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The Impact of Vitamin D, Calcium, Protein Supplementation, and Physical Exercise on Bone Metabolism After Bariatric Surgery: The BABS Study

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ABSTRACT

Laparoscopic Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are common and effective methods to treat severe obesity, but these procedures can adversely influence bone metabolism and areal bone mineral density (aBMD). This was a prospective 24-month single-center interventional two-arm study in 220 women and similarly aged men (median age 40.7 years) with a body mass index (BMI) $>38\ kg/m^2$ after RYGB and SG procedures. Patients were randomized into: 1) an intervention group receiving: 28,000 IU cholecalciferol/wk for 8 weeks before bariatric surgery, 16,000 IU/wk and 1000 mg calciummonocitrate/d after surgery, daily BMI-adjusted protein supplementation and physical exercise (Nordic walking, strength perseverance, and equipment training); 2) a non-intervention group: no preoperative loading, nutritional supplementation, or obligatory physical exercise. At study endpoint, when comparing the intervention group to the non-intervention group, the relative percentage changes of serum levels of sclerostin (12.1% versus 63.8%), cross-linked C-telopeptide (CTX, 82.6% versus 158.3%), 25-OH vitamin D (13.4% versus 18.2%), phosphate (23.7% versus 32%, $p < 0.001$ for all), procollagen type 1 amino-terminal propeptide (P1NP, 12% versus 41.2%), intact parathyroid hormone (iPTH, –17.3% versus –7.6%), and Dickkopf-1 (–3.9% versus –8.9%, $p < 0.05$ for all) differed. The decline in lumbar spine, total hip and total body aBMD, changes in BMI, lean body mass (LBM), as well as changes in trabecular bone score (TBS) values ($p < 0.005$ for all) were less, but significantly, pronounced in the intervention group. We conclude that vitamin D loading and ongoing vitamin D, calcium, and BMI-adjusted protein supplementation in combination with physical exercise decelerates the loss of aBMD and LBM after bariatric surgery. Moreover, the well-known increases of bone turnover markers are less pronounced. © 2015 American Society for Bone and Mineral Research.

KEY WORDS: BIOCHEMICAL MARKERS OF BONE TURNOVER; BONE–FAT INTERACTIONS; DXA; CLINICAL TRIALS

Introduction

Bariatric surgery is a common and effective method to treat severe obesity. Laparoscopic Roux-en-Y gastric bypass (RYGB) and laparoscopic sleeve gastrectomy (SG) are associated with rapid weight loss and a reduction of cardiovascular comorbidities.[1] However, bariatric surgery negatively influences bone metabolism, as has been repeatedly reported.[2] Both RYGB and SG lead to a decline in areal bone mineral density (aBMD), deterioration in bone structure, and an increase in bone resorption up to 6 years after surgery.[3–5] Moreover, prevalent and ongoing vitamin D deficiency is a common side effect after bariatric surgery, leading to secondary hyperparathyroidism followed by increased bone turnover.[6]

A valid explanation for these detrimental effects may be nutritional deficiency and malabsorption after bariatric surgery. A decrease in levels of heat-shock proteins in the jejunal mucosa as co-activators of the vitamin D receptor with negatively impaired calcium absorption and bone metabolism was recently reported.[5]

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To restore bone quality after bariatric surgery, an individualized correction of nutritional deficiency is highly recommended.[7] Calcium and vitamin D replacement should be initiated shortly after the surgical procedure to prevent bone loss and to avoid vitamin D deficiency and secondary hyperparathyroidism.[4,6] Currently, it is unclear if supplemental calcium and vitamin D loading is sufficient to compensate for bone loss and to improve health-related outcomes after bariatric surgery.[3,8,9]

Taking into account the rapid loss not only of fat and bone tissue but also of lean mass, patients would likely benefit from protein supplementation after bariatric surgery. Adequate food intake leads to an acute reduction of elevated bone resorption because of increases of postprandial insulin levels in patients with RYGB.[6,10,11,12] Therefore, maintaining sufficient levels of vitamin D, behavioral management, dietary considerations, patient education, eating habits, protein intake, and physical activity are strongly recommended to improve long-term weight loss in patients after bariatric surgery.[13,14]

Hypothesis

We tested the hypothesis that in obese female and male patients, vitamin D, calcium, and protein supplementation programs in conjunction with moderate physical exercise positively influence changes in bone metabolism, BMD, and body composition after bariatric surgery.

