56.

11. Beck TJ, Ruff CB, Warden KE, Scott WW Jr, Rao GU. Predicting femoral neck strength from bone mineral data. A structural approach. *Invest Radiol.* 1990;25:6-18.

12. Bouxsein ML. Determinants of skeletal fragility. *Best Pract Res Clin Rheumatol.* 2005;19(6):897-911.

13. Turner RT. Skeletal adaptation to external loads optimizes mechanical properties: fact or fiction. *Curr Opin Orthop.* 2001;12(5):384-388.

14. Wolff J. *The Law of Bone Remodelling.* Maquet PG, Furlong R, translators. New York, NY: Springer-Verlag, Berlin; 1986:126.

15. Chappard D, Baslé MF, Legrand E, Audran M. Trabecular bone microarchitecture: A review. *Morphologie.* 2008;92(299):162-170.

16. Callewaert F, Sinnesael M, Gielen E, Boonen S, Vanderschueren D. Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading. *J Endocrinol.* 2010;207(2):127-134.

17. Seeman E. Clinical review 137: sexual dimorphism in skeletal size, density, and strength. *J Clin Endocrinol Metab.* 2001;86(10):4576-4584.

18. Cvijanovié O, LeKié A, Nikolié M, Arbanas J, Bobinac D. Bone quality assessment in individuals of different age, gender and body constitution. *Coll Antropol.* 2010;34(suppl 2):161-168.

19. Seeman E, Delmas PD. Bone quality – The material and structural basis of bone strength and fragility. *N Engl J Med.* 2006;354(21):2250-2261.

20. Olson LE, Ohlsson C, Mohan S. The role of GH/IGF-I-mediated mechanism in sex differences in cortical bone size in mice. *Calcif Tissue Int.* 2011;88(1):1-8.

21. Smith RW Jr, Walker RR. Femoral expansion in aging women: implications for osteoporosis and fractures. *Science.* 1964;145(3628):156-157.

22. Ruff C, Hayes W. Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. *Science.* 1982;217(4563):945-947.

23. Seeman E. Pathogenesis of bone fragility in women and men. *Lancet.* 2002;359(9320):1841-1850.

24. Riggs BL, Melton LJ 3rd, Robb RA, et al. Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. *J Bone Miner Res.* 2004;19(12):1945-1954.

25. Szulc P, Seeman E. Thinking inside and outside the envelopes of bone. *Osteoporos Int.* 2009;20(8):1281-1288.

26. Karim L, Vashishth D. Role of trabecular microarchitecture in the formation, accumulation and morphology of microdamage in human cancellous bone. *J Orthop Res.* 2011;29(11):1739-1744.

27. Heaney RP. Dairy and bone health. *J Am Coll Nutr.* 2009;28(suppl 1):82S-90S.

28. Kogawa M, Findlay DM, Anderson PH, et al. Osteoclastic metabolism of 25(OH)-vitamin D3: A potential mechanism for optimization of bone resorption. *Endocrinology.* 2010;151(10):4613-4625.

29. Anderson PH, Atkins GJ. The skeleton as an intracrine organ for vitamin D metabolism. *Mol Aspects Med.* 2008;29(6):397-406.

30. Bonjour JP. Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr.* 2005;24(6 suppl):526S-536S.

31. Chavassieux P, Seeman E, Delmas P. Insight into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease. *Endocr Rev.* 2007;28(2):151-164.

32. Marini JC. Osteogenesis imperfecta. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* sixth edition. Washington, DC: American Society for Bone and Mineral Research; 2009:418-421.

33. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med.* 1993;94(6):646-650.

34. Cohen A, Dempster DW, Recker RR, et al. Abnormal bone microarchitecture and evidence of osteoblast dysfunction in premenopausal women with idiopathic osteoporosis. *J Clin Endocrinol Metab.* 2011;96(10):3095-3105.

35. Gorski JP. Is all bone the same? Distinctive distributions and properties of non-collagenous matrix proteins in lamellar vs. woven bone imply the existence of different underlying osteogenic mechanisms. *Crit Rev Oral Biol Med.* 1998;9(2):201-223.

36. Mulder L, Koolstra JH, den Toonder JM, van Eijden TM. Relationship between tissue stiffness and degree of mineralization of developing trabecular bone. *J Biomed Mater Res A.* 2008;84(2):508-515.

37. Borah B, Dufresne T, Nurre J, et al. Residronate reduces intracortical porosity in women with osteoporosis. *J Bone Miner Res.* 2010;25(1):41-47.

38. Ma YL, Zeng Q, Donley DW, et al. Teriparatide increases bone formation in modeling and remodeling osteons and enhances IGF-II immunoreactivity in postmenopausal women with osteoporosis. *J Bone Miner Res.* 2006;21(6):855-864.

39. Rizzoli R, Chapurlat RD, Laroche JM, et al. Effects of strontium ranelate and alendronate on bone microstructure in women with osteoporosis. Results of a 2-year study. *Osteoporosis Int.* 2012;23(1):305-315.

40. Jobke B, Burghardt AJ, Muche B, et al. Trabecular reorganization in consecutive iliac crest biopsies when switching from bisphosphonate to strontium ranelate treatment. *PLoS One.* 2011;6(8):e23638.

Keywords: cortical bone; fragility; geometry; microarchitecture; osteoporosis; porosity; remodeling; trabecular bone

LA BEAUTÉ FRAGILE DE L'ARCHITECTURE OSSEUSE

L'os doit être rigide, flexible et léger pour résister aux charges et torsions biomécaniques qui permettent le mouvement. Ces propriétés sont déterminées par un ensemble complexe de facteurs interdépendants, dont la masse osseuse, la géométrie et la composition du matériel osseux, qui définissent la qualité de l'os et maintiennent l'intégrité et la solidité de sa structure. Tout au long de la vie, les propriétés structurales et matérielles de l'os se modulent pour mieux répondre aux contraintes telles que la croissance, la ménopause et le vieillissement. L'incapacité de la macro- et de la microarchitecture à s'adapter à de telles contraintes fragilise l'os et peut être à l'origine de fractures. Cet article est centré sur les composants définissant la macro- et la microarchitecture de l'os. Il décrit leurs différences structurales, leurs relations avec les sites du squelette et leurs rôles respectifs. L'article analyse l'influence des stress vitaux majeurs sur l'adaptation structurale de l'os et traite de la fragilité structurale osseuse en termes d'anomalies dans la composition du matériel osseux observées dans certaines pathologies osseuses. Nous avons besoin de mieux comprendre les propriétés biomécaniques de l'os afin de le maintenir en bonne santé et de prévenir ou de traiter ses pathologies. À cette fin, des méthodes d'évaluation de la qualité globale de l'os en termes de corrélations entre sa composition et sa structure tridimensionnelle, sont nécessaires.

> "There is little doubt that further advances in bone imaging will continue to hold center stage in osteoporosis and related research. As it stands, bone imaging is probably the closest thing to art in medicine, whether this is a visual appreciation of the aesthetic qualities of bone imaging; a conceptual appreciation of how bone imaging links structure to form and function; or an appreciation of how advances in bone imaging have succeeded in bringing a large dose of science to the art of medicine."

Bone imaging – the closest thing to art in medicine

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Advances in bone imaging have had a tremendous impact on our knowledge of skeletal anatomy, physiology, and pathophysiology while at the same time generating images of both aesthetic and scientific interest. Bone imaging for assessing bone quality very much lends itself to multidisciplinary input and collaboration across scientific disciplines, helping to drive technological and analytical advances in the assessment of bone quality. This has allowed a much deeper awareness of the changes that occur in bone quality with increasing age and disease, as well as improved fracture risk prediction and better treatment monitoring. Currently, many high-resolution imaging modalities exist to evaluate bone quality, though all have their particular merits and limitations. The ideal imaging modality, which has yet to fully emerge, would allow an accurate prediction of bone strength, discriminate at-risk individuals, identify which aspects of bone strength are faltering, and precisely monitor the effect of treatment. When this day comes, the occurrence of unheralded debilitating osteoporotic fractures in the middle-aged and elderly will be seen as an unusual, rather than a usual, event. In the meantime, we can look forward to even more aesthetically pleasing images of bone structure, images that help link form to function in the human body and as such administer a helpful dose of science to the art of medicine.

Medicographia. 2012;34:170-177 (see French abstract on page 177)

Over the last 20 years, the digital era has seen an unparalleled expansion in the range and diversity of available imaging modalities and analytical techniques that, for the first time, have allowed detailed, non-invasive 3D assessment of the living human skeleton and soft tissues.[1] These sophisticated imaging modalities are not only successful diagnostic tools but also allow one to appreciate the beauty of human anatomy, especially with isotropic 3D and 4D (3D in real time) multidetector computed tomography (MDCT) and other related imaging techniques.[2] The aesthetics of the natural human form is rarely addressed in anatomical and radiological texts, though it was explored enthusiastically by the esteemed Renaissance painters of the 15th century such as Leonardo Da Vinci and Michelangelo, and later by Andreas Vesalius in the 16th century, all of whom vigorously undertook human dissection to discover and paint the secrets and beauty of human anatomy. Radiological imaging, including bone imaging, has led to a new distinctive type of artwork known as radiological art *(Figures 1 and 2)*. High-resolution imaging provides wide-ranging material for artists to apply their intuition and aesthetic judgment. The pioneers of radiological art have devised means of digitally manipulating and supple-

Figure 1. *Radiological art: Looking from the carpal tunnel down to the fingers.*
From 3D computed tomography data. **Image courtesy of Dr K. H. Fung.**
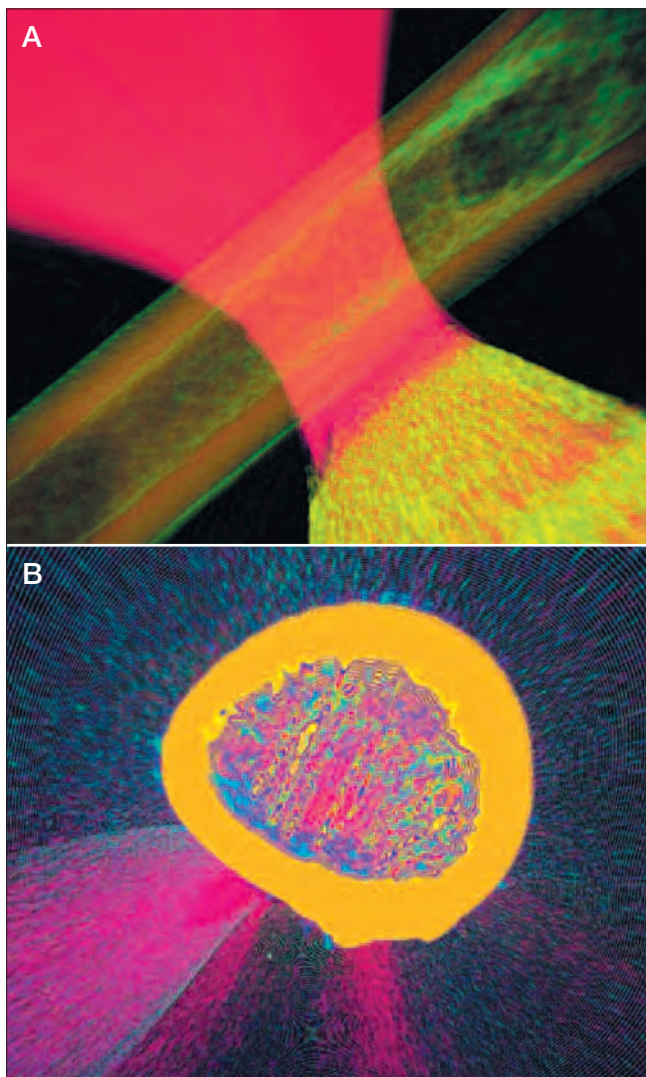
menting clinical radiological images from different body regions to produce visually pleasing images of heightened interest and public appeal. Radiological art draws heavily on the creative qualities and software skills of the producer. Dr Kai-hung Fung, an interventional radiologist from Hong Kong, is one of the leading pioneers of radiological art whose images frequently adorn well-known journals such as *Radiographics* and *Leonardo.* He invented the rainbow technique, first published in 2006, an image-rendering method in which artifacts are stacked between individual image slices to build a 3D image utilizing a contour line effect, with each contour being rendered in a rainbow of colors.[3] In 2009, Dr Fung developed 3D and 4D color Moiré art by enhancing the Moiré interference pattern in 3D computed tomography (CT) and magnetic resonance (MR) datasets.[4] Unlike anatomical dissection, which tends to be objective, analytical, and even disturbing to non-medical observers, radiological imaging provides a palatable means of understanding and appreciating human anatomy, with radiological art often helping to enhance understanding, innuendo, and sentiment. Radiological anatomy, when portrayed as a readily comprehensible image, provides a means of spreading knowledge of the human structure far beyond health-related fields. Further improvements in isotropic 3D imaging and postprocessing digital software will allow increasingly diverse artistic creativity to be applied to baseline imaging data. One can see how the world may well experience an anatomical renaissance with the popularization of digital radiological art, particularly in hospitals, health clinics, and other health-related centers. While a shared inquisitiveness about the natural world drives the pursuit of artists and scientists alike,[5] radiological art allows the creative instincts of the artistic mind to sit happily alongside the analytical instincts of the scientific mind.

Radiological art, in its purest form, deals with portraying human anatomy in an aesthetically pleasing fashion. From a more conceptual perspective, bone imaging also allows one to show and appreciate the amalgamation of human anatomy, form, and function. While traditional bone imaging dealt mainly with bone morphology, modern-day bone imaging correlates structure to function at both microscopic and macroscopic levels. Bone imaging as an art form is best exemplified in the field of bone imaging for bone quality assessment with analytical techniques relating morphology and composition features to functional elements such as strength distribution. Modern bone imaging thereby helps showcase the harmonious combination of form and function in the human skeleton. One cannot help but imagine that if Leonardo Da Vinci, the consummate anatomist/painter/scientist, were alive today, he would be taking a keen interest in radiological imaging anatomy and the analytical techniques that link function to form in the human body.

Figure 2. *Radiological art: the femoral shaft.*
Longitudinal **(A)** and axial **(B)** views of the femoral shaft from computed tomography data. **Image courtesy of Dr K. H. Fung.**

Just as traditional art transcends language and culture, radiological imaging provides a medium not just to make art and medicine interconnect, but also to bridge the gap between basic science, clinical medicine, and other allied scientific fields, and the wider population. Nowhere is this better illustrated than in the field of bone imaging, where scientific input from clinical medicine, anatomy, physiology, chemistry, physics, and computational engineering have contributed to an exponential growth in the knowledge of bone structure and quality over the past 3 decades. It is in part a reflection of this multidisciplinary input that developments in the field of bone imaging have, in many respects, outshone those in many other fields of medicine.[6] Advances in bone imaging techniques such as dual x-ray absorptiometry (DXA), computed tomography, and magnetic resonance imaging (MRI), have provided hard data to further our understanding of medicine and, in addition to providing images of aesthetic quality, helped bring a large dose of science to the art of medicine. This is best illustrated by highlighting some of the recent advances in bone imaging achieved using these modalities.

Dual-x-ray absorptiometry

The widespread clinical use of dual-x-ray absorptiometry (DXA) to diagnose and gauge the severity of osteoporosis has led to osteoporosis being considered, in some circles, as a disease solely of reduced bone mineral density (BMD). This is not correct since osteoporosis is, by definition, a disease characterized not only by reduced BMD but also by "microarchitectural deterioration of bone." This "microarchitectural deterioration of bone" is reflected in the term "bone quality," introduced in 2001 by the Consensus Conference on Osteoporosis of the National Institutes of Health.[7] The capability of DXA machines has been expanded in recent years beyond the measurement of BMD (in g/cm^2) to provide information on aspects of bone quality such as vertebral fracture assessment

SELECTED ABBREVIATIONS AND ACRONYMS

3D-XA	three-dimensional x-ray absorptiometry
BMD	bone mineral density
BV/TV	bone volume fraction (bone volume/total volume ratio)
CT	computed tomography
DXA	dual x-ray absorptiometry
FEA	finite element analysis
HR-pQCT	high resolution peripheral quantitative computed tomography units
MDCT	multidetector computed tomography
MR	magnetic resonance
MRI	magnetic resonance imaging
SNR	signal to noise
Tb.N	trabecular number
vBMD	volumetric bone mineral density
VOI	volume of interest
vQCT	volumetric quantitative computed tomography
VXA	volumetric x-ray absorptiometry

and assessment of proximal femoral bone geometry. This additional information regarding vertebral fracture prevalence can be incorporated into the fracture risk assessment (FRAX®) model along with clinical risk factors to improve prediction of the 10-year probability (%) of major osteoporotic fracture (clinical vertebral, distal radius, proximal femur, or proximal humerus) (http://www.shef.ac.uk/FRAX).[8] For the hip region, advances in DXA software allow automatic calculation of several proximal femoral structural parameters at the "narrow neck" (ie, the narrowest portion of the femoral neck), the intertrochanteric region, and the subtrochanteric femoral shaft region. Parameters such as hip axis length, outer diameter, endosteal diameter, average cortical thickness, cross-sectional moment of inertia, section modulus, and femoral neck shaft angle can be analyzed. These structural parameters compare favorably with similar measurements obtained by volumetric quantitative computed tomography (vQCT) and can be combined with subject height, weight, and age data to calculate the femoral strength index.[9,10] In a study comparing 365 hip fracture patients with more than 2000 control subjects over the age of 50 years, fracture prediction was significantly improved by combining T-score with hip axis length and femoral strength index, compared with T-score alone.[11] Geometric data is best achieved from 3D data and, with this in mind, volumetric x-ray absorptiometry (VXA) has evolved.[12] In human cadaveric specimen, 3D reconstruction of the proximal femur using frontal and lateral acquisitions from a standard DXA unit can be obtained with good accuracy and precision *(Figure 3A).*[12] A more iterative approach that can be applied in vivo has been developed recently *(Figure 3B).*[13] The steps involved in 3D x-ray absorptiometry (3D-XA) include spatial calibration of a commercially available DXA device, acquisition of DXA images in about four different planes (eg, -21, 0, 20, and 30 degree relative to the coronal plane), identification of the specific contours on both views, and deformation of the 3D generic object until its projected contours match the 2D-identified contours.[10] In excised proximal femora, combining areal BMD with 3D geometric parameters (such as femoral head diameter and midfemoral neck cross-sectional area) obtained by 3D-XA improved failure load prediction over density measurements alone.[11] VXA shows excellent correlation with vQCT for shape parameters (femoral neck axis length, cross-sectional slice area) and density parameters (volumetric bone mineral density [vBMD]).[13] Although VXA cannot currently distinguish cortical from trabecular bone and cannot accurately measure cortical thickness, it does show promise as a low-cost, low-radiation, clinically applicable alternative to vQCT in predicting proximal femoral fracture risk, though its clinical usefulness in this respect still has to be determined.
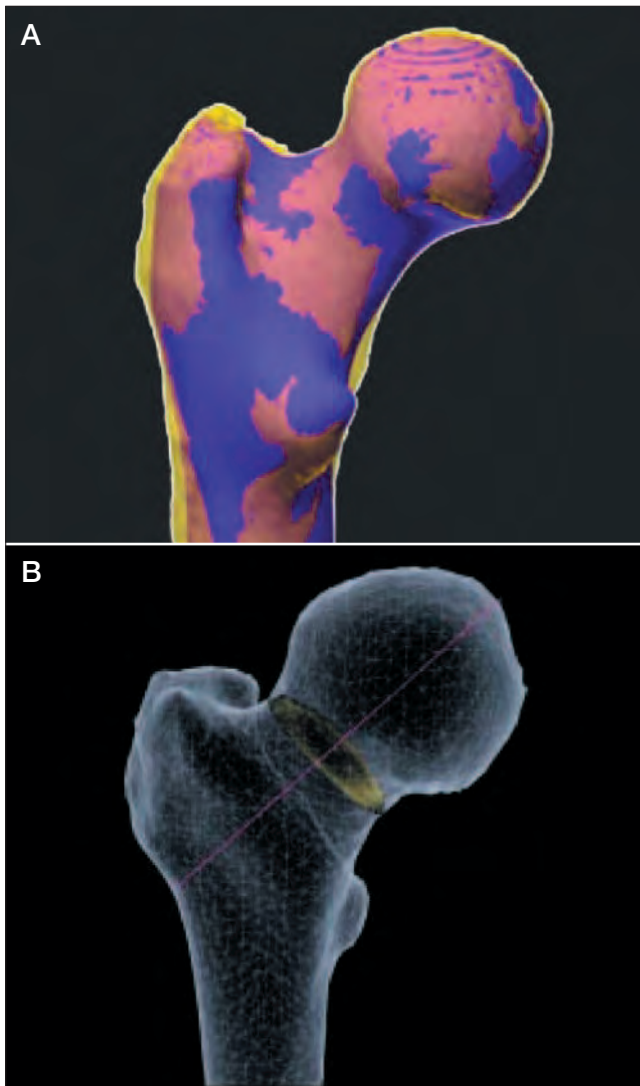
Computed tomography

The high spatial resolution afforded by MDCT facilitates improved delineation of bone architecture with faster acquisition of near-isotropic vQCT datasets than earlier generations of CT scanners. MDCT scanners with 64 multidetector row spiral

Figure 3. *Volumetric x-ray absorptiometry (VXA) images of the proximal femur.*
(A) Superimposition of a VXA image (darker colors) obtained ex vivo and a 3D computed tomography image (yellow color). (B) In vivo iterative-approach VXA reconstruction of the proximal femur. The femoral neck axis (purple line) and narrow neck region (yellow shading with darker yellow indicating higher density) are shown.
*(A) Image courtesy of Dr S. Kolta. (B) Image courtesy of Dr K. Engelke. Reproduced from reference 13: Ahmad et al. J Bone Miner Res. 2010;25:2744-2751.*

technology yield an in-plane resolution of 150-300 µm and a slice thickness of approximately 500 µm. MDCT allows assessment of density, structure, and biomechanical properties separately of trabecular and cortical bone components. It also provides volumetric density measurements (in mg/cm³) as opposed to the areal assessment by standard DXA (in g/cm²). One of the main advantages of whole-body MDCT over smaller, higher-resolution peripheral units is the ability to evaluate bone quality in the biologically relevant central areas of the skeleton that are particularly susceptible to fracture. This is important because changes observed in peripheral bone quality do not necessarily reflect bone quality changes in the central skeleton. MDCT systems correlate highly (R=0.92; P<0.0001)

with reference standards for bone volume fraction (BV/TV) and trabecular spacing, though—as expected—far less well with trabecular thickness and number, as the spatial resolution of MDCT is larger than the average trabecular thickness of 50-150 µm and more comparable to the average trabecular spacing of 200-2000 µm. Structural parameters obtained by MDCT provide a better discriminator of clinical change than DXA and may be detected as early as 12 months postbaseline. This benefit was shown in a study of postmenopausal women, where teriparatide increased vertebral apparent BV/TV by 30.6±4.4% (mean ± SE), and apparent trabecular number (Tb.N) by 19.0±3.2% compared with a 6.4±0.7% increase in DXA-derived areal BMD.[14]

High-precision software, known as medical image analysis framework, facilitates analysis of vQCT datasets through automatic determination of anatomical coordinates to yield predetermined volumes of interest (VOIs) for analysis *(Figure 4, page 174).*[15,16] This automated anatomical coordinate system facilitates the study of the relative contributions of density, geometry, and trabecular and cortical bone to mechanical failure as well as facilitating longitudinal study. An example of how automated anatomical coordinate systems can be used to facilitate CT image analysis was shown in a study by Engelke et al. By comparing predetermined anatomical areas, one can appreciate how ibandronate treatment for 1 year increased volumetric density in the subcortical and extended trabecular areas of the proximal femur, as well as the extended cortical and superior/inferior trabecular regions of the vertebral body, all of which are mechanically significant areas.[17]

Although densitometric and morphometric analysis of high-resolution imaging data improves assessment of fracture risk and treatment efficacy, a more direct measurement of bone strength would be preferable. Finite element analysis (FEA) modeling is a classic engineering computational technique used in design and failure analysis that provides information on parameters such as stiffness, estimated load failure, and stress distribution *(Figure 5, page 174).* This technique has been used in bone imaging to improve estimation of bone strength in vivo. Mechanical properties are assigned to each finite element high-resolution CT (or MRI) model following segmentation and decomposition. The finite elements can be hexagonal, tetrahedral, or curved scaled versions of CT voxels and can employ either linear or quadratic nodal displacement formulation. Load vectors typifying habitual or more spurious overloads, simulating for example a sideways fall, can be used to perform a virtual stress test either to the whole bone or to the cortical or trabecular components separately. Models can be created with or without adjacent soft tissues and bones, and analyses can be run for single or multiple loading conditions.[18] FEA analysis of vQCT data has revealed that vertebral body strength decreases with age twice as much in women than in men and that this sex difference is primarily due to a greater decline in cortical bone strength in women
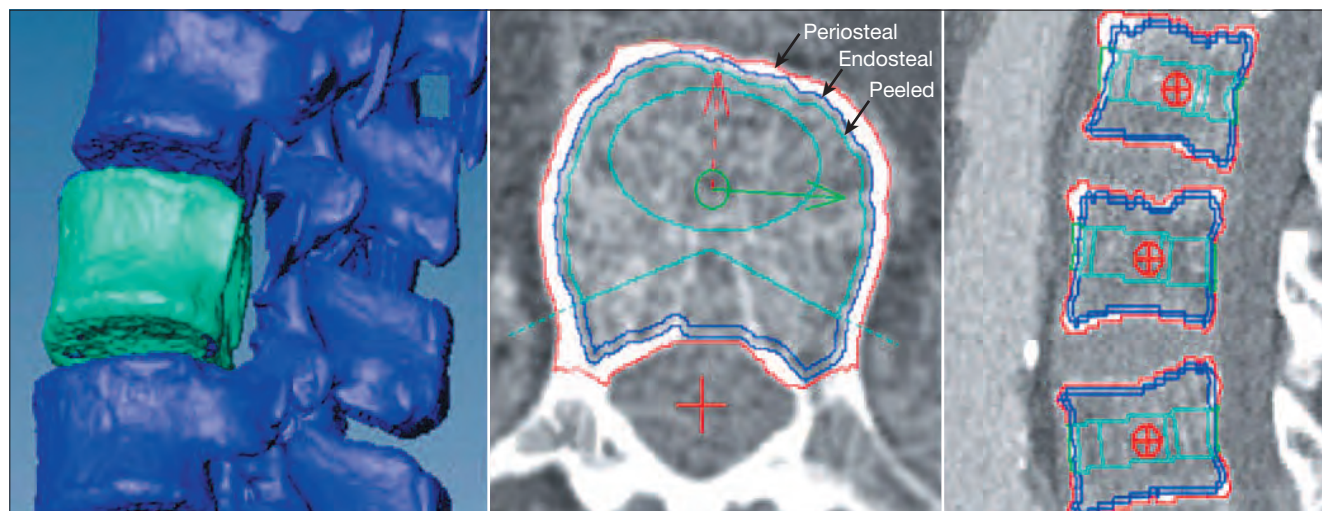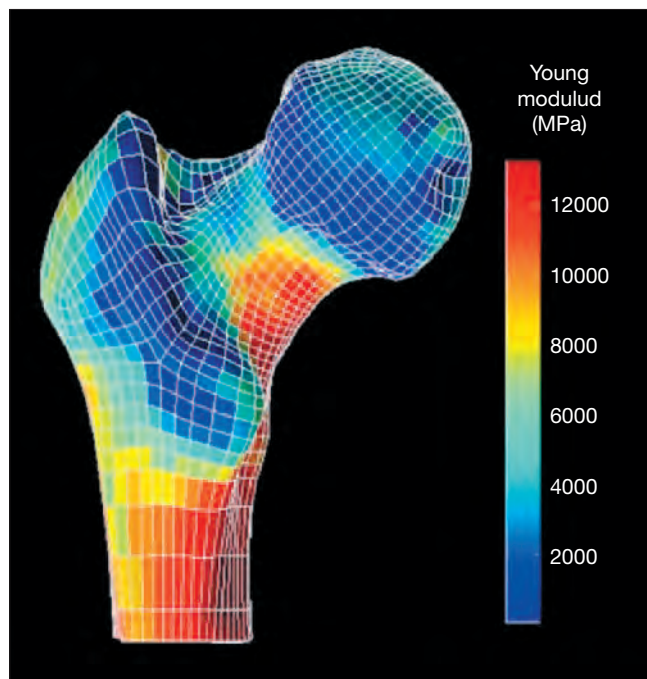
Figure 4. *Volumetric quantitative computed tomography of the lumbar spine.*
Automated anatomical coordinates outline the periosteal, endosteal, and juxtaendosteal ("peeled") contours of the vertebral body. Several different volumes of interest (VOIs) can be evaluated such as the total, trabecular, peeled, elliptical, and Pacman VOIs in the axial plane as well as the superior, midvertebral, and inferior VOIs in the sagittal plane.
Images courtesy of Dr K. Engelke; reproduced from reference 6: Griffith and Genant. Curr Rheumatol Rep. 2011;13:241-250.

while trabecular bone strength declines to a comparable degree in both sexes.[19] In other words, relatively greater cortical bone resorption in women may in part account for their increased vertebral fracture prevalence. Compared with non-fracture control subjects, vertebral vBMD, apparent cortical thickness, compressive strength assessment by FEA, and load-to-strength ratio were shown to be less in females with mild vertebral fracture and least in those with moderate to severe vertebral fracture, emphasizing how fracture severity, in addition to the presence of a fracture per se, is indicative of relative vertebral strength.[20] Using vQCT data and FEA to study age-related changes in proximal femoral strength, Keaveny et al showed how proximal femoral strength declines with aging, much more than would be predicted on the basis of areal BMD changes alone. This study also showed how low proximal femoral strength is much more prevalent in older subjects than osteoporosis as defined by DXA.[21]

High-resolution peripheral quantitative computed tomography

High-resolution peripheral quantitative computed tomography (HR-pQCT) units (Xtreme CT, Scanco Medical AG, Basserdorf, Switzerland) have been developed that can scan the distal radius or distal tibia in 2.8 minutes, acquiring a stack of 110 images over a 9-mm length with a nominal isotropic voxel size of approximately 90 μm. Scan coverage is standardized to a defined distance from the distal radius or distal tibia *(Figure 6)*. This is the only CT system available capable of acquiring high-resolution structural bone detail in humans in vivo. The structural parameters acquired are Tb.N, thickness, separation, structure model index, connectivity, anisotropy, and cortical thickness, all of which are derived from density measurements assuming a fixed mineralization of 1200 mg HA/cm^3. As the analysis programs are density-based, many of the structural parameters will strongly correlate with vBMD, though these structural parameters have been validated against microCT measurements. Limitations of HR-pQCT include movement artifacts, particularly of the radius, and the inability to measure the midshaft of the forearm or leg bones. Structural parameters of cortical and trabecular bone assessed by HR-pQCT at the ultradistal radius can discriminate

Figure 5. *Volumetric quantitative computed tomography provides a basis for finite element analysis of the proximal femur.*
Note how stress distribution as related to color code is highest along the inferomedial aspect of the femoral neck and proximal shaft.
*Image courtesy of Dr K. Engelke; reproduced from reference 6: Griffith and Genant.* Curr Rheumatol Rep. 2011;13:241-250.

between women with and without vertebral fractures, partially independently of DXA results. In a 2-year, randomized, double-blind, prospective study comparing strontium ranelate and alendronate in postmenopausal women with osteoporosis, HR-pQCT monitoring revealed a 6.3% increase in cortical thickness and a 2.5% increase in cancellous BV/TV in those treated with strontium ranelate, while these parameters only increased by 0.9% and 0.8%, respectively, in those treated with alendronate.[23] Estimated failure load also increased with strontium ranelate (+2.1%; *P*<0.005) but not with alendronate (–0.6%; *P*<0.05).[23] In this study, values were not adjusted for strontium content. However, bone strontium content is low after 2 years of treatment (about 1%). Trabecular microarchitectural and biomechanical properties derived from FEA analysis of both the distal radius and tibia are associated (odds ratio, 1.19-2.29) with vertebral and nonvertebral insufficiency fractures in men and women. A similar magnitude of association was seen for these parameters, irrespective of whether they were derived from the distal radius or distal tibia.[24,25]
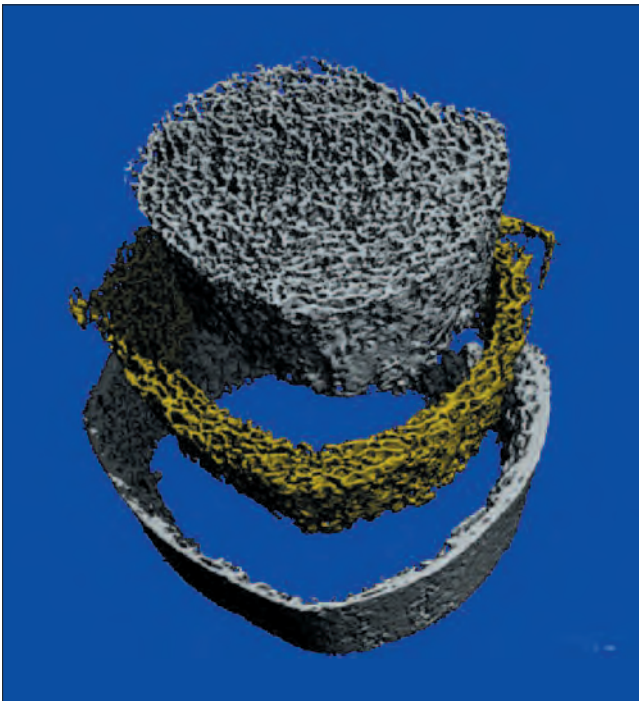
Figure 6. *HR-pQCT image of distal tibia in normal subject.*
The inter trabecular bone, the outer trabecular bone, and the cortical bone components have been separated allowing individual analysis of each component.
Image courtesy of Ling Qin; reproduced from reference 22: Griffith and Genant. Best Pract Res Clin Endocrinol Metab. *2008;22:737-764.*

Magnetic resonance imaging

MRI has several advantages over CT in the assessment of bone quality: no ionizing radiation is involved, thus making it very acceptable in the clinical or research setting; images can be obtained in orthogonal planes directly; and aspects of bone physiology, particularly those related to the marrow cavity such as marrow fat content, marrow diffusion, marrow perfusion, and water content, are obtainable. The disadvantages are

cost, time requirements, and more technically demanding data acquisition and analysis. Due to trade-off issues between spatial resolution, signal to noise ratio (SNR), and radiofrequency signal attenuation, most in vivo MR studies have addressed relatively superficial peripheral sites such as the distal radius, distal tibia, and calcaneus as these trabecular-rich areas are accessible to small high-resolution coils. Nearly all MR-derived structural parameters of the distal radius are better than DXA at differentiating women with and without vertebral fracture.[26]

High-resolution MRI of the central skeleton is limited by SNR and resolution issues due to the persistence of hematopoietic marrow, which contrasts less well with adjacent trabeculae than marrow fat. In an in vitro study of excised proximal femoral specimens, combining MR-derived structural parameters with DXA-derived BMD measures led to improved correlation with bone strength parameters, with *R* values of up to 0.93 being reached.[27] The trabecular structure of the proximal femur has been studied with 3 Tesla MRI, using SNR-efficient sequences with an in-plane resolution of 234 µm x 234 µm and a slice thickness of 1500 µm. Future improvements in resolution and analytical techniques may help advance MRI of biologically relevant sites such as the proximal femur.[28]

To monitor the effects of treatment, MRI of the trabecular structure of the distal radius and trochanteric region of the proximal femur was performed in postmenopausal women. This revealed preservation of apparent BV/TV, apparent Tb.N, and apparent trabecular spacing in patients treated with calcitonin for 2 years, compared with significant loss in a placebo group.[29] Over the same period of time, no significant change in DXA BMD was observed among both groups. This study may help explain the results of an earlier study, which showed substantial reduction in fracture risk with calcitonin treatment despite only a small increase in BMD.[30] The longitudinal effects of alendronate on MRI-based trabecular bone structure parameters have been evaluated.[31] MR-derived apparent Tb.N, as well as 4 topographical parameters, showed treatment effects in the distal tibia after 24 months, especially when fuzzy clustering trabecular bone segmentation, rather than dual thresholding trabecular segmentation, was used, emphasizing the importance of carefully choosing the right computational method for analysis.[31] Surprisingly, no treatment effect was observed by HR-pQCT.[31]

MR-based virtual bone biopsy of peripheral bone has also been advocated as a means to monitor treatment. To this effect, reproducibility was assessed for a 13-mm wide axial slab encompassing the distal radial medullary cavity as well as a for a 5-mm cuboid subvolume. Whole-volume-derived aggregate mean coefficient of variation of all structural parameters was 4.4% (range 1.8%-7.7%) and 4.0% for axial stiffness; while mean coefficients of variation for similar parameters in the corresponding data in the subvolume were 6.5% and 5.5%, respectively.[32]
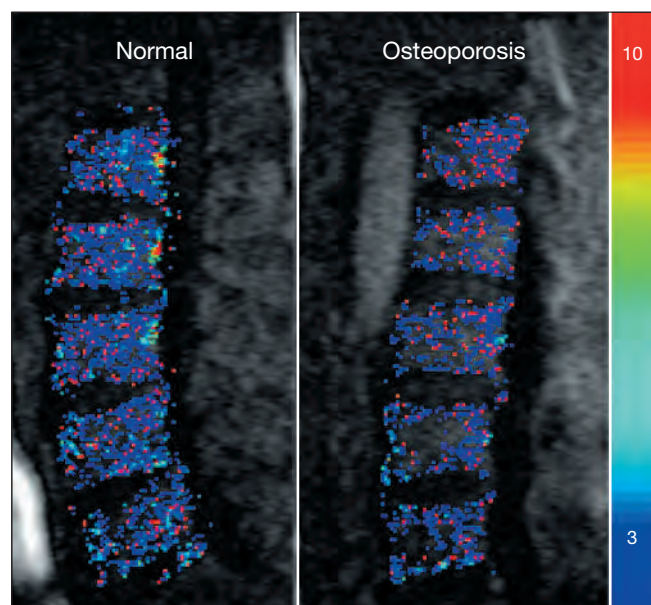
Figure 7. *Color mapping based on amplitude map from pharmacokinetic modeling of lumbar vertebral body MR perfusion data in a subject with normal BMD and one with osteoporosis.*
Note how perfusion parameters are appreciably reduced in the subject with osteoporosis compared with the subject with normal BMD.

As well as being used to examine the trabecular bone component, MRI has been applied to the study of the bone cortex. Cortical bone only accounts for about 10% of vertebral body bone, though it accounts for up to 75% of femoral neck bone and 50% of femoral intertrochanteric bone.[33] Cortical bone seems to have a relatively greater role in proximal femoral bone strength than vertebral body bone strength. With bone loss, cortical bone becomes thinner and more porous.

Cortical porosity is a challenging parameter to measure in vivo, even with HR-pQCT. The wider capability of MRI has allowed a different approach to the assessment of cortical porosity in that cortical water content may serve as a surrogate measure of cortical porosity. Cortical bone water content assessed by ultrashort echo time MRI correlates well with that measured by isotope exchange.[34] Tibial cortical water content in hemodialysis subjects was found to be 135% greater

than in premenopausal women and 43% greater than in postmenopausal women, although no difference in cortical BMD was found between the 3 groups. This indicates that cortical water content may prove to be a better indicator of cortical bone loss and cortical porosity than cortical BMD.[34]

MR also has the capability of assessing marrow fat content, molecular diffusion, and marrow perfusion. MRI studies have shown how perfusion is reduced in nonfractured osteoporotic vertebrae bodies compared with those of normal BMD *(Figure 7)*.[35,36] This reduced perfusion is most likely to be due to atherosclerosis, impaired endothelial function, or reduced demand for tissue oxygenation due to a relative decrease in the amount of hemopoietic marrow within osteoporotic vertebral bodies.[37,38] MR-based perfusion parameters are reduced in osteoporotic vertebral fractures compared with adjacent nonfractured vertebrae.[39] Good perfusion is clearly a prerequisite for normal bone metabolism and fracture healing, including microdamage repair. The smaller the area of enhancing tissue within an acutely fractured vertebral body, the more likely that fractured vertebral body will reduce in height on subsequent follow-up.[40]

Conclusion

The last two decades have seen an exponential growth in bone imaging with new imaging modalities and analytical techniques helping to improve our perception of bone anatomy, physiology, and pathophysiology as well as providing images of aesthetic quality. Bone imaging serves as a focal point for collaboration between clinical and other allied scientific disciplines, which has led to a much better understanding of bone structure and function as well as appreciation of the changes that may occur with age, disease, and treatment. There is little doubt that further advances in bone imaging will continue to hold center stage in osteoporosis and related research. As it stands, bone imaging is probably the closest thing to art in medicine, whether this is a visual appreciation of the aesthetic qualities of bone imaging; a conceptual appreciation of how bone imaging links structure to form and function; or an appreciation of how advances in bone imaging have succeeded in bringing a large dose of science to the art of medicine. ∎

References

1. Link TM. The Founder's Lecture 2009: advances in imaging of osteoporosis and osteoarthritis. *Skeletal Radiol.* 2010;39:943-955.
2. Marinković S, Stošić-Opinćal T, Strbac M, Tomić I, Tomić O, Djordjević D. Neuroradiology and art: a review and personal contribution. *Tohoku J Exp Med.* 2010;222:297-302.
3. Fung KH. The rainbow technique: an innovative approach to the artistic presentation of 3D computed tomography. *Leonardo.* 2006;39:101-103.
4. Fung KH. Creating special visual effects with Moiré patterns in stereoscopic 3D and 4D computed tomographic art. *Leonardo.* 2010;43:306-307.
5. Kemp M. From science in art to the art of science. *Nature.* 2005;434:308-309.
6. Griffith JF, Genant HK. New imaging modalities in bone. *Curr Rheumatol Rep.* 2011;13:241-250.
7. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA.* 2001;285: 785-795.
8. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int.* 2008;19:385-397.
9. Prevrhal S, Shepherd J, Faulkner K, Gaither K, Black D, Lang T. Comparison of DXA hip structural analysis with volumetric QCT. *J Clin Densitom.* 2008;11: 232-236.
10. Faulkner KG, Wacker WK, Barden HS, et al. Femur strength index predicts hip fracture independent of bone density and hip axis length. *Osteoporos Int.* 2006;17:593-599.
11. Le Bras A, Kolta S, Soubrane P, Skalli W, Roux C, Mitton D. Assessment of femoral neck strength by 3-dimensional X-ray absorptiometry. *J Clin Densitom.* 2006;9:425-430.
12. Kolta S, Le Bras A, Mitton D, et al. Three-dimensional X-ray absorptiometry (3D-XA): a method for reconstruction of human bones using a dual X-ray absorptiometry device. *Osteoporos Int.* 2005;16:969-976.

13. Ahmad O, Ramamurthi K, Wilson KE, Engelke K, Prince RL, Taylor RH. Volumetric DXA (VXA): a new method to extract 3D information from multiple in vivo DXA images. *J Bone Miner Res.* 2010;25:2744-2751.

14. Graeff C, Timm W, Nickelsen TN, et al; EUROFORS High Resolution Computed Tomography Substudy Group. Monitoring teriparatide-associated changes in vertebral microstructure by high-resolution CT in vivo: results from the EUROFORS study. *J Bone Miner Res.* 2007;22:1426-1433.

15. Kang Y, Engelke K, Fuchs C, Kalender WA. An anatomic coordinate system of the femoral neck for highly reproducible BMD measurements using 3D QCT. *Comput Med Imaging Graph.* 2005;29:533-541.

16. Engelke K, Mastmeyer A, Bousson V, Fuerst T, Laredo JD, Kalender WA. Reanalysis precision of 3D quantitative computed tomography (QCT) of the spine. *Bone.* 2009;44:566-572.

17. Engelke K, Fuerst T, Dasic G, Davies RY, Genant HK. Regional distribution of spine and hip QCT BMD responses after one year of once-monthly ibandronate in postmenopausal osteoporosis. *Bone.* 2010;46:1626-1632.

18. Keaveny TM. Biomechanical computed tomography-noninvasive bone strength analysis using clinical computed tomography scans. *Ann N Y Acad Sci.* 2010; 1192:57-65.

19. Christiansen BA, Kopperdahl DL, Kiel DP, Keaveny TM, Bouxsein ML. Mechanical contributions of the cortical and trabecular compartments contribute to differences in age-related changes in vertebral body strength in men and women assessed by QCT-based finite element analysis. *J Bone Miner Res.* 2011;26: 974-983.

20. Melton LJ 3rd, Riggs BL, Keaveny TM, et al. Relation of vertebral deformities to bone density, structure, and strength. *J Bone Miner Res.* 2010;25:1922-1930.

21. Keaveny TM, Kopperdahl DL, Melton LJ 3rd, et al. Age-dependence of femoral strength in white women and men. *J Bone Miner Res.* 2010;25:994-1001.

22. Griffith JF, Genant HK. Bone mass and architecture determination: state of the art. *Best Pract Res Clin Endocrinol Metab.* 2008;22:737-764.

23. Rizzoli R, Chapurlat RD, Laroche JM, et al. Effects of strontium ranelate and alendronate on bone microstructure in women with osteoporosis: results of a 2-year study. *Osteoporos Int.* 2011 Sept 10. Epub ahead of print.

24. Vilayphiou N, Boutroy S, Szulc P, et al. Finite element analysis performed on radius and tibia HR-pQCT images and fragility fractures at all sites in men. *J Bone Miner Res.* 2011;26:965-973.

25. Vilayphiou N, Boutroy S, Sornay-Rendu E, et al. Finite element analysis performed on radius and tibia HR-pQCT images and fragility fractures at all sites in postmenopausal women. *Bone.* 2010;46:1030-1037.

26. Krug R, Carballido-Gamio J, Burghardt AJ, et al. Assessment of trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-resolution peripheral quantitative computed tomography ex vivo and in vivo. *Osteoporos Int.* 2008;19:653-661.

27. Link TM, Vieth V, Langenberg R, et al. Structure analysis of high resolution magnetic resonance imaging of the proximal femur: in vitro correlation with biomechanical strength and BMD. *Calcif Tissue Int.* 2003;72:156-165.

28. Krug R, Banerjee S, Han E, Newitt D, Link T, Majumdar S. Feasibility of in vivo structural analysis of high-resolution magnetic resonance images of the proximal femur. *Osteoporos Int.* 2005;16:1307-1314.

29. Chesnut CH 3rd, Majumdar S, Newitt DC, et al. Effects of salmon calcitonin on trabecular microarchitecture as determined by magnetic resonance imaging: results from the QUEST study. *J Bone Miner Res.* 2005;20:1548-1561.

30. Chesnut CH 3rd, Silverman S, Andriano K, et al. A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: the prevent recurrence of osteoporotic fractures study. *Am J Med.* 2000; 109:267-276.

31. Folkesson J, Goldenstein J, Carballido-Gamio J, et al. Longitudinal evaluation of the effects of alendronate on MRI bone microarchitecture in postmenopausal osteopenic women. *Bone.* 2011;48:611-621.

32. Lam SC, Wald MJ, Rajapakse CS, Liu Y, Saha PK, Wehrli FW. Performance of the MRI-based virtual bone biopsy in the distal radius: Serial reproducibility and reliability of structural and mechanical parameters in women representative of osteoporosis study populations. *Bone.* 2011;49:895-903.

33. Krug R, Burghardt AJ, Majumdar S, Link TM. High-resolution imaging techniques for the assessment of osteoporosis. *Radiol Clin North Am.* 2010;48:601-621.

34. Techawiboonwong A, Song HK, Leonard MB, Wehrli FW. Cortical bone water: in vivo quantification with ultrashort echo-time MR imaging. *Radiology.* 2008; 248:824-833.

35. Griffith JF, Yeung DK, Antonio GE, et al. Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. *Radiology.* 2005;236: 945-951.

36. Griffith JF, Yeung DK, Antonio GE, et al. Vertebral marrow fat content and diffusion and perfusion indexes in women with varying bone density: MR evaluation. *Radiology.* 2006;241:831-838.

37. Griffith JF, Wang YX, Zhou H, et al. Reduced bone perfusion in osteoporosis: likely causes in an ovariectomy rat model. *Radiology.* 2010;254:739-746.

38. Griffith JF, Kumta SM, Huang Y. Hard arteries, weak bones. *Skeletal Radiol.* 2011;40:517-521.

39. Biffar A, Schmidt GP, Sourbron S, et al. Quantitative analysis of vertebral bone marrow perfusion using dynamic contrast-enhanced MRI: initial results in osteoporotic patients with acute vertebral fracture. *J Magn Reson Imaging.* 2011;33: 676-683.

40. Kanchiku T, Taguchi T, Toyoda K, Fujii K, Kawai S. Dynamic contrast-enhanced magnetic resonance imaging of osteoporotic vertebral fracture. *Spine.* 2003;28: 2522-2526.

Keywords: bone imaging; computed tomography; dual x-ray absorptiometry; magnetic resonance imaging; osteoporosis; radiological art

L'IMAGERIE OSSEUSE C'EST CE QU'IL Y A DE PLUS PROCHE DE L'ART EN MÉDECINE

Les avancées de l'imagerie osseuse ont eu un impact considérable sur notre connaissance physiopathologique, physiologique et anatomique du squelette ; elles ont également un intérêt à la fois esthétique et scientifique. L'imagerie osseuse se prête largement à des collaborations et participations scientifiques pluridisciplinaires pouvant faire avancer les progrès techniques et d'analyse de l'évaluation de la qualité osseuse. Ceci a permis d'acquérir une connaissance beaucoup plus approfondie des changements dans la qualité osseuse qui interviennent avec l'âge et la maladie, ainsi que l'amélioration de la prévision du risque de fracture et une meilleure prise en charge du traitement. Il existe actuellement de nombreuses modalités d'imagerie à haute résolution pour évaluer la qualité osseuse ; cependant elles ont chacune leurs propres avantages et limites. L'imagerie idéale, qui n'existe pas encore, permettrait de prévoir précisément la résistance osseuse, de distinguer les individus à risque, d'identifier les faiblesses de la résistance osseuse et de contrôler précisément les effets des traitements. Quand tout cela sera possible, la survenue des fractures ostéoporotiques débilitantes sans signes annonciateurs chez les personnes d'âge moyen ou âgées sera un événement plus inhabituel qu'habituel. D'ici là, nous pouvons espérer avoir des images toujours plus agréables esthétiquement de la structure osseuse, qui vont nous aider à établir un lien entre la forme et la fonction dans le corps humain et ainsi ajouter une dose utile de science à l'art de la médecine.

"Exercise during growth primarily influences bone structure (external diameter), rather than mass, to increase bone strength. The intense practice of a physical activity such as baseball induces stimulation of the humerus in torsion, leading to a higher external diameter and increased cortical thickness in men who started playing at a young age. In retired players, a progressive decrease in cortical thickness occurs, whereas the outer diameter remains larger."

Light and resistant: is bone the perfect material?

by P. Ammann, *Switzerland*

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Bone is a fascinating organ and its structure is fully adapted to its function. The presence of cortical and trabecular bone allows perfect mechanical competence with the lowest bone mass, ie, bone weight. Bone is also able to adapt to external constraints, such as repeated stimuli. This is particularly well demonstrated by the modifications of bone structure and geometry occurring in the context of extreme sport performance or in the absence of gravity during space flight. In other examples, the extreme load generated during mastication counteracts the effect of ovariectomy on the mandible. Hormonal modulation leading to progressive decrease in trabecular bone mass and microarchitecture can also be associated with compensatory increase in femoral neck diameter, limiting the decrease in bone strength. Antiosteoporotic treatments restore bone strength by modifying the natural architecture. Modulation of sclerostin action by antisclerostin treatment mimics mechanical loading of the skeleton and induces formation of new bone and improvement of mechanical properties. All these influences selectively modified all determinants of bone strength, such as bone geometry, microarchitecture, and intrinsic bone tissue quality. Bone is not only able to adapt to the environment, but also to repair microdamage, to heal fracture, and to integrate implants.
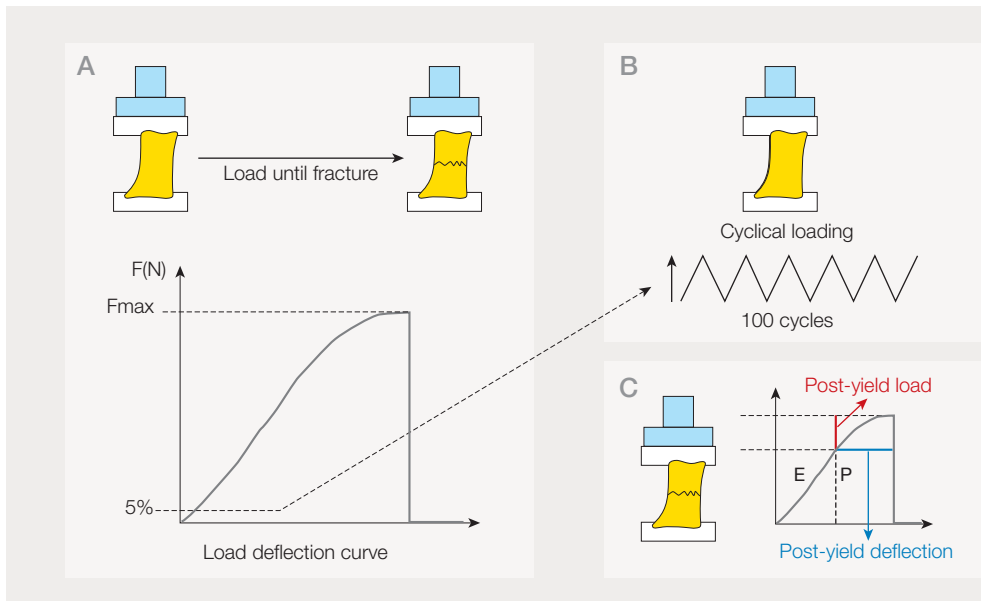
Bone is a fascinating organ. Its structure is fully adapted to its function. Furthermore, it is also able to adapt to new external constraints like repeated stimuli, such as walking, running, and jumping. Bone is not only able to adapt to the environment, but also to repair microdamage, to heal fracture, and to integrate implants. The presence of cortical and trabecular bone allows perfect mechanical competence with the lowest bone mass, ie, bone weight. We now have extensive knowledge on the correlation between bone mechanical properties and bone geometry (diameter of long bone, shape) and microarchitecture (cortical thickness, trabecular bone mass, three-dimensional [3D] distribution, form of the trabeculae [plate vs rods], cortical porosity, microcracks). However, the study of intrinsic bone tissue quality (ie, bone material properties) is only starting to be systematically considered and more investigated at the basic level.

How to evaluate bone mechanical properties and their determinants?

One challenge is to measure bone mechanical properties, which represent an objective measurement of fracture risk. Biomechanical tests of resistance to fracture provide an objective measure of overall bone quality, but these methods are inva-

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sive and cannot be applied in the clinic. Since the ability of bone to withstand stress is controlled by determinants of bone strength—including mass, geometry, and microarchitecture—and intrinsic bone tissue quality, these determinants are used as surrogate measures in the clinical setting.

◆ *Mechanical properties*

Tests are now available to quantify the mechanical properties of bone from different parts of the skeleton.[1] Biomechanical properties of intact cortical and trabecular bone are investigated by axial compression of the vertebral body and proximal tibia. Purely cortical bone is tested by flexion applied at three or four points. The load/deflection curve is used to measure stiffness (the slope of the linear portion of the curve) and maximal load (load at fracture) *(Figure 1)*. The transition point of the load/deflection curve between elastic (linear) and plastic (nonlinear) deformation is defined as the yield point.[1,2] The areas under these sections of the curve represent the energies absorbed during elastic and plastic deformation. Indirect information concerning the material properties can be obtained from the load/deflection curve evaluating the plastic deformation. These values quantify the events occurring from the yield point to the fracture, ie, the plastic deformation. The post-yield load corresponds to the load measured after the yield point to the maximal load (fracture). It is also possible to measure the post-yield deflection corresponding to the deformation imposed on the tested bone sample during plastic deformation. The area under the curve from the yield to the fracture is also of interest. These parameters are influenced by the fatigue (cyclical loading) and modification of intrinsic bone tissue quality, eg, due to low protein intake or ovariectomy. Of note, dissipated energy measured by nanoindentation, ie, the measurement of intrinsic bone tissue quality, is correlated with the plastic energy, but not with other parameters, such as stiffness, which is essentially determined by the geometry of the
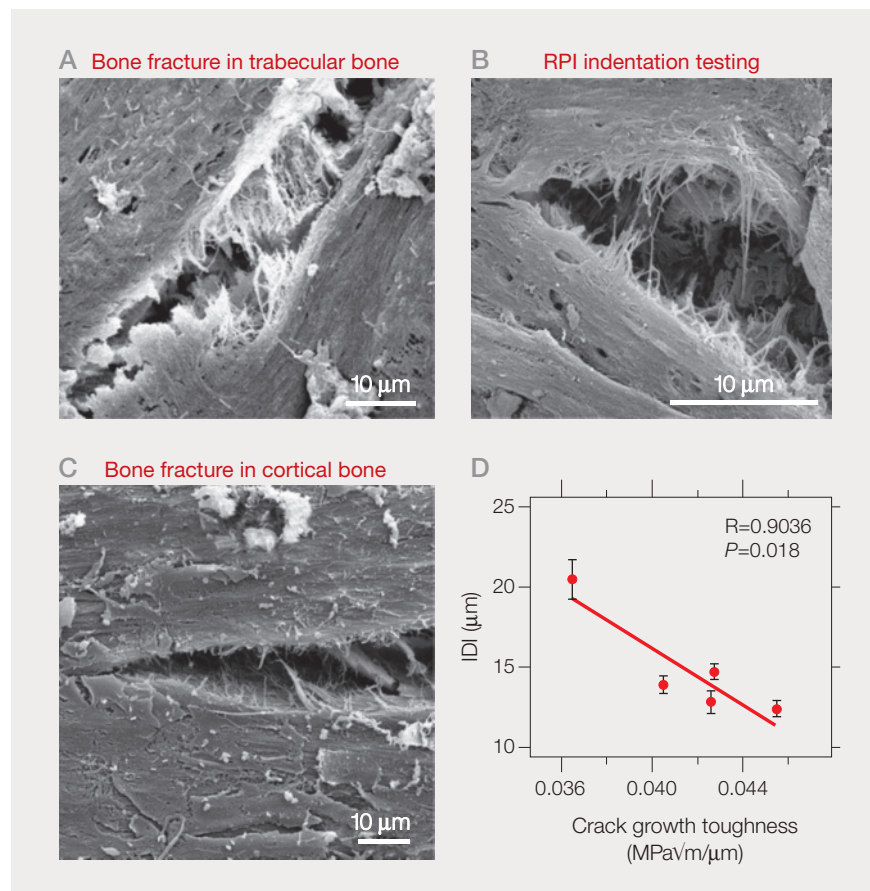
sample. This emphasizes the importance of the post-yield events characterizing intrinsic bone tissue quality. These tests give an excellent quantification of bone mechanical properties and are certainly representative of the load applied on bone during a fall resulting in a fracture. This technical approach allows a very reproducible evaluation of bone strength.

Repeated loading seems to play a role in the genesis of damage and eventually of fracture. In real life, repeated loading could correspond to jumping, running, or hiking. How repeated loading influences mechanical properties or, inversely, how bone tolerates repeated loading, could be of major interest to better understand the risk of fracture occurring not only during bone disease (eg, estrogen deficiency, ovariectomy, and protein malnutrition), but also under the influence of therapeutic agents, particularly those known to influence intrinsic bone tissue quality. We developed an ex vivo dynamic test, for which bone of one site is used as a control value and also to determine the load to apply during the fatigue test *(Figure 1)*. The contralateral bone is cyclically loaded with the load being calculated from the value obtained by measuring the contralateral bone. The load and the number of cycles have to be de-

SELECTED ABBREVIATIONS AND ACRONYMS	
BMU	bone multicellular unit
BSU	bone structural unit
μCT	microcomputed tomography
DXA	dual-energy x-ray absorptiometry
IDI	indentation distance increase
IGF-I	insulin-like growth factor-I
PTH	parathyroid hormone
RPI	reference point indentation

A — Bone fracture in trabecular bone

B — RPI indentation testing

C — Bone fracture in cortical bone

D

IDI (µm)

R=0.9036
P=0.018

Crack growth toughness
(MPa√m/µm)

10 µm

Figure 2. *Microfractures induced by in vivo microindentation are similar to fractures observed in cortical and trabecular bone.*
Abbreviations: IDI, indentation distance increase; R, Pearson correlation coefficient; RPI, reference point indentation.
Modified from reference 19: Diez-Perez et al. J Bone Miner Res. *2010;25(8):1877-1885. © 2010 American Society for Bone and Mineral Research.*

tests, including neck of femur flexion and vertebral compression[4,5]; BMD predicts 60% to 74% of mechanical property variance. As a ratio between hydroxyapatite mineral content and the scanned area, BMD incorporates bone dimensions in addition to mineral quantity. Indeed, the propensity of BMD to predict bone strength is due, at least in part, to its incorporation of bone size.

Dimensions such as external diameter and cortical thickness are key determinants of bone strength.[1,2] Increasing the external diameter of a long bone substantially increases its resistance to flexion.[6-8] Increasing cortical thickness has a lesser effect on bone strength.[7] A 3% to 5% change in diameter can strengthen a long bone by 15% to 20%.

termined in preliminary studies to induce fatigue, but not fracture, during the test and to mimic a load close to the in vivo situation. Alteration of post-yield load and induced deflection are used to monitor bone tissue alteration induced by fatigue *(Figure 1).* This test provides a way to investigate other properties of bone and could be used to test cortical bone in the long bone, or to test both trabecular and cortical bone in the vertebrae.

In patients, the presence of a previous fracture is a risk factor for the occurrence of another fracture.[3] As an example, patients suffering a forearm fracture have to be considered at high risk of osteoporosis, as this type of fracture is the first event occurring in this disease. Thus, a forearm fracture occurring in the context of low-energy trauma could be considered an excellent positive biomechanical test requiring a complete investigation for osteoporosis diagnosis.

◆ *Determinants of bone strength*
The most widely used noninvasive method for the diagnosis of early osteoporosis and to establish fracture risk is dual-energy x-ray absorptiometry (DXA), the conventional determinant of mechanical properties, namely "areal" or "surface" (ie, nonvolumetric) bone mineral density (BMD). In the absence of treatment, ex vivo studies show an excellent correlation between proximal femur BMD and the results of biomechanical

The major features of bone microarchitecture are trabecular bone volume, trabecular density, intertrabecular spacing, trabecular morphology (plate versus column ratio), and the parameters of trabecular connectivity. Changes in any of these features can affect bone strength. Histomorphometry, performed on a horizontal transiliac crest core biopsy, offers a two-dimensional (2D) window and provides information on the degree of mineralization and lamellar organization (lamellar or woven bone).[9] By offering a three-dimensional (3D) window into bone microarchitecture, microcomputed tomography (µCT) appears to be optimal for the evaluation of trabecular microarchitecture.[9] It can also differentiate the trabecular morphology (plates versus columns) that plays a determining role in the transmission and distribution of mechanical stress within bone tissue.[9] In addition, it allows finite element analysis to simulate bone mechanical tests and to analyze the role of each determinant.[10,11] However, being biopsy-based, it also remains invasive.

Newly-developed µCT systems (extreme CT) have sufficient resolution for the noninvasive in vivo measurement of human wrist and tibia microarchitecture.[12] Although the resolution is lower than in ex vivo studies, the technique provides data on trabecular connectivity and morphology. Its major advantage is that it can be used for serial microarchitecture monitoring.[13] Reports have confirmed its accuracy, sensitivity, and repro-

Bone: the perfect material? – *Ammann*

ducibility. Prospective studies indicate that this measurement is able to monitor treatment efficacy and to predict fracture risk, independent of DXA measurement.[11]

The study of intrinsic bone tissue quality (ie, the bone tissue material properties) is only starting to be systematically considered and more investigated at the basic level. Bone is a heterogeneous tissue made up of a mineral component (hydroxyapatite) and an organic collagen component. Theoretically, each is capable of influencing the intrinsic quality of bone tissue. The degree of mineralization has been the more studied aspect of bone tissue to date.[14] Various techniques are available for assessing and quantifying intrinsic bone tissue quality, with respect to both the bone structural unit (BSU) (microindentation)[15,16] and lamella (nanoindentation).[17,18] These give overall information on intrinsic quality as influenced by the mineral and organic components, but only nanoindentation selectively evaluates the influence of each. However, all these approaches are invasive and therefore difficult to apply in clinical strategies and do not allow a longitudinal follow-up. A novel technique is now available for the in vivo measurement of bone tissue strength in a clinical setting.[19] This technique is based on creating microfractures and measuring the bone's overall resistance to their propagation *(Figure 2)*.[19] This represents a direct assessment of fracture pathophysiology and potentially of material properties. The major limitation of this new in vivo technology is that we do not know at the present time whether these measurements are related to bone material properties and/or bone strength.

Bone remodeling could also be considered a determinant of bone strength. Bone turnover allows permanent removal of damaged bone and its replacement by new bone of excellent quality (bone remodeling). This process also allows modification of the size and form of trabeculae, and alteration of bone mass and microarchitecture, occurring in pathological bone loss and during treatments. Bone turnover is considered a determinant of fracture risk, independent of DXA measurement.[20] Bone remodeling can modify the size of the bone, eg, during growth and loading, and allows adaptation of the cortical envelope to mechanical demand. The key role of osteocytes, and their response to loading exerted on bone (through sclerostin regulation), in bone formation have been clearly established.[21] This process allows bone adaptation to external load.

How do bone structures adapt to hormonal and environmental influences?
◆ *Mechanical loading and gravity*
Through the process of remodeling and modeling, bone is able to adapt its geometry to the demand *(Figure 3)*. Exercise performed at a high level, such as in tennis or baseball, adapts not only the cortical thickness, but also the external diameter of the stimulated site, with the greatest bone gain observed during growth. Studies suggest that exercise-induced gains

in bone mass are lost with age.[22,23] However, exercise during growth primarily influences bone structure (external diameter), rather than mass, to increase bone strength. As an example, the intense practice of a physical activity such as baseball (pitchers and catchers) induces stimulation of the humerus in torsion, leading to a higher external diameter and increased cortical thickness in men who started playing at a young age. In retired players, a progressive decrease in cortical thickness occurs, whereas the outer diameter remains larger. This is a good example of skeletal adaptation to external solicitation during growth (increased diameter and cortical thickness) and

Figure 3. *Bone is able to adapt to mechanical loading.*
(A) Cardinals vs Dodgers. October 10, 2004. © Armando/Arorizo/ZUMA/Corbis.
(B) US astronaut in space. © Stocktrek/Corbis.

reduced stimulation (decrease in cortical thickness only). This also emphasizes that exercise during youth has lifelong beneficial effects on cortical bone structure and strength, independent of beneficial effects on bone mass.

In contrast, astronauts living for a period of time in space without gravity's influence lose bone mass in the legs, as they are no longer solicited by any mechanical load (gravity or muscle activity).[24] However, in the arms, the BMD remains normal, possibly explained by muscle demand compensating for the absence of gravity, as the muscles of the upper members are extremely solicited to stabilize the body and perform physical activities.

◆ *Sex hormone deficiency*
Estrogen deficiency is associated with increased bone turnover and results in a negative bone balance. Changes in architecture account for the early decline in bone strength after ovariectomy. Significant decreases in vertebral strength antedate any significant decrease in BMD.[1] The dissociation between these two variables is due to an early change in mi-

diameter leads to progressive recovery of normal femoral neck strength.[26] Though not observed in postmenopausal women, a compensatory increase in femoral neck diameter has been observed in elderly osteoporotic men.[27]

Estrogen deficiency has different effects on the mandible than other regions of the skeleton,[28] but mandible osteoporosis is not always observed after menopause or ovariectomy. This discrepancy is explained by the fact that the extreme load generated during mastication counteracts the effect of ovariectomy. When ovariectomized animals were fed a soft diet (reduction of load), bone loss was observed, but this did not occur in rats fed a hard diet. This shows the capacity of bone to compensate for bone loss and to adapt its structure to environmental conditions.

◆ *Low protein intake*
Protein malnutrition results in increased bone resorption and decreased bone formation, leading to bone loss. In contrast to effects of ovariectomy, no compensatory increment of bone diameter is observed, and alteration of bone strength occurs

Normal-protein diet   Low-protein diet   Low-protein diet and essential amino acid supplements

Figure 4.
Reversibility of isocaloric low-protein diet effect on bone.
Adult female rats were fed a normal or an isocaloric low-protein diet. Reversibility of the effect was evaluated by giving essential amino acid supplements. Scans of the proximal tibia were obtained at the end of the study.
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croarchitecture, such as the perforation and/or disappearance of trabeculae, with no major effect on BMD. In humans, increased vertebral fracture severity, measured semiquantitatively, is associated with deterioration in bone microarchitecture.[25] This continuous and progressive modification of microarchitecture accounts for the accelerated cascade of fractures observed in patients with vertebral fracture. Recent studies indicate that microarchitecture evaluated by extreme CT in humans predicts the risk of fracture, independent of BMD.[12] This observation is a further argument in favor of the importance of the spatial distribution of bone mass. It also underlines that the perturbation of bone metabolism could interfere with the mechanical adaptation of bone.

In the long bone, compensatory expansion of diameter occurs after ovariectomy, and is related to a transient increase in insulin-like growth factor-I (IGF-I) in the presence of estrogen deficiency.[7] In ovariectomized rats, a progressive decrease in bone mass and microarchitecture occurs in the femoral neck,[26] but a subsequent compensatory increase in external neck

also in the long bone. The depressed somatotrope axis probably explains the absence of a compensatory periosteal apposition.[29] Thus, protein supplementation leads to profound modification of the microarchitecture, geometry, and intrinsic bone tissue quality: thickening of the remaining trabeculae and cortex, and normalization of intrinsic bone tissue quality *(Figure 4)*. All these positive effects on determinants of bone strength restore normal[30] bone strength, but the bone architecture is different from that in normal rats. Taken together, this emphasizes potential adaptation of bone geometry and architecture to pathologic situations and the bone's capacity to recover when environmental conditions are restored.

◆ *Antiosteoporotic treatments*
Osteoporosis is defined as a decreased bone mass and alteration of microarchitecture leading to bone fragility and an increased risk of fracture. In other terms, alteration of the determinants of bone strength represents the phenotype of osteoporosis. Therefore, treatments have to induce a modification of these determinants (bone mass, microarchitecture

geometry, and/or intrinsic bone tissue quality) to counteract bone loss and to adapt bone structure to mechanical demand. Different antiosteoporotic treatments are now available. Most of them are classified into two different families according to their cellular bone effects: anticatabolic agents (reduction of bone turnover) and anabolic agents (stimulation of bone formation). Strontium ranelate is a novel compound that can be classified in a third category. Indeed, strontium ranelate influences bone turnover by reducing bone resorption and maintaining a high level of bone formation. The only anabolic agent in clinical use is parathyroid hormone (PTH), which stimulates bone turnover and induces a positive bone balance. Bisphosphonates (anticatabolic agents) reduce bone resorption and, secondarily, bone formation, leading to a prevention of further bone loss and alteration of the microarchitecture and geometry. Strontium ranelate decreases bone resorption, thus maintaining a high level of bone formation and leading to a positive bone balance.

All three treatments decrease fragility and reduce fracture risk. However, they have the opposite effect on bone turnover, which is supposed to be the most important target of antiosteoporotic drugs. They also have variable effects on bone strength determinants, including microarchitecture, geometry, and bone mass.[31] A preclinical study comparing the effects of anticatabolic drugs (pamidronate and raloxifen) and PTH in ovariectomized rats indicates that anticatabolic agents prevent further alteration of microarchitecture and geometry and increase bone material properties as evaluated by nanoindentation. By contrast, PTH increases bone mass, geometry, and microstructure, but does not prevent the alteration of bone material properties induced by the ovariectomy. Strontium ranelate reduces the incidence of fractures independently of severity of osteoporosis, bone turnover, and presence of fracture.[32-34] Since this efficacy cannot be related only to an extent of bone mass modification, an effect on bone material properties could be suspected. Indeed, the deleterious effect of ovariectomy on bone mechanical properties was fully prevented by strontium ranelate administration in adult rats.[32] Thus, microarchitecture deterioration and a decrease in bone mass (both major determinants of bone strength) induced by ovariectomy were prevented by strontium ranelate treatment. Investigation of bone material properties under experimental conditions showed that strontium ranelate treatment

markedly improved hardness and working energy in ovariectomized rats in which a decrease in these properties had been observed.[18] The values measured in strontium ranelate–treated ovariectomized rats after one dose were significantly higher than in sham controls. This suggests that the in vivo modulation of material properties by an antiosteoporotic agent could participate in the improvement of bone strength. Similar positive effects of strontium ranelate salt on bone material properties were observed in humans. A recent study of the vertebral bodies of intact rats treated with strontium ranelate or placebo clearly demonstrated by finite element analysis integrating both microarchitecture parameters and intrinsic bone tissue quality that both determinants of bone strength (bone mass and bone material properties) independently and significantly participate in the determination of bone strength.[10] Bone strength was simulated in the model and also measured in the adjacent vertebra using a compression test. The contribution of both determinants to the prediction of bone strength was equivalent. These clinical and preclinical observations indicate that these bone strength determinants, characterizing bone mass and its spatial distribution, are not enough to explain changes in bone strength and fracture risk. Bone material properties have to be considered and are of major importance.

Antiosteoporotic treatments restore bone strength by modifying the natural architecture. Modulation of sclerostin action by antisclerostin treatment mimics mechanical loading of the skeleton and induces formation of new bone and improvement of mechanical properties.[35] This potential treatment of osteoporosis is an example of therapeutic use of our knowledge of bone adaptation to mechanical loading.

Conclusions

Bone is a fascinating organ and its structure is fully adapted to its function. Furthermore, it is also able to adapt to external constraints, such as repeated stimuli, hormonal modulation, and antiosteoporotic treatment. Modulation of all determinants (geometry, microarchitecture, and intrinsic tissue quality) is implicated in the response to mechanical stimuli and osteoporosis treatments. Hormonal dysregulation partially interferes with skeletal adaptation and further research is warranted to investigate solutions to tailor more adequate treatment options. ■

References

1. Bonjour JP, Ammann P, Rizzoli R. Importance of preclinical studies in the development of drugs for treatment of osteoporosis: a review related to the 1998 WHO guidelines. *Osteoporos Int.* 1999;9(5):379-393.
2. Turner CH. Biomechanics of bone: determinants of skeletal fragility and bone quality. *Osteoporos Int.* 2002;13(2):97-104.
3. Klotzbuecher CM, Ross PD, Landsman PB, Abbott TA III, Berger M. Patients with prior fractures have an increased risk of future fractures: a summary of the literature and statistical synthesis. *J Bone Miner Res.* 2000;15(4):721-739.
4. Dalen N, Hellstrom LG, Jacobson B. Bone mineral content and mechanical strength of the femoral neck. *Acta Orthop Scand.* 1976;47(5):503-508.

5. Granhed H, Jonson R, Hansson T. Mineral content and strength of lumbar vertebrae. A cadaver study. *Acta Orthop Scand.* 1989;60(1):105-109.
6. Ammann P, Shen V, Robin B, Mauras Y, Bonjour JP, Rizzoli R. Strontium ranelate improves bone resistance by increasing bone mass and improving architecture in intact female rats. *J Bone Miner Res.* 2004;19(12):2012-2020.
7. Ammann P, Rizzoli R, Meyer JM, Bonjour JP. Bone density and shape as determinants of bone strength in IGF-I and/or pamidronate-treated ovariectomized rats. *Osteoporos Int.* 1996;6(3):219-227.
8. Ejersted C, Andreassen TT, Oxlund H, et al. Human parathyroid hormone (1-34) and (1-84) increase the mechanical strength and thickness of cortical bone in

rats. *J Bone Miner Res.* 1993;8(9):1097-1101.

9. Ammann P. Bone strength and ultrastructure. *Osteoporos Int.* 2009;20(6):1081-1083.

10. Boyd SK, Szabo E, Ammann P. Increased bone strength is associated with improved bone microarchitecture in intact female rats treated with strontium ranelate: A finite element analysis study. *Bone.* 2011;48(5):1109-1116.

11. Boutroy S, Van Rietbergen B, Sornay-Rendu E, Munoz F, Bouxsein ML, Delmas PD. Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with wrist fracture in postmenopausal women. *J Bone Miner Res.* 2008;23(3):392-399.

12. Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab.* 2005;90(12):6508-6515.

13. Rizzoli R, Chapurlat RD, Laroche JM, et al. Effects of strontium ranelate and alendronate on bone microstructure in women with osteoporosis : Results of a 2-year study. *Osteoporos Int.* 2012;23(1):305-315.

14. Boivin G, Meunier PJ. The mineralization of bone tissue: a forgotten dimension in osteoporosis research. *Osteoporos Int.* 2003;14(suppl 3):S19-S24.

15. Bala Y, Farlay D, Chapurlat R, Boivin G. Modifications of bone material properties in postmenopausal osteoporotic women long-term treated with alendronate. *Eur J Endocrinol.* 2011;165(4):647-655.

16. Bala Y, Depalle B, Douillard T, et al. Respective roles of organic and mineral components of human cortical bone matrix in micromechanical behavior: an instrumented indentation study. *J Mech Behav Biomed Mater.* 2011;4(7):1473-1482.

17. Hengsberger S, Ammann P, Legros B, Rizzoli R, Zysset P. Intrinsic bone tissue properties in adult rat vertebrae: modulation by dietary protein. *Bone.* 2005;36(1):134-141.

18. Ammann P, Badoud I, Barraud S, Dayer R, Rizzoli R. Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality, a determinant of bone strength. *J Bone Miner Res.* 2007;22(9):1419-1425.

19. Diez-Perez A, Guerri R, Nogues X, et al. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. *J Bone Miner Res.* 2010;25(8):1877-1885.

20. Garnero P, Delmas PD. Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. *J Musculoskelet Neuronal Interact.* 2004;4(1):50-63.

21. Rochefort GY, Pallu S, Benhamou CL. Osteocyte: the unrecognized side of bone tissue. *Osteoporos Int.* 2010;21(9):1457-1469.

22. Warden SJ. Extreme skeletal adaptation to mechanical loading. *J Orthop Sports Phys Ther.* 2010;40(3):188.

23. Warden SJ, Fuchs RK, Castillo AB, Nelson IR, Turner CH. Exercise when young provides lifelong benefits to bone structure and strength. *J Bone Miner Res.* 2007;22(2):251-259.

24. Vico L, Collet P, Guignandon A, et al. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. *Lancet.* 2000;355(9215):1607-1611.

25. Delmas PD. The use of bisphosphonates in the treatment of osteoporosis. *Curr Opin Rheumatol.* 2005;17(4):462-466.

26. Bagi CM, DeLeon E, Ammann P, Rizzoli R, Miller SC. Histo-anatomy of the proximal femur in rats: impact of ovariectomy on bone mass, structure, and stiffness. *Anat Rec.* 1996;245(4):633-644.

27. Beck TJ, Looker AC, Mourtada F, Daphtary MM, Ruff CB. Age trends in femur stresses from a simulated fall on the hip among men and women: evidence of homeostatic adaptation underlying the decline in hip BMD. *J Bone Miner Res.* 2006;21(9):1425-1432.

28. Mavropoulos A, Rizzoli R, Ammann P. Different responsiveness of alveolar and tibial bone to bone loss stimuli. *J Bone Miner Res.* 2007;22(3):403-410.

29. Bourrin S, Ammann P, Bonjour JP, Rizzoli R. Dietary protein restriction lowers plasma insulin-like growth factor I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats. *Endocrinology.* 2000;141(9):3149-3155.

30. Ammann P, Laib A, Bonjour JP, Meyer JM, Ruegsegger P, Rizzoli R. Dietary essential amino acid supplements increase bone strength by influencing bone mass and bone microarchitecture in ovariectomized adult rats fed an isocaloric low-protein diet. *J Bone Miner Res.* 2002;17(7):1264-1272.

31. Brennan TC, Rizzoli R, Ammann P. Selective modification of bone quality by PTH, pamidronate, or raloxifene. *J Bone Miner Res.* 2009;24(5):800-808.

32. Bain SD, Jerome C, Shen V, Dupin-Roger I, Ammann P. Strontium ranelate improves bone strength in ovariectomized rat by positively influencing bone resistance determinants. *Osteoporos Int.* 2009;20(8):1417-1428.

33. Collette J, Bruyere O, Kaufman JM, et al. Vertebral anti-fracture efficacy of strontium ranelate according to pre-treatment bone turnover. *Osteoporos Int.* 2010;21(2):233-241.

34. Meunier PJ, Roux C, Seeman E, et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med.* 2004;350(5):459-468.

35. Papapoulos SE. Targeting sclerostin as potential treatment of osteoporosis. *Ann Rheum Dis.* 2011;70(suppl 1):i119-i122.

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LÉGER ET RÉSISTANT : L'OS EST-IL LE MATÉRIAU PARFAIT ?

L'os est un organe étonnant dont la structure est parfaitement adaptée à la fonction. La présence d'os cortical et trabéculaire lui confère une qualité mécanique parfaite avec une masse (c'est-à-dire le poids osseux) très peu élevée. L'os est aussi capable de s'adapter aux contraintes externes, comme les stimuli répétés. Ceci est particulièrement bien illustré par les modifications de la géométrie et de la structure osseuses intervenant dans le contexte des performances sportives extrêmes ou en apesanteur pendant les vols spatiaux. Autre exemple, la charge énorme produite lors de la mastication neutralise l'effet de l'ovariectomie sur les mandibules. La modulation hormonale conduisant à la diminution progressive de la microarchitecture et de la masse osseuse trabéculaires peut aussi être associée à une augmentation compensatoire du diamètre du col fémoral, limitant ainsi la diminution de la solidité osseuse. Les traitements antiostéoporotiques restaurent la solidité osseuse en modifiant son architecture naturelle. La modulation de l'action de la sclérostine par un traitement anti-sclérostine reproduit l'effet de la charge mécanique sur le squelette et induit la formation d'os nouveau et l'amélioration des propriétés mécaniques. Tous ces facteurs d'influence modifient sélectivement l'ensemble des déterminants de la solidité osseuse, comme la géométrie osseuse, la microarchitecture, et la qualité intrinsèque du tissu osseux. Non seulement l'os est capable de s'adapter à l'environnement, mais il répare aussi les microlésions, cicatrise les fractures et intègre les implants.

“ Bone differs from other tissues in that it has the capacity to self-repair after fracture, without leaving a scar; bone continuity and mechanical properties are restored and the repaired bone is similar to the original….Secondary fracture healing is the most common… Though the healing process has been described as comprising four successive phases, in reality it appears that these phases may occur within different timeframes at different sites, and that they sometimes take place simultaneously.”

An architect's dream: a self-repairing structure

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Fracture healing is one of the most fascinating processes in the body as it does not result in a scar, but in reconstitution of the injured tissue in a structure which cannot differ from its original. "Self-regeneration" of bone—its integrity and biomechanical properties—involves a sequence of extremely complex events governed by a variety of cellular elements and stimulating agents. For simpler description of this dynamic process, it has been arbitrarily divided into a succession of phases that immediately follow mechanical insult. Histological features show that fractures heal by a combination of intramembranous and endochondral ossification that is highly dependent on the mechanical environment. The bone repair process looks similar to the normal development of the skeleton during embryogenesis. As knowledge of bone biology has improved over the last decade, we now recognize that many mediators and cellular elements interact at the molecular level in coordination with physiological and mechanical conditions to control bone formation during the fracture healing process. A better understanding of the precise mechanisms of bone formation facilitates the development of new therapeutic strategies to repair damaged bone. Between 5% and 10% of extremity fractures result in delayed union or nonunion with considerable morbidity and economic burden due to the loss of productivity and independence.

F ractures can be classified according to the characteristics of the force that causes them. During injury, single application of a force may generate tensile, compressive, or shear stresses—or some combination thereof—in the bone, leading to bone breakage. The pattern of bone injury depends on the type of force, the mechanical properties of the bone, and the bone's energy absorbing capacity. At the moment of impact, the energy absorbed by the bone leads to mechanical and structural failure. High-energy injuries result in more significant structural changes, that is, greater comminution and displacement as well as larger surrounding lesions in soft tissue and periosteum, which may affect healing capacity. In the osteoporotic bone, the decrease in bone mass, the changes in trabecular architecture modification, and the thinning of cortices result in bone fragility, and the risk of low-energy fracture after a simple fall from height is increased.

Fracture healing

Bone differs from other tissues in that it has the capacity to self-repair after fracture, without leaving a scar; bone continuity and mechanical properties are restored and

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the repaired bone is similar to the original. Fracture healing is a complex physiological process involving biological factors and mechanical principles. For example, fracture stability, depending on the method of fixation chosen by the surgeon, determines the type of bone union.[1] Primary fracture healing, also called direct bone union, occurs in fractures un-

Figure 1. *X-ray views of secondary healing of a tibia after intramedullary nail fixation.*
(A) 1 month post operation. (B) 4 months post operation (hard callus). (C) 1 year post operation (remodeling).

der rigid fixation, providing high stability under loading. Secondary fracture healing, also called indirect bone union, is the most common, occurring when there is relative stability at the fracture level, allowing some degree of motion between the fragments. Loading results in formation of an external callus bridging the fracture gap, and the fracture is considered healed when bone continuity is visible by radiography. This indirect bone healing is characteristically seen with nonoperative fracture treatment and with fixation that preserves some elasticity, such as intramedullary nailing, external fixation, or internal plate fixation in complex and comminuted fractures *(Figure 1).*

◆ *Secondary fracture healing*
The histology of bone following fracture was first described in 1930 by Ham, and McKibbin later emphasized the cellular mechanism involved in fracture healing.[2] In recent decades, better understanding of bone biology has improved our grasp of the molecular control of cellular events.[3]

The healing process is a combination of intramembranous ossification and endochondral ossification similar to bone formation during osteogenesis.[4] Though the healing process has been described as comprising 4 successive phases, in reality it appears that these phases may occur within different timeframes at different sites, and that they sometimes take place simultaneously.[5]

◆ *Hematoma and inflammatory phase*
The hematoma and inflammatory phase is the immediate reaction to fracture: bleeding occurs from the bone and the sur-

rounding soft tissues; the microvascular disruption leads to hypoxia and causes bone necrosis. The hematoma coagulates around the bone extremities and within the medulla, forming a template for callus formation. The fracture hematoma releases inflammatory mediators (cytokines) and initiates the inflammatory response: increased blood flow, increased vessel permeability, and increased cell migration.[6] Osteoclasts are activated to resorb bone debris and vascular proliferation provides stem cells and signaling molecules. This inflammatory response peaks within 24 hours and is complete after 7 days.

◆ *Proliferation and differentiation phase*
The proliferation and differentiation phase is characterized by a proliferation of primitive mesenchymal stem cells (MSCs), which differentiate into cells with osteogenic potential determined by the mechanical environment and biological signals. Tissue formed at the fracture site is called a callu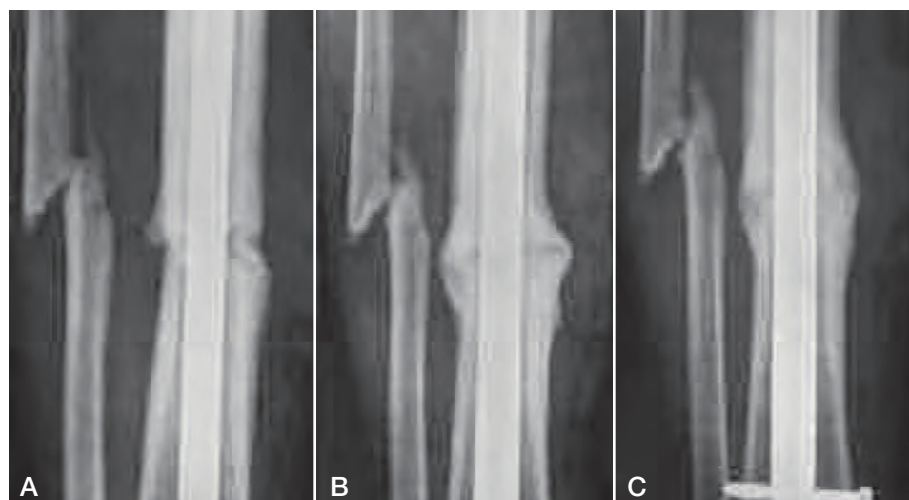s, which becomes stiff as it calcifies. This phase of fracture healing has been classically divided into formation of the soft callus and, through its subsequent calcification, formation of the hard callus.

◆ *Soft callus formation*
Soft callus formation occurs over a 3- to 4-week period. During this process, the clot is invaded by a fibrin-rich granulation tissue. Within this tissue, an endochondral formation develops between the bone extremities, external to the periosteum. This chondroid cartilaginous matrix, rich in proteoglycans and type 2 collagen, is replaced by an osteoid matrix rich in type 1 collagen. The ossified cartilage is replaced progressively by woven bone. Thus, the soft callus enveloping the bone extremities becomes more solid and mechanically rigid *(Figure 1).*

◆ *Hard callus formation*
Hard callus formation occurs over 3 to 4 months. Overlapping the soft callus formation stage, intramembranous ossification occurs in the subperiosteal area adjacent to the distal and proximal ends of the fracture, forming the peripheral

SELECTED ABBREVIATIONS AND ACRONYMS	
BMP	bone morphogenetic protein
MSC	mesenchymal stem cell
PDGF	platelet-derived growth factor
PTH	parathormone
SR	strontium ranelate
TGF	transforming growth factor

hard callus. The inner layer of the periosteum is rich in osteoblasts which synthesize a matrix rich in type 1 collagen, generating calcified tissue.[7] This final central bridging by woven bone provides the fracture with a semi-rigid structure, allowing weight bearing and restoring limb function. At this stage, the woven bone is identical to the secondary spongiosa of the growth plate and the fracture is considered healed *(Figure 2).*

◆ *Remodeling phase*
Once the fracture has been bridged by the callus, the process of fracture repair continues with remodeling slowly replacing the new woven bone with lamellar bone. This remodeling process, resulting in a balanced resorption of hard callus by osteoclasts and lamellar bone deposition by osteoblasts, is initiated as early as the first month and takes years to achieve a fully regenerated bone structure *(Figure 3).*

◆ *Primary fracture healing*
Direct bone union is not common in the natural process of fracture healing, because it requires an anatomical reduction of the fracture, without any gap, and a very rigid fixation. Bone on one side of the cortex can unite with the other side only when the cells within the fracture are subject to zero strain. Primary bone healing can occur by direct remodeling of lamellar bone without formation of a periosteal callus.[5]

Disorders of bone union

The bone healing process fails in 5% to 20% of fractures and the management of nonunion is challenging for an orthopedic trauma surgeon. The diagnosis itself is difficult because of a lack of consensus about the definitions of delayed union and nonunion.[8] This paper discusses aseptic nonunion only, excluding the problem of local infection which is itself a major cause of nonunion.

◆ *Delayed union*
Delayed union is a situation where there are distinct clinical and radiological signs of prolonged fracture healing time. It describes a fracture which has not healed within the expected timeframe and for which the outcome remains uncertain.

◆ *Nonunion*
Nonunion is generally defined as a fracture that has failed to unite within 9 months and that has no radiographic sign of healing for 3 consecutive months. Sometimes, the distinction between delayed union and nonunion is difficult to make and may just reflect the surgeon's hope for healing without further intervention. The diagnosis is based on clinical symptoms such as pain, inability to bear weight, persistence of motion at the fracture site, and absence of bridging callus on x-ray.

X-ray patterns for nonunion have been described according to callus formation.[9] Hypertrophic nonunion is linked with inadequate immobilization and appears to have a normal blood supply and healing response; x-rays show poor callus forma-

Figure 2. *Bifocal fracture of the tibia, reduction and fixation by intramedullary nailing.*
(A) Bifocal fracture of the tibia. (B) Post-operative view. (C) 6 months post operation; complete healing.
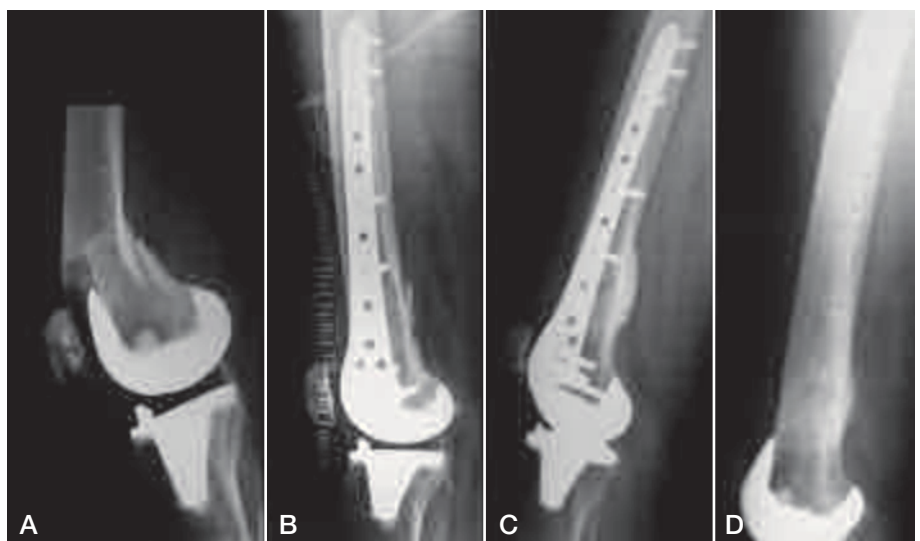
Figure 3. *Periprosthetic fracture in a 72-year-old osteoporotic woman treated by plate fixation.*
(A) Periprosthetic fracture. (B) Plate fixation. (C) Healing at 3 months. (D) View of remodeling, 15 months after plate removal.

tion and an elephant foot configuration. Atrophic nonunion is linked to a poorly vascularized nonunion with very poor healing potential; x-rays show little callus formation, a persistent gap usually filled with fibrous tissue, and resorption of bone cortex.

Understanding the underlining causes of nonunion is key to deciding on the best therapeutic strategy. Giannoudis et al previously described the "diamond concept" of requirements for successful bone healing.[10] The mandatory factors for optimization of fracture repair are not only the fundamental constituents of bone repair—potent osteogenic cell populations, osteoinductive stimulants, and an osteoconductive scaffold—but also mechanical stability. More recently, the same authors emphasized the contribution of other factors, such as vascularization and existing biological variation of the host.[11] Therefore, the progression of fracture healing can be compromised by many physiologic, pathologic, or environmental factors. Surgical treatment has been effective for years, acting only on mechanical and local biological conditions.[12] In hypertrophic nonunion, therapeutic intervention aims to correct the insufficiency of fracture stability with stable fixation, without impairing the blood supply. In atrophic nonunion, the objective of the intervention is to provide, of course, stability, but more-

Figure 4. *Evolution of healing in atrophic nonunion treated with revision surgery.*
(A) Atrophic nonunion of a distal humerus 2 years after fracture, despite several bone grafting procedures.
(B) Evolution of the bone healing process following revision surgery (reduction, stable fixation, and in situ administration of bone morphogenetic protein). (Courtesy of Prof L. Obert).
Abbreviation: m, month.

over to improve the poor biological environment. This includes removal of necrotic bone and fibrous scar tissues, and autologous bone grafting to fill in the bone defect and to provide a sufficient amount of mesenchymal cells, growth and differentiation factors, and a scaffold to enhance bone formation. Noninvasive adjuvant physical therapies like low-intensity pulsed ultrasound, extracorporeal shockwave therapy, and electrical stimulation have had some success, but the amount of evidence is small due to the heterogeneity of results and lack of a sufficient number of randomized control trials.[13-15]

Emerging bone healing therapies

Elucidation of the molecular and cellular basis of bone repair has great potential to improve the treatment of bone union disorders; novel therapies are emerging.[16]

◆ Molecular therapy

A number of key molecules that regulate the fracture healing process, such as growth factors, have been identified and are used in clinical practice or are under investigation.[17] Bone morphogenetic proteins (BMPs) are members of the transforming growth factor superfamily (TGF); they play an important role at the beginning of the process, acting on the MSCs to promote osteoblastic differentiation. Using recombinant DNA technol-

ogy, BMP-2 and BMP-7 have been produced and licensed for clinical use to improve bone healing in restricted indications: open fractures, recalcitrant nonunion *(Figure 4),* or spine fusion. Their use is increasing, more often in association with bone graft in off-label indications, despite some concerns: they have a possible side effect (ectopic bone formation), they are expensive to manufacture, and difficult to handle. Many studies are underway to optimize the delivery process, mini-invasively, and assess the cost effectiveness of the product. Platelet-derived growth factors (PDGFs) can be delivered to the fracture site as platelet-rich plasma: a volume of the plasma fraction of autologous blood with a concentration of platelets is delivered in situ, often mixed with thrombin to create a gel; however, the clinical efficacy of this procedure is unclear.

◆ Bone marrow cells (BMCs)

Bone marrow aspirate, injected into the fracture, has been demonstrated to enhance bone healing.[18] Centrifugation of the aspirate optimizes the procedure by concentrating the marrow, which has osteogenic effect, and discarding fat and cellular aggregate. Treatment of nonunion appears more effective when a minimum of 2600 progenitor cells/mm^3 is used.[19]

◆ Systemic drug delivery

A major concern in clinical practice, the impact of osteoporosis drugs on fracture healing has been widely evaluated in preclinical studies. While the main goal of osteoporosis treatment is fracture prevention, it should ideally have a positive, or at least a neutral, effect on fracture repair. There is no evidence at this time that osteoporosis treatment impairs the fracture healing process.[20-21] The most used, the bisphosphonates, were expected to delay callus formation due to their mode of action. However, experimental data have suggested that callus size and strength were increased in treated animals compared with controls. Late remodeling is delayed, but that doesn't affect long bone fracture healing in the long term. So, there is no evidence-based reason to withhold antiresorptive therapy while a fracture heals, regardless of whether the patient was taking such therapy when the fracture occurred.

Agents with bone-forming properties are expected to improve fracture healing and have been widely studied, showing preclinical evidence of their role in bone repair. Here, two such agents are discussed: parathormone (PTH) and strontium ranelate (SR).

Recent studies in animals and humans have shown compelling evidence of a positive action of PTH on bone fracture healing.[22] Two controlled trials in postmenopausal women have demonstrated that PTH administration accelerates fracture healing time in conservatively treated distal radius fractures[23] and in pubic bone fractures.[24] Positive effects of PTH in fracture healing have been noted in case reports for hip fractures[25] and nonunions.[26]

SR has a dual mechanism with a net bone-forming effect. Preclinical studies have suggested that fractures heal better and faster with SR treatment, showing an increase in the volume and resistance of the callus.[27-29] Case reports also support the beneficial impact of SR on fracture healing and fracture nonunion.[30-31] Recently, there have been reports of SR or PTH treatment of nonunion of atypical femoral fracture result-ing in a similar reversal action on bone-formation markers[32] An ongoing clinical study in male and female osteoporotic patients with distal radius fracture aims to confirm the efficacy of SR on fracture healing. The primary objective of this study is to evaluate whether radiological healing is accelerated under SR treatment compared with placebo.

Conclusions

The role of the orthopedic surgeon in fracture treatment is to reconstruct the normal anatomy of the injured bone to restore normal function. Providing stable fixation of the fracture and preserving its biological environment are essential requirements for satisfactory performance of the physiological and extremely complex healing process. Despite a tremendous improvement in surgical techniques, it is the better knowledge of bone biology that has led to development of new physical therapies and local biological treatment to enhance fracture healing. Future use of systemic drugs that lower the rate of healing disorders and accelerate bone union time is very promising, and would be a major advance in the management of fractures, hopefully minimizing the economic burden of skeletal injury. ■

References

1. Aro HT, Chao EY. Bone-healing patterns affected by loading, fracture fragment stability, fracture type, and fracture site compression. *Clin Orthop Relat Res.* 1993;293:8-17.
2. McKibbin B. The biology of fracture healing in long bones. *J Bone Joint Surg Br.* 1978;60-B(2):150-162.
3. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury.* 2005;36(12):1392-1404.
4. Deschaseaux F, Sensebe L, Heymann D. Mechanisms of bone repair and regeneration. *Trends Mol Med.* 2009;15(9):417-429.
5. Marsell R, Einhorn TA. The biology of fracture healing. *Injury.* 2011;42(6):551-555.
6. Kolar P, Gaber T, Perka C, Duda GN, Buttgereit F. Human early fracture hematoma is characterized by inflammation and hypoxia. *Clin Orthop Relat Res.* 2011;469(11):3118-3126.
7. Ball MD, Bonzani IC, Bovis MJ, Williams A, Stevens MM. Human periosteum is a source of cells for orthopaedic tissue engineering: a pilot study. *Clin Orthop Relat Res.* 2011;469(11):3085-3093.
8. Bhandari M, Guyatt GH, Swiontkowski MF, Tornetta P III, Sprague S, Schemitsch EH. A lack of consensus in the assessment of fracture healing among orthopaedic surgeons. *J Orthop Trauma.* 2002;16(8):562-566.
9. Megas P. Classification of non-union. *Injury.* 2005;36(suppl 4):S30-S37.
10. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. *Injury.* 2007;38(suppl 4):S3-S6.
11. Giannoudis PV, Einhorn TA, Schmidmaier G, Marsh D. The diamond concept–open questions. *Injury.* 2008;39(suppl 2):S5-S8.
12. Harwood P, Newman J, Michael A. An update on fracture healing and non-union. *Orthopaedics and Trauma.* 2010;24(1):9-23.
13. Nelson FR, Brighton CT, Ryaby J, et al. Use of physical forces in bone healing. *J Am Acad Orthop Surg.* 2003;11(5):344-354.
14. Rodriguez-Merchan EC, Forriol F. Nonunion: general principles and experimental data. *Clin Orthop Relat Res.* 2004;419:4-12.
15. Chao EY, Inoue N, Elias JJ, Aro H. Enhancement of fracture healing by mechanical and surgical intervention. *Clin Orthop Relat Res.* 1998;355(suppl):S163-S178.
16. Einhorn TA, Laurencin CT, Lyons K. An AAOS-NIH symposium. Fracture repair: challenges, opportunities, and directions for future research. *J Bone Joint Surg Am.* 2008;90(2):438-442.
17. Axelrad TW, Kakar S, Einhorn TA. New technologies for the enhancement of skeletal repair. *Injury.* 2007;38(suppl 1):S49-S62.
18. Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br.* 2005;87(7):896-902.
19. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am.* 2005;87(7):1430-1437.
20. Goldhahn J, Little D, Mitchell P, et al. Evidence for anti-osteoporosis therapy in acute fracture situations–recommendations of a multidisciplinary workshop of the International Society for Fracture Repair. *Bone.* 2010;46(2):267-271.
21. Gruber R, Koch H, Doll BA, Tegtmeier F, Einhorn TA, Hollinger JO. Fracture healing in the elderly patient. *Exp Gerontol.* 2006;41(11):1080-1093.
22. Della Rocca GJ, Crist BD, Murtha YM. Parathyroid hormone: is there a role in fracture healing? *J Orthop Trauma.* 2010;24(suppl 1):S31-S35.
23. Aspenberg P, Genant HK, Johansson T, et al. Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. *J Bone Miner Res.* 2010; 25(2):404-414.
24. Peichl P, Holzer LA, Maier R, Holzer G. Parathyroid hormone 1-84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. *J Bone Joint Surg Am.* 2011;93(17):1583-1587.
25. Yu CT, Wu JK, Chang CC, Chen CL, Wei JC. Early callus formation in human hip fracture treated with internal fixation and teriparatide. *J Rheumatol.* 2008;35 (10):2082-2083.
26. Rubery PT, Bukata SV. Teriparatide may accelerate healing in delayed unions of type III odontoid fractures: a report of 3 cases. *J Spinal Disord Tech.* 2010; 23(2):151-155.
27. Li YF, Luo E, Feng G, Zhu SS, Li JH, Hu J. Systemic treatment with strontium ranelate promotes tibial fracture healing in ovariectomized rats. *Osteoporos Int.* 2010;21(11):1889-1897.
28. Ozturan KE, Demir B, Yucel I, Cakici H, Yilmaz F, Haberal A. Effect of strontium ranelate on fracture healing in the osteoporotic rats. *J Orthop Res.* 2011;29(1): 138-142.
29. Habermann B, Kafchitsas K, Olender G, Augat P, Kurth A. Strontium ranelate enhances callus strength more than PTH 1-34 in an osteoporotic rat model of fracture healing. *Calcif Tissue Int.* 2010;86(1):82-89.
30. Alegre DN, Ribeiro C, Sousa C, Correia J, Silva L, de Almeida L. Possible benefits of strontium ranelate in complicated long bone fractures. *Rheumatol Int.* 2012;32(2):439-443.
31. Tarantino U, Celi M, Saturnino L, Scialdoni A, Cerocchi I. Strontium ranelate and bone healing: report of two cases. *Clin Cases Miner Bone Metab.* 2010;7:65-68.
32. Carvalho NN, Voss LA, Almeida MO, Salgado CL, Bandeira F. Atypical femoral fractures during prolonged use of bisphosphonates: short-term responses to strontium ranelate and teriparatide. *J Clin Endocrinol Metab.* 2011;96(9):2675-2680.

UN RÊVE D'ARCHITECTE : UNE STRUCTURE AUTORÉPARABLE

La cicatrisation fracturaire est l'un des processus les plus étonnants du corps, car elle n'entraîne pas de cicatrice, mais conduit à la reconstitution du tissu lésé en une structure qui ne diffère pas de l'original. « L'autorégénération » de l'os – de son intégrité et de ses propriétés biomécaniques – nécessite un enchaînement d'événements extrêmement complexes et contrôlés par grand nombre d'éléments cellulaires et d'agents stimulants. Afin de décrire plus simplement ce processus dynamique, il a été arbitrairement divisé en une succession de phases qui suivent directement la lésion mécanique. Les caractéristiques histologiques montrent que les fractures cicatrisent grâce à l'action combinée de l'ossification intramembraneuse et de l'ossification endochondrale, qui est extrêmement dépendante de l'environnement mécanique. Le processus de réparation osseuse est semblable à celui du développement normal du squelette pendant l'embryogenèse. La connaissance de la biologie osseuse s'étant améliorée ces 10 dernières années, on pense maintenant que de nombreux médiateurs et éléments cellulaires interagissent au niveau moléculaire de façon coordonnée en fonction des conditions physiologiques et mécaniques pour contrôler la formation osseuse pendant le processus de cicatrisation fracturaire. Une meilleure compréhension des mécanismes précis de la formation osseuse permet le développement de nouvelles stratégies thérapeutiques pour réparer l'os lésé. Entre 5 % et 10 % des fractures des extrémités conduisent à des consolidations différées ou à l'absence de consolidation, entrainant des coûts et une morbidité considérables liés à une perte de productivité et d'indépendance.

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THE QUESTION

Deciding on whether it is necessary to systematically prescribe vitamin D and calcium to osteoporotic patients is one of the conundrums in the clinical treatment of osteoporosis in current medical practice. On the one hand, calcium and vitamin D are involved in bone physiology; on the other hand, there is no hard evidence to indicate which dosage schedule is the most effective in the prevention of osteoporotic fractures. Clinicians from eleven different countries share their experience with us on whether or not to treat and, if so, at what dosage?

Is systematic supplementation with calcium/vitamin D necessary to treat post-menopausal osteoporosis?

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*C*alcium and vitamin D are important for skeletal homeo-stasis, and postmenopausal women have a higher risk of both calcium and vitamin D deficiencies. Many large population-based studies, including the National Health And Nutrition Examination Survey III (NHANES III; n=13 432), have shown a positive association between serum 25-hydroxy-vitamin D [25(OH)D] levels and bone mineral density (BMD), with BMD increasing monotonically with higher 25(OH)D levels up to at least 32 ng/mL (80 nmol/L).[1,2] Serum 25(OH)D levels in institutionalized individuals and the very elderly (>70 years) have also been found to correlate with muscular strength and postural balance, both important factors for fall risk. Most (including NHANES III),[3] but not all, observational studies have found that lower serum 25(OH)D levels are associated with a higher risk of hip, vertebral, and nonvertebral fractures in postmenopausal women. These studies seem to suggest that serum 25(OH)D levels exceeding 19 to 24 ng/mL (47.5 to 60 nmol/L) are necessary to maintain skeletal health in older individuals.[3]

Randomized placebo-controlled trials have generally reported a beneficial effect of calcium or calcium plus vitamin D supplementation on bone density in postmenopausal women.[4] However, data on fracture prevention have been more conflicting. The two largest trials, WHI (Women's Health Initiative; n=36 282) and RECORD (Randomised Evaluation of Calcium OR vitamin D; n=5292), showed no significant reduction in fracture risk (primary and secondary prevention) with calcium (1000 mg/day) and vitamin D (400-800 IU/day) supplementation.[4,5] Meta-analyses have also yielded variable conclusions depending on the trials included. The largest and probably most comprehensive meta-analysis[6] found that overall there was a statistically significant reduction in the incidence of hip fracture with calcium/vitamin D supplementation (relative risk [RR], 0.84; 95% confidence interval [CI], 0.73-0.96), particularly in institutional residents but not in community dwellers. Overall, there was no statistically significant reduction in the incidence of new nonvertebral fractures, although this was significant in institutional residents (RR, 0.85; 95% CI, 0.74-0.98). No risk reduction was found for clinical vertebral fractures regardless of residential status.

Several factors may be responsible for the conflicting results. These may include differing patient populations (community-dwelling or institutionalized, aged below or above 70-75 years), adherence to therapy, doses of calcium and vitamin D used, baseline vitamin D levels, and target vitamin D levels achieved after treatment. Current data seem to suggest that calcium and vitamin D supplementation probably has the most fracture risk reduction in institutionalized residents and in those who are very elderly and who are compliant with therapy. The optimal daily dose of calcium for women with postmenopausal osteoporosis appears to be 1000-1500 mg/day, with no additional benefit being seen above 1500-2000 mg/day. Vitamin D doses of at least 700-800 IU/day appear to be associated with significant reductions of almost 20% in both hip and nonvertebral fractures, whereas no risk reduction was seen in trials and cohorts using 400 IU/day. Fracture risk reduction appears to be optimal with achieved serum 25(OH)D levels of between 75-110 nmol/L (30-44 ng/mL). This may require between 1800 to 4000 IU of vitamin D per day in individuals with low baseline 25(OH)D level. It has been recommended that the upper safety limits for vitamin D supplementation be revised upwards from 2000 IU/day to 10 000 IU/day, with minimal risk of hypercalcemia.

In conclusion, calcium and vitamin D supplementation remains an important part of therapy for postmenopausal osteoporosis, with optimum efficacy for fracture risk reduction in older women who are compliant with therapy, institutionalized residents, and those with low baseline 25(OH)D levels. Ideal calcium intake should be between 1000-1500 mg/day, while vitamin D doses should be at least 800 IU/day and tailored to achieve 25(OH)D levels between 75-110 nmol/L (30-44 ng/mL). ∎

References
1. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *Am J Med.* 2004;116:634-639.
2. Kuchuk NO, van Schoor NM, Pluijm SM, et al. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective. *J Bone Miner Res.* 2009;24:693-701.
3. Gerdhem P, Ringsberg KA, Obrant KJ, et al. Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. *Osteoporos Int.* 2005;16:1425-1431.
4. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354:669-683.
5. Grant AM, Avenell A, Campbell MK, et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet.* 2005;365:1621-1628.
6. Avenell A, Gillespie WJ, Gillespie LD, O'Connell D. Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis. *Cochrane Database Syst Rev.* 2007;2:1-161.

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Osteoporosis-related fractures are associated with significant morbidity and mortality. The importance of this disease as a worldwide public health problem is evident. Since reduction of fracture risk is the main goal in treating patients with osteoporosis, a complete strategy against this devastating disease should always include trying to reduce the risk of falls. Prerequisites for any therapeutic strategy to prevent osteoporosis and fractures are sufficient calcium and vitamin D intake, physical activity, and fall prevention. Vitamin D supplements today play a key therapeutic role. The efficacy of specific drugs for osteoporosis has been shown only when such supplements are given concurrently. Guidelines therefore recommend adequate calcium and vitamin D intake in addition to antiresorptive medications for the prevention of osteoporotic fractures.

Vitamin D deficiency and insufficiency afflict approximately one billion individuals worldwide. Vitamin D has a proven impact on bone mineral density and bone quality.[1] It is important to determine the optimal intake of calcium and vitamin D to minimize the risk of falls and fractures. In older men and women, higher 25-hydroxyvitamin D [25(OH)D] levels have been associated with better muscle performance and balance, and vitamin D supplementation has reduced body sway—a measure of balance—and improved grip strength. Desirable levels of 30 ng/ml have been shown to reduce the risk of falls and fractures.[2] The function of vitamin D is not limited to maintaining normal bone mineralization, but involves different organs and tissues containing specific receptors.[3] Maintaining sufficient levels of 25(OH)D (>30 ng/ml) helps prevent several pathologies and maintain good general health.[4] Routine screening of 25(OH)D levels is not recommended for those at normal risk, but is advisable for higher-risk older adults.

Calcium and vitamin D are recommended as a baseline supplemental therapy to sustain bone health, rather than to treat osteoporosis. Evidence suggests that vitamin D/calcium supplementation may have favorable effects on bone mineral density and even reduce the risk of fracture,[5] although some recent randomized controlled trials have shown no evidence of a reduced fracture risk with vitamin D/calcium supplementation. In a recent meta-analysis in men and women aged 50 years or older, a combination of calcium and vitamin D was shown to significantly reduce the risk of nonvertebral fracture, and to reduce bone loss at the hip and at the spine.[6] In this study, treatment effects were greatest with daily doses of calcium ≥1200 mg and of vitamin D ≥800 IU. Additional benefits of vitamin D/calcium supplementation in postmenopausal women are a notable reduction in the incidence of falls, which may be attributed to effects on muscle strength and balance.

The American Society for Bone and Mineral Research has issued a statement recommending the use of combined vitamin D and calcium supplementation instead of calcium-only supplementation, and a preference for increased dietary uptake of calcium over calcium supplements.

In conclusion, adequate intake of vitamin D and calcium is recommended as an inexpensive baseline therapy for the prevention and treatment of postmenopausal osteoporosis, and is included in most clinical trials evaluating newer therapeutic osteoporosis agents. ■

References

1. Bischoff-Ferrari HA, Willett WC, Wong JB, et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials. *Arch Intern Med.* 2009;169:551-561.
2. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ.* 2009;339:b3692.
3. Hollick MF. Vitamin D deficiency. *N Engl J Med.* 2007;357:266-281.
4. Freedman BI, Wagenknecht LE, Hairston KG, et al. Vitamin D, adiposity, and calcified atherosclerotic plaque in African-Americans. *J Clin Endocrinol Metab.* 2010; 95:1076-1083.
5. Jackson RD, La Croix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354:669-683.
6. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* 2007;370:657-666.

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One of the major components of the skeleton is calcium. Calcium absorption and its proper incorporation into bone tissue are stimulated by vitamin D. Based on these simple facts, calcium and vitamin D supplementation appears to be a logical step in the treatment of osteoporosis. Especially if we consider that a large proportion of the population worldwide has low calcium intake as well as vitamin D deficiency/insufficiency.

In the case of calcium, the necessary intake for maintenance of calcium balance was previously determined and is 1000-1500 mg/day, depending on the physiological condition (during growth, in the elderly, menopause, pregnancy, etc). Reduced calcium intake may lead to a negative calcium balance and deterioration of bone mass. Thus, supplementation in patients with osteoporosis should be beneficial. However, five large-scale, randomized, controlled trials have questioned the benefits of calcium in reducing fracture risk.[1] Calcium users have also been suspected of being at increased risk for renal stones and gastrointestinal problems. Nevertheless, these studies had important limitations, including selection bias, high baseline calcium intake, and low adherence to treatment regimens. Moreover, in some of the studies, vitamin D was not included in the treatment protocol or was not used at levels sufficient to optimize calcium absorption. In trials with the most treatment-adherent participants, significant reduction in osteoporotic fracture risk with calcium supplement use was found.[1]

High calcium intake (>2000 mg daily) may, however, result in an increased number of fractures.[2] To increase the turmoil further, some evidence has been published showing an association between calcium supplementation of more than 500 mg/day and increased risk of cardiovascular disease.[3,4] However, a number of criticisms can be brought against these studies, thus the data need validation in prospective, randomized, placebo-controlled trials.[4,5] Vitamin D has been in existence for about 500 million years, which suggests that the primary target of this compound is not the skeleton. During the last two decades, a number of other functions for vitamin D have been reported, including antitumor, immune-modulatory, endocrine, and neural effects. With the change in our lifestyles, ie, not staying long enough in the sunshine, vitamin D deficiency/insufficiency has become extremely widespread.[6] According to recent reports, 50%-70% of the elderly populations of developed countries suffer from this condition. Vitamin D supplementation alone or in combination with calcium results in a consistent bone-protective effect. Daily administration of 800-1000 IU vitamin D in those with osteoporosis inevitably reduces fracture rate. However, very high doses of vitamin D once per year may have adverse effects. The extraskeletal effects of vitamin D are in addition to the beneficial effect on bone, but these are still under investigation.

Anti-osteoporotic drugs cannot exert their therapeutic effects unless proper calcium and vitamin D supply is present. When patients with osteoporosis are treated with a bisphosphonate, they should receive a vitamin D and calcium supplement to avoid secondary hyperparathyroidism caused by markedly decreased calcium efflux from bone. Analysis of all the available data suggest that in postmenopausal osteoporotic patients receiving therapy, we should obtain and maintain a serum 25(OH)D level of between 30-50 ng/mL and a calcium intake of 1200 mg/day, by supplementation if needed. ■

References
1. Spangler M, Phillips BB, Ross MB, Moores KG. Calcium supplementation in postmenopausal women to reduce the risk of osteoporotic fractures. *Am J Health Syst Pharm.* 2011;68:309-318.
2. Klompmaker TR. Lifetime high calcium intake increases osteoporotic fracture risk in old age. *Med Hypotheses.* 2005;65:552-558.
3. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ.* 2011;342:d2040.
4. Miller PD. Vitamin D, calcium, and cardiovascular mortality: a perspective from a plenary lecture given at the annual meeting of the American Association of Clinical Endocrinologists. *Endocr Pract.* 2011;17:798-806.
5. Meier C, Kränzlin M. Calcium supplementation, osteoporosis and cardiovascular disease. *Swiss Med Wkly.* 2011;141:w13260.
6. van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab.* 2011;25:671-680.

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V itamin D and calcium play key roles in bone physiology. Inadequate serum 25-hydroxyvitamin D [25(OH)D] concentrations have been shown to be very common in postmenopausal osteoporosis. Vitamin D deficiency leads to decreased intestinal calcium absorption, which, in turn, results in secondary hyperparathyroidism, increased bone turnover, and accelerated bone loss, and can eventually increase fracture risk. In addition, declining serum levels of vitamin D in elderly people are associated with muscle weakness and sarcopenia, resulting in reduced physical performance and higher propensity to falls. Vitamin D thus affects fracture risk through its effects on both bone metabolism and falls. As such, vitamin D supplementation may provide additional benefits given on top of other anti-osteoporotic therapy, because the latter does not influence muscle strength and balance and therefore does not reduce the risk of falls. Moreover, it has been shown that adequate vitamin D supplementation is necessary for optimal response to antiresorptives.[1] As a result, there is no doubt that vitamin D/calcium supplementation is necessary in all cases of postmenopausal osteoporosis. However, the available data on the antifracture efficacy of vitamin D, as well as its dosage, are still controversial. Vitamin D supplementation has been inconsistently shown to reduce the risk of nonvertebral fractures, particularly hip fractures. By contrast, it does not seem to influence the risk of vertebral fracture, and cannot be used in the treatment of osteoporosis alone without other approved antiresorptive or anabolic agents.

In recent years, several meta-analyses have been performed to study the effect of vitamin D on fracture risk and falls. The majority have found that the degree of reduction in falls and fracture risk was dose dependent and higher in those who received higher doses of vitamin D. Supplementation of at least 700 IU of vitamin D, preferably cholecalciferol, was required to achieve risk reduction. At doses of <400 IU/day, no significant benefit was found for reduction of falls and fracture rate. Bischoff-Ferrari et al[2] suggest a daily dose of vitamin D providing a 25(OH)D concentration of ≥75 nmol/L (in the range 1800-4000 IU), although other guidelines are more conservative. The International Osteoporosis Foundation Working Group[3] proposes 2000 IU daily for people at risk of vitamin D deficiency, while Osteoporosis Canada recommends 800-2000 IU daily for osteoporosis patients over 50 years old, emphasizing that in some cases, the dosage of vitamin D can be even higher.[4] The absence of a clear consensus stems from the fact that the efficacy and safety of doses >800 IU for fracture and 1000 IU for falls have not been evaluated in randomized controlled trials. We should be cautious in interpreting the results of studies showing the usefulness of large loading doses of vitamin D in patients with severe vitamin D deficiency. Such an approach might be effective in achievement of rapid repletion and normalization of vitamin D status; however, it was reported that an annual oral intake of 500 000 IU of Vitamin D3 resulted in an increased risk of falls and fractures.[5]

In my view, at present, daily doses of vitamin D should not exceed 2000 IU. Doses should be determined based on the initial 25(OH)D level, body size, age, presence of obesity, and sun exposure limitation, etc. The International Osteoporosis Foundation Working Group recommends estimating the required dose of cholecalciferol based on the measured 25(OH)D level: each 100 IU of added vitamin D will increase serum 25(OH)D by about 2.5 nmol/L.[3] In addition, it is reasonable to perform a retest about 3 months after starting supplementation in order to confirm that the target concentration has been reached. We need further robust data from randomized controlled trials using high-dose vitamin D supplementation. ■

References
1. Adami S, Giannini S, Bianchi G, et al. Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int.* 2009;20:239-244.
2. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, et al. Benefit-risk assessment of vitamin D supplementation. *Osteoporos Int.* 2010;21:1121-1132.
3. Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement: vitamin D recommendations for older adults. *Osteoporos Int.* 2010;21:1151-1154.
4. Hanley DA, Cranney A, Jones G, et al. Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. *CMAJ.* 2010;182: E610-E618.
5. Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA.* 2010;303:1815-1822.

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The point of treating osteoporosis is to reduce fractures. From basic physiology, ionized calcium must be maintained within a relatively narrow range for optimal sensitivity to depolarization of excitable tissues, nerve, and muscle. Calcium is also an essential component of bone mineral. Calcium is not plentiful in food, however, and ingested calcium is inefficiently absorbed. There is an obligatory daily loss of 150-200 mg calcium in urine, higher in postmenopausal women. If blood calcium is to be maintained, the lost calcium will have to come from absorption of ingested calcium, or from bone. Generally, neutral calcium balance requires ingestion of around 1000-1200 mg calcium, with the higher amounts required for postmenopausal women.[1] Vitamin D, or at least its hormonal form, 1,25-dihydroxyvitamin D [1,25(OH)2D] is also required for active gut calcium absorption.

If either ingested calcium is inadequate, or 25-hydroxyvitamin D [25(OH)D] levels are low, more parathyroid hormone is secreted and bone turnover increases. High bone turnover is itself a risk factor for fracture. Continued resorption of bone, particularly in older individuals, whose capacity to replace bone is limited, will reduce bone density and impair bone architecture. There is some evidence that low calcium intake, malabsorption, and/or raised parathyroid hormone levels also accelerate the degradation of vitamin D.[2] Recently, local 1,25(OH)2D production in osteoblasts has been reported, with somewhat different physiological outcomes described when 1,25(OH)2D is produced within bone cells compared with exogenous addition.[3] This provides another theoretical basis for ensuring adequate circulating concentrations of substrate to optimize bone function. With moderate consistency, cross-sectional studies in older individuals show that bone density generally increases with 25(OH)D up to around 50 nmol/L.[4] There is also evidence, at least in older individuals, that optimal lower extremity muscle function increases with 25(OH)D up to around 50 nmol/L,[4] which could contribute to fewer falls and fewer fractures. From this physiology, it is reasonable to propose that adequate calcium intake and vitamin D levels sufficient to suppress parathyroid hormone levels ought to be a minimum base in postmenopausal women. A third pillar, adequate weight-bearing exercise, would also be physiologically ideal.

Trials of supplemental vitamin D and calcium have produced mixed results. But calcium and vitamin D are not like ordinary pharmacological agents, and are normally present in the body. So any increases above "optimal" levels may not show a benefit. Benefits are more likely to be seen if the study population is substantially deficient in vitamin D and/or has low calcium intake at baseline. Often forgotten is that even 1000 IU vitamin D per day is only likely to raise 25(OH)D by 10-20 nmol/L, so smaller doses of 400 IU/day may not achieve much. Compliance issues are well recognized. Several meta-analyses have mostly concluded that despite a relatively large number of trials showing no benefit, there is a moderate consensus that supplementation with vitamin D, mostly with calcium, modestly decreases fractures, provided the caveats are kept in mind. While there are theoretical reasons, outlined above, as to why the combination of vitamin D and calcium might be more effective than vitamin D alone, it should be noted that the vast majority of the individual study participants were enrolled in trials of vitamin D with calcium, rather than vitamin D alone.

Given the myriad of other problems in postmenopausal women, it is hardly surprising that additional pharmacological intervention might be needed to more robustly decrease fracture risk. There is limited evidence that the effectiveness of agents such as bisphosphonates and selective estrogen receptor modulators is reduced if calcium and vitamin D levels are less than optimal.[5,6] Accordingly, it is reasonable to propose that calcium and vitamin D repletion might form a reasonable baseline on which to add further pharmacological interventions. ∎

References
1. Nordin BE. Calcium and osteoporosis. *Nutrition.* 1997;13:664-686.
2. Davies M, Heys SE, Selby PL, Berry JL, Mawer EB. Increased catabolism of 25-hydroxyvitamin D in patients with partial gastrectomy and elevated 1,25-dihydroxyvitamin D levels. Implications for metabolic bone disease. *J Clin Endocrinol Metab.* 1997;82:209-212.
3. Atkins GJ, Anderson PH, Findlay DM, et al. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D3. *Bone.* 2007;40:1517-1528.
4. Lips P, Bouillon R, van Schoor NM, et al. Reducing fracture risk with calcium and vitamin D. *Clin Endocrinol.* 2010;73:277-285.
5. Adami S, Giannini S, Bianchi G, et al. Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int.* 2009;20:239-244.
6. Ishijima M, Sakamoto Y, Yamanaka M, et al. Minimal required vitamin D level for optimal increase in bone mineral density with alendronate treatment in postmenopausal women. *Calcif Tissue Int.* 2009;85:398-404.

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There is a very high prevalence of calcium, protein, and vitamin D insufficiency in the elderly. Calcium and vitamin D supplementation reverses secondary hyperparathyroidism, has beneficial effects on bone density, and additionally, improves body sway and lower extremity strength, reducing the risk of falls.[1-3] Conflicting results—both positive and negative—regarding the effect of supplementation on fracture risk raise questions as to the rationale behind its indication. Some of the discrepancies reported in the existing literature may arise from the fact that subjects entering clinical trials with these agents show great differences in baseline calcium intake and vitamin D status, different doses during the trials, and limited compliance, resulting in controversial conclusions.[1-3] Meta-analysis of many of these trials reports a modest reduction of fracture risk in compliant patients.[4]

Practically all of the evidence-based guidelines on prevention and treatment of osteoporosis support the need to supplement calcium and vitamin D in women with a deficiency or at risk of having a deficiency, and in all of those who receive antiresorptive or anabolic therapy. Supplementation is widely accepted as a preventive therapy in women at risk, particularly in those with dietary insufficiencies, but should not be recommended as a single therapy for women with fragility fractures or women who fulfill criteria for osteoporosis, or those who have an elevated 10-year absolute fracture risk as defined by FRAX. In those patients, currently-approved antiresorptive and bone forming agents have been shown to significantly decrease the risk of both vertebral and nonvertebral fractures when compared with patients receiving placebo plus calcium and vitamin D in randomized controlled clinical trials.[1-3]

Maintaining adequate intake of calcium and vitamin D through diet modification and/or supplementation should be considered part of the standard care of patients with postmenopausal osteoporosis. Dairy products constitute the main nutritional source of calcium in the Western diet. Most guidelines state the need to reach a daily intake of 1200-1500 mg of calcium in postmenopausal women. Rarely will patients include such an intake in their regular diet, and therefore estimation of consumption (with simple questionnaires on sources of calcium in the usual diet) may help in practically defining the dose to add in the form of supplements for each patient. Calcium carbonate and citrate are the most commonly used forms, with some advantages for the latter in terms of digestive tolerance.[1-3]

Based on the relationship between serum 25-hydroxyvitamin D [25(OH)D], bone mineral density, bone turnover, lower extremity function, and falls, it has been suggested that 50 nmol/L is the appropriate serum 25(OH)D concentration threshold to define vitamin D insufficiency. The aim of supplementation should therefore generally be to increase 25(OH)D levels to within the 50-75 nmol/L range. The estimated average vitamin D requirements for older adults to reach an "optimal" serum 25(OH)D concentration of 75 nmol/L (30 ng/ml) is 20-25 μg/day (800 to 1000 IU/day of vitamin D3 or cholecalciferol).[1-6]

Although gastrointestinal upset with most calcium forms is not uncommon, serious toxicity is rare. Concerns about the relationship between calcium supplementation and kidney stones, atherosclerosis, and colorectal cancer have been reasonably ruled out by several studies. Recommended doses of vitamin D also show a very low incidence of side effects.

In conclusion, every woman with postmenopausal osteoporosis should receive supplementation with calcium and vitamin D simultaneously alongside adequate pharmacological therapy, and should be properly followed to ensure compliance. ■

References
1. Kanis JA, Burlet N, Cooper C, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int.* 2008; 19:399-428.
2. Body JJ, Bergmann P, Boonen S, et al. Evidence-based guidelines for the pharmacological treatment of postmenopausal osteoporosis: a consensus document by the Belgian Bone Club. *Osteoporos Int.* 2010;21:1657-1680.
3. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA.* 2001;285: 785-795.
4. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* 2007;370: 657-666.
5. Mithal A, Wahl DA, Bonjour JP, et al; IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int.* 2009;20:1807-1820.
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Calcium plays a fundamental role in promoting bone health, as well as in blood coagulation, muscle contraction, and regulation of nerve excitability. 1200 mg/day of calcium for men and women aged over 50 years has been proposed by the US National Academy of Sciences as an adequate intake, whereas 1000 mg/day is considered sufficient for younger adults.[1] European recommendations indicate lower doses: 800 mg/day for women aged 50-65 years. Many studies report that healthy adults have calcium intakes below both these benchmarks. Vitamin D is essential for maintaining calcium homeostasis, mainly through regulation of intestinal calcium absorption. Circulating 25-hydroxyvitamin D [25(OH)D] concentration indicative of a deficiency state is typically defined as ≤25 nmol/L. In the presence of inadequate vitamin D levels, calcium absorption is reduced and there is a homeostatic increase in parathyroid hormone levels with a consequent stimulation of bone resorption and accelerated bone loss.[2] In elderly people, vitamin D deficiency is common because of decreased exposure to sunshine and reduced capacity of the skin to synthesize vitamin D. Muscular strength is also regulated by vitamin D: vitamin D levels lower than 25-30 nmol/L in older individuals are associated with muscular weakness, decreased physical performance, and increased propensity to falls. Moreover, falls are considered one of the main risk factors for pathological fractures.[3]

Many randomized controlled trials have been performed over the past two decades investigating the efficacy of calcium plus vitamin D in the prevention of fractures, with conflicting results. The daily oral dose of calcium used in the trials has ranged from 500 mg to 1200 mg, and from 400 IU to 800 IU for vitamin D. In terms of fracture risk reduction or decrease in fall incidence, in general, more positive results have been observed using vitamin D 800 IU/day. The percentage reduction in parathyroid hormone levels does not seem to correlate with antifracture efficacy.[2] A recent meta-analysis performed on 12 randomized controlled trials reported that in 8 of the 12 trials, together with the correction of secondary hyperparathy-

roidism, there was a relative risk of 0.86 for nonvertebral fractures and 0.91 for hip fractures, underlining the relationship between efficacy and the optimal dose of 800 IU/day of vitamin D.[3] As regards calcium intake, calcium supplementation is particularly important when baseline calcium intake is low. In our experience, mean calcium intake in elderly people may sometimes be less than 600 mg/day.[4] New estimates from the National Health And Nutrition Examination Survey (NHANES) show that American adults are characterized by an age-related decline in calcium intake, partly explained by a concurrent decline in energy intake, while supplemental calcium use is highest in older age groups.[5] Besides the need for vitamin D, another crucial problem with calcium supplementation is compliance and persistence: adherence with medication in osteoporosis is frequently less than optimal and this may modify the treatment effect. In our opinion, the use of calcium-dense foods could be encouraged to maintain adequate calcium intake across the lifespan. Considering that low calcium intake and poor vitamin D status play a significant role in increasing osteoporotic fracture risk, we believe that calcium and vitamin D must be considered determinant components in the prevention of bone loss and falls, and in the treatment of osteoporosis together with antiresorptive or bone-forming agents.

Finally, there is a growing issue as regards calcium supplementation and the risk of cardiovascular events. A recent reanalysis of the Women's Health Initiative CaD study suggests that calcium supplements taken with or without vitamin D modestly increase the risk of cardiovascular events, especially myocardial infarction.[6] The question remains under discussion. ■

References
1. Heaney RP. The importance of calcium intake for lifelong skeletal health. *Calcif Tissue Int.* 2002;70:70-73.
2. Lips P, Bouillon R, van Schoor N, et al. Reducing fracture risk with calcium and vitamin D. *Clin Endocrinol.* 2010;73:277-285.
3. Nuti R, Martini G, Valenti R, et al. Vitamin D status and bone turnover in women with acute hip fracture. *Clin Orthop Relat Res.* 2004:208-213.
4. Bischoff-Ferrari HA, Willett WC, Wong JB, et al. Prevention of non-vertebral fractures with oral vitamin D and dose dependency. A meta-analysis of randomized controlled trials. *Arch Inter Med.* 2009;169:551-561.
5. Mangano K, Walsh S, Insogna K, Kenny A, Kerstetter J. Calcium intake in the United States from dietary and supplemental sources across adult age groups: new estimates from the National Health and Nutrition Examination Survey 2003-2006. *J Am Diet Assoc.* 2011;111:687-695.
6. Bolland M, Grey A, Avenell A, Gamble G, Reid I. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ.* 2011;342: d2040.

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The rationale for calcium and vitamin D supplementation in the prevention and treatment of osteoporosis is that low dietary calcium intake and/or vitamin D deficiency or insufficiency may contribute to bone loss.[1] Additionally, supplementation is generally recommended as an adjunct to other osteoporosis therapies (antiresorptive and anabolic agents, and strontium ranelate).

Nevertheless, it is still unclear whether the addition of calcium/vitamin D supplements leads to an incremental benefit in patients taking these bone-active drugs. It is also unclear as to what extent osteoporosis treatment maintains its efficacy in patients with an inappropriate intake of calcium or with vitamin D deficiency.

Patients receiving treatment for osteoporosis in a routine clinical setting are often older and frailer than those recruited in clinical trials. Thus, individuals placed on pharmacological treatment for osteoporosis are likely to be at the highest risk of vitamin D deficiency.[2] This high proportion of patients on pharmacological treatment for osteoporosis with vitamin D deficiency may sound somewhat surprising, since all guidelines recommend that any pharmacological intervention should include calcium and vitamin D supplements. Implementation of these guidelines, however, is hampered by a number of factors.

Recently, there have been concerns in the literature about potential risks (ie, excess cardiovascular events) with calcium supplementation and high normal serum calcium levels. Additionally, concerns have been expressed that higher treatment doses of vitamin D than those conventionally used may induce vitamin D toxicity.

The Institute of Medicine has issued guidance on vitamin D and calcium intake. Their consensus report found strong support for the use of vitamin D for bone health, but not for other conditions. Vitamin D is involved in calcium homeostasis, and calcium-associated toxicities at high concentrations include kidney and tissue damage.[3] Resolving whether the benefits outweigh the risks will determine the appropriateness of supplemental nondietary calcium in fracture prevention.

The most obvious reason to supplement a patient's anti-osteoporosis medication with calcium and vitamin D is that all clinical trials having demonstrated antifracture efficacy have been performed by adding—in both groups (placebo and treated)—a combination of calcium and vitamin D.

Additionally, it has been demonstrated that differences in vitamin D status may affect the anticatabolic response to anti-osteoporotic treatment,[4] and that optimal vitamin D repletion appears to be a prerequisite for maximizing the response to antiresorbers in terms of both bone mineral density changes and antifracture efficacy. Greater benefits can even be achieved when blood vitamin D levels are higher than those recently recommended by the Institute of Medicine to maintain bone health.[5]

In conclusion, it is evident that calcium and vitamin D supplementation should be a prerequisite for maximizing the response to anti-osteoporosis drugs in terms of both bone mineral density changes and antifracture efficacy. However, the treatment adherence to these calcium and/or vitamin D formulations is modest as a result of poor tolerability.[6] The consequence of this is that many patients receive neither calcium nor vitamin D. Given this issue, more effort may be usefully applied to encourage persistence with treatment. ■

References
1. Pérez-López FR, Chedraui P, Fernández-Alonso AM. Vitamin D and aging: beyond calcium and bone metabolism. *Maturitas.* 2011;69:27-36.
2. Adami S, Giannini S, Bianchi G, et al. Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int.* 2009;20:239-244.
3. Institute of Medicine. Dietary reference intakes for calcium and vitamin D: Washington, DC. December 15, 2010. Available at: www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx. Accessed December 18th, 2011.
4. Mastaglia SR, Pellegrini GG, Mandalunis PM, Gonzales Chaves MM, Friedman SM, Zeni SN. Vitamin D insufficiency reduces the protective effect of bisphosphonate on ovariectomy-induced bone loss in rats. *Bone.* 2006;39:837-844.
5. Shieh A, Carmel A, Bockman R. Vitamin D insufficiency is associated with decreased bisphosphonate response. Presented at The Endocrine Society's 93rd Annual Meeting & Expo; June 4-7, 2011; Boston, MA. Abstract #P1-228.
6. Quesada JM, Blanch J, Díaz-Curiel M, Díez-Pérez A. Calcium citrate and vitamin D in the treatment of osteoporosis. *Clin Drug Investig.* 2011;31:285-298.

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Calcium is the most important mineral in bone, and the skeleton comprises its biggest store; when serum levels of calcium decline, parathyroid hormone is released and osteoclastic activity increases, mobilizing calcium from bone to blood in an attempt to maintain calcium homeostasis. Thus, it is easy to see that adequate calcium intake and metabolism is needed to maintain healthy bones. It has never been proven, however, that calcium alone is enough to prevent osteoporotic fractures—despite the demonstration that calcium supplements can reduce bone resorption markers and produce slight increases in bone mineral density.

Vitamin D is of growing interest to the medical community, as an increasing volume of data is becoming available on its functions related to protecting bones, preventing falls, maintaining and improving equilibrium, and reducing mortality rates, as well as its antineoplastic functions and immune properties. Its efficacy as an isolated treatment against fractures has not been demonstrated, however, other than in institutionalized elderly patients also taking calcium supplements.[1]

All available anti-osteoporotic treatments have only shown their efficacy in clinical trials in association with calcium and vitamin D; additionally, the placebo in the comparison groups was not really placebo, but rather it was calcium and vitamin D. But are the available anti-osteoporotic drugs still effective in the absence of supplementation with calcium and vitamin D? There are few data to answer this question, but in a randomized controlled trial of 2 years' duration in about 700 postmenopausal women treated with either 400 UI of vitamin D and alendronate, alendronate plus calcium, alendronate alone, or calcium alone, the authors failed to demonstrate any differences in bone mineral density increases in the group taking alendronate plus calcium compared with those taking alendronate alone. However, there was a significant difference in the excretion of urinary N-terminal telopeptide (NTX), suggesting an optimization of antiresorptive action.[2] It has been shown in pharmacological studies that the efficacy of strontium ranelate is not dependent on supplementation with calcium and vitamin D.[3-5] In phase 3 clinical studies, all patients (strontium ranelate–treated patients and placebo groups) were given supplements with calcium and vitamin D according to their needs, as required for the treatment of postmenopausal osteoporosis in current medical practice.

Questions have been raised about the cardiovascular safety of calcium supplements. A recent meta-analysis of 26 clinical trials with about 20 000 participants showed that the use of calcium supplements alone was associated with a modest increase in the risk of myocardial infarction (about 30%), but not with an increased risk of stroke or mortality.[6] Analysing the data more carefully, one can see that this risk is bigger in patients with the biggest intake of calcium; the dangerous cutoff seems to be 1300 mg of calcium a day (combining dietary and supplemental intake). Moreover, the majority of the trials were conducted with considerable amounts of calcium given in supplements, some of them with about 1.5 g/day. We also do not know the cardiovascular effects of the combination of calcium and vitamin D, although some data (for instance, the Women's Health Initiative population) suggest that vitamin D can be protective.

In conclusion, this really is a controversial point, and the question posed deserves two different answers: (i) Yes, for systematic supplementation with vitamin D to treat postmenopausal osteoporosis; and (ii) No, for systematic supplementation with calcium to treat postmenopausal osteoporosis. Particularly, no for calcium alone (without vitamin D), and no if dietary calcium intake is sufficient (avoid a total calcium intake greater than 1300 mg/day). ■

References
1. DIPART Group. Patient level pooled analysis of 68 500 from seven major vitamin D fracture trials in US and Europe. *BMJ.* 2010;340:b5443.
2. Bonnick S, Broy S, Kaiser F, et al. Treatment of alendronate plus calcium, alendronate alone, or calcium alone for postmenopausal low bone mineral density. *Cur Med Res Opin.* 2007;23:1341-1349.
3. Hurtel AS, Mentaverri R, Caudrillier A, et al. The calcium-sensing receptor is involved in strontium ranelate-induced osteoclast apoptosis: new insights into the associated signalling pathways. *J Biol Chem.* 2009;284:575-584.
4. Caverzasio J. Strontium ranelate promotes osteoblastic cell replication through at least two different mechanisms. *Bone.* 2008;42:1131-1136.
5. Bonnelye E, Chabadel A, Saltel F, Jurdic P. Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. *Bone.* 2008;42:129-138.
6. Bolland M, Avenell A, Baron J. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ.* 2010;341:c3691.

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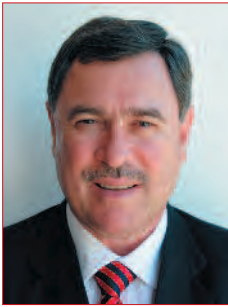
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Systemic supplementation of calcium and vitamin D in the treatment and prevention of postmenopausal osteoporosis has, until recently, been a knee-jerk reaction based on physiological considerations and the belief that this practice was safe. This was in spite of the fact that the results of fracture prevention studies of calcium supplementation were inconsistent and complicated by population differences, differences in calcium and vitamin D baseline status, differences in doses, and poor compliance.

In a recent meta-analysis, it was reported that calcium supplements are associated with an increased risk of myocardial infarction that is greater than the expected reduction in fracture risk. The authors warned that as calcium supplements are widely used, these modest increases in risk of cardiovascular disease might translate into a large burden of disease in the population. More prospective studies are needed to quantify the risk.

Since food sources of calcium produce similar benefits for bone density as do supplements, and dietary calcium intake does not seem to be related to adverse cardiovascular effects, calcium intake from nutritional sources needs to be encouraged. According to the latest 2010 guidelines of the Institute of Medicine (IOM), postmenopausal women need a dietary reference intake (DRI) of 1200 mg of elemental calcium. The best dietary source of calcium is dairy products because of their favorable elemental calcium content, their ability to be absorbed, and their cost effectiveness. Doses of supplemental calcium should be restricted to cover the shortfall between dietary intake and the DRI of 1200 mg. This should equate in most instances to not more than 500 mg of daily elemental calcium.

The role of vitamin D in bone homeostasis has been reassessed in the past few years. As many as 60% of older patients may have inadequate levels of vitamin D, caused by the age-related inability of the skin and kidney to produce the active form of vitamin D, and a general trend of less sunlight exposure. Dietary supplementation is a practical option, as the normal diet contains very little vitamin D. It is possible to directly determine vitamin D status by measuring the blood level of 25-hydroxyvitamin D [25(OH)D]. The latest recommendation of the IOM is a DRI of 600 IU (previously 400 IU) of vitamin D for women aged 51-70 years and 800 IU after age 70 years. The target 25(OH)D level was set at 50 nmol/L (20 ng/ml). Expert opinion and the International Osteoporosis Foundation disagree with these values, and recommend a DRI of 800-1000 IU of vitamin D in order to achieve a target serum 25(OH)D level of 75 nmol/L (30 ng/ml). Vitamin D supplementation has been shown to independently lower the risk of falling in elderly patients.

In conclusion, routine supplementation of calcium and vitamin D cannot be supported in the treatment of osteoporosis. It should rather be replaced by a case-specific approach according to the principles discussed. ■

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Osteoporosis is a disorder of bone metabolism characterized by microarchitectural deterioration, low bone mass, and an increased risk of fractures. Bone is made up of one third organic material such as collagen and two-thirds inorganic material consisting of carbonated hydroxyapatite; ie, calcium and phosphate salts—$Ca_{10}(PO4)_6(OH)_2$. As 99% of the body's calcium stores are in bone and teeth, it makes intuitive sense that calcium plays an important role in bone diseases. Vitamin D is also critical in calcium homeostasis; too little vitamin D leads to increased parathyroid hormone secretion, which increases intestinal calcium absorption but also increases bone resorption to mobilize bone calcium stores, thus reducing bone mineral density (BMD).

In the clinical setting, in population groups above the age of 50 years, calcium and/or vitamin D on their own have been shown to have beneficial effects on BMD and the risk of fractures. Daily intake of calcium at a dose of 1200 mg elemental calcium reduced the risk of fractures.[1] Calcium or calcium and vitamin D supplementation has been shown to reduce BMD loss, and is associated with a reduced risk of osteoporotic fractures.[2] Vitamin D supplementation has been shown to reduce the risk of falling at doses of 700-1000 IU daily,[3] which thus reduces one of the risk factors for fracture. Aside from this, vitamin D supplementation has also been shown to reduce the risk of vertebral and nonvertebral fractures[4] at doses above 400 IU daily. Thus, maintaining an adequate calcium/vitamin D intake is recommended as part of the standard of care in all osteoporosis guidelines, with a suggested intake of 1000-1200 mg elemental calcium and 800-1000 IU vitamin D daily.[5]

However, not all studies have been uniformly positive. A meta-analysis showed that calcium intake was not related to hip fracture risk.[6] Other studies showed that calcium and vitamin D supplementation was no better than placebo in improving BMD[7] or preventing fractures.[8] An important factor for calcium/vitamin D efficacy may hinge on the degree of compliance with the supplements; when only compliant subjects were considered in the otherwise negative studies, there was an improvement in BMD[9] and a reduction in hip fracture.[8] More worryingly, there have been recent concerns that taking these supplements can actually cause harm. One study showed that an annual oral administration of high-dose vitamin D paradoxically resulted in an increased risk of falls and fractures.[9] In addition, there has been some concern about the adverse side effects of high-dose calcium supplementation, especially with regard to an increased risk of myocardial infarction.[10]

Last, but not least, in trials of osteoporosis treatments with either bisphosphonates, strontium, or parathyroid hormone, supplementary calcium and vitamin D were given in both the placebo and treatment arms. Thus, all the data that is available on the efficacy of osteoporosis treatments is data that included calcium and vitamin D supplements, and it is not known if the efficacy of these drugs would be reduced without adequate calcium/vitamin D intake.

Therefore to conclude, the answer is yes, systematic supplementation with calcium/vitamin D is necessary in the treatment of postmenopausal osteoporosis. However, in view of the possible adverse effects, it is suggested that they are not used alone, but taken together with the pharmacological treatment options for osteoporosis. ■

References
1. Bischoff-Ferrari HA, Rees JR, Grau MV, Barry E, Gui J, Baron JA. Effect of calcium supplementation on fracture risk: a double-blind randomized controlled trial. *Am J Clin Nutr.* 2008;87:1945-1951.
2. Tang BMP, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* 2007; 370:657-666.
3. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomized controlled trials. *BMJ.* 2009;339:b3692.
4. Bischoff-Ferrari HA, Willett WC, Wong JB, et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency. A meta-analysis of randomized controlled trials. *Arch Intern Med.* 2009;169:551-561.
5. Body JJ, Bergmann P, Boonen S, et al. Evidence-based guidelines for the pharmacological treatment of postmenopausal osteoporosis: a consensus document by the Belgian Bone Club. *Osteoporos Int.* 2010;21:1657-1680.
6. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, et al. Calcium intake and hip fracture risk in men and women: a meta-analysis of prospective cohort studies and randomized controlled trials. *Am J Clin Nutr.* 2007;86:1780-1790.
7. Kärkkäinen M, Tuppurainen M, Salovaara K, et al. Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65-71 years: a 3-year randomized population-based trial (OSTPRE-FPS). *Osteoporos Int.* 2010;21:2047-2055.
8. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354:669-683.
9. Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women. A randomized controlled trial. *JAMA.* 2010;303:1815-1822.
10. Bolland MJ, Avenell A, Baron JA, et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ.* 2010;341: c3691.

> "Osteoporosis is often considered a women's disease, appearing after menopause. However, men are affected as well. In fact, approximately 20% of osteoporotic patients are men, and this percentage is expected to increase with longer life expectancy for men. Moreover, 1-year mortality due to hip fracture is greater for men than women… But, while several therapies are available for women, only a few studies on osteoporosis treatment have been performed in men."

Comprehensive antifracture efficacy, improvement of bone quality: Protelos, a first-line treatment in osteoporosis

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*O*steoporosis is a chronic disease generally linked to aging. For a long time, it was considered a normal process and later considered a fatal disease. Nowadays, many treatment options are available to delay or even stop the process. Several of them have demonstrated efficacy on specific patient profiles or fracture sites. An ideal treatment, however, should be able to prevent/treat osteoporosis at any fracture site (vertebral or nonvertebral, including hip) and in different types of patients: aged or young, osteoporotic or osteopenic, with or without previous fracture, regardless of fracture risk, whatever the sex, etc. In this article, we will show that Protelos, an original antiosteoporotic treatment, prevents fracture in all these kinds of patients. Moreover, Protelos has demonstrated its safety and efficacy against fractures for the longest period ever followed for an antiosteoporotic treatment, up to 10 years. In addition, the specific mode of action of Protelos and its effect on microarchitecture will be reviewed, in order to explain why Protelos is a first-line treatment, as acknowledged in several national and international guidelines.
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*O*steoporosis was long considered a physiological phenomenon due to aging. Nowadays, we know that this disease is linked to a substantial increase in morbidity and mortality, but fortunately, there are several treatments that can prevent it. These treatments, however, have different modes of action and different levels of efficacy and safety. Hence, it can be difficult to choose the best treatment that is the most effective and safe over the long term and for different patient profiles. In this article, we provide a full update of the numerous data recently published on Protelos, an antiosteoporotic treatment with a unique mode of action.

Protelos is effective whatever the site: vertebral, nonvertebral, and hip sites

In order to be really effective in the treatment of osteoporosis a drug must prevent both vertebral and nonvertebral fractures. Surprisingly, only a few antiosteoporotic treatments have shown efficacy against these two kinds of fractures. Protelos has demonstrated its efficacy to prevent both vertebral and nonvertebral fracture (including hip fracture) in 2 pivotal international, multicenter, double-blind studies: SOTI (the Spinal Osteoporosis Therapeutic Intervention study) and TROPOS (TReatment Of Peripheral Osteoporosis Study). These studies included 6740 postmenopausal women. All of them received calcium/vitamin D supplementation at a dose tailored to their degree of deficiency (500/1000 mg of calcium and 400/800 international units [IU] of vitamin D3) as well as either Protelos 2 g/day or placebo.
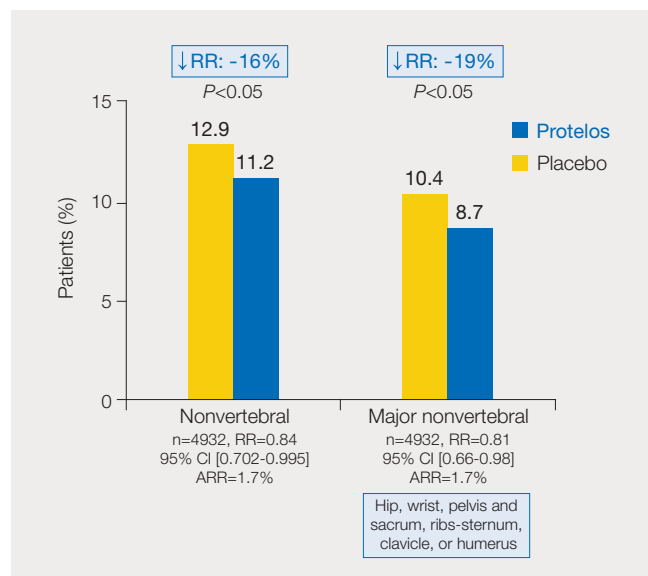
Figure 1. *TROPOS study: Effect of Protelos on relative risk of nonvertebral and major nonvertebral fractures versus placebo after 3 years of treatment.*
Abbreviations: ARR, absolute risk reduction; CI, confidence interval; RR, relative risk. Modified after reference 1: Reginster et al. J Clin Endocrinol Metab. 2005;90: 2816-2822. © 2005, The Endocrine Society.

Both studies demonstrate that, in osteoporotic patients, Protelos significantly reduced the risk of new vertebral fracture as well as new clinical vertebral fracture (vertebral fracture associated with back pain and/or height loss ≥1 cm) after three years of treatment compared with placebo. In the SOTI study, Protelos reduced the vertebral risk of fractures by 41% (relative risk [RR], 0.59; 95% confidence interval [CI], 0.48-0.73; $P<0.001$) and clinical vertebral fractures by 38% (RR, 0.62; 95% CI, 0.47-0.83; $P<0.001$).[2] In the TROPOS study, the risk of new vertebral fractures was decreased by 39% (RR, 0.61; 95% CI, 0.51-0.73; $P<0.001$).[1]

The TROPOS study shows that, after 3 years of treatment, Protelos also significantly decreased the risk of nonvertebral fracture by 16% (RR, 0.84; 95% CI, 0.702-0.995; $P<0.05$) and of major nonvertebral fractures (hip, wrist, pelvis, sacrum, ribs-sternum, clavicle, and humerus) by 19% (RR, 0.81; 95% CI, 0.66-0.98; $P<0.05$) *(Figure 1).*[1] An analysis of the subgroup of patients with the highest risk of hip fracture (>74 years with femoral neck T-score ≤-3 standard deviations [SD]) shows that Protelos significantly decreased the risk of hip fractures by 36% (RR, 0.64; 95% CI, 0.412-0.667; $P=0.046$).

Altogether, these studies confirmed the strong efficacy of Protelos against different kinds of osteoporotic fractures, including vertebral and hip fractures.

Protelos is effective in a wide range of patient profiles

The idea that the risk of fracture depends on several different factors is relatively new. Indeed, until recently, the fracture risk was only assessed in postmenopausal women and was based on bone mineral density (BMD) measurement. Nowadays, we know that it is more complicated and that several other factors can be involved, such as age, existence of previous fracture, family history, and individual habits. The existence of osteoporosis is now even proven in men and a growing number of men are treated for this disease. The goal of this chapter is to demonstrate the efficacy of Protelos, whatever the patient profile.

SELECTED ABBREVIATIONS

AE	adverse event
ARR	absolute risk reduction
bALP	bone-specific alkaline phosphatase
BFR	bone formation rate
BMD	bone mineral density
BSU	bone structural unit
BV/TV	bone volume per trabecular volume
CI	confidence interval
CTh	cortical thickness
DMB	degree of mineralization of bone
DRESS	drug rash with eosinophilia and systemic symptoms
FEA	finite element analysis
HR-pQCT	high-resolution peripheral quantitative computed tomography
IU	international units
MAR	mineral apposition rate
MS/BS	mineralizing surface per bone surface
NNT	number needed to treat
OPG	osteoprotegerin
PTH	parathyroid hormone
RANKL	receptor activator of nuclear factor kappa B ligand
RR	relative risk
RRR	relative risk reduction
S-CTX-I	serum c-terminal cross-linked telopeptide of type I collagen
SD	standard deviation
VTE	venous thromboembolic events

SELECTED ACRONYMS

CASIMO	Comparing Alendronate and Strontium ranelate In Male Osteoporosis
EMA	European Medicines Agency
ESCEO	European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis
FDA	Food and Drug Administration
FRAX®	World Health Organization Fracture Risk Algorithm
IOF	International Osteoporosis Foundation
MALEO	MALE Osteoporosis (study)
SOTI	Spinal Osteoporosis Therapeutic Intervention study
TROPOS	TReatment Of Peripheral Osteoporosis Study

◆ *Protelos is effective in patients with main fracture risk factors at baseline*

Even with implication of several fracture risk factors, diagnosis of osteoporosis is still based on BMD level. A subanalysis from the SOTI and TROPOS studies demonstrates that, in osteoporotic women for which hip/lumbar spine T-score is under -2.5 SD, Protelos significantly decreased vertebral fracture risk by 39% (RR, 0.61; 95% CI, 0.53-0.70; *P*<0.001). Moreover, Protelos efficacy was also independent of the previous number of fractures. Indeed, with 0, 1, or 2 prevalent fractures, Protelos decreased vertebral fracture risk by 48% (RR, 0.52; 95%CI, 0.40-0.67; *P*<0.001), 45% (RR, 0.55; 95% CI, 0.41-0.74; *P*<0.001), and 33% (RR, 0.67; 95% CI, 0.55-0.81; *P*<0.001), respectively.[3]

Protelos also significantly reduced vertebral fracture in osteopenic women (hip/lumbar spine T-score between -1 and -2.5 SD) with a decrease as high as 72% (RR, 0.28; 95% CI, 0.07-0.99; *P*=0.045). Furthermore, in these osteopenic women, the risk of vertebral fracture was decreased by 38% and 59% in patients with and without previous fracture, respectively (with previous fracture: RR, 0.62; 95% CI, 0.44-0.88; *P*=0.008; without previous fracture: RR, 0.41; 95% CI, 0.17-0.99; *P*=0.039).[4]

Protelos is also effective in both young and older postmenopausal women. Women over 80 years account for more than 60% of hip fracture, while they only represent around 8% of the postmenopausal population. Moreover, the elderly are the most affected by osteoporotic fracture consequences, including delayed fracture healing, loss of autonomy, and increased morbidity and mortality. Hence, it is very important to treat them with an adapted treatment. After only 1 year of treatment, in patients over 80, Protelos significantly decreased the risk of vertebral fracture (-59%), clinical fractures (-37%), and nonvertebral fractures (-41%) (vertebral fractures: RR, 0.41; 95% CI, 0.22-0.75; *P*=0.002; clinical fractures: RR, 0.63; 95% CI, 0.44-0.91; *P*=0.012; nonvertebral fractures: RR, 0.59; 95% CI, 0.37-0.95; *P*=0.027). Even after 5 years, vertebral fracture risk remained decreased by 31% and nonvertebral fracture risk by 26% (RR, 0.69; 95% CI, 0.52-0.92; *P*=0.010; and RR, 0.74; 95% CI, 0.57-0.95; *P*=0.019, respectively). Hence, Protelos has demonstrated long-term efficacy against vertebral and nonvertebral fractures in the elderly.[5]

Similarly, Protelos has shown its antifracture efficacy in young postmenopausal women. After 4 years of treatment, vertebral fracture risk is decreased by 40% (RR, 0.60; 95% CI, 0.39-0.92; *P*=0.017).[6]

◆ *Protelos is effective whatever the FRAX® score at baseline*

Very recently, the group of John Kanis demonstrated Protelos efficacy whatever the 10-year probability of fracture at baseline. Patients from SOTI and TROPOS treated for 3 years were included. The results show, for the first time, association of a treatment with a significant reduction in clinical osteoporotic fractures across the full range of baseline 10-year probability of fracture. Moreover, the interaction between treatment effect and fracture probability was not significant for both clinical vertebral fracture and morphometric vertebral fracture (*P*>0.3 and *P*=0.1, respectively; *Figure 2),* with or without inclusion of BMD in the FRAX® (World Health Organization Fracture Risk Algorithm) model.[7] These data show that the effectiveness of Protelos was comparable over the whole range of FRAX® probability. Using the same methodology, treatments such as clodronate, bazedoxifene, and denosumab seem to be more effective in women with higher FRAX® probabilities.
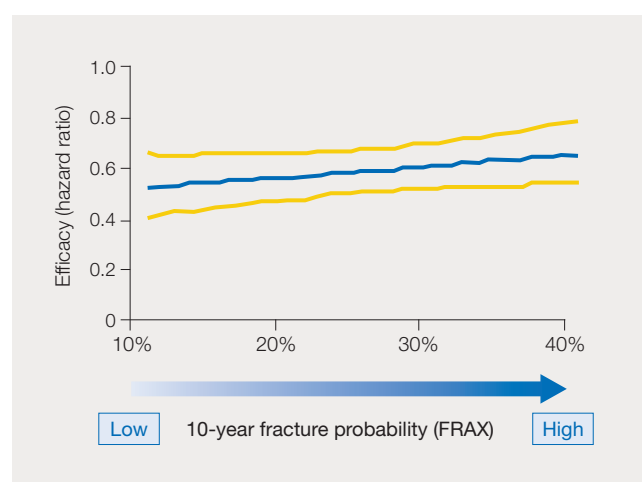
Figure 2. *Probability of morphometric vertebral fracture (computed with bone mineral density included) and the effect of Protelos on morphometric vertebral fractures (HR+95% confidence intervals).* The interaction between efficacy and 10-year fracture probability is not significant (P=0.1).
Modified from reference 7: Kanis et al. Osteoporos Int. 2011;22(8):2347-2355. © 2011, International Osteoporosis Foundation and National Osteoporosis Foundation.

◆ *Protelos is effective in male osteoporosis*

Osteoporosis is often considered a women's disease, appearing after menopause. However, men are affected as well. In fact, approximately 20% of osteoporotic patients are men, and this percentage is expected to increase with longer life expectancy for men. Moreover, 1-year mortality due to hip fracture is greater for men than women (20.7% for men versus 7.5% for women over 75 years of age with hip fracture).[8] But, while several therapies are available for women, only a few studies on osteoporosis treatment have been performed in men.

Two studies were performed with Protelos. The first study, CASIMO (Comparing Alendronate and Strontium ranelate In Male Osteoporosis), an open-label study (n=150), compared the effect of Protelos and alendronate on BMD after 12 months of treatment. Treatment with Protelos increased lumbar spine BMD by 5.8±3.7% and total hip BMD by 3.5±2.8% (*P*<0.001 compared with baseline for both parameters). Treatment with alendronate increased lumbar spine BMD by 4.5±3.4% and the total hip BMD by 2.7±3.2% (*P*<0.001 compared with
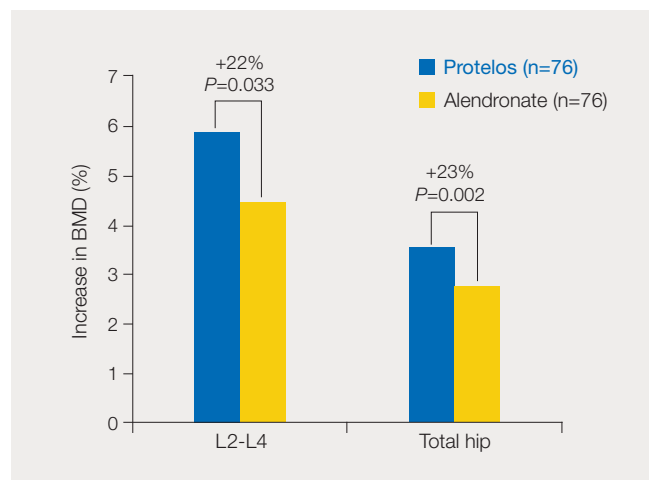
Figure 3. *Mean percentage change (from baseline) in bone mineral density of the lumbar spine and total hip with Protelos and alendronate therapy.*
Abbreviation: BMD, bone mineral density.
After reference 9: Ringe et al. Arzneimittelforschung. 2010;60(5):267-272. © 2010, ECV Edit Cantor Verlag, Aulendorf, Germany.

Figure 4. *Mean percentage change (from baseline) in bone mineral density of the lumbar spine and femoral neck with Protelos therapy. ***P<0.001 versus baseline*
Abbreviation: BMD, bone mineral density.
Modified from reference 10: Kaufman et al. Ann Rheum Dis. 2011;70(suppl 3): 224. Abstract 11-4358.

baseline for both parameters). The mean increases in BMD were significantly higher for patients treated with Protelos: 22% greater for lumbar spine BMD (*P*=0.033) and 23% greater for total hip BMD (*P*=0.002) *(Figure 3).*[9] Moreover, even though both treatments reduce back pain, significantly higher pain relief is observed with Protelos compared with alendronate (69% and 47% decrease, respectively; *P*=0.001).[9]
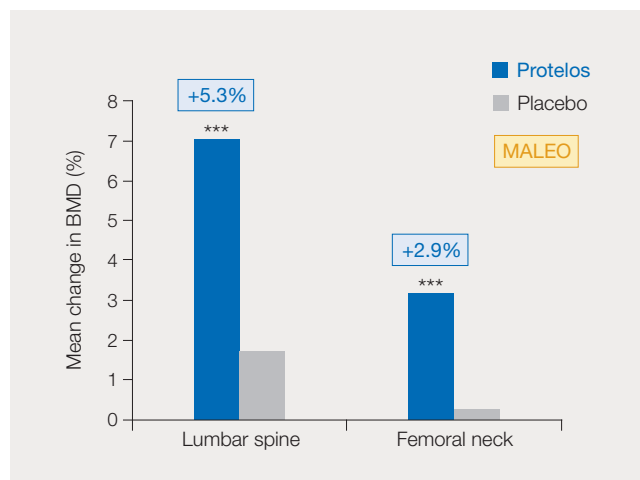
The second study, MALEO (MALE Osteoporosis), is a 2-year, double-blind, placebo-controlled, randomized trial which included 243 men with osteoporosis (ratio of Protelos/placebo, 2:1). This study demonstrates that, after 1 year of treatment, Protelos significantly increased lumbar spine and femoral neck BMD compared with placebo (5.3±0.75%; *P*<0.001 and 2.9± 0.62%; *P*<0.001, respectively) *(Figure 4).*[10]

Considering the present results in men and the previously established relationship between change in BMD and reduction in fracture risk in women treated with Protelos, a similar antifracture effect in men can reasonably be expected. In addition, safety results did not reveal any unexpected adverse events in men treated with Protelos.[10]

Table I. *Protelos is the only antiosteoporotic treatment to have demonstrated efficacy against vertebral, nonvertebral, and hip fractures, whatever the severity of the disease.*
Abbreviations: HRT, hormone replacement therapy; NA, no evidence available; PTH, parathyroid hormone.
Adapted from reference 11: Kanis et al. Osteoporos Int. 2008;19:399-428. © 2008, International Osteoporosis Foundation and National Osteoporosis Foundation.

These new results from the MALEO study have been submitted to the European Medicines Agency (EMA) as a basis for the new indication of Protelos in male osteoporosis.

Position of Protelos, in terms of efficacy, among antiosteoporotic treatments

A large number of antiosteoporotic treatments have been available on the market for several years. The diversity in their modes of action and the different characteristics of the populations included in clinical trials do not make it easy to compare their efficacies. However, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ES-CEO) published in 2008 the *European Guidance for the Treatment and Management of Osteoporosis in Postmenopausal*

	Prevention of vertebral fracture		Prevention of nonvertebral fracture	
	Women with osteoporosis	Women with osteoporosis + vertebral fracture	Women with osteoporosis	Women with osteoporosis + vertebral fracture
Protelos	+	+	+ (including hip)	+ (including hip)
Alendronate	+	+	NA	+ (including hip)
Risedronate	+	+	NA	+ (including hip)
Ibandronate	NA	+	NA	+*
Zoledronic acid	+	+	NA	NA (+)†
HRT	+	+	+	+
Raloxifene	+	+	NA	NA
Teriparatide and PTH	NA	+	NA	+

* In subsets of patients only (post hoc analysis).
† Mixed group of patients with or without prevalent vertebral fractures.
+ = effective drug.

P R O T E L O S

Women, which compared the efficacy of antiosteoporotic treatments on the basis of data available from large trials. These data are summarized in *Table I* and show that Protelos, in comparison with other treatments, has demonstrated efficacy at vertebral and nonvertebral (including hip) levels, even in the presence of previous fractures.[11]

Another way to compare these different treatment efficacies would be to analyze all clinical studies in depth and to consider every parameter that reflects efficacy, such as relative risk reduction (RRR), absolute risk reduction (ARR), and number needed to treat (NNT).[12] A recent study from Ringe and Doherty has compared the different treatments with regard to the number of patients needed to treat in order to avoid 1 fracture. They demonstrated that Protelos has a very low NNT, with only 9 patients needed to be treated over 3 years to avoid 1 vertebral fracture, compared with 21 for ibandronate, and 48 patients needed to be treated to avoid hip fracture, compared with 91 for alendronate, risedronate, or zoledronate *(Figure 5)*.[13] These results fully confirm the position of Protelos as a first-line treatment.

Protelos is effective whatever the duration of the treatment

Another new event this year was the publication of the 10-year efficacy of Protelos. We have already seen that Protelos action is rapid (as soon as 1 year) and maintained for 3 years at both vertebral and nonvertebral sites. But osteoporosis is a chronic disease. Hence, treatments need to maintain long-term efficacy and safety. To date, there is no evidence showing that conventional antiosteoporotic treatments, even bisphosphonates, are able to decrease fractures beyond 3 to 4 years of treatment. Moreover, there are controversial data showing possible association of prolonged bisphosphonate use with atypical femoral fractures.[14] On the contrary, Protelos has now shown its safety and efficacy at vertebral and nonvertebral levels over a 10-year follow-up period, the longest evaluation ever, made possible by the 4- and 5-year SOTI and TROPOS studies and their extensions.

◆ Protelos efficacy after 5 years of treatment

The results of the preplanned 5-year TROPOS study show that Protelos significantly reduced the risk of vertebral fracture by 24% versus placebo (RR, 0.76; 95% CI, 0.65-0.88; *P*<0.001), the risk of nonverte-

bral fracture by 15% (RR, 0.85; 95% CI, 0.73-0.99; *P*=0.032), and the risk of new major nonvertebral fracture by 18% (RR, 0.82; 95% CI, 0.69-0.98; *P*=0.025).

In a subgroup of patients with a high risk of hip fractures (n=1128; ≥74 years; lumbar/femoral neck T-score ≤-2.4 SD), Protelos reduced hip fracture risk by 43% versus placebo over 5 years (RR, 0.57; 95% CI, 0.33-0.97; *P*=0.036). This means that only 21 patients need to be treated with Protelos for 5 years in order to prevent 1 new osteoporotic fracture.[15]

◆ Protelos efficacy after 10 years of treatment

At the end of the TROPOS study, Protelos long-term efficacy and safety was assessed by 3- and 5-year extension studies (open-labeled), leading to a full follow-up of 10 years.
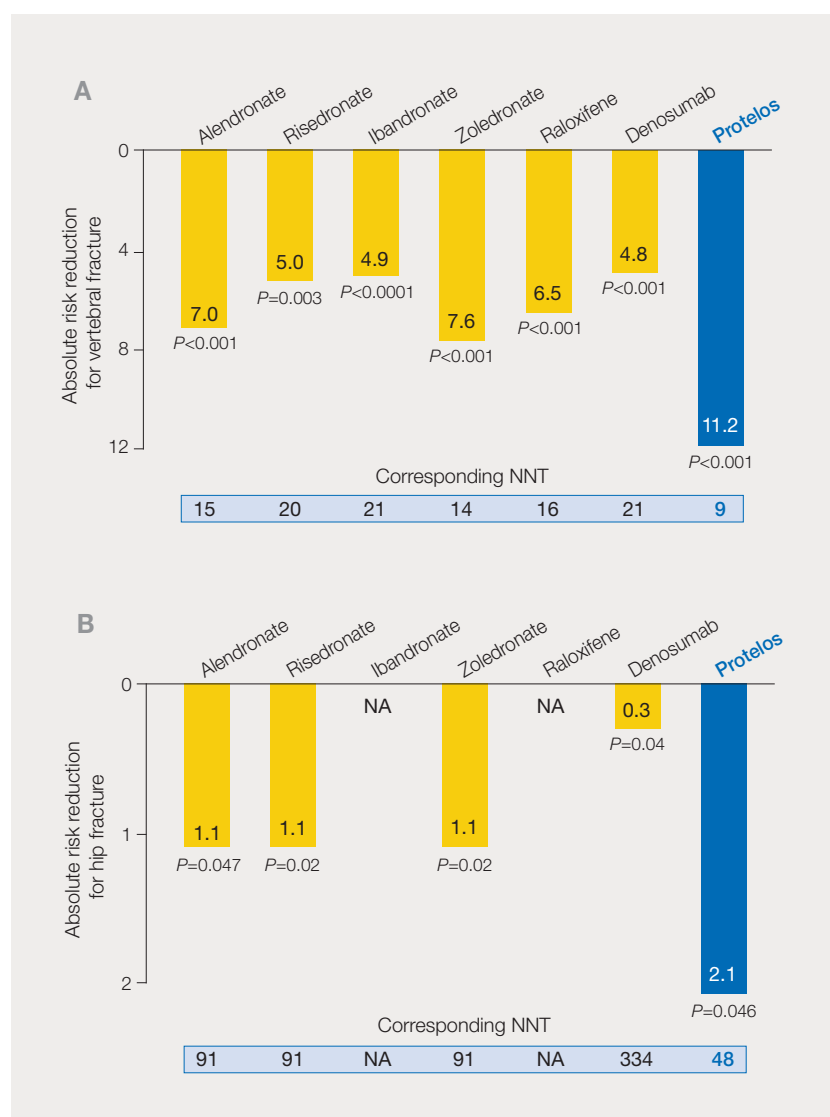
Figure 5. Comparison of the efficacy of different treatments on **(A)** vertebral and **(B)** hip fractures.
Abbreviations: NA, no evidence available; NNT, number needed to treat.
Adapted from reference 13: Ringe and Doherty. Rheumatol Int. 2010;30:863-869. © 2009, Springer-Verlag.

Protelos, a first-line treatment in osteoporosis – *Hueber*   MEDICOGRAPHIA, Vol 34, No. 2, 2012   **207**

The studies included 237 patients. The cumulative incidence of new vertebral or nonvertebral fractures over the 5-year extension period (6-10 years, TROPOS extension) was fully comparable with that in the first 5-year study period (0-5 years, TROPOS study): 20.6% versus 18.5%, respectively, for vertebral fractures and 13.7% versus 12.9% for nonvertebral fractures *(Figure 6).*[16]

Another interesting way to assess treatment efficacy would be to compare the 10-year long-term antifracture efficacy of Protelos with a placebo group. As, for ethical reasons, it isn't allowed to treat patients with placebo for 10 years, the authors sought a matching population in the placebo group of

Protelos efficacy is explained by its unique mode of action, building strong and healthy new bone

◆ *Protelos efficacy through its mechanism of action*

Although fracture risk reduction remains the logical main objective of each antiosteoporotic treatment, the long-term effect of treatment on bone is crucial. Bone is a living tissue. Due to everyday actions, such as walking and climbing stairs for example, bones are damaged by microcracks and need to be continuously repaired through bone remodeling; otherwise, bone fragility is increased, leading to fracture. Conventional treatments, including antiresorptive treatments, are not able to prevent fracture risk in the long term. This is probably due to their mechanisms of action which decrease bone resorption,
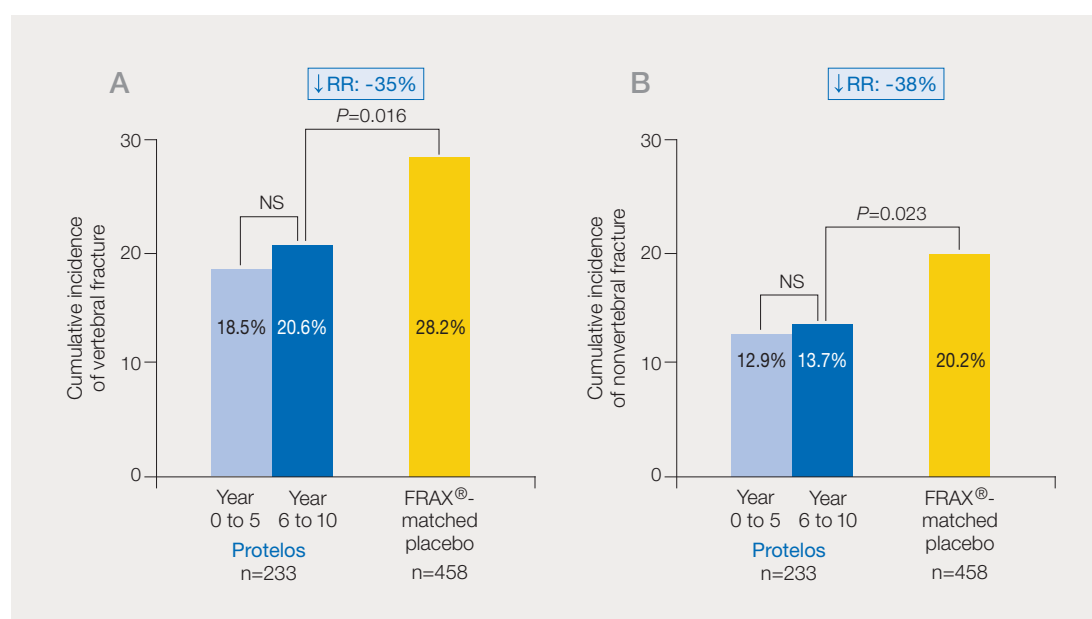
Figure 6. *10-Year efficacy of Protelos on (A) vertebral and (B) nonvertebral fractures.*

Fracture incidence does not increase between years 0 to 5 and 6 to 10, but is significantly decreased compared with FRAX®-matched placebo. **Abbreviations:** *NS, nonsignificant; RR, relative risk.* **Adapted from reference 16:** *Reginster et al. Osteoporosis Int. 2012;23(3): 1115-1122. © 2011, The Author(s). This article is published with open access at Springerlink.com.*

TROPOS (0-5 years). 10-Year fracture probabilities for major osteoporotic fracture in the extension population at year 6 and in the placebo population at year 0 were calculated by FRAX®. To ensure the comparability of the two populations, patients with the same FRAX® score in the 2 groups were identified. This FRAX®-matched placebo population comprised 458 patients.

The comparison of fracture incidence between the patients treated with Protelos for 10 years in the 5-year extension study and the patients from the FRAX®-matched placebo group shows that the cumulative incidence of new fractures was significantly decreased in the treated group. Indeed, the risk decreased by 35% in the treated group for nonvertebral fracture (13.7±2.3% versus 20.2±2.2%; *P*=0.023) and by 38% for new vertebral fracture (20.6±3.0% versus 28.2±2.4%; *P*=0.016) *(Figure 6).* These results are particularly important as it is the first time that an antiosteoporotic treatment has been compared with a placebo group over such a long follow-up period. Altogether, these results confirm the long-term efficacy of Protelos, whatever the site.[16,17]

but also bone formation. Hence, bone remodeling is stopped, as well as microcrack reparation, leading to an increased risk of atypical fractures. These events remain rare, but are dangerous and are a growing concern for the EMA and US Food and Drug Administration (FDA).

Protelos has a very different mechanism of action, allowing formation of new and strong bone. Indeed, studies in various models have shown that Protelos increases osteoblast replication, differentiation, and activity,[18-21] and decreases osteoclast differentiation and activity.[21-24] It has already been demonstrated that Protelos action is mediated by osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL),[25,26] and a recent article has shown new in vivo evidence of this mechanism, showing that Protelos action is partly due to OPG increase.[27]

Another new nonclinical study from Rybchyn and colleagues has demonstrated that the effect of Protelos is also mediated by its action on the Wnt canonical pathway, inducing sclerostin decrease, thus allowing microcrack repair.[28]

◆ Protelos builds strong new bone

Protelos is the first antiosteoporotic treatment to have demonstrated benefits, compared with bisphosphonate, on bone formation in the largest double-blind, international bone biopsy study ever, including 268 postmenopausal women with osteoporosis. Women were around 63 years of age and were treated with Protelos or alendronate (ratio 2:1). There were 2 biopsies taken for each woman: 1 at baseline and 1 after 6 or 12 months of treatment.

After 6 and 12 months of treatment, mineralizing surface per bone surface (MS/BS), which reflects tissue activity, was 2.9±3.7% and 4.9±4.2%, respectively, with Protelos and 0.2±0.9% and 0.3±0.6%, respectively, with alendronate. The difference between groups increased up to 4.7% ($P<0.001$) at 12 months. The mineral apposition rate (MAR), which reflects cellular activity, was also significantly higher with Protelos at 6 months (0.630±0.127 µm/day) and 12 months (0.624±0.094 µm/day), compared with alendronate (0.553± 0.108 µm/day at both time points, $P=0.003$ and $P=0.009$ between groups at 6 and 12 months, respectively). Bone formation rate (BFR=MAR×MS/BS) was also significantly different between groups ($P<0.001$) (Protelos: 0.021±0.024 and 0.033±0.027 µm^3/µm^2/day at 6 and 12 months, respectively; alendronate: 0.003±0.003 µm^3/µm^2/day at both time points). This study demonstrates that bone forming activity was much higher in patients treated with Protelos, with an amplified effect after 12 months of treatment *(Figures 7 and 8).*[29]

It also confirms the results obtained previously in a smaller study which had demonstrated increased cortical thickness (CTh), trabecular number, and decreased trabecular separation after 3 years of treatment with Protelos.[30]

Other evidence of the bone forming activity of Protelos is provided by a new study from Rizzoli and colleagues, which compared alendronate and Protelos activity in a head-to-head randomized, double-dummy, double-blind study in 88 postmenopausal osteoporotic women. This study used high-resolution peripheral quantitative computed tomography (HR-pQCT, SCANCO Medical). This device gives three-dimensional datasets providing bone geometry, cortical, and trabecular structures. It also allows noninvasive quantification of bone strength and mechanical features of cortical and trabecular bone, determined by finite element analysis (FEA). The results show that, at the distal tibial level, 1 year of treatment with Protelos led to a significant increase in CTh (+5.3%, $P<0.001$) and BV/TV (+2.0%, $P=0.002$) compared with baseline, while alendronate had no significant effect.[31] After 2 years of treatment, the CTh increased from baseline by 6.3% ($P<0.0001$) in the Protelos treated group, while it did not significantly change in the alendronate treated group ($P<0.005$ for between groups comparison). In the same way, trabecular bone volume per trabecular volume (BV/TV) increased by 2.5% from baseline with Protelos ($P<0.0001$), compared with 0.8% with alendron-
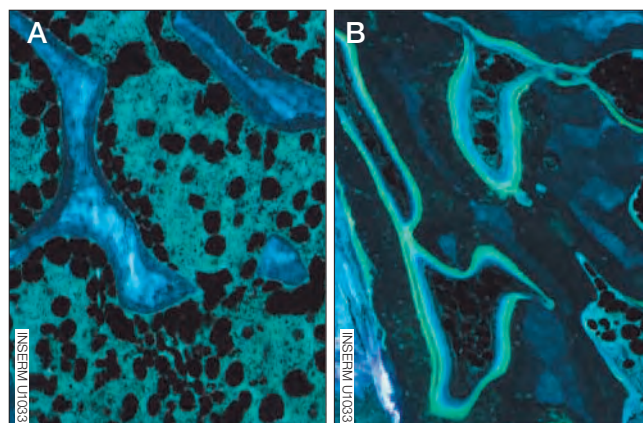
Figure 7. *Illustration of bone forming activity after 6 months of treatment with (A) alendronate and (B) Protelos.*
Bone forming activity is represented by tetracycline labeling. (Courtesy of R. Chapurlat)
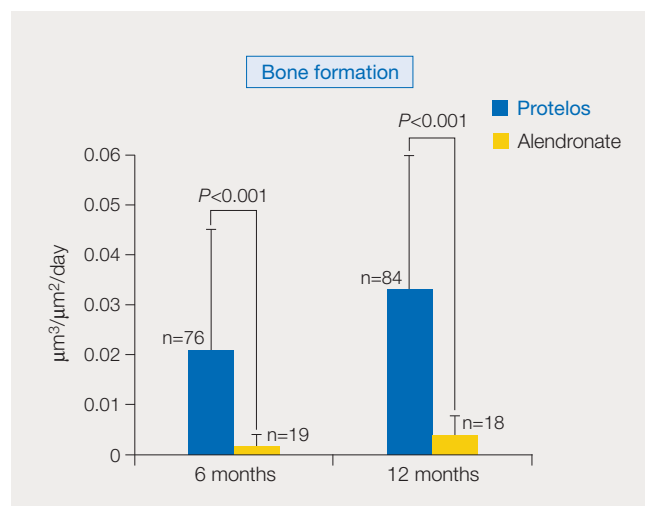
Figure 8. *Comparison of bone formation rate after 6 and 12 months of treatment with alendronate and Protelos.*
Data from reference 29: *Chavassieux et al.* Osteoporosis Int. 2011;22(suppl 1): S104. Abstract OC16.

ate (nonsignificant [NS]) ($P<0.05$ for between groups comparison). The increase compared with alendronate was significant from 3 months *(Figure 9, page 210).*[32] FEA results show that, with Protelos, failure load increased by 2.1% ($P<0.005$ versus baseline) after 2 years of treatment, versus no change with alendronate (-0.6%, NS) ($P<0.01$ between groups).

This study also confirms the effect of Protelos on bone turnover markers. Indeed, serum c-terminal cross-linked telopeptide of type I collagen (s-CTX-I) decreased by 16% and 59% for Protelos and alendronate, respectively. The CTX-I value was significantly decreased from baseline at months 3 and 6 ($P<0.05$) and 18 and 24 ($P<0.005$) with Protelos treatment. In contrast, bone-specific alkaline phosphatase (bALP) increased with Protelos from 3 months, while it significantly decreased with alendronate for all time points (+18% and -31%, respectively, at year 2) *(Figure 10, page 210).*[32]
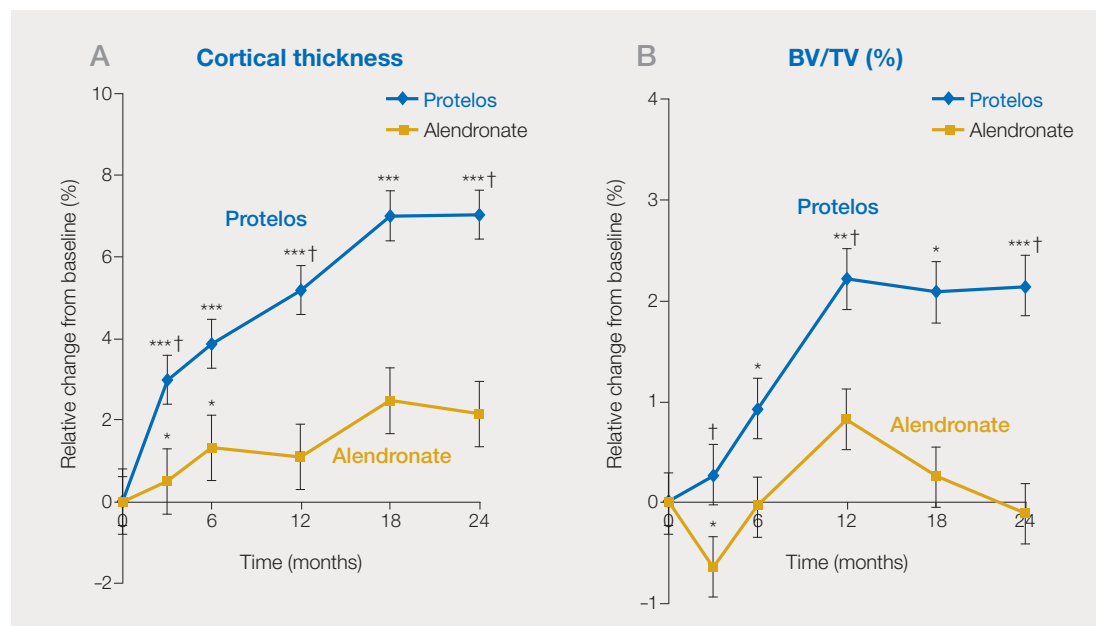
Figure 9. *Comparison between the evolution of (A) cortical thickness and (B) BV/TV during 2 years of treatment with Protelos and alendronate.*

*P<0.05; **P<0.01; ***P<0.001 versus baseline; †P<0.05 strontium ranelate versus alendronate.
Abbreviations: *BV/TV, bone volume per trabecular volume; SR, strontium ranelate.*
Modified from reference 32: *Rizzoli et al. Osteoporos Int. 2012;23(1): 305-315. © 2011, International Osteoporosis Foundation and National Osteoporosis Foundation.*
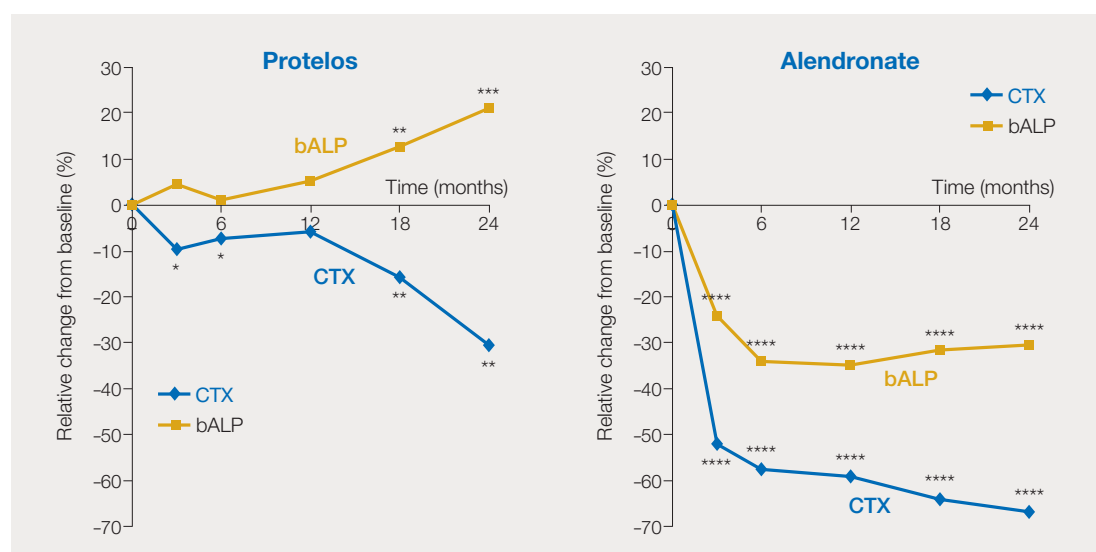
Figure 10. *Comparison between the evolution of bone turnover markers during 2 years of treatment with Protelos and alendronate.*

*P<0.05; **P<0.005; ***P<0.0005; ****P<0.0001 versus baseline.
Abbreviations: *bALP, bone-specific alkaline phosphatase; CTX, c-terminal cross-linked telopeptide of type I collagen.*
Modified from reference 32: *Rizzoli et al. Osteoporos Int. 2012;23(1): 305-315. © 2011, International Osteoporosis Foundation and National Osteoporosis Foundation.*

◆ *Protelos builds strong new bone, remains safe, and preserves bone mineralization*

It is known that the degree of mineralization of bone (DMB) is influenced by the rate of bone remodeling and the mean duration of secondary mineralization. For example, when the bone remodeling rate increases, like during treatment with anabolic agents such as parathyroid hormone (PTH), the birthrate of new bone structural units (BSUs) increases, resulting in less time to complete secondary mineralization, and, ultimately, DMB decreases. On the contrary, when the bone remodeling rate is decreased, like during treatment with antiresorptive agents, the birthrate of BSUs is greatly decreased, their lifespan increases, the duration of secondary mineralization is greatly increased, and the DMB increases. Hence, it could be hypothesized that due to its specific mode of action, Protelos preserves the level of secondary mineralization.

A recent study addressed this hypothesis and also investigated the distribution of strontium in bone during treatment with Protelos. Transiliac bone biopsies from 31 patients treated for 36, 48, or 60 months with Protelos were analyzed and demonstrated that, up to 60 months, DMB, reflecting the secondary mineralization of bone, remains constant in cortical (1.12± 0.08), cancellous (1.13±0.07), and total bone (1.13±0.07).

Global x-ray mapping of these biopsies shows that, contrary to calcium and phosphorus, strontium was always heterogeneously distributed and almost exclusively present in new bone formed during treatment *(Figure 11).*[33] Whatever the duration of treatment, the percentage of bone area with strontium was higher in cancellous bone (36.25±28.52%) than in cortical bone (24.70±15.34%). Moreover, focal strontium content measured in total bone was constant whatever the treat-

ment duration. Indeed, the BSUs containing strontium had mean strontium contents that did not significantly differ over time: 2, 12, 24, 36, 48, and 60 months (P=0.81). In recent bone, the ratio Sr/(Ca+Sr) showed between 0 and 6% (mean: 1.6%) replacement of Ca by Sr. Altogether, these results confirm that long-term treatment with strontium ranelate remains very safe for bone: strontium is absent from old bone built before initiation of the treatment, the focal strontium content is constant in bone formed during treatment, and secondary mineralization is maintained.[33]
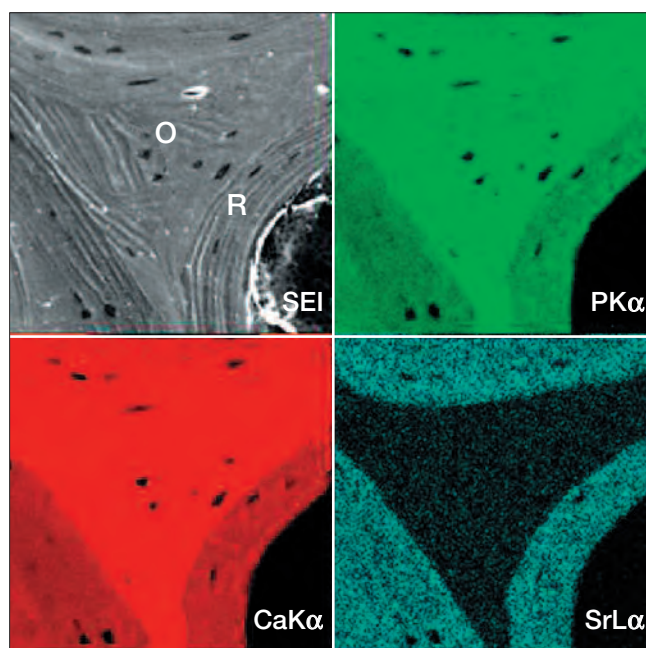
Figure 11. *Distribution and content of strontium in bone.*
Abbreviations: *Ca Kα, calcium; O, old bone; P Kα, phosphorus; R, recent bone; SEI, secondary electron image; Sr Lα, strontium.*
Adapted from reference 33: *Doublier et al.* Eur J Endocrinol. *2011;165(3):469-476. Copyright © 2011, European Society of Endocrinology.*

Protelos remains safe after long-term use

Data obtained from the developmental studies are very reassuring concerning Protelos safety. Indeed, during the 5 years of the placebo-controlled TROPOS study, the incidence of adverse events (AEs) and serious AEs were well balanced between the 2 groups (95.3% and 30.9%, respectively, in the Protelos treated group versus 94.9% and 30.0%, respectively, in the placebo treated group).[1] AEs usually reported are nausea (7.8% in the Protelos group versus 4.8% in the placebo, TROPOS study), diarrhea (7.2% versus 5.45%), headache (3.6% versus 2.7%), dermatitis (2.3% versus 2.0%), and ecze-

ma (2.0% versus 1.5%). The incidence of venous thromboembolic events (VTE) was 2.7% in the Protelos group versus 2.1% in the placebo group after 5 years.[1] However, according to the new summary of product characteristics, Protelos should not be used in patients with current or previous VTE as well as in patients that are permanently or temporarily immobilized. Long-term treatment with Protelos has not demonstrated an increase in AE rates. During the marketing development of Protelos, cases of severe hypersensitivity syndromes, including, in particular, drug rash with eosinophilia and systemic symptoms (DRESS) were described. DRESS incidence is 1/13 725 treated patients. Hence, patients should be informed to stop Protelos therapy immediately and permanently if a rash occurs.

The future of Protelos

Protelos has largely demonstrated its efficacy in postmenopausal women, but the story does not end there. A combination of Protelos and vitamin D is under development. Moreover, the proven efficacy of Protelos in men should lead very soon to a new indication in male osteoporosis. Furthermore, more and more clinical data are being published on Protelos efficacy in fracture healing in many circumstances: osteoporotic fractures, atypical fractures linked to long-term use of bisphosphonates, and also traumatic fracture in younger patients (male and female) due to accidents.[34,35]

Finally, during the last International Osteoporosis Foundation (IOF) congress in Dubai, the rationale and design for a new phase 3 clinical study assessing Protelos efficacy in osteoarthritis were presented. Due to the lack of effective treatment for structural progression of osteoarthritis, a structure-modifying drug would be a real answer to a medical need.

Conclusion

Again this year, several very interesting results were published demonstrating the long-term efficacy and safety of Protelos. Its antifracture efficacy has been demonstrated over a 10-year period, the longest period ever studied for an antiosteoporotic drug. New data have also shown the long-term safety of Protelos in bone without bone accumulation or change in mineralization. The mechanism of action for Protelos is progressively well known as a result of new nonclinical studies and clinical results from biopsies. Moreover, a new therapeutic area should be opened to Protelos during the coming years. Altogether, these findings confirm that Protelos should be considered a first-line treatment for osteoporotic patients. ■

References

1. Reginster JY, Seeman E, de Vernejoul MC, et al. Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab.* 2005;90:2816-2822.
2. Meunier PJ, Roux C, Seeman E, et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med.* 2004;350:459-468.
3. Roux C, Reginster JY, Fechtenbaum J, et al. Vertebral fracture risk reduction with strontium ranelate in women with postmenopausal osteoporosis is independent of baseline risk factors. *J Bone Miner Res.* 2006;21:536-542.
4. Seeman E, Devogelaer JP, Lorenc R, et al. Strontium ranelate reduces the risk of vertebral fractures in patients with osteopenia. *J Bone Miner Res.* 2008;23:433-438.
5. Seeman E, Boonen S, Borgström F, et al. Five years treatment with strontium

ranelate reduces vertebral and nonvertebral fractures and increases the number and quality of remaining life years in women over 80 years of age. *Bone.* 2010; 46:1038-1042.

6. Devogelaer J, Fechtenbaum J, Kolta S, et al. Strontium ranelate demonstrates efficacy over 3 and 4 years against vertebral fracture in young postmenopausal women (50-65 years) with severe osteoporosis. *Osteoporos Int.* 2008;19:S14-S15.

7. Kanis JA, Johansson H, Oden A, McCloskey EV. A meta-analysis of the effect of strontium ranelate on the risk of vertebral and non-vertebral fracture in postmenopausal osteoporosis and the interaction with FRAX®. *Osteoporos Int.* 2011;22(8):2347-2355.

8. Gennari L, Bilezikian JP. Osteoporosis in men: pathophysiology and treatment. *Curr Osteoporos Rep.* 2007;5(1):22-28.

9. Ringe JD, Dorst A, Farahmand P. Efficacy of strontium ranelate on bone mineral density in men with osteoporosis. *Arzneimittelforschung.* 2010;60(5):267-272.

10. Kaufman JM, Audran M, Bianchi G, et al. Efficacy and safety of strontium ranelate in the treatment of male osteoporosis. *Ann Rheum Dis.* 2011;70 (suppl 3):224. Abstract.

11. Kanis JA, Burlet N, Cooper C, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int.* 2008; 19:399-428.

12. MacLaughlin EJ, Raehl CL. ASHP therapeutic position statement on the prevention and treatment of osteoporosis in adults. *Am J Health Syst Pharm.* 2008; 65:343-335.

13. Ringe JD, Doherty JG. Absolute risk reduction in osteoporosis: assessing treatment efficacy by number needed to treat. *Rheumatol Int.* 2010;30:863-869.

14. Black DM, Kelly MP, Genant HK, et al. Bisphosphonates and fractures of the subtrochanteric or diaphyseal femur. *N Engl J Med.* 2010;362(19):1761-1771.

15. Reginster JY, Felsenberg D, Boonen S, et al. Effects of long-term strontium ranelate treatment on the risk of nonvertebral and vertebral fractures in postmenopausal osteoporosis: results of a five-year, randomized, placebo-controlled trial. *Arthritis Rheum.* 2008;58(6):1687-1695.

16. Reginster JY, Kaufman JM, Goemaere S, et al. Maintenance of antifracture efficacy over 10 years with strontium ranelate in postmenopausal osteoporosis. *Osteoporosis Int.* 2012;23(3):1115-1122.

17. Reginster JY, Kaufman JM, Devogelaer JP, Benhamou CL, Roux C. Long-term treatment of postmenopausal osteoporotic women with strontium ranelate : results at 10 years. *Ann Rheum Dis.* 2011;70(suppl 3):167. Abstract.

18. Canalis E, Hott M, Deloffre P, et al. The divalent strontium salt S12911 enhances bone cell replication and bone formation in vitro. *Bone.* 1996;18:517-523.

19. Choudhary S, Halbout P, Alander C, et al. Strontium ranelate promotes osteoblastic differentiation and mineralization of murine bone marrow stromal cells: involvement of prostaglandins. *J Bone Miner Res.* 2007;22:1002-1010.

20. Zhu LL, Zaidi S, Peng Y, et al. Induction of a program gene expression during osteoblast differentiation with strontium ranelate. *Biochem Biophys Res Com-*

mun. 2007;355:307-311.

21. Bonnelye E, Chabadel A, Saltel F, et al. Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. *Bone.* 2008;42:129-138.

22. Baron R, Tsouderos Y. In vitro effects of S12911-2 on osteoclast function and bone marrow macrophage differentiation. *Eur J Pharmacol.* 2002;450:11-17.

23. Takahashi N, Sasaki T, Tsouderos Y, et al. S 12911-2 inhibits osteoclastic bone resorption in vitro. *J Bone Miner Res.* 2003;18:1082-1087.

24. Wattel A, Hurtel-Lemaire A, Godin C, Mentaverri R, Kamel S, Brazier M. Strontium ranelate decreases in vitro human osteoclastic differentiation. *Bone.* 2005; 36:S400-S401. Abstract P585.

25. Atkins GJ, Welldon KJ, Halbout P, Findlay DM. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. *Osteoporos Int.* 2009;20:653-664.

26. Brennan TC, Rybchyn MS, Green W, Atwa S, Conigrave AD, Mason RS. Osteoblasts play key roles in the mechanism of action of strontium ranelate. *Br J Pharmacol.* 2009;157:1291-1300.

27. Peng S, Liu XS, Zhou G, et al. Osteoprotegerin deficiency attenuates strontium-mediated inhibition of osteoclastogenesis and bone resorption. *J Bone Miner Res.* 2011; 26(6):1272-1282.

28. Rybchyn MS, Slater M, Conigrave AD, Mason RS. An Akt-dependent increase in canonical Wnt signaling and a decrease in sclerostin protein levels are involved in strontium ranelate-induced osteogenic effects in human osteoblasts. *J Biol Chem.* 2011;286(27):23771-23779.

29. Chavassieux P, Brixen K, Zerbini C, et al. *Osteoporosis Int.* 2011;22(suppl 1): S104. Abstract OC16.

30. Arlot ME, Jiang Y, Genant HK, et al. Histomorphometric and microCT analysis of bone biopsies from postmenopausal osteoporotic women treated with strontium ranelate. *J Bone Miner Res.* 2008;23:215-222.

31. Rizzoli R, Laroche M, Krieg MA, Frieling I, et al. Strontium ranelate and alendronate have differing effects on distal tibia bone microstructure in women with osteoporosis. *Rhumatol Int.* 2010;30:1341-1348.

32. Rizzoli R, Chapurlat RD, Laroche JM, et al. Effects of strontium ranelate and alendronate on bone microstructure in women with osteoporosis: results of a 2-year study. *Osteoporos Int.* 2012;23(1):305-315.

33. Doublier A, Farlay D, Khebbab MT, Jaurand X, Meunier P, Boivin G. Distribution of strontium and mineralization in iliac bone biopsies from osteoporotic women long-term treated with strontium ranelate. *Eur J Endocrinol.* 2011;165(3):469-476.

34. Alegre DN, Ribeiro C, Sousa C, et al. Possible benefits of strontium ranelate in complicated long bone fractures. *Rheumatol Int.* 2012;32(2):439-443.

35. Carvalho NN, Voss LA, Almeida MO, Salgado CL, Bandeira F. Atypical femoral fractures during prolonged use of bisphosphonates: short-term responses to strontium ranelate and teriparatide. *J Clin Endocrin Metab.* 2011;96(9):2675-2680.

Keywords: bone; efficacy; formation; osteoporosis; Protelos; resorption; strontium ranelate

EFFICACITÉ ANTIFRACTURAIRE GLOBALE, AMÉLIORATION DE LA QUALITÉ DE L'OS : PROTELOS, UN TRAITEMENT DE PREMIÈRE INTENTION DANS L'OSTÉOPOROSE

L'ostéoporose est une maladie chronique généralement liée au vieillissement. Elle a longtemps été considérée comme un processus normal, puis comme une maladie fatale. De nos jours, de nombreux traitements sont disponibles pour différer ou même stopper son évolution. Nombre d'entre eux ont montré leur efficacité sur des profils de patients ou des sites de fracture spécifiques. Le traitement idéal devrait cependant pouvoir prévenir/traiter l'ostéoporose à n'importe quel site de fracture (vertébral ou non vertébral, y compris la hanche) et chez différents types de patients : vieux ou jeunes, ostéoporotiques ou ostéopéniques, avec ou sans antécédents de fracture, indépendamment du risque de fracture, quel que soit le sexe, etc. Dans cet article, nous montrerons que Protelos, un traitement antiostéoporotique original, prévient les fractures chez tous ces patients. En outre, Protelos a démontré sa sécurité d'emploi et son efficacité contre les fractures au cours du suivi le plus long pour un traitement antiostéoporotique, jusqu'à 10 ans. De plus, nous allons examiner le mode d'action spécifique de Protelos et son effet sur la microarchitecture, afin d'expliquer pourquoi Protelos est un traitement de première intention selon plusieurs recommandations nationales et internationales.

"Bone mass at any given age is determined by the amount of bone accumulated at the end of skeletal growth—the so-called peak bone mass—and by the amount of bone that is subsequently lost. There is a large body of evidence linking nutritional intakes—particularly calcium and protein—to bone growth and to bone loss later in life, with both processes influencing fracture risk. Optimal dietary calcium and protein are necessary for bone homeostasis during growth as well as in the elderly."

Nutrition influence on bone health

Interview with R. Rizzoli, *Switzerland*

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*N*utritional intake is an environmental factor influencing both bone capital accumulation—which is fully achieved by the end of the second decade of life—and bone loss occurring during the second half of life. Nutrients may directly modify bone turnover, or do so indirectly through changes in calciotropic hormone levels. Studies of the association between nutrition and bone phenotypic expression may provide inconsistent results, partly because of the low accuracy and reproducibility of the various tools to assess dietary intakes. Dietary calcium and protein are nutrients affecting bone growth and age-related bone loss. An optimal intake of both is mandatory for the maintenance of bone health.

What are the contributions of genetics and nutrition to bone mass?

*M*ore than 60% of bone mass variance is determined by genetic factors.[1] Environmental factors account for the nongenetic influences, among them nutritional intakes and lifestyle. Nutrition can modulate the effects of genetics. Conversely, genetic background can determine the response to nutrition. Dietary intakes can influence bone metabolism and structure through different mechanisms. Products of nutrient metabolism may directly modify bone turnover, or do so indirectly through influence on secretion and circulating concentrations of calciotropic hormone, which affects bone remodeling and bone balance. Some effects of nutrition may even be more indirect. For instance, general malnutrition is associated with muscle wasting, thus submitting bone structure to less constraint. Moreover, the relationship between dietary intakes and bone health could be transient, and differ from long-term effects.

Furthermore, a clear relationship between bone variables and nutrition may be difficult to firmly establish, because of the poor accuracy and low reproducibility of the various methods to assess dietary intakes (eg, food diary, last 24-hour recall, food frequency questionnaire or nutritional intakes history) since all rely on the subject's memory. In evidence-based medicine, randomized controlled trials with homogenous results are considered to provide a higher level of evidence than observational studies,[2] the opinion of experts and/or personal clinical experience being at the bottom of the hierarchy. In the field of nutrition and bone, many concepts are based on association studies or, even worse, on experts' personal beliefs. A causal relationship based on associations is weak. Nutrient intervention studies with a specif-

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ic bone outcome are difficult to carry out and require large cohorts of subjects. Furthermore, the outcomes could be influenced by interaction with a large variety of confounding factors including social or genetic factors.

Bone mass at any given age is determined by the amount of bone accumulated at the end of skeletal growth—the so-called peak bone mass—and by the amount of bone that is subsequently lost.[3] There is a large body of evidence linking nutritional intakes—particularly calcium and protein—to bone growth and to bone loss later in life, with both processes influencing fracture risk. Optimal dietary calcium and protein are necessary for bone homeostasis during growth as well as in the elderly.

When does bone mass accrual occur?

In most parts of the skeleton, peak bone mass is achieved by the end of the second decade of life.[4] Total body mineral mass nearly doubles during puberty, through an increase in the size of the skeleton, with minor changes in volumetric bone density, ie, the amount of bone in bone.[3] A very small proportion of bone consolidation may occur during the third decade, particularly in males. Puberty is the period during which the sex difference in bone mass observed in adult subjects becomes fully expressed. The important gender difference in bone mass that develops during pubertal maturation appears to result from a greater increase in bone size.[5] There is no sex difference in volumetric trabecular density at the end of the period of maturation, ie, in young healthy adults in their third decade. The significantly greater mean areal bone mineral density (BMD) values observed in the lumbar spine and in the midfemoral or midradial diaphysis in young healthy adult males as compared with females appear to be essentially due to a more prolonged period of pubertal maturation rather than a greater maximal rate of bone accretion.[6] It is estimated that a 10% increase in peak bone mass could reduce the risk of osteoporotic fractures during adult life by 50%, or be equivalent to a 14-year delay in the occurrence of menopause. Children with upper limb fractures may have a 1% to 5% reduction in BMD as compared with controls.[7-9]

What role does calcium play in bone development and what is the effect of calcium supplementation on bone mass?

Calcium plays major roles in the regulation of various cell functions, in the central and peripheral nervous systems, in muscle, and in the function of exo-/endocrine glands.[10] In addition, this cation is implicated in the process of bone mineralization, by the formation of hydroxyapatite crystals. Extracellular calcium concentration has to be maintained as constant as possible, because of the high sensitivity of many cell systems or organs to small variations in extracellular calcium concentrations.

Several prospective randomized, double-blind, placebo-controlled intervention trials have concluded that calcium supplementation increases bone mass gain, although the magnitude of the calcium effects appears to vary according to the skeletal sites examined, the stage of pubertal maturation at the time of the intervention, and the spontaneous dietary calcium intake.[11-14] The effects of calcium could be modulated by an interaction with vitamin D receptor genotype.[15] The positive effects of calcium supplementation have essentially been ascribed to a reduction in bone remodeling. Indeed, in one
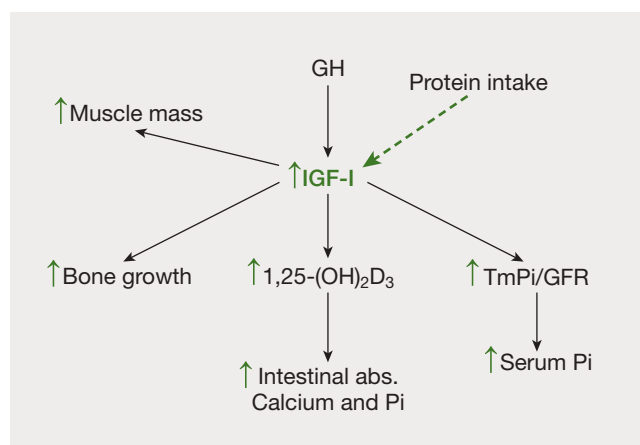
Figure 1. *Influence of dietary protein on mineral and muscle homeostasis through the growth hormone IGF-I system.*
Abbreviations: *abs, absorption; GH, growth hormone; IGF-I, insulin-like growth factor I; Pi, inorganic phosphate; TmPi/GFR, renal tubular reabsorption of Pi.*

of the above-mentioned studies, the plasma level of osteocalcin, a biochemical marker of bone remodeling in adults, was significantly reduced in the calcium-supplemented children.[14] Some effects of calcium supplements on bone modeling have been described as well.[11,16-18] In a double-blind, placebo-controlled study on the effects of calcium supplementation in prepubertal girls, changes in projected scanned bone area and in standing height have suggested that calcium supplementation may influence bone modeling in addition to bone remodeling.[11] Morphometric analysis of the changes observed in the lumbar spine and in femoral diaphysis suggests that calcium could enhance both the longitudinal and the cross-sectional growth of the bone. When bone mineral density was measured 7.5 years after the end of calcium supplementation—ie, in young adult girls—it appeared that menarche occurred earlier in the calcium-supplemented group, and that the persistent effects of calcium were mostly detectable in those subjects with an earlier puberty.[19]

Most of the studies carried out over 1 to 3 years in children and adolescents have shown that supplementation with either calcium or dairy foods enhances the rate of bone mineral acquisition, compared with unsupplemented (or placebo) control groups. In general, these intervention trials increased the usual calcium intake of the supplemented children from about 600-800 mg/day, to around 1000-1300 mg/day. A recent

meta-analysis has reviewed 19 calcium intervention studies involving 2859 children,[20] with doses of calcium supplementation varying between 300 and 1200 mg/day, using either calcium citrate malate, calcium carbonate, calcium phosphate, calcium lactate gluconate, calcium phosphate milk extract, or milk minerals. Calcium supplementation had a positive effect on total body bone mineral content and upper limb bone mineral density, with standardized mean differences (effect size) of 0.14 for both. At the upper limb, the effect persisted for up to 18 months after cessation of calcium supplementation. In the same study, calcium supplementation had no significant effect on weight, height, or body fat.

What is the effect of protein intake on bone growth and bone mass accrual?

In children and adolescents, protein intakes influence bone growth and bone mass accumulation.[3] In "well-nourished" children and adolescents, variations in the protein intake within the "normal" range may have a significant effect on skeletal growth and thereby modulate the genetic potential in peak bone mass attainment (Figure 1).

Changes in BMD and bone mineral content (BMC) in prepubertal boys are positively associated with spontaneous protein intake. Furthermore, higher protein intake enhances the positive influence of physical activity on BMC in prepubertal boys (Figure 2).[13] Nutritional environmental factors seem to affect bone accumulation at specific periods during infancy and adolescence. In a prospective survey carried out in a cohort of female and male subjects aged 9 to 19 years, food intake was assessed twice, at a one-year interval, using a 5-day dietary diary method that consisted in weighing all consumed foods.[21] In this cohort of adolescents, we found a positive correlation between yearly lumbar and femoral bone mass gain, and calcium or protein intake.[22] This correlation was mainly detectable in prepubertal children, but not in those having reached a peri- or postpubertal stage. It remained statistically significant after adjustment for spontaneous calcium intakes.

In a prospective longitudinal study performed in healthy children and adolescents of both sexes, between the ages of 6 and 18, dietary intakes were recorded over 4 years, using an annually assessed 3-day diary.[23] Long-term protein intakes were found to be significantly positively associated with periosteal circumference, cortical area, bone mineral content, and calculated strength strain index. In this cohort with a Western-style diet, protein intakes were around 2 g/kg body weight per day in prepubertal children, and they were around 1.5 g/kg per day in pubertal individuals. There was no association between bone variables and intakes of nutrients with high sulfur-containing amino acids, or intake of calcium. Overall, protein intakes accounted for 3% to 4% of bone parameter variance. It is quite possible that protein intake could be to a large extent related to growth requirement during childhood and adolescence. Only intervention studies would be able to reliably address this question. To our knowledge, there is no large randomized controlled trial that has specifically tested the effects of dietary protein supplements—other than milk or dairy products—on bone mass accumulation.

What is the effect of dairy product intake on bone growth?

In addition to calcium, phosphorus, calories, and vitamins, one liter of milk provides 32 to 35 g of protein, mostly casein, but also whey protein, which contains numerous growth-promoting elements. In growing children, long-term milk avoidance is associated with smaller stature and lower bone mineral mass, either at specific sites or for the whole body.[24] Low

FN BMC **FN Area** **FN BMD**
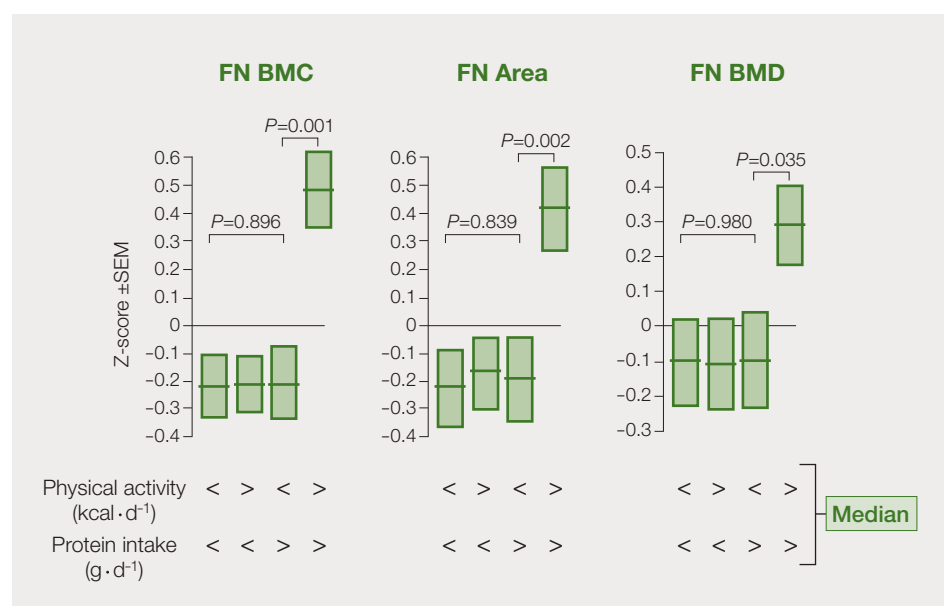
Figure 2. Influence of protein intake on the impact of increased physical activity on bone mineral content, projected scanned bone area and areal bone mineral density of the femoral neck in prepubertal boys aged 7.4±0.4 years.

Data are presented in Z-scores (± SEM). Increased physical activity is associated with a significant increase in femoral neck BMC, area and aBMD in subjects having protein intake above (>), but not below (<) the median. Analyzed by ANOVA, the interaction between physical activity and protein intake was P=0.012 at the FN BMC, P=0.040 at the FN area, and P=0.132 at the FN aBMD. Abbreviations: aBMD, areal bone mineral density; BMC, bone mineral content; FN, femoral neck; SEM, standard error of the mean.
After reference 13: Chevalley et al. J Bone Miner Res. 2008;23(1):131-142. © 2008, American Society for Bone and Mineral Research.

milk intake during childhood and/or adolescence increases the risk of fracture before puberty (a 2.6-fold higher risk has been reported) and possibly later in life.[8] In a 7-year observational study, there was a positive influence of dairy product consumption on bone mineral density at the spine, hip, and forearm in adolescents, thereby leading to a higher peak bone mass.[25] In this study, calcium supplements did not affect spine BMD. However, higher dairy product intakes were associated with a greater total and cortical proximal radius cross-sectional area. Based on these observations, it was suggested that, whereas calcium supplements could influence volumetric BMD, and thus the remodeling process, dairy products may have an additional effect on bone growth and periosteal bone expansion, ie, an influence on modeling.[25] In agreement with this observation, milk consumption frequency and milk intake at age 5-12 and 13-17 years were significant predictors of the height of 12-18 year-old adolescents, studied in the 1999-2002 NHANES survey (National Health and Nutrition Examination Survey).[26]

The earliest milk intervention controlled studies were by Orr,[27] and Leighton and Clark.[28] In British school children, 400 to 600 mL/day of milk had a positive effect on height gain over a 7-month period. Numerous intervention trials have demonstrated a favorable influence of dairy products on bone health during childhood and adolescence.[17,29] In an open randomized intervention controlled trial, 568 mL/day milk supplement for 18 months in 12-year-old girls[17] provided an additional 420 mg/day calcium and 14 g/day protein intakes at the end of the study. In the milk-supplemented group, serum insulin-like growth factor-I (IGF-I) levels were 17% significantly higher. Compared with the control group, the intervention group had greater increases of whole body BMD and BMC.

In another study, cheese supplements appeared to be more beneficial for cortical bone accrual than a similar amount of calcium supplied in the form of tablets.[29] The positive influence of milk on cortical bone thickness may be related to an effect on the modeling process, since metacarpal periosteal diameter was significantly increased in Chinese children receiving milk supplements.

What is the importance of calcium intake in adults?

After menopause, changes in sex hormone levels and nutrition are associated with an increase in bone remodeling and bone fragility. In adults, obligatory calcium losses have to be offset by sufficient calcium intakes and efficacious intestinal absorption *(Table I)*. Otherwise, bone is used as a source of calcium to maintain homeostasis of extracellular calcium concentration. This homeostatic mechanism is altered in the elderly.[30] With increased remodeling rate, the number of resorption cavities in cancellous tissue is higher, influencing bone strength and stiffness independently of bone mass.[31] Thus, slowing down the rate of activation of new

- ↓ Calcium intake
- ↓ Vitamin D intake and endogenous production
- ↓ Intestinal calcium absorption
- ↓ Adaptation to a low calcium diet
- ↓ Renal tubular reabsorption of calcium

Table I. *Changes in calcium metabolism in the elderly.*

remodeling sites should be associated with a decrease in bone fragility. The effect of calcium on bone remodeling is usually ascribed to an inhibition of the secretion of parathyroid hormone, whose plasma level tends to increase with aging.[32-34] Mineral waters with high calcium content could provide useful quantities of bioavailable calcium, independently from their sulfate content.[35]

What is the effect of calcium supplementation on fracture risk?

The antifracture efficacy of specific bone turnover or bone mass modifying agents has always been tested in vitamin D and calcium replete patients,[36] except for hormone replacement therapy. Thus, any antifracture efficacy demonstrated with these agents is above that achieved with calcium and vitamin D. The doses of calcium used in these trials varied between 500 and 1500 mg daily.

Earlier studies have shown a reduction in nonvertebral, or hip fracture risk associated with calcium and vitamin D.[34,37] Two subsequently published large trials have challenged these conclusions by being unable to detect significant antifracture effect in calcium and vitamin D treated individuals.[38,39] Neither study targeted individuals at high fracture risk, and in both studies the adherence was poor. The clinical trial of the Women's Health Initiative was carried out in healthy postmenopausal women with an average calcium intake above 1000 mg/day, 80% of whom were under 70 years of age. When the analysis was carried out in only the compliant subjects, a significant (29%) reduction in hip fracture risk compared with the placebo group was found.

Similarly, whereas in a prevention trial conducted in women over the age of 70, randomized to calcium 1200 mg daily or placebo for 5 years, there was no fracture risk reduction in an intention-to-treat analysis,[40] a 34% fracture risk reduction was detected in the 57% of the patients who took at least 80% of the medication. In contrast, in another prevention trial performed for 5 years in healthy postmenopausal women with a mean age of 74 years, the favorable effects of calcium on bone loss or bone turnover were not associated with any antifracture efficacy, even in a per-protocol analysis.[41] Persistence and compliance with calcium supplementation regimens were low, with poor compliance impairing efficacy.

A meta-analysis of 9 randomized clinical trials, including a total of 53 260 patients, found that whereas supplementation with vitamin D alone was not sufficient to significantly reduce the risk of hip fracture in postmenopausal women, combined supplementation with vitamin D and calcium reduced the risk of hip fracture by 28% and the risk of nonvertebral fracture by 23% compared with supplementation with vitamin D alone.[42] Calcium supplements may be associated with mild gastrointestinal disturbances such as constipation, flatulence, nausea, gastric pain, and diarrhea. Calcium may also interfere with the intestinal absorption of iron and zinc. Recently, it has been reported that calcium supplementation in healthy postmenopausal women was associated with an increased risk of cardiovascular events,[43] mainly in those with a high spontaneous calcium intake.

What is the effect of protein intake on fracture risk?

Virtually all studies assessing a possible association between bone mass at various skeletal sites and spontaneous protein intake, have found a positive, rather than a negative, relationship in children or adolescents,[13,23] pre- or postmenopausal women,[44] and men. Unadjusted BMD was greater in the group with the higher protein intake in a large series of data collected within the framework of the Study of Osteoporotic Fracture.[45] Dietary protein accounted for as much as 2% of bone mineral mass variance. A longitudinal follow-up within the framework of the Framingham study has demonstrated that the rate of bone mineral loss was inversely correlated with dietary protein intake.[46] In contrast, very few surveys have reported that high protein intake was associated with lower bone mass. In a cross-sectional study, a protein intake close to 2 g/kg body weight was associated with reduced BMD only at one out of the two forearm sites measured in young college women.[47]

The large Nurse Health Study reported an inversely related trend for hip fracture incidence to protein intake.[48] The same study reported an increase in the risk of forearm fracture in the subjects with the highest protein intake of animal origin. In a prospective study carried out on more than 40 000 women in Iowa, higher protein intake was associated with a reduced risk of hip fracture.[49] The protective effect was observed with dietary protein of animal origin. In a case-control study, increasing protein intake was associated with a 65% lower hip fracture risk in the highest quartile in the 50- to 69-year-old age group.[50]

In another study, fracture risk was increased when a high protein diet was accompanied by a low calcium intake, in agreement with the requirement of sufficient calcium intake to detect a favorable influence of dietary protein on bone.[51] In a longitudinal study, hip fracture incidence was positively related to a higher ratio of animal-to-vegetal protein intake, whereas protein of vegetable origin was protective.[45]

Does high protein intake affect bone metabolism?

Whereas high protein intake has been claimed to be a risk factor for osteoporosis, further studies indicate that a reduction in dietary protein may lead to a decline in calcium absorption and to secondary hyperparathyroidism (Figure 3).[52,53]

A low (0.7 g/kg body weight), and not a high, protein intake (2.1 g/kg), was associated with an increase in biochemical markers of bone turnover as compared with a diet containing 1.0 g/kg of protein.[54] High meat diets (1.6 g/kg body weight of protein compared with 0.9 g/kg) for 8 weeks did not affect calcium retention nor indices of bone metabolism.[55]
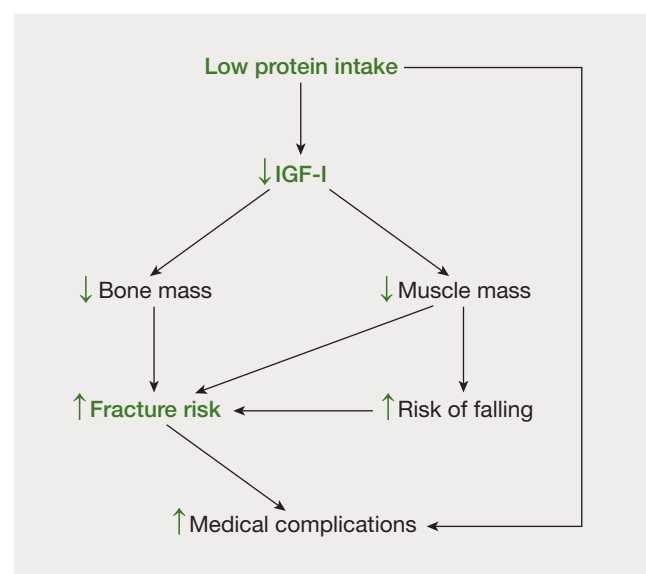
Figure 3. Low protein intake and fracture risk.

Do animal and vegetal proteins have different effects on calcium metabolism?

It has been claimed that the source of proteins, animal versus vegetal, differently affect calcium metabolism. This is based on the hypothesis that animal proteins may generate more sulfuric acid from sulfur-containing amino acids than a vegetarian diet. A vegetarian diet with protein derived from grains and legumes may deliver as many millimoles of sulfur per gram proteins as would a purely meat-based diet. In a cross-sectional survey, BMD was higher in subjects with diets rich in fruits and vegetables, which are presumably rich in alkali.[56,57]

However, this issue is further complicated by the fact that the vegetable intake–induced decrease in bone resorption has been shown to be independent of acid-base changes and that potassium, but not sodium, bicarbonate (ie, the same anion),[58] or citrate administration, reduces urinary calcium excretion.

What is the relationship between dietary protein intake and IGF-I?

Dietary proteins influence both the production and action of IGF-I, particularly the growth hormone (GH)–insulin-like growth factor (IGF) system.[59] Protein restriction has been shown to reduce IGF-I plasma levels by inducing a resistance to the action of GH at the hepatic level, and by an increase in the metabolic clearance rate of IGF-I. In addition to calcium, magnesium, and other multivalent cations, calcium-sensing receptors sense amino acids, specifically L-amino acids, thereby modulating parathyroid hormone secretion.[60] In humans, increased intake of aromatic, but not branched-chain, amino acids is associated with increases in serum IGF-I, intestinal calcium absorption, and 24-hour urinary calcium excretion, without any change in the biochemical markers of bone turnover.[61]

In an adult female rat experimental model of selective protein deprivation, with isocaloric low protein diets supplemented with identical amounts of minerals,[62-64] a decrease in BMD was observed at skeletal sites formed by trabecular or cortical bone in animals fed a low casein diet, but receiving the same amount of energy. This was associated with a marked and early decrease in plasma IGF-I of 40%. Protein replenishment with essential amino acid supplements in the same relative proportion as in casein caused an increase in IGF-I to a level higher than in rats fed the control diet, and improved bone strength more than bone mineral mass, in relation with an increase in cortical thickness, as demonstrated by microquantitative computerized tomography.[62] Intrinsic bone tissue properties were modified by protein intake changes.[65]

Thus, in the elderly, a restoration of the altered GH-IGF-I system by protein replenishment is likely to favorably influence not only BMD, but also muscle mass and strength, since these two variables are important determinants of the risk of falling.

Intervention studies using a simple oral dietary preparation that normalizes protein intake improved the clinical outcome after hip fracture.[66-68] It should be emphasized that a 20-g protein supplement, as administered in these studies, brought the intake from low to a level still below RDA (0.8 g/kg body weight), thus avoiding the risk of an excess of dietary protein.

Follow-up showed a lower rate of complications (bedsore, severe anemia, intercurrent lung or renal infections), and deaths were still observed at six months.[67] The total length of stay in the orthopedic ward and convalescent hospital was significantly shorter in supplemented patients than in controls. In a double blind, placebo-controlled study, protein repletion with 20 g protein supplement daily for 6 months as compared with an isocaloric placebo, produced greater gains in serum prealbumin, IGF-I, and IgM, and an attenuated proximal femur BMD decrease.[68] In a multiple regression analysis, baseline IGF-I concentrations, biceps muscle strength, together with protein supplements accounted for more than 30% of the variance of the length of stay in rehabilitation hospitals (R^2=0.312, P<0.0005), which was reduced by 25% in the protein supplemented group.[68] In another controlled trial, dietary protein supplements favorably influenced bone metabolism in the elderly.[69] In a short-term study on the kinetics and determinants of the IGF-I response to protein supplements in a situation associated with low baseline IGF-I levels, such as the frail elderly, or patients with a recent hip fracture, we found that a 20 g/day protein supplement increased serum IGF-I and IGF-binding protein-3 starting after one week, with a maximal response after 2 weeks.[70,71]

Taken together, these results indicate that a reduction in protein intakes may be detrimental for maintaining bone integrity and function in the elderly. ■

References

1. Rizzoli R, Ammann P, Bourrin S, Chevalley T, Bonjour JP. Protein intake and bone homeostasis. In: Burckhardt P, Dawson-Hughes B, Heaney RP, eds. *Nutritional aspects of osteoporosis.* San Diego, CA: Academic Press; 2001:219-235.
2. Guyatt GA. Evidence-based management of patients with osteoporosis. *J Clin Densitom.* 1998;1:395-402.
3. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass during growth for the prevention of fractures in the adolescents and the elderly. *Bone.* 2010;46(2):294-305.
4. Rizzoli R, Bonjour JP. Determinants of peak bone mass acquisition. In: Adler RA, ed. *Osteoporosis: Pathophysiology and Clinical Management, Second Edition.* New York, NY: Humana Press; 2010:1-22.
5. Seeman E. The structural and biomechanical basis of the gain and loss of bone strength in women and men. *Endocrinol Metab Clin North Am.* 2003;32(1):25-38.
6. Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab.* 1992;75(4):1060-1065.
7. Ferrari SL, Chevalley T, Bonjour JP, Rizzoli R. Childhood fractures are associated with decreased bone mass gain during puberty: an early marker of persistent bone fragility? *J Bone Miner Res.* 2006;21(4):501-507.
8. Goulding A, Rockell JE, Black RE, Grant AM, Jones IE, Williams SM. Children who avoid drinking cow's milk are at increased risk for prepubertal bone fractures. *J Am Diet Assoc.* 2004;104(2):250-253.
9. Ma D, Jones G. The association between bone mineral density, metacarpal morphometry, and upper limb fractures in children: a population-based case-control study. *J Clin Endocrinol Metab.* 2003;88(4):1486-1491.
10. Rizzoli R, Bonjour JP. Physiology of calcium and phosphate homeostases. In: Seibel MJ, Robins SP, Bilezikian JP, eds. *Dynamics of Bone and Cartilage Metabolism.* 2nd ed. San Diego, CA: Academic Press; 2006:345-360.
11. Bonjour JP, Carrie AL, Ferrari S, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *J Clin Invest.* 1997;99(6):1287-1294.
12. Chevalley T, Rizzoli R, Hans D, Ferrari S, Bonjour JP. Interaction between calcium intake and menarcheal age on bone mass gain: an eight-year follow-up study from prepuberty to postmenarche. *J Clin Endocrinol Metab.* 2005;90(1):44-51.
13. Chevalley T, Bonjour JP, Ferrari S, Rizzoli R. High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. *J Bone Miner Res.* 2008;23(1):131-142.

14. Johnston CC, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. *N Engl J Med*. 1992;327(2):82-87.

15. Ferrari S, Rizzoli R, Manen D, Slosman D, Bonjour JP. Vitamin D receptor gene start codon polymorphisms (FokI) and bone mineral density: interaction with age, dietary calcium, and 3'-end region polymorphisms. *J Bone Miner Res*. 1998;13(6):925-930.

16. Bonjour JP, Ammann P, Chevalley T, Rizzoli R. Protein intake and bone growth. *Can J Appl Physiol*. 2001;26(suppl):S153-S166.

17. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. *BMJ*. 1997;315(7118):1255-1260.

18. Prentice A, Ginty F, Stear SJ, Jones SC, Laskey MA, Cole TJ. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. *J Clin Endocrinol Metab*. 2005;90(6):3153-3161.

19. Chevalley T, Bonjour JP, Ferrari S, Hans D, Rizzoli R. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebo-controlled trial in prepubertal boys. *J Clin Endocrinol Metab*. 2005;90(6):3342-3349.

20. Winzenberg T, Shaw K, Fryer J, Jones G. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. *BMJ*. 2006;333(7572):775-778.

21. Clavien H, Theintz G, Rizzoli R, Bonjour JP. Does puberty alter dietary habits in adolescents living in a western society? *J Adolesc Health*. 1996;19(1):68-75.

22. Bonjour JP, Chevalley T, Ammann P, Slosman D, Rizzoli R. Gain in bone mineral mass in prepubertal girls 3.5 years after discontinuation of calcium supplementation: a follow-up study. *Lancet*. 2001;358(9289):1208-1212.

23. Alexy U, Remer T, Manz F, Neu CM, Schoenau E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am J Clin Nutr*. 2005;82 (5):1107-1114.

24. Opotowsky AR, Bilezikian JP. Racial differences in the effect of early milk consumption on peak and postmenopausal bone mineral density. *J Bone Miner Res*. 2003;18(11):1978-1988.

25. Matkovic V, Landoll JD, Badenhop-Stevens NE, et al. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. *J Nutr*. 2004;134(3):701S-705S.

26. Wiley AS. Does milk make children grow? Relationships between milk consumption and height in NHANES 1999-2002. *Am J Hum Biol*. 2005;17(4):425-441.

27. Orr J. Milk consumption and the growth of school-children. *Lancet*. 1928;1: 202-203.

28. Leighton G, Clark M. Milk consumption and the growth of school-children. *Lancet*. 1929;1:40-43.

29. Cheng S, Lyytikainen A, Kroger H, et al. Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. *Am J Clin Nutr*. 2005 Nov;82(5):1115-1126;quiz47-48.

30. Fordtran JS, Walsh JH. Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *J Clin Invest*. 1973;52(3):645-657.

31. Hernandez CJ, Gupta A, Keaveny TM. A biomechanical analysis of the effects of resorption cavities on cancellous bone strength. *J Bone Miner Res*. 2006;21 (8):1248-1255.

32. Boonen S, Rizzoli R, Meunier PJ, et al. The need for clinical guidance in the use of calcium and vitamin D in the management of osteoporosis: a consensus report. *Osteoporos Int*. 2004;15(7):511-519.

33. Chevalley T, Rizzoli R, Nydegger V, et al. Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin-D-replete elderly patients. *Osteoporos Int*. 1994;4(5):245-252.

34. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med*. 1997;337(10):670-676.

35. Burckhardt P. The effect of the alkali load of mineral water on bone metabolism: interventional studies. *J Nutr*. 2008;138(2):435S-437S.

36. Hauselmann HJ, Rizzoli R. A comprehensive review of treatments for postmenopausal osteoporosis. *Osteoporos Int*. 2003;14(1):2-12.

37. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N Engl J Med*. 1992;327(23):1637-1642.

38. Grant AM, Avenell A, Campbell MK, et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet*. 2005;365(9471):1621-1628.

39. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med*. 2006;354(7):669-683.

40. Prince RL, Devine A, Dhaliwal SS, Dick IM. Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double-blind, placebo-controlled trial in elderly women. *Arch Intern Med*. 2006;166(8):869-875.

41. Reid IR, Mason B, Horne A, et al. Randomized controlled trial of calcium in healthy older women. *Am J Med*. 2006;119(9):777-785.

42. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: evidence from a comparative metaanalysis of randomized controlled trials. *J Clin Endocrinol Metab*. 2007;92(4):1415-1423.

43. Bolland MJ, Avenell A, Baron JA, et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ*. 2010;341: c3691.

44. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. *Am J Clin Nutr*. 2009;90(6):1674-1692.

45. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. *Am J Clin Nutr*. 2001;73(1):118-122.

46. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. *J Bone Miner Res*. 2000;15(12):2504-2512.

47. Metz JA, Anderson JJ, Gallagher PN Jr. Intakes of calcium, phosphorus, and protein, and physical-activity level are related to radial bone mass in young adult women. *Am J Clin Nutr*. 1993;58(4):537-542.

48. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. Protein consumption and bone fractures in women. *Am J Epidemiol*. 1996;143(5):472-479.

49. Munger RG, Cerhan JR, Chiu BC. Prospective study of dietary protein intake and risk of hip fracture in postmenopausal women. *Am J Clin Nutr*. 1999;69(1): 147-152.

50. Wengreen HJ, Munger RG, West NA, et al. Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. *J Bone Miner Res*. 2004;19 (4):537-545.

51. Meyer HE, Pedersen JI, Loken EB, Tverdal A. Dietary factors and the incidence of hip fracture in middle-aged Norwegians. A prospective study. *Am J Epidemiol*. 1997;145(2):117-123.

52. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein affects intestinal calcium absorption. *Am J Clin Nutr*. 1998;68(4):859-865.

53. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr*. 2003;78(suppl3):584S-592S.

54. Kerstetter JE, Mitnick ME, Gundberg CM, et al. Changes in bone turnover in young women consuming different levels of dietary protein. *J Clin Endocrinol Metab*. 1999;84(3):1052-1055.

55. Roughead ZK, Johnson LK, Lykken GI, Hunt JR. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *J Nutr*. 2003;133(4):1020-1026.

56. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. *Am J Clin Nutr*. 1997;65(6):1831-1839.

57. New SA. Intake of fruit and vegetables: implications for bone health. *Proc Nutr Soc*. 2003;62(4):889-899.

58. Muhlbauer RC, Lozano A, Reinli A. Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanism independent of their base excess. *J Bone Miner Res*. 2002;17(7):1230-1236.

59. Rizzoli R, Bonjour JP. Dietary protein and bone health. *J Bone Miner Res*. 2004; 19(4):527-531.

60. Conigrave AD, Brown EM, Rizzoli R. Dietary protein and bone health: roles of amino acid-sensing receptors in the control of calcium metabolism and bone homeostasis. *Annu Rev Nutr*. 2008;28:131-155.

61. Dawson-Hughes B, Harris SS, Rasmussen HM, Dallal GE. Comparative effects of oral aromatic and branched-chain amino acids on urine calcium excretion in humans. *Osteoporos Int*. 2007;18(7):955-961.

62. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. *J Bone Miner Res*. 2000;15(4):683-690.

63. Bourrin S, Toromanoff A, Ammann P, Bonjour JP, Rizzoli R. Dietary protein deficiency induces osteoporosis in aged male rats. *J Bone Miner Res*. 2000;15(8): 1555-1563.

64. Bourrin S, Ammann P, Bonjour JP, Rizzoli R. Dietary protein restriction lowers plasma insulin-like growth factor I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats. *Endocrinology*. 2000;141(9):3149-3155.

65. Ammann P, Bourrin S, Brunner F, et al. A new selective estrogen receptor modulator HMR-3339 fully corrects bone alterations induced by ovariectomy in adult rats. *Bone.* 2004;35(1):153-161.

66. Delmi M, Rapin CH, Bengoa JM, Delmas PD, Vasey H, Bonjour JP. Dietary supplementation in elderly patients with fractured neck of the femur. *Lancet.* 1990; 335(8696):1013-1016.

67. Tkatch L, Rapin CH, Rizzoli R, et al. Benefits of oral protein supplementation in elderly patients with fracture of the proximal femur. *J Am Coll Nutr.* 1992;11(5): 519-525.

68. Schurch MA, Rizzoli R, Slosman D, Vadas L, Vergnaud P, Bonjour JP. Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med.* 1998;128(10):801-819.

69. Hampson G, Martin FC, Moffat K, et al. Effects of dietary improvement on bone metabolism in elderly underweight women with osteoporosis: a randomised controlled trial. *Osteoporos Int.* 2003;14(9):750-756.

70. Rodondi A, Ammann P, Ghilardi-Beuret S, Rizzoli R. Zinc increases the effects of essential amino acids-whey protein supplements in frail elderly. *J Nutr Health Aging.* 2009;13(6):491-497.

71. Chevalley T, Hoffmeyer P, Bonjour JP, Rizzoli R. Early serum IGF-I response to oral protein supplements in elderly women with a recent hip fracture. *Clin Nutr.* 2010;29(1):78-83.

Keywords: dietary calcium; dietary protein; fracture; IGF-I; osteoporosis; peak bone mass

INFLUENCE DE LA NUTRITION SUR LA SANTÉ OSSEUSE

L'alimentation est un facteur environnemental qui influe à la fois sur l'accumulation du capital osseux (qui est totalement atteint à la fin de la deuxième décennie de la vie) et sur la perte osseuse survenant lors de la seconde moitié de la vie. Les nutriments peuvent modifier directement le taux de renouvellement osseux ou indirectement au travers de variations des taux d'hormones calciotropes. Les résultats d'études d'association entre la nutrition et l'expression phénotypique osseuse sont parfois contradictoires, en partie en raison des fiabilité et reproductibilité faibles des différents outils d'évaluation de la prise alimentaire. Les protéines et le calcium alimentaires sont des nutriments qui influent sur la croissance osseuse et la perte osseuse liée à l'âge. Les prendre de façon optimale est indispensable au maintien de la santé osseuse.

"In its different layers, the periosteum contains an entire smorgasbord of skeletal cell types at different stages of skeletal development, from mesenchymal stem cells, to chondrocytes, fibroblasts, and cells of the entire osteoblastic lineage. This accounts for its broad potential to create and shape the bone throughout growth, and for its utility as a source of cells for orthopedic procedures such as resurfacing of cartilage surfaces in degenerating joints."

Almost invisible, often ignored: periosteum, the living lace of bone

by D. B. Burr and G. M. Guillot, *USA*

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The periosteal membrane is thin and fibrous, and its deep layer is the source of cells responsible for the growth, development, modeling/remodeling, and fracture repair of our bones. It is highly vascularized and innervated by both sympathetic and pain-sensitive fibers. It arises from condensation of the general mesenchyme during fetal development, and is continuous with the Sharpey's fibers that insert into bone and anchor it. The fibrous portion is composed of several collagen species as well as elastin. The cellular composition is diverse, including undifferentiated mesenchymal stem cells that can differentiate into fibroblasts, chondrocytes, or osteoblasts, the latter communicating extensively with the osteocytes in bone. These cells are under local control and are highly responsive to growth factors (eg, transforming growth factor beta) and several of the bone morphogenetic proteins, sex steroids (both estrogens and androgens), mineral-regulating hormones (eg, parathyroid hormone), and to other proteins associated with bone formation that are modulated through the Wnt pathway (eg, sclerostin). During fracture repair, the periosteum participates in, and provides cells for, both the intramembranous ossification that bridges and stabilizes the fracture, as well as the process of endochondral ossification and remodeling that eventually reestablishes the bone's load bearing properties. It is a multifunctional tissue that permits our bone to adapt throughout life to changing mechanical, hormonal, and pathological circumstances.

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The periosteum is a thin fibrocellular membrane that surrounds bone throughout life. Perhaps because of its relative delicacy, its structure and importance are often overlooked in deference to the more obvious processes that occur on the periosteal surface of the bone itself. Yet, these processes are regulated by the structure and cellular composition of the periosteal membrane. The cells of this thin layer contribute to the growth and development of the bone, repair of the bone following fracture, and the regulation of bone adaptation to mechanical stimuli. Without the periosteal membrane, none of these processes could occur because the cellular diversity and necessary coordination that underlies them would not exist.

Periosteal origins and the regulation of growth

The periosteum arises as a condensation of general mesenchyme that forms a perichondrial sheath around the cartilage anlage during development *(Figure 1, page 222)*. It ends at the joint space, forming a perichondrial ring around the end of the

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The entire periosteum is 70-μm to 150-μm thick in growing individuals,[4] but thins with age as growth and appositional formation slow.[2,5,6] It is typically thicker close to the metaphysis and thinner over the diaphysis *(Figure 2).* The periosteum in the adult is composed of two layers, an outer fibrous sheath of axially aligned collagen fibers that contains both fibroblasts and mesenchymal cells[7] and which is composed of types I, III and VI collagen[8,9] and elastin.[10,11] Type III collagen is found in abundance in blood vessels, and may reflect the vascularity of periosteum, but because it cross-links rapidly may also function to reduce the extensibility of the tissue and enhance stability.[12] The inner osteogenic or "cambium" layer contributes to the appositional growth of the bone throughout life, and includes mesenchymal stem cells, osteoblasts, and endothelial pericytes, the latter probably providing an additional pool of osteoprogenitor cells *(Figure 2).*[13] It is also possible that some of the cells in the fibrous portion migrate into the cambium layer and contribute to bone formation. The osteoblasts of this layer are connected by their cellular processes to osteocytes within the bone. Some have suggested that there is an intermediate elastic layer containing capillaries,[4,10] but this may disappear with maturity. Whether this constitutes another layer or not, it is true that the periosteum is highly vascularized and highly innervated[14,15] by both sympathetic and sensory fibers.[16]

During growth, the periosteum migrates to cover new bone as it grows longitudinally. This migration involves the cellular layer as well as the outer fibrous layer.[6] There is some evidence that the insertion of the periosteum into the mineralized bone by Sharpey's fibers helps to regulate the longitudinal growth of the bone by constraining it, and that release of the periosteum allows additional growth.[16-18] This was presumed to be a physical process because the periosteal membrane is highly prestressed and physically retracts and shortens by about 3 fold when incised from the bone.[19] However, the tension generated by the fibrous periosteum and its insertions into bone has been shown to be insufficient for physical constraint.[20] More recent evidence suggests that constraint may occur through cell-regulated mechanotransduction pathways

cartilage model where the epiphyseal cartilage will develop. As the bone develops, osteoblast progenitor cells differentiate into osteoblasts in the deep layers of the periosteum, contributing to mineralization of an osseous ring around the anlage, and eventually to the enlargement of the bone diaphysis and apposition of new bone by intramembranous ossification. The periosteum is continuous with Sharpey's fibers that insert into the bone and attach it firmly,[1] although the strength and size of these connections are reduced with age.[2] The periosteum is thought to grade into tendons and ligaments as they insert into bone,[3] but there is still some debate about this.

SELECTED ABBREVIATIONS AND ACRONYMS

BMP	bone morphogenetic protein
CDMP	cartilage-derived morphogenic protein
ER	estrogen receptor
GFP	green fluorescent protein
GH	growth hormone
IGF-I	insulin-like growth factor I
PLF	periostin-like factor
PTH	parathyroid hormone
PTHrP	parathyroid hormone–related protein
rhPTH(1-34)	recombinant 1-34 fragment of human parathyroid hormone
TGF-β	transforming growth factor beta

that sense intracellular tension[21] and promote the release of soluble inhibitory factors by periosteal cells.[22,23] When the periosteum is released, bone morphogenetic proteins (BMPs) such as BMP-2 and BMP-4 are produced,[24] which stimulates a proliferative reaction that causes growth.

Cells of the periosteum

In its different layers, the periosteum contains an entire smorgasbord of skeletal cell types at different stages of skeletal development, from mesenchymal stem cells, to chondrocytes, fibroblasts, and cells of the entire osteoblastic lineage. This accounts for its broad potential to create and shape the bone throughout growth, and for its utility as a source of cells for orthopedic procedures such as resurfacing of cartilage surfaces in degenerating joints.

The cells in the cambial layer of the periosteum are highly osteogenic, and respond to mechanical stimulation, infection, and tumors. They are highly proliferative and capable under these conditions of forming either highly organized lamellar bone, or highly disorganized woven bone in pathological situations. Because mesenchymal cells are also present, however, cells in the deep layer of the periosteum can also differentiate into chondroblasts, and form cartilage, most notably in adults during the fracture healing process. The diversity of tissue-forming potential in this area is critically important during fracture healing (see below).

Cells in the cambium layer of the periosteum express markers for both osteogenic and chondrogenic lineages. Like progenitors and fully differentiated bone and cartilage cells in other locations, these cells also are responsive to regulation by a wide range of growth factors and other proteins. Perhaps most prominently, transforming growth factor beta (TGF-β) appears to promote chondrogenic activity, but may inhibit differentiation of osteoblast progenitors.[25] The cartilage-derived morphogenic proteins (CDMP) are also known to drive periosteal cells into the chondrogenic pathway,[26] whereas both CDMP and parathyroid hormone–related protein (PTHrP), the latter in response to Indian hedgehog expressed by hypertrophic chondrocytes, may contribute to chondrocyte regulation during the early stages of growth, or in fracture healing.[27] Bone morphogenetic proteins, most notably BMP-2 and BMP-4, may promote the proliferation and differentiation of osteogenic cells,[28] and are known to be expressed particularly during fracture healing. BMP-7 is expressed during periods of endochondral ossification, both in growth and in fracture healing.[29]

Although osteoclasts are blood-derived rather than bone-derived, modeling of the bone during growth requires the presence of cells that can develop into osteoclasts. Periosteum is highly vascularized, and these vessels can transport monocytes that are found in the mesenchyme of the developing limb. Type IV collagenase, which is a marker for preosteoclast

development, has been immunolocalized within the deep and fibrous layers of the periosteum. Osteoclasts are thought to migrate from the more superficial layers of the periosteum, through the deeper layers to the bone surface where they can begin to shape the bone. This migration is prevented by TGF-β[30] and by matrix metalloproteinases,[31] suggesting again an antagonistic relationship between chondrogenic and osteogenic processes during growth.

Periostin is a protein important during development that, in the skeletal tissues, is localized to the periosteal membrane and to the periodontal ligament. It is an intriguing, but not yet well understood, candidate to regulate cellular processes within the periosteum, and controls the osteogenic potential of the
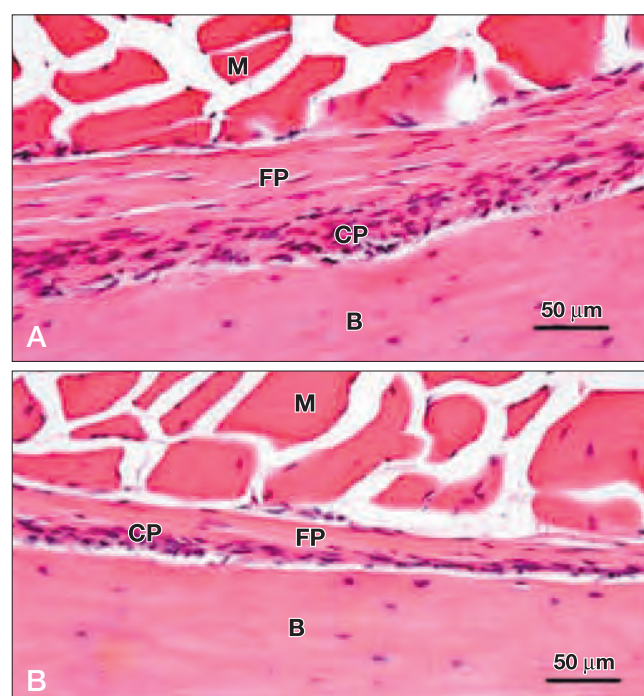
Figure 2. *Photomicrographs of periosteum from rat.*
A, The thickness of the periosteum varies along the length of the bone, being thicker closer to the metaphysis and B, thinner along the diaphysis. Stained with hematoxylin and eosin.
Abbreviations: B, bone; CP, cambium layer of periosteum; FP, fibrous periosteum; M, muscle.

periosteum. Periostin regulates cellular adhesion and recruitment,[32] and may be either a positive[33] or a negative[34] regulator of osteoblast differentiation. When its promoter binds to Twist, a transcription factor important in osteogenesis and also important in determination of cell type and cell differentiation, it prevents the conversion of preosteoblasts to fully differentiated osteoblasts capable of making bone.[35] Thus, upregulation of Twist and expression of periostin prevent intramembranous ossification and periosteal apposition of lamellar bone.

Periostin has a variety of isoforms, not all of which are localized to the same location or behave in like manner, making their role in the regulation of cellular differentiation and bone for-

mation a complex matter. One of these, periostin-like factor (PLF) has been detected during embryogenesis both in mesenchymal cells in the periosteum, and in osteoblasts along trabecular bone, a location where periostin protein itself is not found.[36] Moreover, PLF accelerates the differentiation of precursors into functioning osteoblasts, and promotes bone formation,[37] which may be different from the action of periostin itself.[34] Also, PLF is upregulated during fracture repair, whereas periostin seems to negatively regulate mineralization of the newly forming callus.[34] Thus, periostin likely prevents differentiation of osteoblasts and reduces bone formation, whereas its isoform PLF appears to promote differentiation and osteogenesis.

Bone fracture healing is commonly described to be divided into four stages: an inflammatory stage, during which a hematoma is formed and the initial molecular signals for repair are generated; periosteal woven bone formation, which bridges and stabilizes the fracture gap; cartilage formation and endochondral ossification; and finally, bone remodeling to return the bone to its original lamellar structure and external shape (Figure 3). The initial inflammatory stage incorporates a hematoma that extends into the periosteum and stimulates the proliferation of periosteal osteoprogenitors within the first two days. This may initially be under the stimulus of insulin-like growth factor I (IGF-I) and PTHrP and their receptors, which are sensitive to inflammatory mediators in the hematoma, and which are
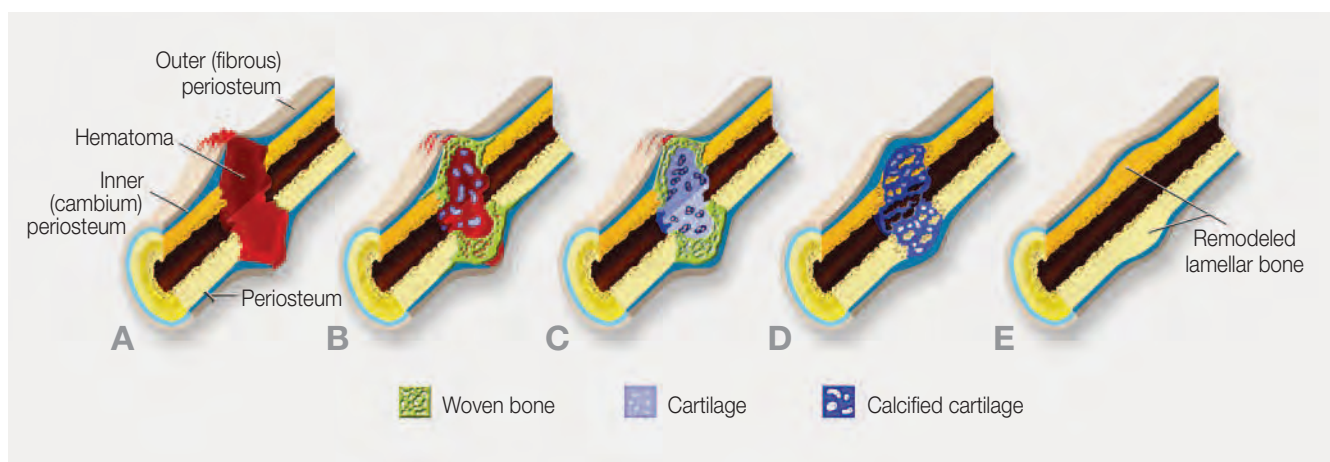
Figure 3. *Periosteal contributions to fracture healing.*
A, *Within hours after the fracture occurs, a hematoma develops between the fractured surfaces and within the marrow cavity. This hematoma will provide cells to reconstruct the damaged periosteum.* **B,** *Woven bone forms through intramembranous ossification and the periosteum begins to repair. The woven bone stabilizes the fracture so that pockets of cartilage can form, produced by chondroblasts that originate from mesenchymal stem cells provided by the periosteum.* **C,** *The fracture continues to heal through a process of endochondral ossification. Chondrocytes in the cartilage begin to hypertrophy, allowing calcification to begin.* **D,** *Cartilage continues to calcify, and is internally remodeled to form pockets of bone.* **E,** *The outer callus begins to remodel and reshape the bone. Cells for this process come from the vascularized periosteum and from bone-forming cells in the cambium layer. Thus, the periosteum is involved in all phases of fracture healing, providing cells for intramembranous ossification and woven bone, endochondral ossification, and finally for bone modeling and remodeling.*

Although the number of osteogenic cells in the cambium layer declines with age,[24] this seems to have little effect on its ability to respond to a mechanical stimulus. It is well known that periosteal apposition of bone continues throughout life, partially compensating for the loss of bone from other surfaces. In animal models, the significant reduction of cells in the cambium layer of the periosteum is associated with reduced chondrogenesis with age,[38] but the potential to heal a fracture is not known to diminish with age in humans, in the absence of other metabolic abnormalities.

The periosteal role in fracture repair

The periosteum plays a central and multifaceted role in the processes of fracture repair. The plethora of mesenchymal stem cells can differentiate into either osteoblasts or chondroblasts under the multiple molecular signals that are released during the initial inflammatory stage of repair.[39,40] The periosteum thereby participates in both the intramembranous bone formation and the processes of endochondral formation and ossification that occur during the healing process.[41]

upregulated in the cambium layer of the periosteum within 24 hours following the fracture.[27] By day 3, the proliferative response is at its peak, concurrent with expression of BMP-2,3,4,5,8, and noggin within the periosteum.[29] There is also an early (within 3 days) upregulation of periostin during fracture healing. Subsequently, committed progenitor cells from the periosteum migrate and differentiate to begin forming woven bone a few millimeters from the fracture site.[42,43] This process eventually creates a bridge between the two ends of the broken bone, and forms a cortical collar that will later be remodeled.

Recent studies using green fluorescent protein (GFP) reporter mice have shown the sequence of events leading to this intramembranous ossification, but it is still unclear whether the cells involved are only osteoprogenitor cells or also include pericytes and dedifferentiated lining cells.[43] Concurrently, or shortly after this, multipotent periosteal progenitor cells proliferate[26] and migrate to the fracture site, become chondrogenic, and begin the process of cartilage formation within the frac-

ture gap.[44] By days 4-7 after the fracture, osteoblasts adjacent to the fracture site in the subperiosteal region upregulate the expression of osteocalcin and osteonectin within the zone of intramembranous ossification. These periosteal cells also produce types I and V collagen, the latter possibly contributing to the assembly and orientation of the type I collagenous matrix. At the same time, both osteonectin and osteopontin are highly expressed at the junction between the intramembranous and endochondral ossification fronts, probably signaling ossification. By day 14, periosteal osteoprogenitor cells are no longer proliferating in the area of intramembranous ossification, but the process of ossification of cartilage in the fracture gap continues. Subsequently, the calcified cartilage within the gap and the woven bone adjacent to the fracture begin to remodel and reshape the bone. Thus the periosteum is involved in all phases of the fracture repair process, providing cells for both osteogenesis and chondrogenesis, participating in both the intramembranous and endochondral ossification processes, and ultimately reshaping the bone and reestablishing normal microstructure and material properties by modeling the external shape and regulating diaphyseal curvature.

Bone modeling, remodeling, and periosteal apposition

Although the periosteal membrane thins and becomes less cellular with age, it maintains the capability for apposition of new lamellar bone throughout life. Periosteum is highly mechanosensitive, and the pluripotent osteo- and chondroprogenitor cells that reside in it are more mechanically sensitive even than mesenchymal stem cells. Periosteal apposition occurs in both men and women as they age, although the amount of apposition that occurs in women is insufficient to offset the large losses of bone from trabecular and endocortical compartments, or to maintain premenopausal bone strength.

It was an adage for years that the periosteal surface of bone is immune to bone resorption or to coupled remodeling, except perhaps during modeling processes near the bony metaphysis during growth. Although it is true that for most of adult life, periosteal bone is more osteogenic than resorptive, remodeling involving resorption does occur on this surface, particularly in older people.[45] It is not difficult to find erosion cavities on the surface of the femur, for instance, in people in their 9th decade. This is likely part of the life-long adaptive process.

The periosteum is very responsive to a number of hormones, but often responds to them differently than do other bone envelopes (eg, endocortical, trabecular, and intracortical). During the period of growth and maturation, the periosteal surface of bone is particularly responsive to growth hormone (GH) and IGF-I, both of which promote appositional growth during development. However, the estrogens and androgens are also important influences on appositional growth both before and after puberty, and both are probably required for periosteal

expansion. Androgens stimulate periosteal apposition in both sexes, but low levels of estrogen increase the sensitivity of androgens on the periosteal surface, even in boys.[46,47] This may be the reason that aromatase-deficient boys with normal androgen levels have smaller bones. This interaction of estrogen and androgen on periosteal apposition may persist throughout life.[48]

It is well documented that postmenopausal estrogen deficiency is associated with periosteal apposition, and that estrogen supplementation reduces expansion,[49] although it is not clear whether this is a direct effect of estrogen or a mechanical compensation for the loss of bone on the endocortical surface. However, the picture is complicated by the presence of two estrogen receptor (ER) subtypes, ER-α and ER-β, that may be antagonistic. Some animal experiments suggest that interaction of estradiol with ER-α promotes periosteal expansion,[50] whereas ER-β inhibits periosteal apposition.[50,51]

Mice in which ER-α is inactive have thinner bones,[52] but this is not necessarily the case in animals in which ER-β is knocked out.[53] Whether this is a direct effect, or an indirect one involving coregulation with IGF-I is not clear, as ER-α knockout mice have lower IGF-I levels, whereas ER-β knockout mice have higher levels.[53] This has led some to speculate that the compensatory effects of IGF-I on GH may be more important than direct effects of estrogen on periosteal osteoblasts.

The idea that ER-β is a negative regulator of periosteal apposition is consistent with the observation that apposition is suppressed in estrogen-replete women and that this inhibition is removed in estrogen deficiency. However, the picture is complicated by the fact that in humans, unlike in mice, ER-α predominates over ER-β,[54] and so the antiapoptotic[55] and pro-osteogenic effects of ER-α are inconsistent with the observation of the normal premenopausal suppression of periosteal apposition in women, or the postmenopausal periosteal expansion. It is possible that the two receptors interact in ways that cause different effects when only one is present,[56] or that the relative importance of the receptors is gender-specific, with ER-α achieving greater effect in the male skeleton, but the presence of both being a requirement for apposition in the female skeleton.[57] There may also exist a more complex relationship in which receptor signaling depends on higher or lower threshold levels of estrogen.

Likewise, the responsiveness of cells in the periosteum to IGF-I may help to explain the stimulatory effect of parathyroid hormone (PTH) on periosteal apposition.[58,59] Intermittent delivery of the recombinant 1-34 fragment of human PTH (rhPTH [1-34]) is suspected to promote periosteal apposition, and its effect on bone strength has been partly explained by this phenomenon. PTH (1-34) is known to prevent apoptosis of periosteal osteoblasts,[60] which could partly account for its effect on the cells in the osteogenic layer of the periosteum. PTH sig-

naling also downregulates *Sost* expression in osteocytes,[61] which has been shown in mice with a constitutively active PTH receptor to increase periosteal bone formation. Sclerostin, the protein which the *Sost* gene encodes, downregulates bone formation through the Wnt pathway. The inhibition of *Sost* expression in osteocytes by PTH increases bone formation on the periosteal surface. This demonstrates that the osteogenic cells in the periosteum are in communication with cortical osteocytes, which help to regulate periosteal bone formation through a Wnt-dependent pathway.

Conclusion

The periosteal membrane provides cells for growth, development, maturation, adaptation, and repair of our bones throughout our entire life. It is an important target tissue that maintains our skeletal health and well-being by adapting to our changing developmental, hormonal, and mechanical needs over the many decades of our life. Although often overlooked, it is vital to our skeletal health. ■

References

1. Weiner S, Wagner HD. The material bone: structure mechanical function relations. *Ann Rev Mater Sci.* 1998;28:271-298.
2. Al-Qtaitat A, Shore RC, Aaron JE. Structural changes in the ageing periosteum using collagen III immuno-staining and chromium labelling as indicators. *J Musculoskelet Neuronal Interact.* 2010;10:112-123.
3. Turner RT, Colvard DS, Spelsberg TC. Measurement of mRNA levels in the periosteum of aging rats. *Cells Mater.* 1991;suppl 1:47-51.
4. Squier CA, Ghoneim S, Kremenak CR. Ultrastructure of the periosteum from membrane bone. *J Anat.* 1990;171:233-239.
5. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. *J Orthop Res.* 2001;19:95-103.
6. Ochareon P, Herring SW. Growing the mandible: role of the periosteum and its cells. *Anat Rec.* 2007;290:1366-1376.
7. Ueno T, Kagawa T, Mizukawa N, Nakamura H, Sugahara T, Yamamoto T. Cellular origin of endochondral ossification from grafted periosteum. *Anat Rec.* 2001;264:348-357.
8. Saino H, Luther F, Carter DH, et al. Evidence for an extensive collagen type III proximal domain in the rat femur. II. Expansion with exercise. *Bone.* 2003;32:660-668.
9. Luther F, Saino H, Carter DH, Aaron JE. Evidence for an extensive collagen type III/VI proximal domain in the rat femur. I. Diminution with ovariectomy. *Bone.* 2003;32:652-659.
10. Taylor JF. The periosteum and bone growth. In: Hall BK, ed. *Bone Growth VI.* Boca Raton: CRC Press. 1992:21-52.
11. Aaron JE, Skerry TM. Intramembranous trabecular generation in normal bone. *Bone Miner.* 1994;25:211-230.
12. Burgeson RE, Nimni ME. Collagen types. Molecular structure and tissue distribution. *Clin Orthop Relat Res.* 1992;282:250-272.
13. Diaz-Flores L, Gutierrez R, Lopez-Alonso A, Gonzalez R, Varela H. Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. *Clin Orthop Relat Res.* 1992;275:280-286.
14. Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL. Innervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. *Science.* 1986;232:868-871.
15. Ellender G, Feik SA, Carach BJ. Periosteal structure and development in rat caudal vertebra. *J Anat.* 1988;158:173-187.
16. Forriol F, Shapiro F. Bone development: interaction of molecular components and biophysical forces. *Clin Orthop Relat Res.* 2005;432:14-33.
17. Wilde GP, Baker GC. Circumferential periosteal release in the treatment of children with leg-length inequality. *J Bone Joint Surg Br.* 1987;69:817-821.
18. Hernandez JA, Serrano S, Mariñoso ML, et al. Bone growth and modeling changes induced by periosteal stripping in the rat. *Clin Orthop Relat Res.* 1995;320:211-219.
19. McBride SH, Evans SF, Knothe Tate ML. Anisotropic mechanical properties of ovine femoral periosteum and the effects of cryopreservation. *J Biomech.* 2011;44:1954-1959.
20. Foolen J, van Donkelaar CC, Murphy P, Huiskes R, Ito K. Residual periosteum tension is insufficient to directly modulate bone growth. *J Biomech.* 2009;42:152-157.
21. Foolen J, van Donkelaar CC, Ito K. Intracellular tension in periosteum/perichondrium cells regulates long bone growth. *J Orthop Res.* 2011;29:84-91.
22. Bertram JE, Polevoy Y, Cullinane DM. Mechanics of avian fibrous periosteum: tensile and adhesion properties. *Bone.* 1998;22:669-675.
23. Di Nino DL, Long F, Linsenmayer TF. Regulation of endochondral cartilage growth in the developing avian limb: cooperative involvement of perichondrium and periosteum. *Dev Biol.* 2001;240:433-442.

24. Simon TM, Van Sickle DC, Kunishima DH, Jackson DW. Cambium cell stimulation from surgical release of the periosteum. *J Orthop Res.* 2003;21:470-480.
25. Iwasaki M, Nakata K, Nakahara H, et al. Transforming growth factor-beta 1 stimulates chondrogenesis and inhibits osteogenesis in high density culture of periosteum-derived cells. *Endocrinology.* 1993;132:1603-1608.
26. Sanyal A, Sarkar G, Saris DB, Fitzsimmons JS, Bolander ME, O'Driscoll SW. Initial evidence for the involvement of bone morphogenetic protein-2 early during periosteal chondrogenesis. *J Orthop Res.* 1999;17:926-934.
27. Okazaki K, Jingushi S, Ikenoue T, Urabe K, Sakai H, Iwamoto Y. Expression of parathyroid hormone-related peptide and insulin-like growth factor I during rat fracture healing. *J Orthop Res.* 2003;21:511-520.
28. Ishidou Y, Kitajima I, Obama H, et al. Enhanced expression of type I receptors for bone morphogenetic proteins during bone formation. *J Bone Miner Res.* 1995;10:1651-1659.
29. Yu YY, Lieu S, Lu C, Miclau T, Marcucio RS, Colnot C. Immunolocalization of BMPs, BMP antagonists, receptors, and effectors during fracture repair. *Bone.* 2010;46:841-851.
30. Dieudonne SC, Foo P, van Zoelen EJ, Burger EH. Inhibiting and stimulating effects of TGF-beta 1 on osteoclastic bone resorption in fetal mouse bone organ cultures. *J Bone Miner Res.* 1991;6:479-487.
31. Blavier L, Delaisse JM. Matrix metalloproteinases are obligatory for the migration of preosteoclasts to the developing marrow cavity of primitive long bones. *J Cell Sci.* 1995;108:3649-3659.
32. Horiuchi K, Amizuka N, Takeshita S, et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res.* 1999;14:1239-1249.
33. Bonnet N, Standley KN, Bianchi EN, et al. The matricellular protein periostin is required for sost inhibition and the anabolic response to mechanical loading and physical activity. *J Biol Chem.* 2009;284:35939-35950.
34. Fuchs RK, Galley M, Doyle J, et al. The extracellular matrix protein periostin regulates periosteal apposition. *J Bone Miner Res.* 2008;23(suppl 1):S51. Abstract.
35. Oshima A, Tanabe H, Yan T, Lowe GN, Glackin CA, Kudo A. A novel mechanism for the regulation of osteoblast differentiation: transcription of periostin, a member of the fasciclin I family, is regulated by the bHLH transcription factor, Twist. *J Cell Biochem.* 2002;86:792-804.
36. Zhu S, Barbe MF, Amin N, et al. Immunolocalization of periostin-like factor and periostin during embryogenesis. *J Histochem Cytochem.* 2008;56:329-345.
37. Zhu S, Barbe MF, Liu C, et al. Periostin-like-factor in osteogenesis. *J Cell Physiol.* 2009;218:584-592.
38. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. *J Orthop Res.* 2001;19:95-103.
39. Nakahara H, Bruder SP, Haynesworth SE, et al. Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. *Bone.* 1990;11:181-188.
40. Tatsuyama K, Maezawa Y, Baba H, Imamura Y, Fukuda M. Expression of various growth factors for cell proliferation and cytodifferentiation during fracture repair of bone. *Eur J Histochem.* 2000;44:269-278.
41. Gruber R, Mayer C, Bobacz K, et al. Effects of cartilage-derived morphogenetic proteins and osteogenic protein-1 on osteochondrogenic differentiation of periosteum-derived cells. *Endocrinology.* 2001;142:2087-2094.
42. Einhorn TA. The cell and molecular biology of fracture healing. *Clin Orthop Relat Res.* 1998;355(suppl):S7-S21.
43. Ushiku C, Adams DJ, Jiang X, Wang L, Rowe DW. Long bone fracture repair in mice harboring GFP reporters for cells within the osteoblastic lineage. *J Orthop Res.* 2010;28:1338-1347.

44. Sandberg M, Aro H, Multimaki P, Aho H, Vuorio E. In situ localization of collagen production by chondrocytes and osteoblasts in fracture callus. *J Bone Joint Surg Am.* 1989;71:69-77.

45. Orwoll ES. Toward an expanded understanding of the role of the periosteum in skeletal health. *J Bone Miner Res.* 2003;18:949-954.

46. Bouillon R, Bex M, Vanderschueren D, Boonen S. Estrogens are essential for male pubertal periosteal bone expansion. *J Clin Endocrinol Metab.* 2004;89: 6025-6029.

47. Vanderschueren D, Venken K, Ophoff J, Rouillon R, Boonen S. Clinical review: sex steroids and the periosteum – reconsidering the roles of androgens and estrogens in periosteal expansion. *J Clin Endocrinol Metab.* 2006;91:378-382.

48. Khosla S, Melton LJ III, Riggs BL. Clinical Review 144: Estrogen and the male skeleton. *J Clin Endocrinol Metab.* 2002;87:1443-1450.

49. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. *Endocr Rev.* 1994;15:275-300.

50. Lee KC, Jessop H, Suswillo R, Zaman G, Lanyon LE. The adaptive response of bone to mechanical loading in female transgenic mice is deficient in the absence of oestrogen receptor-α and β. *J Endocrinol.* 2004;182:193-201.

51. Saxon LK, Robling AG, Castillo AB, Mohan S, Turner CH. The skeletal responsiveness to mechanical loading is enhanced in mice with a null mutation in estrogen receptor-β. *Am J Physiol Endocrinol Metab.* 2007;293:E484-E491.

52. Vidal O, Lindberg MK, Hollberg K, et al. Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. *Proc Natl Acad Sci U S A.* 2000;97:5474-5479.

53. Lindberg MK, Alatalo SL, Halleen JM, Mohan S, Gustafsson JA, Ohlsson C. Estrogen receptor specificity in the regulation of the skeleton in female mice. *J Endocrinol.* 2001;171:229-236.

54. Riggs BL, Khosla S, Melton LJ III. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev.* 2002;23:279-302.

55. Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: Dissociation from transcriptional activity. *Cell.* 2001;104:719-730.

56. Sims NA, Dupont S, Krust A, et al. Deletion of estrogen-receptors reveals a regulatory role for estrogen receptors-beta in bone remodeling in females but not in males. *Bone.* 2002;30:18-25.

57. Vico L, Vanacker JM. Sex hormones and their receptors in bone homeostasis: insights from genetically modified mouse models. *Osteoporos Int.* 2010;21: 365-372.

58. Parfitt AM. Parathyroid hormone and periosteal bone expansion. *J Bone Miner Res.* 2002;17:1741-1743.

59. Elis S, Courtland HW, Wu Y, et al. Elevated serum IGF-1 levels synergize PTH action on the skeleton only when the tissue IGF-1 axis is intact. *J Bone Miner Res.* 2010;25:2051-2058.

60. Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J Clin Invest.* 1999;104:439-446.

61. Rhee Y, Allen MR, Condon K, et al. PTH receptor signaling in osteocytes governs periosteal bone formation and intracortical remodeling. *J Bone Miner Res.* 2011;26:1035-1046.

PRESQUE INVISIBLE, SOUVENT IGNORÉ : LE PÉRIOSTE, LA DENTELLE VIVANTE DE L'OS

La membrane périostée est mince et fibreuse, et de sa couche profonde proviennent des cellules responsables de la croissance, du développement, du modelage/remodelage et de la réparation des fractures de nos os. Elle est largement vascularisée et innervée par des fibres sympathiques et nociceptives. Elle provient de la concentration du mésenchyme général pendant le développement fœtal et se situe dans la continuité des fibres de Sharpey qui s'insèrent dans l'os pour l'arrimer. La partie fibreuse est composée de plusieurs sortes de collagène ainsi que d'élastine. La composition cellulaire est diverse et comprend des cellules souches mésenchymateuses indifférenciées qui peuvent se différencier en fibroblastes, en chondrocytes ou ostéoblastes, ces dernières communiquant de façon importante avec les ostéocytes de l'os. Ces cellules sont sous contrôle local et sont très sensibles aux facteurs de croissance (par ex. au transforming growth factor beta*) et à plusieurs des protéines morphogéniques osseuses, aux stéroïdes sexuels (estrogènes et androgènes), aux hormones de croissance (par ex. l'hormone parathyroïdienne) et à d'autres protéines associées à la formation osseuse qui sont modulées par la voie Wnt (par ex. la sclérostine). Le périoste participe à la réparation fracturaire et fournit des cellules à la fois pour l'ossification intramembraneuse qui comble et stabilise la fracture, et pour le processus d'ossification endochondrale et de remodelage qui rétablit la capacité de charge de l'os. C'est un tissu multifonctionnel qui permet à nos os de s'adapter tout au long de notre vie aux différentes conditions pathologiques, hormonales et mécaniques.*

"Although the mature osteocyte is surrounded by a mineralized matrix and may therefore appear isolated from its neighboring cells both within the matrix and on the bone surface, these cells, in fact, display a high degree of connectivity. The cell processes, which lie within the narrow canaliculi, connect osteocytes to other osteocytes, osteoblasts, and lining cells via gap junctions. This unique morphology of the osteocyte allows for the passage of nutrients and biochemical signals from one cell to the next and, as such, forms a functional network of cells…"

The osteocyte: doing the hard work backstage

by M. Prideaux and L. F. Bonewald, *USA*

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Osteocytes are the most numerous and long-lived of all bone cells; however, relatively little is known about their function when compared with osteoblasts and osteoclasts. Although originating from osteoblast precursors, they display dramatic differences in morphology and gene expression, hence suggesting their functions differ from those of osteoblasts. So far, roles have been determined for the osteocyte in processes such as mechanotransduction and bone homeostasis, via modulation of osteoblast and osteoclast activity. In addition, the osteocyte network has been shown to act as part of an endocrine system, targeting organs such as the kidney and skeletal muscle. Expression of phosphate regulatory genes by osteocytes controls phosphate metabolism within and beyond bone and plays a key role in mineralization of the bone extracellular matrix. Osteocytes are also capable of expressing markers of bone resorption and can form new bone matrix, suggesting that they are capable of remodeling their microenvironment. Clearly, the osteocyte, far from being a passive cell trapped within the mineralized bone matrix plays a highly active and functional role in the maintenance of bone strength and viability.

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Although the biology and function of both osteoblasts and osteoclasts have been well documented, the osteocyte remains more of a mystery. For years, the study of osteocytes has been stymied by their location within the mineralized bone matrix and their relative inaccessibility, compared with the cells situated on the bone surface. In addition, their relative lack of abundance of cellular organelles such as the Golgi apparatus and endoplasmic reticulum,[1] when compared with osteoblasts and osteoclasts,[2] previously led to the assumption that these cells were metabolically inactive and of little importance during bone growth and development. The multiple roles of the osteocyte, both within and beyond the bone microenvironment are, however, starting to be revealed. The ability to delete genes specifically within osteocytes in animal models, coupled with the development of several osteocyte-like cell lines,[3-5] has generated increased interest in these once-forgotten cells. No longer merely considered as "placeholders" within the bone matrix, osteocytes have been shown to exhibit complex functions that are both numerous and vital in the development and maintenance of bone health. This review will focus on these functions in light of recent discoveries, which demonstrate the importance of the osteocyte as an orchestrator of bone modeling and remodeling and as part of an endocrine system, targeting organs outside of the bone environment.

Figure 1. *The process of osteoblast-to-osteocyte differentiation.*

(A) Tetrachrome staining of murine cortical bone. The osteoid seam is demonstrated with light blue staining and the mineralized bone is stained black with von Kossa. The stages of differentiation are described as follows: 1) A mature osteoblast on the surface of the osteoid. 2) An osteoid-osteocyte, which is embedded in the unmineralized osteoid. 3) A mineralizing osteocyte, which is partially surrounded by mineral. 4) A mineralizing osteocyte, completely surrounded by mineral. 5) A mature osteocyte, embedded deep within the mineralized extracellular matrix. (B) A schematic diagram showing the differentiation process outlined in (A) and the expression of known genes at each of these stages of differentiation. The numbers in brackets correspond to the numbers in (A).
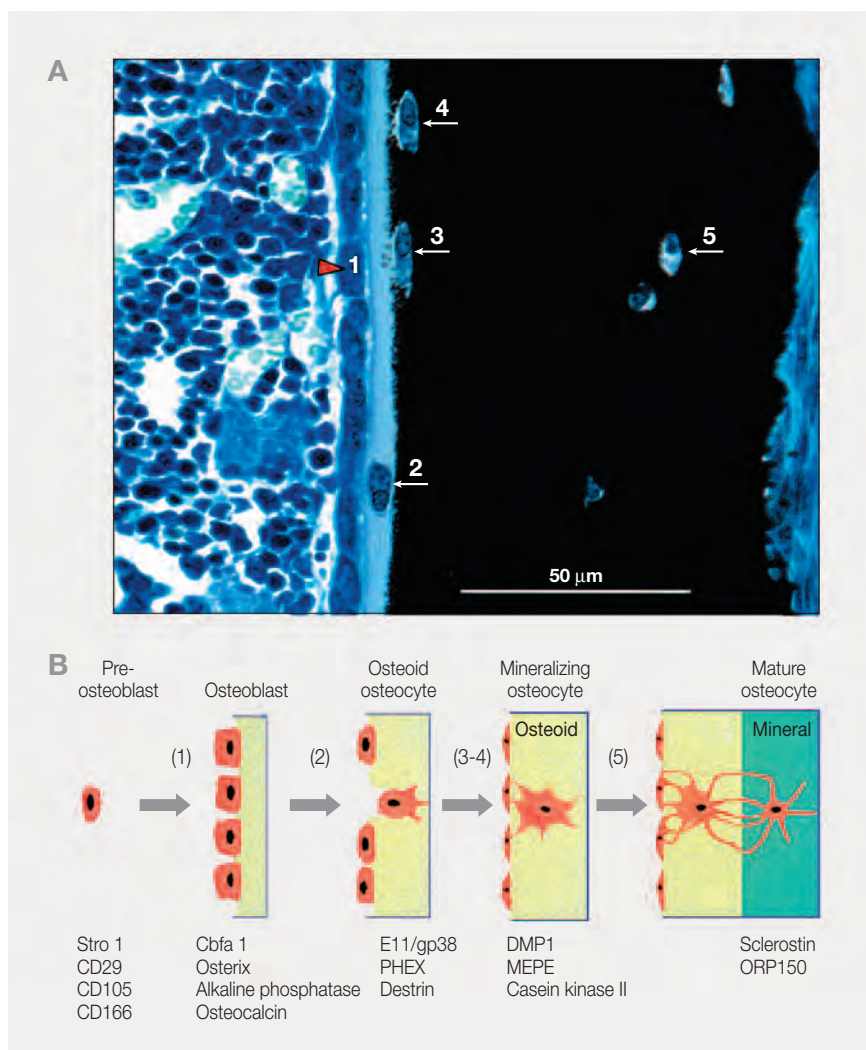
Osteocyte differentiation and morphology

While it has been known for decades that osteocytes are descended from terminally differentiated osteoblasts,[1] the mechanisms that govern this process of differentiation are still poorly understood. The morphological changes associated with this transition have, however, been well characterized[6] and are summarized in *Figure 1*. During differentiation, the osteoblast changes from a polygonal morphology toward a dendritic appearance, accompanied by the development and elongation of numerous cellular projections, or processes. Concurrently, there is a reduction in cell volume (of up to 70%) and cellular organelles as the cell becomes embedded within the bone

matrix. This embedding cell, termed an osteoid-osteocyte,[6] is responsible for mineralizing its surrounding extracellular matrix (ECM)[7] and exhibits polarity with regard to its process formation[6] as it further differentiates into a mature osteocyte. Osteocyte differentiation has commonly been regarded as a passive process, whereby an osteoblast slows its matri-synthesizing capacity and becomes buried by the matrix produced by its neighboring cells.[8] Research by others, however, has suggested that the embedding of an osteoid-osteocyte is an active process, as demonstrated by the requirement of collagenase activity for the formation of cell processes and the development of the lacunocanalicular system.[9,10] In particular, modification of the ECM by membrane type 1 matrix metalloproteinase (MT1-MMP) appears to be essential for development of the cell processes.[9] In addition, recent studies have demonstrated that osteocytes embedded within the bone have the capacity to extend their processes and indeed, are capable of forming new connections with neighboring osteocytes and osteoblasts.[11] This therefore suggests, that rather than simply being a static cell within the mineralized bone matrix, osteocytes are, in fact, highly dynamic.

SELECTED ABBREVIATIONS AND ACRONYMS

DKK	dickkopf-related protein
DMP1	dentin matrix protein 1
ECM	extracellular matrix
ERα	estrogen receptor α
FFSS	fluid flow shear stress
FGF23	fibroblast growth factor 23
GSK3-β	glycogen synthase kinase 3-β
HYP	hypophosphatemic
MEPE	matrix extracellular phosphoglycoprotein
NO	nitric oxide
OPG	osteoprotegerin
PGE$_2$	prostaglandin E$_2$
PHEX	phosphate-regulating endopeptidase homolog, X-linked
PTH	parathyroid hormone
RANKL	receptor activator of nuclear factor κB ligand
SFRP	secreted frizzled-related protein

Until recently, characterizing the molecular and genetic changes that an osteoblast undergoes as it differentiates into an osteocyte has proved challenging due to the lack of specific osteocyte marker genes. Such genes are, however, now being identified. The onset of expression of genes such as E11/gp38 *(E11),* fibroblast growth factor 23 *(FGF23),* and sclerostin *(SOST),* concurrent with the upregulation of dentin matrix protein 1 *(DMP1),* phosphate-regulating gene with homologies to endopeptidases on the X chromosome *(PHEX),* and matrix extracellular phosphoglycoprotein *(MEPE),* and the downregulation of alkaline phosphatase and type I collagen are all indicative of transition toward an osteocyte phenotype.[12] Such genes have functions ranging from the regulation of mineralization *(DMP1),* phosphate homeostasis *(PHEX, MEPE, FGF23),* and cytoskeletal arrangement and process development (E11/gp38), which will be discussed later in this review.

Although the mature osteocyte is surrounded by a mineralized matrix and may therefore appear isolated from its neighboring cells both within the matrix and on the bone surface, these cells, in fact, display a high degree of connectivity, as is demonstrated in *Figure 2.*

The cell processes, which lie within the narrow canaliculi, connect osteocytes to other osteocytes, osteoblasts, and lining cells via gap junctions.[6,13] This unique morphology of the osteocyte allows for the passage of nutrients and biochemical signals from one cell to the next and, as such, forms a functional network of cells, facilitating communication and maintaining cell viability.[14] Indeed, proper osteocyte function is dependent on a viable network of cells and disruption of this network can have devastating consequences for bone health.
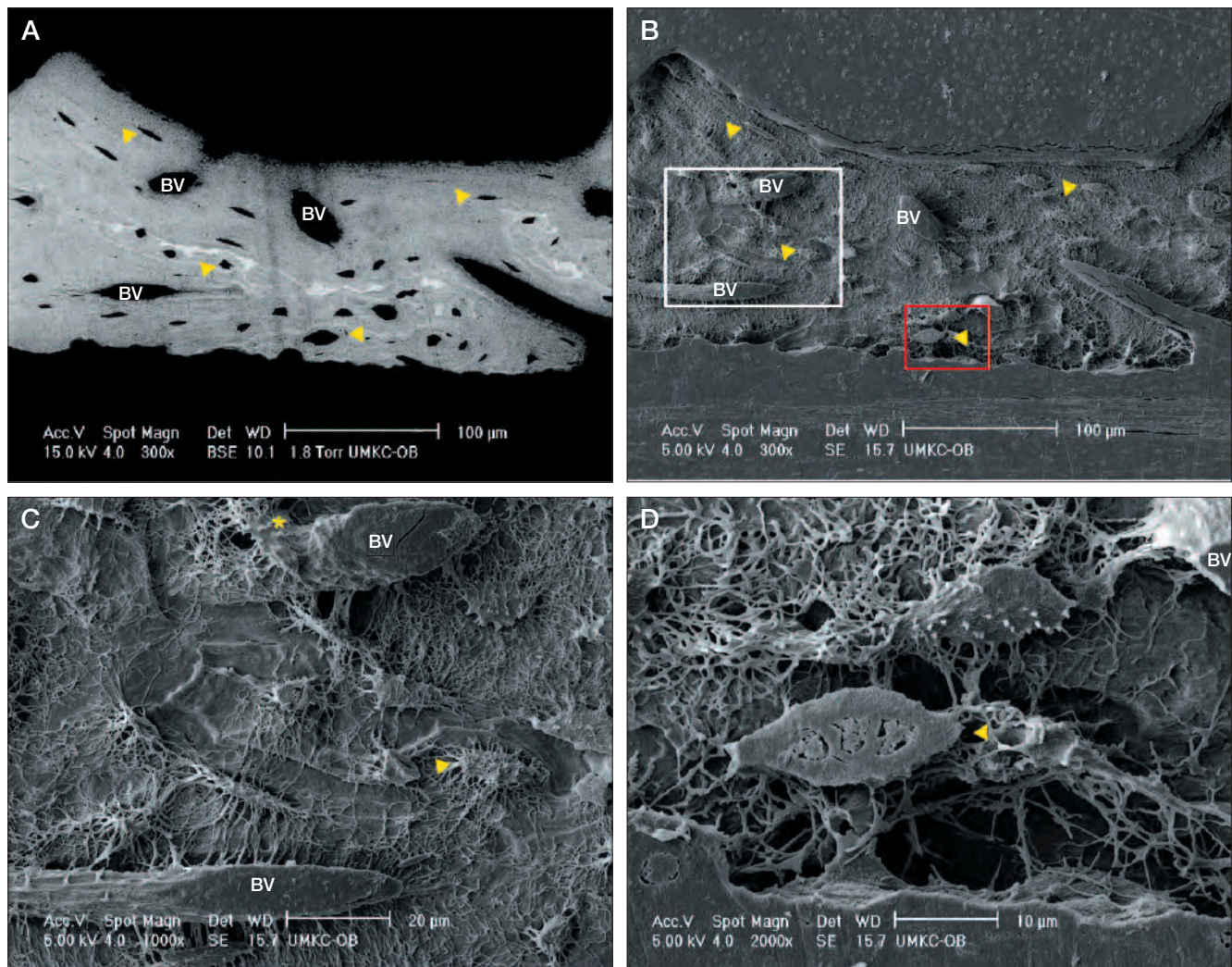
Figure 2. *Scanning electron microscopy of bone microstructure.*
(A) Scanning electron microscopy of mouse cortical bone, showing osteocyte lacunae (arrowheads) and blood vessels (BV). The osteocytes appear isolated from each other and the blood vessels. (B) Scanning electron microscopy of the same area of cortical bone after acid-etching of the bone surface. The same osteocyte lacunae are marked by arrowheads as in (A). (C) A higher-magnification image of the area outlined by a white box in (B). The degree of connectivity between the processes of the osteocytes with other osteocytes and the blood vessels is readily apparent. An osteocyte in close proximity to a blood vessel is marked by an asterisk. (D) A higher magnification image of the red box in (B) showing an occupied lacuna (arrowhead), connected to other osteocytes and the bone surface via numerous processes.
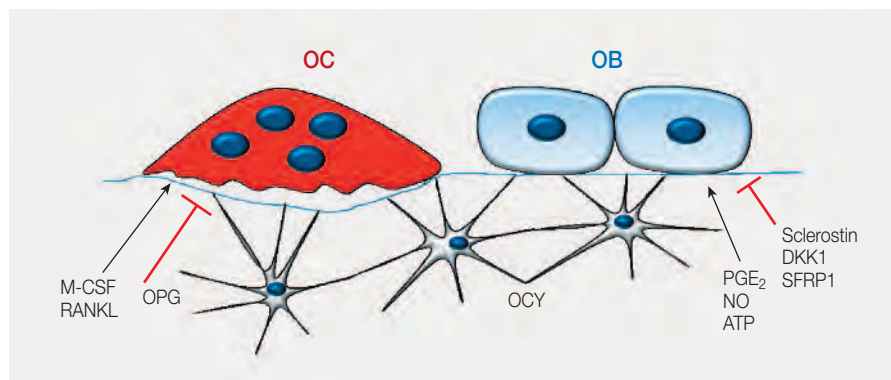
Figure 3. *Regulation of bone resorption and formation via osteocytes.*
Osteocyte (OCY) expression of RANKL and M-CSF promotes, whereas the expression of OPG inhibits, osteoclast (OC) activity and subsequent bone resorption. Osteocytes also secrete factors that activate Wnt/β-catenin signaling in osteoblasts (OB), such as PGE2, NO, and ATP, to promote bone formation. However, osteocytes also release factors such as sclerostin, DKKs, and SFRPs, which have an inhibitory effect on Wnt/β-catenin signaling and result in decreased osteoblast activity.

Osteocyte functions – regulators of bone modeling

As previously discussed, osteocytes are, via their processes, connected to osteoblasts on the bone surface. This connectivity suggests a role for the osteocyte in regulating osteoblast activity.[2] It has previously been demonstrated that conditioned media from MLO-Y4 osteocyte-like cells[15] and primary chick osteocytes[16] enhances early osteoblast differentiation and alkaline phosphatase activity. Osteocytes in vivo are also known to recruit mesenchymal stem cells to fracture sites, via secretion of osteopontin.[17] These data would suggest positive regulation of osteoblast activity via the osteocyte; however, it is the ability of these cells to negatively control bone formation that is receiving the most attention. The Wnt signaling pathway plays an important role in promoting early osteoblast differentiation and osteocytes express known inhibitors of the Wnt signaling pathway such as the dickkopf-related proteins (DKKs), secreted frizzled-related proteins (SFRPs), and sclerostin.[18] Inhibition of Wnt signaling either by direct binding to Wnt ligand (SFRP1), or binding of the coreceptor LRP5/6 (DKK1, sclerostin) results in phosphorylation of β-catenin by glycogen synthase kinase 3-β (GSK3-β) and subsequent degradation by the proteasome.[18] Therefore, β-catenin is unable to translocate to the nucleus and activate the transcription factors required for inducing osteoblast differentiation and subsequent bone formation. While all of these factors are known to target the Wnt pathway, it is the activity of sclerostin that is garnering the most interest. Since its discovery as the secreted protein product of the SOST gene, which was identified by its absence in patients suffering from the sclerosing bone disorders van Buchem disease and sclerosteosis,[19,20] sclerostin has emerged as one of the most important therapeutic targets for bone disorders. Inactivating mutations in the SOST gene leads to an increase in bone mass and resistance to fracture.[21] Using this knowledge, specific targeting of sclerostin catabolic activity using monoclonal antibodies has demonstrated beneficial effects in animal models and human trials.[22] Recent studies have also suggested that inhibiting sclerostin activity can aid with fracture healing[23] and that the anabolic effects observed with parathyroid hormone (PTH) treatment are mediated by down-regulation of SOST expression.[24,25]

In addition to regulation of osteoblast activity, there is increasing evidence to suggest a role for the osteocyte during osteoclastogenesis. The differentiation of a mature osteoclast requires binding of receptor activator of nuclear factor κB ligand (RANKL), found on the surfaces of cells of the osteoblast lineage, to its receptor, RANK, expressed by osteoclast precursors.[26] RANKL expression has been demonstrated in osteocytes, along with expression of the decoy receptor, osteoprotegerin (OPG).[27,28] The ratio of RANKL/OPG expression is responsible for regulation of osteoclast development and activity. Previous studies have demonstrated that MLO-Y4 osteocyte-like cells support the activation of osteoclasts in vitro and express RANKL and OPG.[28] However, others have suggested that osteocytes only induce osteoclast formation and activity when undergoing apoptosis.[29] Additionally, mice in which the diphtheria toxin receptor is conditionally expressed in osteocytes, display dramatic increases in osteoclast number and activity following osteocyte death after diphtheria toxin injection,[30] suggesting that osteocyte death induces osteoclastogenesis. Conversely, stimulation of osteocytes by mechanical loading has been shown to increase OPG expression and decrease osteoclastogenesis,[31] suggesting a dual role for osteocytes in the regulation of bone resorption. Interestingly, deletion of β-catenin specifically in osteocytes in mice using the DMP1-Cre system, resulted in significantly decreased OPG expression and enhanced osteoclast activity.[32] These mice were characterized by a dramatic reduction in both cancellous and cortical bone volume, with no effect on osteoblast or osteocyte number or viability. These results suggest an important role for the Wnt signaling pathway in osteocytes in the negative regulation of bone resorption. The importance of osteocyte signaling in both bone formation and resorption is summarized in *Figure 3*.

Mechanotransduction

The idea of a "mechanostat", a sensor of mechanical loading within the bone, was first proposed by Harold Frost in 1987.[33] This mechanostat would have the ability to sense deformation of the bone due to mechanical stress and regulate changes in bone mass, accordingly. The osteocyte, within the mineralized bone would appear to be ideally located to sense such changes in bone loading and, because of its unique cellular

morphology, be able to communicate such strains to osteo-blasts and osteoclasts. Indeed, osteocytes have been shown to respond to fluid flow shear stress (FFSS)[34] and membrane stretching,[35] two different mechanisms inducing deformation of the osteocyte dendrites or/and cell body in vitro. Ex vivo, it has been shown that deformation of the osteocyte lacunae and canaliculi occurs in response to mechanical loading of cortical bone,[36] and osteocyte predominant genes such as the DMP1, E11/gp38, MEPE, and sclerostin genes have been shown to be regulated by mechanical loading in vivo. Until recently, however, it was unknown how the osteocyte was able to sense this mechanical stress but recent studies have suggested that primary cilia, which play a mechanosensory role in many cell types, are known to be expressed by osteocytes.[37] Moreover, deletion of *Pkd1,* an integral component of the cilia signaling pathway, attenuated increases in bone mass observed in mechanically loaded mice,[38] suggesting the importance of the cilia in modulating the response of osteocytes to load.

The importance of the osteocyte processes in sensing strain induced by fluid flow was demonstrated in a recent study by Burra et al.[39] It was observed that disruption of the glycocalyx—the protective coating of glycoproteins secreted by the cell—of the processes diminished the ability of the osteocytes to sense and respond to FFSS. No such effect was observed when the glycocalyx of the cell body was similarly disturbed, suggesting that the processes, and not the cell body, are responsible for detecting mechanical strain.

Whichever way mechanical loading is sensed by osteocytes, it needs to be translated into biochemical signals to induce an appropriate biological response. The Wnt/β-catenin signaling pathway has been widely implicated in modulating the anabolic effects of mechanical loading and the catabolic effects of unloading (for review, see reference 18). In vivo loading of bone results in decreased production of sclerostin by osteocytes, promoting Wnt/β-catenin signaling, whereas unloading increases sclerostin expression. Regulation of bone mass by estrogen signaling has also been suggested, as translocation of estrogen receptor α (ERα) to the nucleus was observed in osteocytes in response to mechanical strain.[40] Such translocation was found to be necessary for transportation of β-catenin to the nucleus in osteoblasts,[41] suggesting cross-talk between ERα and the Wnt signaling pathway. This therefore indicates a mechanism for postmenopausal bone loss, whereby a decline in circulating estrogen levels leads to decreased expression of ERα and, subsequently, an attenuated response to mechanical loading via Wnt/β-catenin signaling.

It is also known that mechanical strain leads to the rapid release of factors such as prostaglandin E_2 (PGE$_2$)[35,36] and nitric oxide (NO).[34,35,42] Release of PGE$_2$ in response to mechanical strain has been demonstrated to be dependent on connexin 43 hemichannels,[43] with the opening of these hemichannels

essential for the passage of PGE$_2$ and other soluble factors between cells. PGE$_2$ has recently been shown to promote β-catenin nuclear translocation in osteocytes and this occurs via inactivation of GSK3-β,[43] suggesting a regulatory role for prostaglandin signaling on the Wnt/β-catenin pathway. NO is also believed to play a similar regulatory role, as inhibitors of NO synthase attenuate the stabilization of β-catenin observed after mechanical stimulation and prevent the activation of Wnt target genes.[44]

Phosphate homeostasis and matrix mineralization

The importance of the osteocyte in the regulation of phosphate homeostasis has been clearly demonstrated in diseases such as X-linked hypophosphatemic rickets (XLH) and autosomal dominant hypophosphatemic rickets (ADHR), in which defects in osteocyte-specific or predominant proteins result in decreased circulating levels of inorganic phosphate (P_i). FGF23, a phosphaturic hormone that is synthesized primarily by osteocytes, inhibits reabsorption (and therefore increases excretion) of phosphate by the kidney and prevents phosphate uptake in the intestine (for review, see reference 45). FGF23 expression can be regulated by diet, 1,25-dihydroxyvitamin D3 levels, and circulating PTH. In addition, other osteocyte-secreted proteins are known to regulate FGF23 activity and, as a consequence, serum Pi levels. Inactivating mutations in PHEX such as those observed in the hypophosphatemic (HYP) mouse model, result in increased circulating FGF23 levels and hypophosphatemia,[45,46] and deletion of FGF23 is able to reverse the HYP phenotype.[46] PHEX has been shown to co-localize with FGF23 in osteocytes,[45] although the mechanism by which PHEX regulates FGF23 remains to be fully elucidated.

MEPE is another osteocyte-secreted protein that is responsible for elevating FGF23 levels. MEPE is not known, however, to act on FGF23 directly but instead induces hypophosphatemia via inhibition of PHEX enzymatic activity.[45] Cleavage of MEPE by cathepsin B releases the ASARM peptide, which, in addition to antagonizing PHEX, can bind directly to hydroxyapatite to inhibit mineralization.[47] DMP1 is another small integrin-binding ligand N-linked glycoprotein (SIBLING) that is produced by osteocytes and regulates P_i upstream of FGF23.[46,48] *Dmp1*-null mice show increased FGF23 levels in osteocytes, decreased serum P_i levels, and have an osteomalacic phenotype.[48] In addition, these mice display defective ECM mineralization around the osteocyte lacunae and impaired osteoblast-to-osteocyte differentiation.[48]

These data suggest the importance of DMP1, not only in P_i homeostasis but also in ECM mineralization and indicate a role for the early osteocyte in mineralizing its surrounding matrix. In addition, preosteocytes in vitro and in vivo have been shown to initiate mineralization by depositing calcospherulites along collagen fibrils,[7] and mineralization of primary osteoblasts cultured in vitro was found to be associated with cells

that were expressing osteocyte markers.[11] All in all, these results suggest both localized and endocrine roles for the osteocyte in regulating phosphate homeostasis.

Microenvironment remodeling by osteocytes

Much debate has occurred regarding the ability of an osteocyte to resorb bone from its perilacunar surface and therefore remodel its surrounding microenvironment. Initially suggested as a mechanism for transiently increasing the bioavailability of calcium,[49] "osteocytic osteolysis" has been reported in response to space flight in rats,[50] and PTH administration in rats induced an increase in osteocyte organelle number and activity, concomitant with osteolysis.[51] Others, however, have denied such activity, claiming that osteocytes do not have the capacity to remodel their lacunae.[52]

Improved histomorphometric analysis, combined with the advent of molecular biology techniques has enabled further investigation into this area and evidence is growing to suggest the ability of the osteocytes to remodel their surrounding matrix. Continuous administration of PTH was found to induce osteocyte expression of acid phosphatase and increase osteocyte lacunar area.[53] In addition, increases in lacunar size and expression of osteoclast marker genes such as acid phosphatase and cathepsin K were observed in lactating rats.[54] These results suggest a mechanism whereby supplemental sources of calcium can be utilized by osteocytes during periods when excess calcium is required.

In addition to the removal of their lacunar matrix, osteocytes have also been demonstrated to synthesize new bone matrix. Tetracycline labeling has been observed around osteocyte lacunae and canaliculi in response to decreased PTH[55] and deposition of new matrix was observed in the osteocyte lacunae of egg-laying hens.[56] The participation of osteocytes in such remodeling is a further indication of the activity of these cells. Unlike the substantial bone resorbing capabilities of the osteoclast, the role of the osteocyte in such remodeling is likely to enable the transient mobilization of factors and minerals stored within the ECM. The lacunocanalicular system would allow the rapid transport of such factors to their required targets. It has long been known that many bioactive proteins and minerals are stored within the ECM. The osteocytes may therefore act as the "gatekeepers" for these factors, allowing access to them when required.

Perspective

For a cell that was once considered to act as little more than a "placeholder" within the bone matrix, the diverse functions of the osteocyte demonstrate a dynamic, active role for this cell, both within and outside the bone environment. Recent research has identified novel functions of the osteocyte, such as control of phosphate homeostasis and osteoclastogenesis, while confirming long-held hypotheses such as osteocytic osteolysis and the ability to sense mechanical strain.

However, these studies may only be the "tip of the iceberg" as regard osteocyte activity. Recently, it has been shown that osteocytes may regulate the differentiation of skeletal muscle cells by the release of soluble factors[57] and that factors released by muscle cells may influence osteocyte activity and viability,[58] suggesting cross-talk between bone and muscle. In addition, the expression of osteocyte-specific marker genes have been observed in calcified regions of the aorta, suggesting that differentiation of vascular smooth muscle cells toward an osteocyte phenotype may promote pathological calcification.[59]

Although, clearly, there is still much to learn about the osteocyte, it is becoming apparent that it shares equal importance with its more illustrious neighboring bone cells. Future therapeutics, rather than directly targeting osteoblast or osteoclast activity, could instead be directed against osteocytes to regulate bone mass. Indeed, targeting of sclerostin activity using such treatments has already proved successful. It is, therefore, time for the osteocyte to move from the back of the stage to the center and share the limelight that its crucial role deserves. ■

References

1. Jande SS, Belanger LF. Electron microscopy of osteocytes and the pericellular matrix in rat trabecular bone. *Calcif Tissue Res.* 1971;6:280-289.
2. Dudley HR, Spiro D. The fine structure of bone cells. *J Biophys Biochem Cyto.* 1961;11:627-649.
3. Kato Y, Windle JJ, Koop BA, Mundy GR, Bonewald LF. Establishment of an osteocyte-like cell line, MLO-Y4. *J Bone Miner Res.* 1997;12:2014-2023.
4. Kato Y, Boskey A, Spevak L, Dallas M, Hori M, Bonewald LF. Establishment of an osteoid preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. *J Bone Miner Res.* 2001;16:1622-1633.
5. Woo SM, Rosser J, Dusevich V, Kalajzic I, Bonewald LF. Cell line IDG-SW3 replicates osteoblast-to-late-osteocyte differentiation in vitro and accelerates bone formation in vivo. *J Bone Miner Res.* 2011;26:2634-2646.
6. Palumbo C, Palazzini S, Zaffe D, Marotti G. Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes. *Acta Anat (Basel).* 1990;137:350-358.
7. Barragan-Adjemian C, Nicolella D, Dusevich V, Dallas MR, Eick JD, Bonewald LF. Mechanism by which MLO-A5 late osteoblasts/early osteocytes mineral-ize in culture: similarities with mineralization of lamellar bone. *Calcif Tissue Int.* 2006;79:340-353.
8. Nefussi JR, Sautier JM, Nicolas V, Forest N. How osteoblasts become osteocytes: a decreasing matrix forming process. *J Biol Buccale.* 1991;19:75-82.
9. Holmbeck K, Bianco P, Pidoux I, et al. The metalloproteinase MT1-MMP is required for normal development and maintenance of osteocyte processes in bone. *J Cell Sci.* 2005;118:147-156.
10. Zhao W, Byrne MH, Wang Y, Krane SM. Osteocyte and osteoblast apoptosis and excessive bone deposition accompany failure of collagenase cleavage of collagen. *J Clin Invest.* 2000;106:941-949.
11. Dallas, SL, Vena PA, Rosser JL, et al. Time lapse imaging techniques for comparison of mineralization dynamics in primary murine osteoblasts and the late osteoblast/early osteocyte-like cell line MLO-A5. *Cells Tissues Organs.* 2009; 189:6-11.
12. Franz-Odendaal TA, Hall BK, Witten PE. Buried alive: how osteoblasts become osteocytes. *Dev Dyn.* 2006;235:176-190.
13. Bonewald LF. Osteocytes: a proposed multifunctional bone cell. *J Musculos-*

kelet Neuronal Interact. 2002;2:239-241.

14. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. *Int J Biochem Cell Biol.* 2004;36:1-8.

15. Heino TJ, Hentunen TA, Vaananen HK. Osteocytes inhibit osteoclastic bone resorption through transforming growth factor-beta: enhancement by estrogen. *J Cell Biochem.* 2002;85:185-197.

16. Vezeridis PS, Semeins CM, Chen Q, Klein-Nulend J. Osteocytes subjected to pulsating fluid flow regulate osteoblast proliferation and differentiation. *Biochem Biophys Res Commun.* 2006;348:1082-1088.

17. Raheja LF, Genetos DC, Yellowley CE. Hypoxic osteocytes recruit human MSCs through an OPN/CD44-mediated pathway. *Biochem Biophys Res Commun.* 2008;366:1061-1066.

18. Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. *Bone.* 2008;42:606-615.

19. Balemans W, Ebeling M, Patel N et al. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet.* 2001;10:537-543.

20. Brunkow ME, Gardner JC, Van Ness J, et al. Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. *Am J Hum Genet.* 2001;68:577-589.

21. Li X, Ominsky MS, Niu QT, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner Res.* 2008;23:860-869.

22. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J Bone Miner Res.* 2011;26:19-26.

23. Ominsky MS, Li C, Li X, et al. Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. *J Bone Miner Res.* 2011;26:1012-1021.

24. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone.* 2005;37:148-158.

25. O'Brien CA, Plotkin LI, Galli C, et al. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PLoS One.* 2008;3:e2942.

26. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev.* 2000;21:115-137.

27. Zhao S, Zhang YK, Harris S, Ahuja SS, Bonewald LF. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. *J Bone Miner Res.* 2002;17:2068-2079.

28. Silvestrini G, Ballanti P, Patacchioli F, et al. Detection of osteoprotegerin (OPG) and its ligand (RANKL) mRNA and protein in femur and tibia of the rat. *J Mol Histol.* 2005;36:59-67.

29. Gu G, Mulari M, Peng Z, Hentunen TA, Vaananen HK. Death of osteocytes turns off the inhibition of osteoclasts and triggers local bone resorption. *Biochem Biophys Res Commun.* 2005;335:1095-1101.

30. Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab.* 2007;5:464-475.

31. You L, Temiyasathit S, Lee P, et al. Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading. *Bone.* 2008;42:172-179.

32. Kramer I, Halleux C, Keller H, et al. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol.* 2010;30:3071-3085.

33. Frost H M. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner.* 1987;2:73-85.

34. Klein-Nulend J, Semeins CM, Ajubi NE, Nijweide PH, Burger EH. Pulsating fluid flow increases nitric oxide (NO) synthesis by osteocytes but not periosteal fibroblasts—correlation with prostaglandin upregulation. *Biochem Biophys Res Commun.* 1995;217:640-648.

35. Pitsillides AA, Rawlinson SC, Suswillo FR, Bourrin S, Zaman G, Lanyon LE. Mechanical strain-induced NO production by bone cells: a possible role in adaptive bone (re)modeling? *FASEB J.* 1995;9:1614-1622.

36. Bonivtch AR, Bonewald LF, Nicolella DP. Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis. *J Biomech.* 2007;40:2199-2206.

37. Xiao Z, Zhang S, Mahlios J, et al. Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and Runx2 expression. *J Biol Chem.* 2006;281:30884-30895.

38. Xiao Z, Dallas M, Qiu N, et al. Conditional deletion of Pkd1 in osteocytes disrupts skeletal mechanosensing in mice. *FASEB J.* 2011;25:2418-2432.

39. Burra S, Nicolella DP, Jiang JX. Dark horse in osteocyte biology: Glycocalyx around the dendrites is critical for osteocyte mechanosensing. *Commun Integr Biol.* 2011;4:48-50.

40. Zaman G, Jessop HL, Muzylak M, et al. Osteocytes use estrogen receptor alpha to respond to strain but their ERalpha content is regulated by estrogen. *J Bone Miner Res.* 2006;21:1297-1306.

41. Armstrong VJ, Muzylak M, Sunters A, et al. Wnt/beta-catenin signaling is a component of osteoblastic bone cell early responses to load-bearing and requires estrogen receptor alpha. *J Biol Chem.* 2007;282:20715-20727.

42. McGarry JG, Klein-Nulend J, Prendergast PJ. The effect of cytoskeletal disruption on pulsatile fluid flow-induced nitric oxide and prostaglandin E2 release in osteocytes and osteoblasts. *Biochem Biophys Res Commun.* 2005;330:341-348.

43. Xia X, Batra N, Shi Q, Bonewald LF, Sprague E, Jiang JX. Prostaglandin promotion of osteocyte gap junction function through transcriptional regulation of connexin 43 by glycogen synthase kinase 3/beta-catenin signaling. *Mol Cell Biol.* 2010;30:206-219.

44. Santos A, Bakker AD, Zandieh-Doulabi B, de Blieck-Hogervorst JM, Klein-Nulend J. Early activation of the beta-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. *Biochem Biophys Res Commun.* 2010;391:364-369.

45. Liu S, Quarles LD. How fibroblast growth factor 23 works. *J Am Soc Nephrol.* 2007;18:1637-1647.

46. Liu S, Tang W, Fang J, et al. Novel regulators of Fgf23 expression and mineralization in Hyp bone. *Mol Endocrinol.* 2009;23:1505-1518.

47. Rowe PS, Matsumoto N, Jo OD, et al. Correction of the mineralization defect in hyp mice treated with protease inhibitors CA074 and pepstatin. *Bone.* 2006;39:773-786.

48. Feng JQ, Ward LM, Liu S, et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet.* 2006;38:1310-1315.

49. Belanger LF. Osteocytic osteolysis. *Calcif Tissue Res.* 1969;4:1-12.

50. Foldes I, Rapcsak M, Szilagyi T, Oganov VS. Effects of space flight on bone formation and resorption. *Acta Physiol Hung.* 1990;75:271-285.

51. Krempien B, Friedrich E, Ritz E. Effect of PTH on osteocyte ultrastructure. *Adv Exp Med Biol.* 1978;103:437-450.

52. Mercer RR, Crenshaw MA. The role of osteocytes in bone resorption during lactation: morphometric observations. *Bone.* 1985;6:269-274.

53. Tazawa K, Hoshi K, Kawamoto S, Tanaka M, Ejiri S, Ozawa H. Osteocytic osteolysis observed in rats to which parathyroid hormone was continuously administered. *J Bone Miner Metab.* 2004;22:524-529.

54. Qing H, Pajevic PD, Barry K, Dusevich V, Wysolmerski J, Bonewald LF. PTHR1 in osteocytes plays a major role in perilacunar remodeling through the activation of osteoclastic genes in osteocytes. *J Bone Miner Res.* 2010;25(suppl 1):S25.

55. Yajima A, Inaba M, Tominaga Y, Nishizawa Y, Ikeda K, Ito A. Increased osteocyte death and mineralization inside bone after parathyroidectomy in patients with secondary hyperparathyroidism. *J Bone Miner Res.* 2010;25:2374-2381.

56. Zambonin Zallone A, Teti A, Primavera MV, Pace G. Mature osteocytes behaviour in a repletion period: the occurrence of osteoplastic activity. *Basic Appl Histochem.* 1983;27:191-204.

57. Lara N, Hall T, Touchberry C, et al. Bone-muscle crosstalk is demonstrated by morphological and functional changes in skeletal and cardiac muscle cells in response to factors produced by MLO-Y4 osteocytes. *J Bone Miner Res.* 2010;25(suppl 1):S112.

58. Lara N, Hall T, Brotto L, et al. Muscle derived factors influence the MLO-Y4 osteocyte response to shear stress. *J Bone Miner Res.* 2010;25(suppl 1):S99-S100.

59. Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE. The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PLoS One.* 2011;6:e19595.

Keywords: bone; mechanotransduction; mineralization; osteocyte; phosphate

L'OSTÉOCYTE FAIT LE TRAVAIL DIFFICILE EN COULISSES

Les ostéocytes sont les cellules les plus nombreuses et celles qui vivent le plus longtemps parmi toutes les cellules osseuses ; cependant, nous savons peu de choses sur leur fonction par rapport aux ostéoblastes et aux ostéoclastes. Bien qu'ayant pour origine les précurseurs des ostéoblastes, les différences considérables observées dans leur morphologie et l'expression de leurs gènes suggèrent que leurs fonctions diffèrent de celles des ostéoblastes. Nos connaissances actuelles portent sur les processus apparentés à la mécanotransduction et à l'homéostasie osseuse, par l'intermédiaire de la modulation de l'activité des ostéoblastes et des ostéoclastes. De plus, le réseau des ostéo-cytes semble agir comme un système endocrine, en prenant pour cible des organes comme le rein et le muscle squelettique. L'expression des gènes régulateurs des phosphates par les ostéocytes contrôle le métabolisme des phosphates à l'intérieur et au-delà de l'os et joue un rôle clé dans la minéralisation de la matrice extracellulaire de l'os. Les ostéocytes sont aussi capables d'exprimer des marqueurs de la résorption osseuse et peuvent former une nouvelle matrice osseuse, ce qui suggère qu'ils peuvent remodeler leur microenvironnement. Clairement, l'ostéo-cyte, loin d'être une cellule passive bloquée dans la matrice osseuse minéralisée, joue un rôle extrêmement actif et fonctionnel dans le maintien de la viabilité et de la solidité osseuses.

How did Gallo-Roman physicians treat their patients?

A look into the earliest pharmacopoeias of France

D. Gourevitch, *France*

Medicine in Roman Antiquity.
Fresco from the "House of Siricus"
(VII.1.47) in Pompeii.

Depicted is a scene from the poet Virgil in the Aeneid: the injured Trojan Aeneas, accompanied by Menestheus and Achates, leans on the shoulder of his weeping son, Ascanius, while his mother, Aphrodite, looks on and the surgeon Iapix removes an arrowhead from his thigh. Naples Archeological Museum. © Bridgeman Art Library.

Lutetia, the Gallo-Roman ancestor of Paris

G. Coulon, *France*

Map of Lutetia in the 4th century AD.
Watercolor by Jean-Claude Golvin.
(Detail)
© Éditions Errance, Paris.

The inventorying of plants used by Gallo-Roman physicians dispels the myth of a medical practice indifferent to the pain suffered by the patient. Whereas medical practitioners did not routinely seek the reversible abolition of sensitivity to disease-related and surgical pain (essentially because of the influence of Stoic doctrine), they did not ignore it either, witness the widespread use of the famous triad of poppy, henbane, and mandrake, well-known for their painkilling properties.

How did Gallo-Roman physicians treat their patients? A look into the earliest pharmacopoeias of France

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Danielle Gourevitch, Chevalier de la Légion d'Honneur *(Knight of the National Order of the Legion of Honor); Professor of Grammar; Graduate of the* École Normale Supérieure; *Past Member of the* École Française de Rome; *Emeritus Director of the* École Pratique des Hautes Études; *Member of the Institute for Advanced Study (IAS) in Princeton. Specialist of Greek and Roman history of medicine, with studies based on textual accounts, archeological finds, and human remains (paleopathology). Author of more than 300 articles and 15 books and exhibition catalogs. Main publications (in French): Critical edition of a gynecological treatise by Soranus of Ephesus, a Greek physician of the 1st/2nd century AD, who practiced medicine in Alexandria and Rome, in 4 volumes;* The Hippocratic Triangle in the Greco-Roman World: The Patient, his Disease, and his Physician; Women as Patients: Women and Medicine in Ancient Rome; Diseases in Art in Antiquity – Galen's Young Patients: Pathocenosis of the Roman Empire (in Italian); The Daily Life of Women in Ancient Rome. *Most recent book (2011):* Archeology of Roman Medicine. Women in Medicine, *by V. Boudon-Millot, V. Dasen, and B. Maire, was published as a homage to Danielle Gourevitch (De Boccard Editions, Paris 2008).*

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Traditional study of the pharmacopoeia of Roman antiquity, in Gaul as in the rest of the Empire, was long based solely on textual accounts, mostly medical and magical, sometimes historical, rarely epigraphic. The rise of new forms of archaeology (rescue, preventive, underwater, etc) has focused attention on subjects hitherto uncharted or misconstrued: the chemistry of dry collyria from Lyon (La Favorite necropolis), the petrographic analysis of collyrium stamps, which were particularly frequent in Gaul, comparison between these stamps and their collyria, botanical investigation of carbonized plants at medical sites (particularly in Switzerland and Germany), examination of the contents of shipwrecks or burned out medical premises (the Surgeon's House in Rimini), chemical analysis of the contents of terracotta ware and glassware (notably in France, Germany, and Belgium), chance discoveries like that in London of an intact pyxis containing a skin cream, scientific investigation of the preparation of ancient remedies of the Roman era, application of today's pharmaceutical formulation (simple or compound drugs) to ancient remedies, study of medicinal clays (Lemnian earth), virtual object displays, and the organization of archeological exhibitions and colloquia. All these methodological novelties in a way created a new historical material—ancient remedies—which were especially present in the Gallo-Roman, Germanic and Romano-British worlds.

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Medicine in Roman Antiquity. *Fresco from the "House of Siricus" (VII.1.47) in Pompeii. Depicted is a scene from the poet Virgil in the* Aeneid: *the injured Trojan Aeneas, accompanied by Menestheus and Achates, leans on the shoulder of his weeping son, Ascanius, while his mother, Aphrodite, looks on and the surgeon Iapix removes an arrowhead from his thigh. Naples Archeological Museum.*
© Bridgeman Art Library.

MEMPHI   GLEGORI

Oculist examining a patient. *2nd-century AD relief. Museo della Civiltà Romana, Rome.*
© Giraudon/Bridgeman Art Library.

The Gallo-Roman world

Gallo-Roman is a notion both geographical and cultural, like Romano-British, its counterpart for the British Isles. "Gallo" derives from *Gallia,* the Latin name for Gaul. At the end of the Roman Republic, when Julius Caesar conquered vast swathes of land beyond the Alps, following his victory over Vercingetorix at the famous battle of Alesia in 52 BC, he recounted his exploits in his *Commentaries on the Gallic War,* a paean to his own glory and to the glory of Rome. The regions conquered, from the Alps to Brittany and the Rhine, were soon transformed into an administrative circumscription bearing the name of *provincia: Gallia,* covering present-day France—minus Narbonese Gaul, which was already colonized—and a large part of Belgium and Switzerland. Under Caesar Augustus, the first *princeps,* the Empire followed the Republic and boldly organized administrative and religious bodies and built roads: in the Rhône Valley, an essential trading route since prehistoric times, at the confluence of the Saône and Rhône rivers, *Lugdunum* (modern-day Lyon), founded as a Roman town in 43 BC, became the capital of the Gauls in 27 BC, a vital crossroads and river route.

Diseases and physicians

This new civilization of free movement had all manner of effects. As for contagious diseases, it changed their epidemiology. The most striking example is the spread of the plague known as the "Antonine Plague" or "Plague of Galen," in fact likely smallpox,* the dissemination of which was hastened by the return of troops from battlefields in Mesopotamia to their bases, notably various posts on the *Limes Germanicus,* the frontier fortifications dividing the Roman Empire and the unsubdued Germanic tribes. But Romanization happened more or less spontaneously and deeply depending on the region, with armies stationed at frontiers playing a major role in this acculturation, which in daily life was most manifest in private religious practice, eating habits, and medicine.

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*Mirko Grmek (1924-2000), a French scientist of Croatian origin and one of the founders of the discipline of history of medicine would have described this as a change in "pathocenosis," a term he coined and which refers to the coexistence of all diseases in a specific time, place, and society.

Medicine box. *Roman ivory box with six compartments, originally for keeping pills and salves, dated ca 400 AD, with sliding lid featuring Asclepius holding a book in his left hand, and a serpent-entwined staff in his right hand.*
The box was discovered in 1943 in the sepulchrum of the main altar of the Cathedral of Chur, Switzerland, where it served as a reliquary, and is now part of the Churer Domschatz (Treasure of the Cathedral of Chur). Photo © Rätisches Museum Chur/Courtesy of Kathedralstiftung der Diözese Chur (Cathedral Foundation of the Diocese of Chur, Switzerland).

In Roman Britain, at Poundbury, a doctor pulled off the remarkable feat of performing an embryotomy that seems not to have killed the mother; at Vindolenda, in Northern Britain, a report shrewdly distinguishes among the unfit for daily duties the three categories of the sick, the wounded, and *lippientes,* ie, those suffering from a contagious eye-disease (the exact nature of which is unknown). *Oculari,* Roman oculists or eye-specialists, traveled throughout Gaul, while divinities, specialized or not in the treatment of diseases, received from grateful patients votive offerings, often anatomic in nature, the wording clearly expressing their motivation: *votum solvit libens merito,* abbreviated to V.S.L.M. and meaning "willingly and deservedly fulfilled his vow." Some of the best-known examples in Gaul have been found at temples at the sources of the River Seine in Burgundy (Dijon Museum), Chamalières near Clermont-Ferrand, and in the Halatte Forest (Senlis Museum).

Whereas military doctors were recruited taking certain precautions and subject to special requirements, civilian doctors were self-proclaimed and had no diploma. Some were well trained, thanks to their own self-imposed professional and philosophical standards, and in their youth had had the financial wherewithal to spend extended periods in the great centers of learning under the Empire (Alexandria in Egypt, and Pergamon in Asia Minor, notably). Others, whether honest physicians or quacks, were self-taught, and in the best cases had studied with a master.

Many, too many, practiced in the large towns, including Rome where the competition was frantic and cut-throat, while in the country physicians were few and far between, whence peripatetic medical practitioners, as witnessed by the stamps, discovered all over that they used to authenticate remedies, notably collyria.

The pharmacopoeia of ancient Gaul

The pharmacopoeia of Roman antiquity, in Gaul as in the rest of the Empire, was long studied using only textual sources, above all medical and magical, at times historical, seldom epigraphic. The emergence of

new forms of archaeology (rescue, preventive, underwater, etc) has shifted attention to subjects previously uncharted or misconstrued: the chemistry of dry collyria from Lyon (La Favorite), petrographic analysis of collyrium stamps, which were rather common in Gaul, comparison between these stamps and their collyria, botanical study of carbonized plants at medical sites, the examination of shipwreck cargoes or of equipment from burned out doctors' houses, systematic sampling for analysis of the contents of medical containers made of terracotta and above all glass, but also metal (as in the case of the sensational discovery in London of a largely intact small tin canister, referred to as a pyxis, containing an ointment—about which more later), many examples of which have been unearthed in the Gallo-Roman, Germanic, and Romano-British regions. All these methodological novelties have contributed to highlight a new historical material—ancient remedies—and create a new science, the study of processes used in the manufacture of remedies during the Roman era, with attempts to apply the formulation techniques of today's remedies and medicinal clays to early remedies, virtual object displays, and the organization of archeological exhibitions and colloquia.

Medicinal plants: St John's wort, henbane, mandrake, and others

The use of certain plants predated the Roman era, by when they were highly prized by the general public and by technical authors writing Greek or Latin: Pliny the Elder, Scribonius Largus (court physician to the Roman emperor Claudius), Quintus Gargilius Martialis (Roman writer on horticulture), Pseudo-Apuleius (author of a 5th-century herbal or book about plants and their medicinal and other virtues), Dioscorides (a physician in the Roman army and author of *De Materia Medica,* a herbal treatise produced ca 65 AD, which was influential for the next thousand years), Galen, and others. The Swiss Bronze Age site of Hauterive-Champréveyres yielded an exceptional concentration of St John's wort seeds, the use of which can only have been medicinal: the bright yellow flowers yield fruit, which on drying split to release myriad small seeds. The flowers and seeds were used to treat wounds and injuries, internal infections, neuralgia, and for sedation in some mental disorders. Empirical knowledge of certain pharmacological effects, now scientifically acknowledged, and magical beliefs generally went hand in hand when plants were being chosen in the fields. Other plants, though attested in literary and archaeological terms, remain mysterious today. *Herba Britannica* or *Radix Britannica,* for instance, is supposed to have saved Roman sailors from what seems to have been scurvy, and its name figures on a box of medicines from Haltern (North Rhine–Westphalia). It may have been sorrel or broad-leaved dock, the root of which was reduced to powder and ingested.

When their properties were known, wild plants were cultivated, although gathering continued as before because domestication of plant species was believed to weaken their powers. It is not always easy to distinguish for which purpose plants were used: perfumes, medicines, or food. In the Netherlands, at Uitgeest, was discovered a fine 3rd-century AD bronze bottle filled with seeds evidently deemed precious: radish, celery, oregano, and mallow. Again in *Gallia Belgica,* a bronze container may have held either cleansing soap or medicated soap, used for some or other skin complaint. It was found together with a strigil, an instrument used by ancient Romans

Four medicinal plants used by Gallo-Roman physicians.

Clockwise from top: Hypericum perforatum *(Saint John's wort)* - © Steven Foster; Hyoscyamus niger *(henbane)* - © Steve Klics/Corbis; berries of Lycium barbarum - © Steven Foster; Papaver somniferum *(opium poppy)* and seed pod - © Joe Petersburger/National Geographic Society/ Corbis.

Two strigils and container.
In Roman baths, the skin was softened with perfumed oil from the container and was scraped with the curved metal strigil, thereby removing the dirt. 1st century AD, Glyptothek Munich © Matthias Kabe, all rights reserved.

to scrape moisture off the skin after bathing. The container is finely worked, was tightly closed, and still contained a creamy substance based on animal fat. This discovery tends to confirm Pliny the Elder's assertion that *sapo* (soap) was invented in Gaul.

Shipwrecks have yielded a good deal of valuable information. In one ship, which foundered at the end of the 1st century BC off Ladispoli, some 40 kilometers north of Rome, a large wooden box is of special interest. Perfectly intact, its lid closed by a minuscule bronze lock, it once contained cumin and coriander seeds in bags, traces of which remain. These two *Umbelliferae,* greatly appreciated as culinary ingredients, were also prized for their medicinal virtues, to stimulate and improve the digestion. It is unlikely that this modest vessel had a ship's surgeon, so the doctor was probably a passenger, on call if needed. Unfortunately, for various reasons (primarily technical and financial), the analyses have yet to be done.

The inventorying of anesthetic plants dispels a myth regarding the history of ancient medicine—that of a medical practice indifferent to the pain suffered by the patient. Here we shall confine ourselves to the famous triad of poppy, henbane, and mandrake, the three herbs most used to assuage pain. Whereas Roman medical practitioners did not routinely seek the reversible abolition of sensitivity to disease-related and surgical pain (for technical as well as for moral reasons, the latter essentially because of the influence of Stoic doctrine on the social mores of the time), they did not ignore it either. Often combined in a compound remedy, these three plants contain, as we now know, scopolamine, atropine, and hyoscyamine, and are unquestionably effective. Dangerous too, as they provoke transient or lasting hallucinatory effects, delirium, dulling of the senses, obnubilation, headache, and fits of "madness." Mandrake has long been associated with dreamlike states, delirium, and hallucinations, and was used in magic rituals. Its name may possibly have been adopted through folk etymology from mandragora, since the roots tend to resemble the human form and because "drake," believed by some to derive from the Old English "draca," ultimately from dracō, the Latin for dragon), is suggestive of magical qualities. It is also known as *circaeum,* the plant of Circe, the Greek enchantress who, having turned half her crew into pigs, attempted to bewitch Ulysses, but failed as he protected himself using a magic herb provided by Hermes.

Dioscorides wrote that:

The bark of the root is pounded and juiced while it is fresh, and placed under a press. After it is stirred the beaters should bottle it in a ceramic jar. The apples are also juiced in a similar way, but the juice from them becomes weakened. The bark from the root is peeled off, pierced with a thread, and hanged up in storage. Some boil the roots in wine until a third remains, strain it, and put it in jars. They use a winecupful of it for those who cannot sleep, or are seriously injured, and whom they wish to anesthetize to cut or cauterize. Twenty grains of the juice (taken as a drink with honey and water) expel phlegm and black bile upward like hellebore, but when too much is taken as a drink it kills.

Dioscorides himself practiced anesthesia not only for sedation during surgery, but also to soothe chronic pain, but it was up to the patients to decide whether or not to avail themselves of it.

Galen considered opium, or poppy juice, to be "the strongest of the drugs which numb the senses and induce a deadening sleep." He sounded a cautionary note though: "Dulling intense pain may be beneficial … but if more potent or more liberally administered narcotics are used, the body becomes cold and dies." As for henbane, it is well represented in the medical texts. The priestesses of Apollo allegedly used henbane, also known as *herba Apollinaris,* to yield oracles. The plant derived added prestige from its Greek name, υοσκυαμος (from "uos," pig, and "kuamos," bean), *Hyoscyamus* in Latin, which was associated with the Erymanthian Boar, a monster that roamed the Arcadian highlands and the capture of which constituted the fourth Labor of Hercules. According to some accounts, alone among all animals—who carefully avoid grazing on the highly toxic henbane—the Erymanthian Boar fed on its seed pods ("beans"), which explained its aggressive behavior. The

speed of the toxic effect depends upon the dosage, the season, where the plant was collected, and its freshness. We have archaeological evidence of the medical use of henbane. At the ancient site of *Novaesium* (present-day Neuss am Rhein), historical studies have greatly benefitted from two accidents, one in the 1st century AD, when a fire ravaged a military hospital, and the other in 1962, when bulldozers uncovered the site. Archaeological digs revealed vessels containing foodstuffs, lentils, and carbonized peas, and also a burnt sort of hay, composed in fact of centaury, plus thirty-nine perfectly recognizable henbane seeds.

Medicinal clays: snake bites and counterfeit

Roman physicians also made much use of clays, dried and cut into pieces for storage. It is unlikely these were of much value for diseases of the internal organs, but could be genuinely useful, once moistened and softened, for treating certain wounds. Famous clays included those from Kimolos, one of the Cyclades islands (called Cimolian earth), Samos (an is-

"seal" in Greek). He described how, observing a local rite, the priestess took the earth to the town of Hephaistias, mixed it with water to produce a slurry, which she stirred and left to settle.

> The supernatant liquid was then decanted, and the earth deposited was removed, freed from stones, and dried into a soft mass which was afterwards cut into tablets and stamped with the sacred seal of Diana [an image of the goddess or of a deer, her sacred animal]. The priestess then placed the tablets in the shade, where they were allowed to remain until all moisture had evaporated and they had become hard and dry.

Galen read a book by a local singing the praises of Lemnian earth, and was convinced: "I was pleased to experiment with them and took away with me twenty thousand of those seals." Unfortunately, these and other drugs and instruments, and a good part of Galen's library, all of which he had deposited for safe-keeping in the Temple of Peace in Rome, were destroyed when the temple burned to the ground in 191.

Terra sigillata. Tablets of "sealed earth" (terra sigillata) produced in Germany, dated 14th century or later, very similar to the stamped Lemnian earth tablets used during the Gallo-Roman period.
Medicinal earths and clays are attested as early as 2500 BC in Mesopotamia and as late as the 19th century in Europe. Science Museum London © Wellcome Images.

land in the eastern Aegean Sea), Eretria (on the Aegean island of Euboea), Chios (an island near the coast of present-day Turkey), and Selinunte (on the south coast of Sicily), but the most renowned of all was the clay of Lemnos, collected at the foot of Mosychlos, a mountain on this island in the northern Aegean. This pale red Lemnian earth was smooth, and soft to the touch, had a styptic and astringent taste, and as the most sought after medicinal clay was also the costliest and therefore the most commonly counterfeited. In the 160s, Galen, intrigued by strange rumors and wishing to procure authentic products, went to Lemnos to witness at first hand the making of the famous tablets, called Lemnian *sphragis* (meaning

Galen also bought *lyceum,* or boxthorn, as a liquid medicament, in Cyprus, Syria-Palestine, and Phoenicia, as well as aloe from India.

Roman medical practitioners used Lemnian earth in ointment (to treat watering eyes, lacrimal fistula, eye pain), in a potion with vinegar or wine (for hemoptysis, spleen and kidney ailments, hypermenorrhea), as an antidote (sometimes combined with other antidotes) to bites by snakes and other venomous creatures, sea hares *(Aplysia),* blister beetles, and even rabid dogs, and in topical application (slow-healing wounds, bites, including by rabid dogs, old wounds). A collyrium stick

How did Gallo-Roman physicians treat their patients? – *Gourevitch*

found in Reims was used to smooth asperities on the inside of the eyelids, and its active ingredient was Lemnian earth, in this case called *fragis*.

Compound medicines: from painkillers to theriac, the cure-all

The literature, medical or paramedical, presents interesting mixtures. Galen refers to an "anodyne" in the old sense, a painkiller (*an,* without; *odynē*, pain), and writes of three goals: "to dull sensitivity to pain, to leave no damage around the affected part, and to be as effective as possible in countering with the morbific tendency." He continues by speaking of a mixture of henbane and poppy juice made by Philo who wanted to produce a sleep redolent of deep coma and to numb the capacity to feel pain.

Compounding ingredients was hampered by a great lack of precision in weighing them out. The Romans of course knew how to weigh, but there was never-ending rivalry between their system of weights and measures and those of the Greeks, and this was problematical for the ancients as it is for us today. Accurate dosing was therefore hard to achieve as was mastery of the drug's effects on individual patients. There was no coherent pharmacodynamics, despite a certain awareness of its necessity and the use of a system, which to us may seem strange, to classify the strength and quality of simple medicines as a function of the characteristics ascribed to the human body and of the finished product, considering that the properties of each ingredient may not only be additive, but also somehow potentiate each other. The most famous of this type of remedy was theriac, recipes for which vary greatly, and a find of what might be a jar of it revealed fifty-four ingredients: forty-seven plant species and some animal remains, including the essential snake or viper flesh. Whatever the final presentation, there was almost always a phase of grinding and/or cremation, cleaning and purification of individual ingredients, and then the mixture was finely powdered.

Pharmaceutical stamps: Gallo-Roman brand names and "advertising"

The practice of stamping was important for authentication of precious simples and for remedies like Lemnian earth, compound medicines that carried a risk, and certain specific remedies. There were several types of pharmaceutical stamps, rings, seals of hard wood, like boxwood, of bronze, and so forth. The collyrium stamps are the most informative, but care should be taken over the exact meaning of the word collyrium, which in those times designated not a liquid, but a convenient storage form in the shape of a small or elongated bread roll easy to transport and cut in small portions, kept in the physician's case or in the pharmacy, usually for eye diseases (whence the modern meaning), but not always. More than 300 collyrium stamps have been recorded to date, and archaeological excavations are constantly adding new ones to the list. They shed light on how physicians practiced their art, and en-

Gallo-Roman pharmacy.
Pharmacist (a woman) with a bowl in her right hand, above a cauldron in which medicinal plants are brewing, stirred with a caduceus-shaped ladle; on her left knee, she holds a recipe tablet. Her assistant (upper right) examines a cylindrical vial. 2nd century AD. Museo de la Civiltà Romana. Rome © Bridgeman Art Library.


Oculist's collyrium stamp.
This four-sided stamp was used to mark semi-solid sticks of eye ointments (collyria). *The inscriptions are for four remedies prepared with saffron by Junius Taurus from a prescription of Pacius. Stone (serpentine?), 1st-3rd centuries AD, Naix-aux-Forges, France. © British Museum.*

Oculist's kit from the necropolis of La Favorite in Lyon.

Brass sheet box containing twenty dried collyria (displayed) shown open with its lid on the right; beveled slate plate to grind the collyria; sheath made of two brass cylinders fitting into each other, containing the three brass instruments displayed. Dated end 2nd to beginning 3rd century AD. © Musée gallo-romain de Lyon-Fourvière.
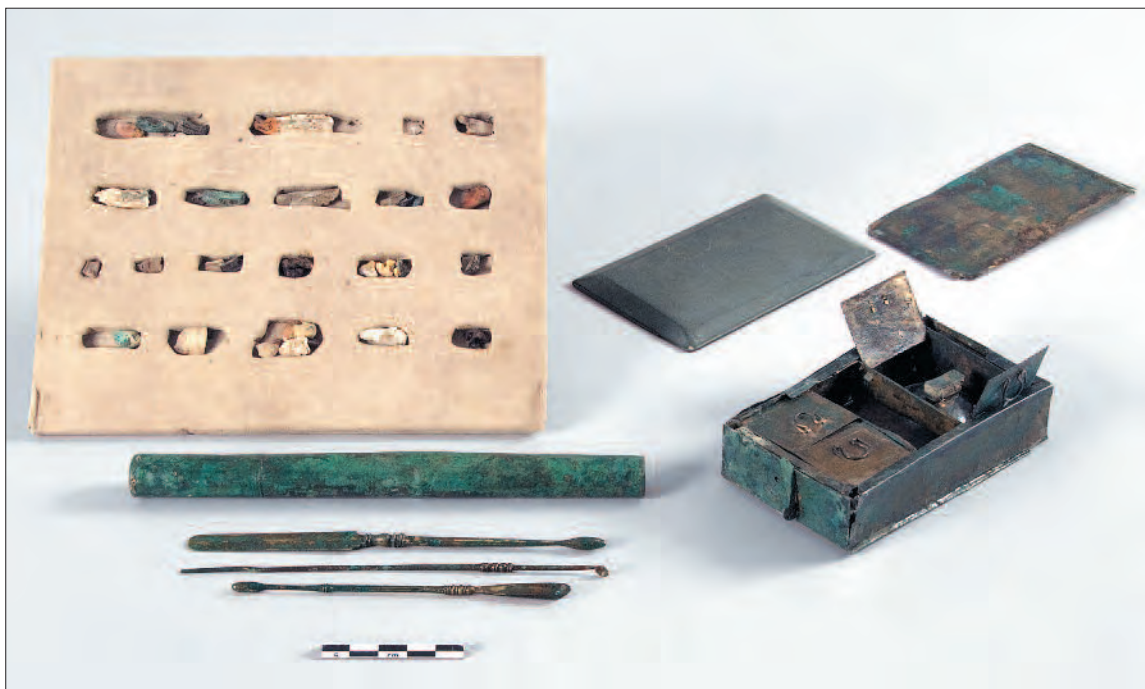
Gallo-Roman oculist's instruments.

3rd century AD. Musée Crozatier, Le Puy-en-Velay, France. © Giraudon/Bridgeman Art Library.

able comparisons between medicinal products advocated in the literature, the constituents indicated on tablets and dry remedies, and the ingredients actually included. They are authenticating medicine stamps, and at the same time genuine prescriptions on stone, generally greenish schist or steatite, since green was thought of as a restful color, good for the eyes, judging from those that have come down to us, which date from the first half of the 1st century AD to the 4th century AD. The inscriptions on the four edges of these small quadrilateral or oblong medicine stamps are cut retrograde (read from right to left), so when pushed into soft or doughy compound medicines their impress usually reads from left to right, and indicates information such as the name of the remedy (attributed according to its color, appearance, and effectiveness ["marvelous success"]), the indication (for this or against that), its effect (soothing, etc), the category of patients targeted (babies, soldiers, etc), the method of use, the diluent, the key ingredient, the name of the inventor or of the person presumed to be, the name of the prescribing physician. The large faces, sometimes slightly hollow, were used to grind the dry drug or to mix it with an excipient just before use. Many questions remain regarding these collyrium stamps and local scholars should be vigilant when new discoveries are made.

Much rarer than the *collyrium* stamps are collyria that are themselves stamped during drying, and hence fragile, divisible, and friable. They are usually submitted to chemical analysis, a practice started notably by the great Marcellin Berthelot (1827-1907) for medicines found at Reims. But it was a superb discovery in Lyon, in a cremation tomb in the necropolis of La Favorite in 1983-1985, that greatly advanced our knowledge of these compound drugs: in a box with compartments

belonging to an oculist, dated end of 2nd, beginning of 3rd century AD, were arranged twenty collyria, eleven of which were inscribed, in Greek and in Latin. Chemical analysis was used to classify the constituents by families, depending on the main ingredient, which is not necessarily the real active principle: clayey constituents, lead, zinc, copper, gum resins, iron, arsenic, carbon black. Pollen analysis can be used to detect eyebrights *(Euphrasia)* and mugworts *(Artemisia).* It is interesting to note the presence of blackcurrant because, given the period, it must have been imported as it was not yet grown in the Gallo-Roman world. Another surprise is the *crocodes* collyrium, which contains copper, zinc, potassium, iron, and lead, but no trace of the pollen of crocus, or saffron, its name suggests. Yet this pollen, obtained from the stigmata of *Crocus sativus* L. flowers, figures in the recipes of numerous collyria because of its astringent and anti-inflammatory properties. Perhaps we should not take *crocodes* literally, but rather as a simple reminder of the color yellow, tantamount to pharmaceutical fraud, to allay the customer's mistrust, a type of falsification that was not rare and which was facilitated by the distant provenance and complex circulation of certain products. Overall, the scientific analysis of collyria has yielded results consonant with what we know from textual sources of the tendency of the ancients to a sort of polypharmacy, to wit the use of highly complex remedies in which it is difficult to know what is expected from the various ingredients. Inscribed collyrium stamps and collyria have been found above all in the Roman West, Gaul, *Germania,* and *Britannia,* though quite why is unclear, since what we know does not suggest that eye diseases were more prevalent in these regions.

Soft and liquid drugs

It is quite exceptional for a centuries-old soft medicinal form to be preserved, and the dermatological cream stored in a securely closed, intact, cylindrical tin pyxis discovered in 2003 in London, in the remains of a temple in Southwark, on the south bank of the Thames, created a sensation. It was opened at the Museum of London, with all due precautions, and, to everyone's amazement, the small canister, which dates from the middle of the 2nd century AD, was virtually full of a white creamy substance in which could still be seen the user's finger marks. After studies at Bristol and Bradford, it was established that the white translucency of this cream, which was intended for whitening of women's skin or to heal sores and cuts, was due to tin oxide, which was mixed with starch and animal (cattle or sheep) fat that had been heated. Ancient cosmetics more often contain ceruse or lead acetate, and it may be that the tin (relatively easy to procure for the Romano-British who occupied the Cassiterides, meaning Tin Islands, traditionally thought to refer to the British Isles, because of tin deposits in Cornwall) was introduced into the mixture because of confusion with ceruse, or was deliberately used in a pharmaceutical fraud of the kind we saw in the case of saffron. Doubt therefore remains regarding the use made of such creams, cosmetic or dermatological, sometimes even culinary.

Thus, the one hundred thirty-six cylindrical, turned boxwood containers discovered in a ship wrecked off the coast of Populonia (Tuscany, central Italy), around 100 BC, contained cinnamon, vanilla, and cumin. On the other hand, Egyptian make-up containing lead could have had beneficial effects in the treatment of eye ailments.

Among liquid drugs, sometimes viscous or syrupy, the most renowned was doubtless *lycium,* in Greek *lycion,* which Pliny the Elder and Dioscorides applied to a type of boxthorn which was in vogue from the 5th century BC onwards throughout the Mediterranean Basin. The best, from the Indies, was carried in camel skin or rhinoceros hide, whereas other products

***"Stela of the Medica"* portraying a Gallo-Roman female physician.** *Funerary stela discovered in Metz, France.*
Only part of the inscription remains, but still shows the word "MEDICA." The medica, standing draped in her palla, holds a rectangular object in her left hand, either a medicine box or a book. 2nd century AD. Photo courtesy of Musée de La Cour d'Or Metz Métropole. © Kieffer Laurianne - Musée de La Cour d'Or - Metz Métropole.
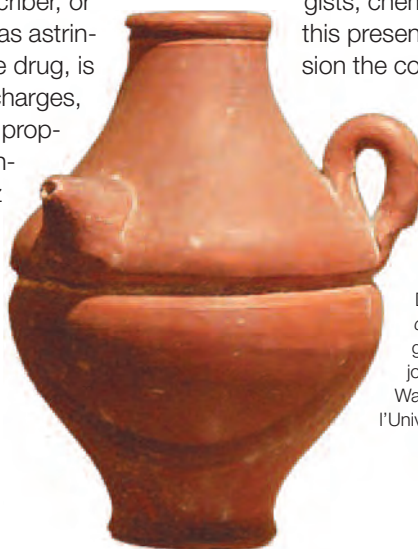
The London pyxis.
Tin pyxis (diameter 6 cm, height 5.2 cm) dated 2nd century BC, discovered in 2003 in London (Southwark) containing a well-preserved dermatological cream. On permanent display in the Museum of London's Roman Gallery. Photo courtesy of Museum of London. © Museum of London.

were transported in amphorae or bags, or in large dried balls. *Lycium* was extracted from the branches and roots of box-thorn from *Lycia* (part of present-day Anatolia, Turkey), or sometimes in preference from India. It was sold to Western customers in glass or terra cotta bottles marked with the name of the remedy or of its maker or prescriber, or with both, after the fashion of dry drugs. It has astringent properties and, without being a miracle drug, is effective against certain ulcerations and discharges, since its constituent berberine has antibiotic properties, although it is slightly toxic in some conditions. But from Pliny we learn that ersatz and counterfeit versions circulated in the Gallo-Roman world. The minuscule bottles discovered in Athens, Catania (Sicily), Tarento (Southern Italy), and elsewhere show that *lycium* was not cheap, and some bear medicine stamps analogous to those for dry collyria.

Conclusion

With ancient medicinal remedies, studying the link between textual sources and archaeological finds is especially crucial and its elucidation can only benefit from multidisciplinary research and collaboration between archaeologists, philologists, chemists, botanists, and others. To complete this presentation of products we also need to envision the containers, to which we have only alluded, and the instruments used to make them. These have much to teach us about the preparation, labeling, denomination, storage, circulation, and utilization of the remedies. ∎

Further reading

Danielle Gourevitch, *Pour une archéologie de la médecine romaine,* De Boccard, Paris, 2011. La Coupe d'Hygie : *Médecine et Chimie dans l'Antiquité,* actes de la journée d'études de juin 2011, organisée par Philippe Walter et Muriel Labonnelie, sous presse aux Presses de l'Université de Dijon.

Gallo-Roman terra-cotta so-called "baby-feeding bottle."
Although such "bottles" were indeed intended to contain milk, it is now believed that they may have been placed for ritual purposes in children's tombs. 1st century AD. History of Medicine Museum, Paris V René Descartes University, Paris. © Musée d'Histoire de la Médecine, Paris.

COMMENT LES MÉDECINS GALLO-ROMAINS TRAITAIENT-ILS LEURS PATIENTS ? UN APERÇU DES PHARMACOPÉES DE LA FRANCE PRIMITIVE

L'étude traditionnelle de la pharmacopée antique, en Gaule comme dans le reste de l'Empire, a longtemps reposé sur le seul témoignage des textes, médicaux et magiques surtout, parfois historiques, très rarement épigraphiques. Le grand développement de formes nouvelles de l'archéologie (archéologie de sauvegarde, archéologie de prévention, archéologie subaquatique en mer et dans les fleuves etc…) a attiré l'attention sur des objets totalement inconnus ou très grandement méconnus : collyres secs de Lyon (nécropole de La Favorite), étude pétrographique des cachets à collyres particulièrement fréquents en Gaule, comparaison entre les collyres et les cachets qui les estampillent, étude botanique de plantes carbonisées sur des sites médicaux (particulièrement en Suisse et en Allemagne), étude des contenus de vaisseaux naufragés ou de maison médicale incendiée (Rimini), étude chimique systématique du contenu de récipients, en terre-cuite et en verre (en particulier en France, en Allemagne et en Belgique), découvertes aléatoires comme celle d'une pyxide londonienne contenant une crème dermatologique pour ainsi dire intacte, étude « scientifique » des processus de fabrication de remèdes d'époque romaine, essai d'application aux remèdes anciens de la « formulation » des remèdes d'aujourd'hui (simples ou composés), étude des argiles thérapeutiques (terre de Lemnos), regroupement virtuel d'objets sur ce thème et organisation d'expositions archéologiques et de colloques… Toutes ces nouveautés méthodologiques ont créé en quelque sorte un matériau historique nouveau : le remède antique, particulièrement présent en milieu gallo-romain, germanique et romano-british.

Even the humblest towns of Gaul had at least one public bathhouse. Lutetia boasted three. A stroll there before the evening meal was not to be missed, through rooms, corridors, gardens, and porticos, keeping in shape by exercising in the *palaestra,* improving the mind in libraries. People conversed, spread the latest tittle-tattle, listened to the improvised diatribes of orators, talked business. It was a place of encounters and sociability, all the more so because admission was free or the charge nominal.

Lutetia, the Gallo-Roman ancestor of Paris

by G. Coulon, *France*

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The Gallo-Roman town of Lutetia was the chief settlement of the Parisii (Gallic tribe). It stretched along the left bank of the River Seine, on what is now Sainte-Geneviève hill and the Île de la Cité *(natural island in the Seine). A network of orthogonal roads divided the town into blocks* (insulae) *containing public spaces and dwellings. This street plan was organized around a major north-south thoroughfare, the present-day rue Saint-Jacques. At its apogee in the late 2nd century AD, Lutetia was home to almost 10 000 people, a modest population among the towns of Gaul. Lutetia boasted a forum with its basilica and probably a temple, places of entertainment (a theater and above all the amphitheater), and public baths, in the south, the east, and the north (those called Cluny). Its craftsmen and tradespeople generated the town's wealth and its influential guild of boatmen controlled navigation on the River Seine and its tributaries. These boatmen, the* nautae parisiaci, *played a major role in town life, and in the early days of the Roman Empire even erected a monument to Emperor Tiberius, the famed Pillar of the Boatmen. In the 4th century, barbarian incursions, rural malcontents, and political upheaval prompted the inhabitants of Lutetia to abandon the left bank and withdraw to the* Île de la Cité, *around which they erected ramparts. Paradoxically, as the town's fortunes waned, its military importance grew, and by the year 360 when Julian's soldiers proclaimed him Emperor there, his beloved Lutetia was well on the way to becoming Paris.*

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Greek geographer Strabo, in the 1st century BC, wrote "On the banks of the river *Sequanas* (Seine) lived the *Parisii* who occupied an island in the river and had for a city *Lucotocia* (Lutetia)." Later the town grew and its people erected public monuments, but it was never more than a modest town of Roman Gaul. In short, its origins are commonplace, like many urban centers in Antiquity. Yet the town that was to emerge as the capital of France needed to take pride in glorious beginnings, so from the Middle Ages onwards all manner of legendary origins were dreamed up. One such outlandish story linked it to the fall of Troy, after which displaced Trojans were said to have settled on the banks of the Seine in a place that was "beautiful and delectable, plentiful and fertile and well placed for living."*

As for the *Parisii,* it was claimed that their name came from Paris himself, the son of Priam and lover of Helen of Troy. Such a filiation, fanciful as it was, conferred on Lutetia a mythical origin comparable to that of Rome, which in one tradition was founded by the Trojan Aeneas. And to further extol its beginnings, it was even professed that Lutetia was founded well before the Eternal City, a view completely at odds with current archaeological opinion, which holds that the oldest traces of a Roman presence in the soil of Paris go no further back than 30 BC.

Lutetia: from Gallic to Roman

In his *Commentaries on the Gallic War* (Book VII, 57), Julius Caesar mentions Lutetia "town of the Parisii, situated on an island in the Seine," but archaeological excavations have never uncovered significant Gallic remains on the *Île de la Cité.* To the point that researchers are beginning to wonder whether Lutetia was located elsewhere, at Nanterre, where a site has recently yielded substantial traces of Celtic occupation. All the more so since the Nanterre site was abandoned early in the reign of Emperor Augustus, just at the time of the first signs of a Roman presence in Paris. According to this hypothesis, Lutetia was transferred to the Sainte-Geneviève hill, where the Gallo-Roman town was founded and then grew during the 1st century AD.

Without falling prey to simplistic determinism, it is legitimate to underscore the advantages of the location of Paris. First there is the Seine, a major waterway extended by a whole series of navigable tributaries. Situated at the nexus of several complementary regions, the site was also favorable for water-land transfers. Swampy, dotted with small islands and channels, the alluvial plain is surrounded by heights and hills conducive to human settlement. Roman city planners little by little mastered this environment and laid out the pattern of the town.

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*Raoul de Presles, *Description de Paris sous Charles V.* 1371. In: Le Roux de Lincy and L. M. Tisserand. *Paris et ses Historiens aux XIVe et XVe Siècles. Documents et Écrits Originaux.* Paris. 1867:103-104.
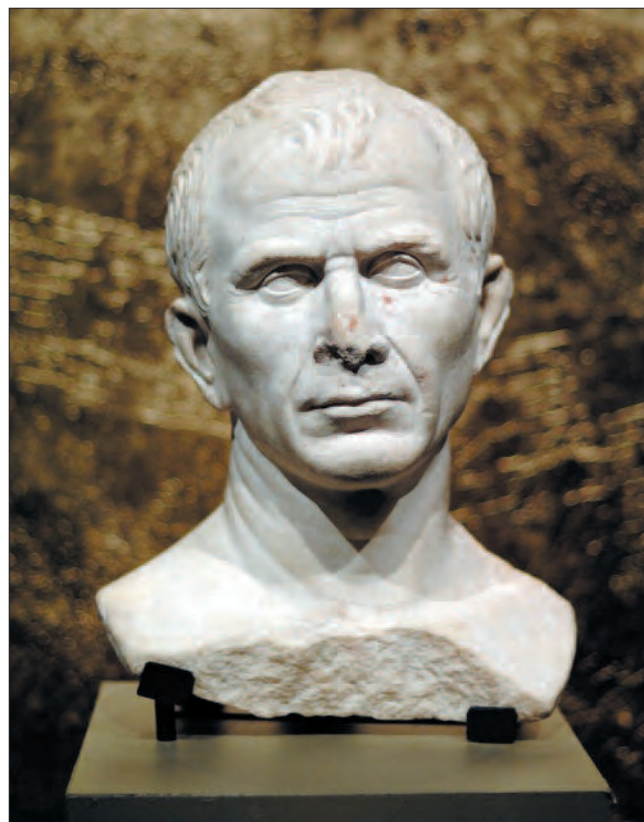
Marble bust of Julius Caesar, *dated 46 BC, claimed to be a true likeness.*
The bust was discovered in 2007, during an archeological diving mission. Caesar wrote extensively on Gaul in his Commentarii de Bello Gallico (Commentaries on the Gallic War)*. © Chris Hellier/Corbis.*

A town with a grid plan

At Timgad in Algeria, Cologne and Trier (formerly called Treves) in Germany, Avenches in Switzerland, Orange, Amiens, Limoges, and Autun in France, Roman planners laid out the town in a more or less regular grid plan. The streets cross at right angles and the two main thoroughfares intersect at the town center. The north-south road was the *cardo maximus;* the east-west road the *decumanus maximus.* Parallel to these were the secondary roads, which gave the town its orthogonal pattern, creating *insulae,* like apartment buildings, laid out as if on the squares of a chessboard.

The heart of Lutetia is no exception. The *cardo maximus,* which runs perpendicular to the Seine, was the town's principal thoroughfare. Its course has remained unchanged over the centuries and today corresponds to the rue Saint-Jacques, the rue de la Cité, and the rue Saint-Martin. Using a theoretical layout, yet adapting with pragmatism to the local terrain, Roman planners built the town on the left bank of the river, on the heights and slopes of the Sainte-Geneviève hill, away from areas liable to flooding. In the whole of the center of Lutetia, the blocks or units defined by the *decumanus* and *cardo* corresponded to exactly 300 Roman feet, that is to squares close to 89 x 89 meters. In the center of this ur-

Map of Lutetia in the 4th century AD.
From the Île de la Cité *(right page), continuing the southern
bridge, is the main* cardo maximus *thoroughfare (now* rue
St Jacques)*, intersecting at right angles with the* decumanus
maximus *at the* Forum's Basilica *(the largest and longest building
on the left page). Close to the fold of the page, the round dome
of the* Thermal baths of Cluny, *with a wisp of white smoke.
On the right page, bottom, the* Arènes de Lutèce *amphitheater.*
*Watercolor by Jean-Claude Golvin. © Éditions Errance, Paris.*
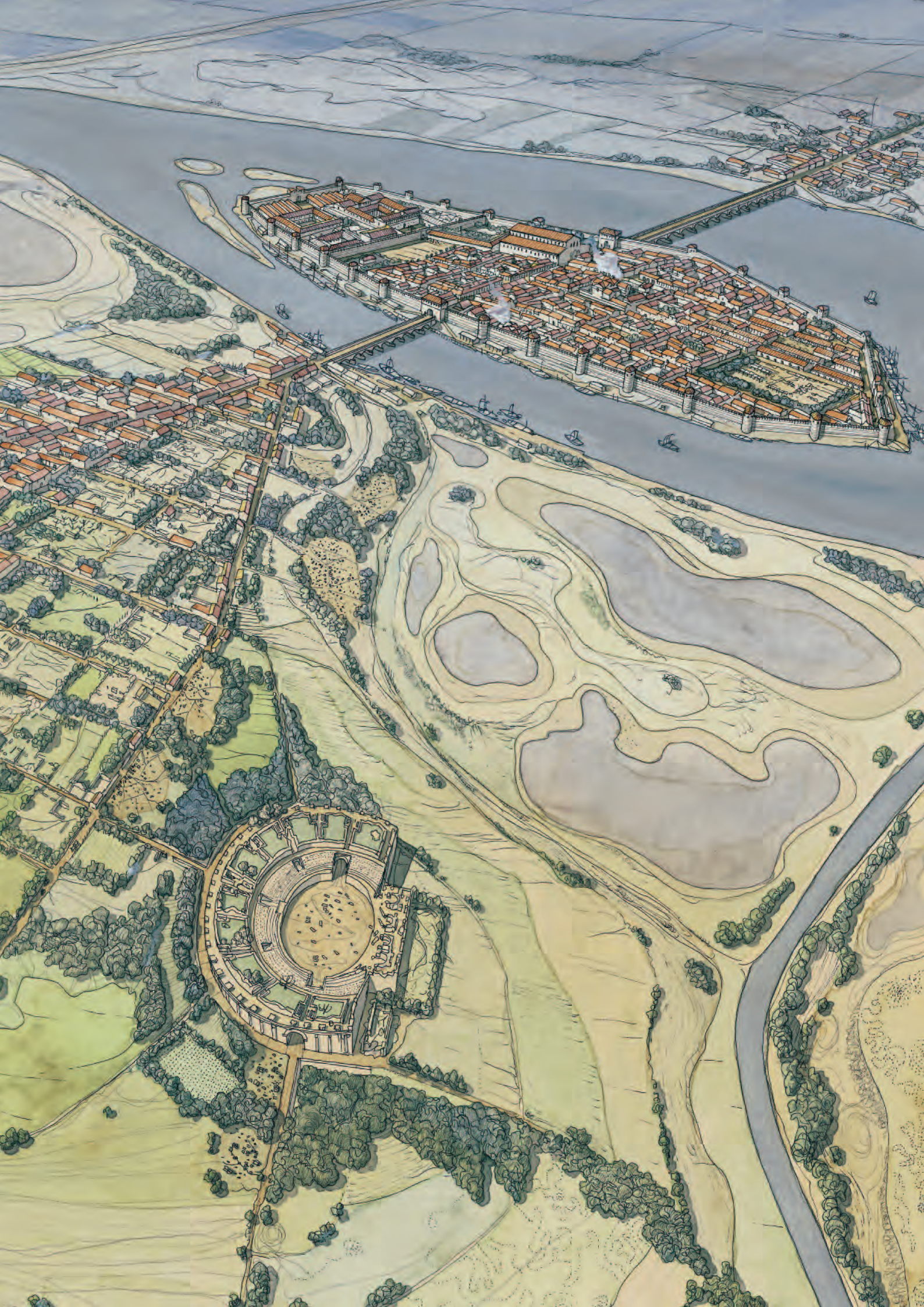
ban network were public monuments and private housing, and the grid pattern was not immutable since the forum, for example, occupied two blocks. Lutetia, which must have covered an area of 60 to 70 hectares, extended also to the Île de la Cité and a small stretch of the right bank of the Seine. Despite this modest size—Nîmes occupied more than 220 hectares, Lyon 350, and Reims 600—Lutetia was chosen as the main town of the Parisii. This political and administrative function distinguished Lutetia from the other towns of Gaul and it was provided with the buildings and public spaces typical of all provincial capitals, which prided themselves on being in the image of Rome, the urbs par excellence.

The town's forum and monumental trappings

"Deep in the Seine valley, imagine the ancient monumental town at its apogee being laid down in stages, to the great pride of its worthies who thus gave expression to their membership of the Empire: above the forum and its thermal baths, halfway down the slope, the theater, the thermal baths of the *Collège de France* and the amphitheater, and lastly, down below, the Cluny baths forming the monumental façade." This panorama of Lutetia, evoked by Didier Busson, archaeologist at the Department of the History of Architecture and Archaeology of the City of Paris,* gives a fair idea of how the town's monuments and public spaces were laid out.

Generally rectangular, Gallo-Roman forums consisted of an esplanade surrounded by portico colonnades and organically linked to a basilica, curia (place of assembly), temple, and shops. The civil basilica served at the same time as the lawcourts, the trading exchange, a covered market, and a waiting hall used during inclement weather or heat waves. Contiguous with the basilica was the curia, the assembly room for the decurions (members of the city senate), who formed a sort of town council. The shops were run by merchants, craftsmen, businessmen, and sometimes even teachers. As for the plaza itself, the statues of notables and their honorific inscriptions made this the symbolic repository of the town's collective memory. This juxtaposition of buildings conferred on this public space legal, political, administrative, and religious functions, and to a lesser degree social and economic importance. In short, forums were the busiest and liveliest of places in which the town's heart beat *(See Box, right).*

Established opposite the Luxembourg Gardens, between the present-day boulevard Saint-Michel and rue Saint-Jacques, aligned with the rue Soufflot, Lutetia's forum covered an area greater than that of today's Pantheon. On three sides, the cen-
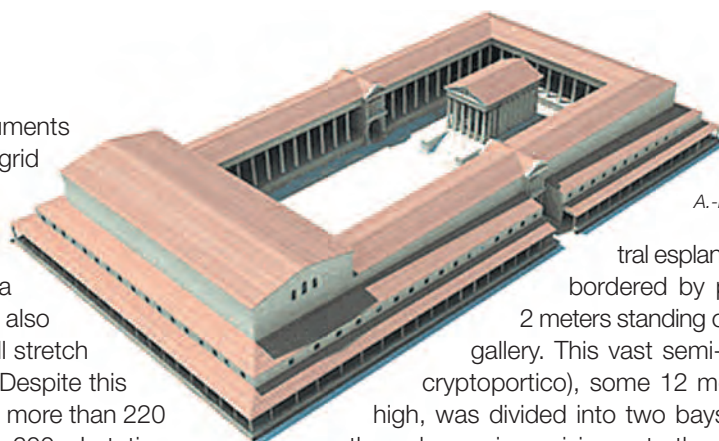
3-D reconstruction of the Forum of Lutetia.
© Infographie M.-O. Agnès et A.-B. Pimpaud. With kind permission.

tral esplanade (118×43 meters) was bordered by porticos raised by about 2 meters standing on an underlying U-shaped gallery. This vast semi-underground gallery (the cryptoportico), some 12 meters wide and 6 meters high, was divided into two bays, into which light filtered through openings giving onto the esplanade. By raising the monumental porticos, the cryptoportico displayed them to full effect and, because of the sloping terrain, ensured the stability of the artificial terrace constructed when laying out the forum. The presence of a curia remains controversial, but the basilica, comprising a nave and two aisles, closed the eastern end of the esplanade. Facing it stood a temple, probably devoted to the cult of the Emperor. A few vestiges of its podium and decoration have survived and suggest that it was of classical type with columns, capitals, and pediment.

The Cluny thermal baths

Even the humblest towns of Gaul had at least one public bathhouse. Lutetia boasted three. Such a proliferation of thermal baths—the use of which was introduced by Rome—cannot be explained by concern for hygiene and bodily cleanliness alone. They appeared in the decades following Caesar's conquest of Gaul, as the expression of a new art of living. A stroll there before the evening meal was not to be missed. An aimless wander through rooms, corridors, gardens, and porticos, chance meetings, relaxation, keeping in shape by exercising in the *palaestra* (a rectangular court surrounded by colonnades), improving the mind in libraries and conference rooms. People conversed, spread the latest tittle-tattle, listened to the improvised diatribes of orators, talked business. It was a place of encounters and sociability, all the more so because admission was free or the charge nominal.

THE LAW-COURTS, AN IDEAL PLACE FOR WOOING...

In his famous treatise *The Art of Love* (Book I), the poet Ovid (43 BC-AD 17 or 18) wrote:

"And the law-courts (who'd believe it?) they suit love:
A flame is often found in the noisy courts:
Where the Appian waters pulse into the air,
From under Venus's temple, made of marble,
There the lawyer's often caught by love,
And he who guides others, fails to guide himself:
In that place of eloquence often his words desert him,
And a new case starts, his own cause is the brief.
There Venus, from her neighboring temples, laughs:
He, who was once the counsel, now wants to be the client."

*Paris Villa Antique, Guides Archéologiques de la France, Monum, Éditions du Patrimoine, 2001:39.

Thermes de Cluny, also called Thermes du Nord. Present-day aspect.

3-D reconstruction of the Thermes de Cluny, by Renou Laurent.

Thermal baths were not the place for a man in a hurry. Rather, he tarried and enjoyed an unalloyed bathing ritual. After a few physical exercises in the *palaestra,* the visitor left his clothes in the *apodyterium* (changing room) and entered the *tepidarium* (warm bathroom), where a slave anointed him with olive oil and then scraped it off with a strigil (a curved metal tool designed for the purpose, *see illustration in preceding article, page 243).* The renowned 1st-century Roman physician Aulus Cornelius Celsus wrote that "He who has a weak head should stay there without undressing until he perspires slightly, and only then can he submit to high temperature without danger." The next step was in the hot room *(caldarium),* where the temperature was about 55°C and the humidity 95%, and where, in his *Satires,* Juvenal warns of the dangers run by a lordly and gluttonous patron: "But you will soon pay for it, my friend, when you take off your clothes, and with distended stomach carry your peacock into the bath undigested! Hence a sudden death, and an intestate old age" (Satire I. Translated by G. G. Ramsay for the Loeb Classical Library [1918]). After intense sweating, and a relaxing massage, the bather returned to the *tepidarium* before plunging into a pool of cold water in the *frigidarium.* Thermal baths were identified in the 19th century at the site of the *Collège de France* and immediately south of the forum, at the corner of the rue Gay-Lussac and the rue Le Goff, but unquestionably the largest were those in the north, known as the Cluny thermal baths. They originally covered a little over one hectare, and their exceptional state of preservation—the full height of the frigidarium, for in-

stance, is today part of the *Musée National du Moyen Âge* (National Museum of the Middle Ages)—allows us to imagine the grandiose proportions of the edifice and the richness of its decoration. Its ribbed vault, extended in three directions by barrel vaults, rose to 14.5 meters, making it one of the tallest still visible in the Roman West. The groins rest on consoles

Marble bathtub, 2nd century AD. Paris, Musée National du Moyen-Âge, Thermes de Cluny. © RMN/Jean-Claude Berizzi.

sculpted in the shape of the prows of ships, about which more later. And to appreciate fully the vastness of this bath complex, it should not be forgotten that there were two levels unseen by the bathers: beneath the bath complex were the lower level of the water conveyance, with its tanks and sewerage system, and the intermediate level for the staff and technical facilities.

The *Arènes de Lutèce*

This amphitheater is the second largest monument of Gallo-Roman Paris that has survived to the present day. It was uncovered during Baron Haussmann's vast urban renovation, which modernized Paris during the Second Empire (1852-1870) and beyond. In 1867, the building of the rue Monge enabled Théodore Vacquer, the inspector of the capital's archaeological sites, to identify and then excavate the amphitheater. Three years later, the building of a depot for the *Compagnie Générale des Omnibus* revealed a good half of the amphitheater. There followed a virulent campaign to save the archaeological treasure. "The destruction of such a historic monument would shame Paris in the eyes of all of scholarly Europe" the historian Henri Martin indignantly proclaimed in a letter to the newspaper *Le Siècle.*

Politics entered the fray and Napoleon III in person visited the site, but was unenthused. It's true the Emperor had other things on his mind: three months later the Franco-Prussian War broke out! In the confused aftermath of the conflict, the amphitheater was at risk of being razed. In 1883, Victor Hugo, then 81 years of age, wrote an impassioned plea to the President of the City Council: "Paris, the city of the Future, cannot renounce the living proof that it was also the city of the Past. The Past brings about the Future. The *Arènes* are the ancient mark of the Great City. They are a unique monument. A city council that destroys them would so to speak destroy itself! Save the *Arènes de Lutèce!* You will be doing a valuable deed, and, what is still more worthwhile, you will be setting a great example." The old man's moral authority won the day and the monument was saved. And just before the Great War, the architect Jules Formigé restored it, or rather reconstructed it, given the liberties he took. This operation, conducted jointly with the laying out of a square, was carried out under the watchful scientific eye of Dr Louis Capitan *(see Box, below).*

DR LOUIS CAPITAN (1854-1929) PHYSICIAN, PREHISTORIAN, AND ARCHAEOLOGIST

Graduate of the Paris Medical School, Dr Louis Capitan also worked in the fields of prehistory and anthropology without ever abandoning his medical vocation. In 1898, he succeeded his professor, Gabriel de Mortillet, as Chair of Anthropology at the Paris School of Anthropology, and in 1908 was appointed Professor of American Antiquities at the *Collège de France.* The following year he entered the Academy of Medicine and then, as Vice-President of the *Commission du Vieux Paris,* became scientific director of the restoration of the *Arènes de Lutèce* undertaken by the architect Jules Formigé. He published two major articles on the archaeological excavations of the amphitheater in 1915 in the *Comptes Rendus des Séances de l'Académie des Inscriptions et Belles-Lettres.* In the disciplines of both medicine and prehistory he published some 250 titles. Above all, with his friends Denis Peyrony and Henri Breuil he discovered the Paleolithic cave art at Les Combarelles and Font-de-Gaume in the Périgord.

Joseph Louis Capitan (1854-1929). © Roger-Viollet.

Present-day aspect of the Arènes de Lutèce.

One of the hidden marvels of Paris: the entrance, 49 rue Monge in the 5th arrondissement, is easily overlooked. In the background, on the right, is the tower of the Faculté de Jussieu (Jussieu University): the university tradition of the "Quartier Latin" lives on!

3-D reconstruction of the Arènes de Lutèce by Cyrille Castellant/Riches Heures.

A 3RD-CENTURY SURGEON'S INSTRUMENT CASE

Discovered in 1880 in a tomb at 180 avenue de Choisy (13th arrondissement in Paris), this surgeon's case contained small bronze instruments, some silver-plated. As was common at the time, the instruments are dual purpose, with a tool at each end. Their uniform decoration seems to indicate that the practitioner bought them in one lot rather than acquired them over time. Dated to the 3rd century by the coins within, the case contained a small crucible, two cupping glasses, a box and tubes once used for medicines (some with metal ingredients), a round spoon with a lip, tools for blowing powder into the nose and throat, two flat stones used as a mortar and pestle, forceps (one with a curved blade), scalpels with iron blades, a curette, spatulas with olive-shaped tips used as a probe or cauterizer, two tourniquets… We cannot tell from this exceptional find, conserved in the Carnavalet Museum (dedicated to the history of Paris), whether or not the owner was specialized in a particular medical practice. It does, however, seem clear that he practiced surgery, since ancient medical texts indicate that some of the forceps in the case were used for the ablation of tumors and that stylets and blunt-edged spatulas were at that time used for various operations.

Surgical instruments belonging to a Gallo-Roman physician, discovered in the 13th arrondissement of Paris in 1880. Musée Carnavalet, Paris. © Patrick Pierrain/Musée Carnavalet/Roger-Viollet.

Left: reconstitution of the Pilier des Nautes (Pillar of the Boatmen) at the Musée Carnavalet (1991).
© R. Briant et L. Degrâces/ Musée Carnavalet/ Roger-Viollet.

*Right: one of the sculpted stones of the Pilier des Nautes, repre*senting the Gallic god Esus (see inscription on the top of the stone). 14-17 AD.
Paris, Musée National du Moyen-Âge, Thermes de Cluny. © RMN/ Jean-Giles Berizzi/ Gérard Blot.

To take advantage of the natural slope of the south-east side of the Sainte-Geneviève hill, the *Arènes* were built outside the town. The plan combined an amphitheater and a theater, so scenes could alternate between gladiatorial combats and hunts, and theatrical productions, dances, and pantomimes. From the outside, the *Arènes de Lutèce* presented almost all the characteristics of a classical amphitheater, and measured 100×130.4 meters. The elliptical arena (52×46 meters) was encircled by tiers of seating, interrupted on the east side by the emplacement of a 41.2-meter long theater stage. The wall circling the arena was 2.2 meters high, suggesting that hunts and fights between big cats were staged there, an impression reinforced by the presence underneath the seating of five subterranean cells for wild animals.

(see Box, left page), building tradesmen (masons, painters, mosaicists, carpenters), not forgetting shoemakers, weavers, basket makers, cabinet-makers and woodworkers, carters, and others. We have evidence of three workshops, which were located in the outlying neighborhoods to limit the coming and going of carts through the town's streets and to minimize the risk of fire. One such workshop, in what is now the rue des Lombards, produced amphorae for the transport of wine in the 3rd century; the two others made crockery for the table and the kitchen.

The *nautae Parisiaci* of Lutetia—the boat owners and river pilots—oversaw transshipments on the Seine and its tributaries. The junction of the Seine and Rhône river basins was a

Detail of the Gallo-Roman segment of the Peutinger Table, a facsimile on vellum of the 3rd-century original. The magnified inset shows the word "Parisi," ie, Paris. The map was discovered in 1494 in Worms (Palatinate) by Conrad Celtes and bequeathed in 1508 to Konrad Peutinger. It consists of 11 sheets of velum, forming a 6.82×0.34-strip, featuring 200 000 km of Roman roads (the cursus publicus), from Spain and the British Isles to India. It is conserved at the Österreichische Nationalbibliothek in the Hofburg, in Vienna. © Musée de la Poste, Paris France/Archives Charmet/Bridgeman Art Library.

Inland water transport and the Pillar of the Boatmen

Lutetia in the Gallo-Roman era may have been a small town, with an estimated population of just under 10 000*, but it was lively and prosperous and its craftsmen and tradespeople were quite able to meet the daily needs of its inhabitants. A few funerary stelae yield useful indications suggesting the presence of fishmongers, blacksmiths, tanners, and shopkeepers. We lack direct accounts, yet it is easy to imagine the caterers (bakers, butchers, wine merchants), medical professionals

major strategic point in Gaul which Lutetia proclaimed as a mandatory port of call for merchandise shipped westwards and to the Atlantic. Locally too the Seine was vital for supplying those living on its banks with food (wine, olive oil, cereals) and materials (stones for building), and for shipping fresh supplies to the legions in the Lower Roman Empire. Controlled by the powerful guild of *nautae Parisiaci,* the river port of Lutetia had an elaborate infrastructure with wharfs, approach ramps, and landing stages. Such was the guild's influence that it offered to the Emperor Tiberius (14-37) a pillar covered with bas-relief depictions of Greco-Roman and Celtic deities and "paid for out of our joint fund." Several blocks of this Pillar of the Boatmen collected in 1711 from under the

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*Figure put forward by Sylvie Robin and Didier Busson who have overseen all archaeological digs in Paris for the last 20 years or so.

chancel of Notre-Dame Cathedral in Paris today have pride of place in the antiquities collection of the *Musée National du Moyen Age* (National Museum of the Middle Ages). This is the oldest sculpted monument dated by an imperial inscription discovered on French soil. This museum houses further evidence of the prosperity of Lutetia's boatmen: the large room of the *frigidarium* of the thermal baths—to which we have already referred—is remarkably decorated by four stone consoles sculpted with the prows of ships, an allusion suggesting that the watermen probably helped pay for the bathhouse.

And Lutetia became Paris...

Lutetia reached its apogee in the late 2nd and early 3rd centuries, after which came the first Barbarian incursions, political upheaval, and the uprisings of the *bagaudae,* peasant insurgents. After the fashion of numerous towns facing this climate of insecurity, the people of Lutetia erected ramparts around the *Île de la Cité,* abandoned the left bank of the Seine, and withdrew into this first enclosure of Paris, which extended over just 10 hectares. As was common practice in Antiquity, they used stones from demolished monuments and necropolises and abandoned buildings as foundations for the ramparts. The strategic value of the island in the Seine was thus turned to good account and this settlement became the new heart of the town.

At the same time Lutetia started to play an important part in the defenses of northern Gaul. In 357, Julian was appointed supreme commander of operations in Gaul and set up his headquarters there. Three years later, his soldiers and people acclaimed him emperor and he was clad in Tyrian purple. Julian, known as Julian the Apostate, loved Lutetia, as is clear from his writings: "I happened to be in winter quarters at my beloved Lutetia—for that is how the Celts call the capital of the Parisians. It is a small island lying in the river; a wall entirely surrounds it, and wooden bridges lead to it on both sides. The river seldom rises and falls, but usually is the same depth in the winter as in the summer season, and it provides water which is very clear to the eye and very pleasant for one who wishes to drink. For since the inhabitants live on an island they have to draw their water chiefly from the river. The winter too is rather mild there, […]. And a good kind of vine grows thereabouts, and some persons have even managed to make fig-trees grow by covering them in winter with a sort of garment of wheat straw and with things of that sort, such as are used to protect trees from the harm that is done them by the cold wind" (*Misopogon, or Beard-Hater,* a satirical essay. Translated by Wilmer Cave Wright for the Loeb Classical Library [1913]).

A basilica and a palace were erected and, in 365 and in 366, welcomed Emperor Valentinian I. These and other visits by influential figures augured well for the destiny of Lutetia, a town which in the early 4th century acquired a second name. On a milliary column (milestone) dated to 305-308, the town is referred to as *civitas parisiorum,* the city of Paris, a name which coexisted with that of Lutetia until the early Middles Ages when the latter fell into disuse. Lutetia had become Paris. ■

Further reading
– Busson D. *Paris ville antique, Guides archéologiques de la France,* Paris, Monum Editions du Patrimoine, 2001.
– Busson D, Robin S (dir.). *Les grands monuments de Lutèce.* Premier projet urbain de Paris, Catalogue d'exposition, Paris-Musées 2009.
– de Carbonnières P. *Lutèce. Paris ville romaine,* Paris, Gallimard, Découvertes 1997.
– Collectif. *Construire à Lutèce,* Catalogue d'exposition, Paris-Musées, 2007.
– Mousseaux RM, Robin S (dir.). *Et Lutèce devint Paris. Métamorphoses d'une cité au IVe siècle,* Catalogue d'exposition, Paris-Musées, 2011.
– Velay P. *De Lutèce à Paris : l'île et les deux rives,* Paris, CNRS éditions, 2009.

LUTÈCE, L'ANCÊTRE GALLO-ROMAINE DE PARIS

Chef-lieu du peuple des Parisii, *la ville gallo-romaine de Lutèce s'étendait principalement sur la rive gauche de la Seine, sur les pentes de la montagne Sainte-Geneviève et dans l'île de la Cité. Un réseau de rues orthogonales découpait la ville en îlots* (insulae) *dans lesquels s'inséraient espaces publics et quartiers d'habitation. Cette trame s'organisait autour d'un axe majeur nord-sud, l'actuelle rue Saint-Jacques. À la fin du IIe siècle, cette ville, modeste à l'échelle de la Gaule, atteignit son apogée et devait regrouper un peu moins de 10 000 habitants. Plusieurs monuments l'embellissaient : le forum avec sa basilique et son temple probable, ses édifices de spectacle (théâtre et surtout les « Arènes ») et ses thermes publics, ceux du sud, de l'est et du nord, dits de Cluny. L'agglomération tirait sa prospérité de ses artisans et de ses marchands. La navigation sur la Seine et sur ses affluents était contrôlée par la puissante corporation des nautes. Ces* nautae parisiaci *jouaient un rôle majeur dans la vie municipale. Au début de l'Empire, ils dressèrent même un monument à l'empereur Tibère, le célèbre Pilier des nautes. Au IVe siècle, confrontés à l'insécurité, les habitants de Lutèce délaissent la rive gauche et se replient sur l'île de la Cité qu'ils entourent d'un rempart. Paradoxalement, alors que la ville semble connaître un certain déclin, elle joue un rôle militaire de plus en plus important. En 360, Julien y est proclamé empereur par ses soldats. Mais déjà la ville commence à changer de nom et Lutèce devient Paris.*

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