Objectives

The primary objective of this study was to investigate differences in serum markers of bone turnover (BTM) between the intervention group (loading/supplementation of vitamin D, calcium, protein, and muscle exercise) and the non-intervention group (without any supplementation or exercise) after 24 months.

Secondary objectives included the evaluation of differences between both groups for changes in areal lumbar spine, total hip and total body BMD, trabecular bone score (TBS), as well as changes in lean body mass, body composition, and quality-of-life scores.

Materials and Methods

Study design

This was a prospective interventional two-arm open-label single-center study in premenopausal women and similarly aged men with morbid obesity. The study was performed at the St. Vincent Hospital, Medical Department II, in Vienna, Austria. The first patient entered the study in October 2011 and the last patient completed the study in March 2015.

Patients were recruited at the Department of Visceral Surgery at the St. Vincent Hospital in Vienna, a specialized referral center for bariatric surgery in Austria. The decision regarding the respective surgical method—RYGB or SG—was based on the determination of the department of surgery and the patient's own judgment.

A 1:1 randomization table was generated by an independent statistician and given to clinical nurses not involved in the study who kept these data confidential. When an informed consent document had been signed, the investigators assigned consecutive numbers to the patients in chronological order. Study-related medications were assigned according to the randomization table provided by the clinical nurse at the request of the investigators.

The study was approved and supervised by the local ethics committee. All patients signed a written informed consent form before any study-related procedures. The study has been registered in Clinical Trials: NCT01739855.

Inclusion and exclusion criteria

Obese premenopausal women and men (minimum age 25 years) with a body mass index (BMI) $\geq$38 kg/m^2 and a total body weight $\leq$160 kg were included. A cortisol stress test was performed before surgery. Patients were excluded if they had received any prior oral calcium and/or vitamin D supplementation consistent with recommended dosages to prevent osteoporosis (>500 mg calcium/d and/or >800 IU vitamin D), any antiresorptive or anabolic bone-specific therapy, as well as cessation of menstrual bleeding. Further exclusion criteria were any ongoing therapy with insulin, oral anti-diabetic drugs, elevation of liver enzymes (ASAT >45 IU/L, ALAT >45 IU/L, GGT >60 IU/L), eGFR <90 mL/min/1.73m^2, elevation of alkaline phosphatase, systemic or inhalative glucocorticoid use, hypogonadism, any systemic inflammatory disease, 25-hydroxyvitamin D deficiency <10 ng/mL, or alcohol use >3 units/d.

Intervention and non-intervention groups

In the intervention group, all patients received 28,000 IU of cholecalciferol/wk delivered as sublingual drops for 8 weeks before surgery. After surgery, the maintenance sublingual dosage was 16,000 IU/wk for 24 months. A daily oral supplement of 1000 mg calciummonocitrate was also initiated. Patients were instructed to ingest the calcium supplement in 4 units/d throughout the entire study period. After the first postoperative week, patients were additionally instructed to add protein powder to their food intake 4 times/d. The amount of daily protein supplementation was adjusted on BMI and the individual basal metabolic rate (BMR; the amount of energy required to maintain the body's normal metabolic activity at rest with no additional activity) based on the Harris-Benedict-Formula: females: $BMR = 655.096 + (9.563 \times \text{weight in kg}) + (1.850 \times \text{height in cm}) - (4.676 \times \text{age in years})$; males: $BMR = 66.473 + (13.752 \times \text{weight in kg}) + (5.003 \times \text{height in cm}) - (6.755 \times \text{age in years})$.[15] Protein supplementation ranged from 35 to 60 g/d.[16,17] All patients initiated an individualized aerobic exercise program 2 weeks after surgery, which was monitored and documented by experts of physical medicine: Nordic walking adapted to the individual's target heart rate for a minimum of 45 minutes at least 3 times/wk, as well as strength perseverance and equipment training for 30 minutes at least 2 times/wk. At each study visit, patients received instructions for the optimization of their physical exercise by experts from the department of physical medicine and rehabilitation at the hospital.[18] Each patient received a treatment diary to document the date and time of all study-related supplements and procedures: intake of cholecalciferol drops, calciummonocitrate and protein powder, nutritional behavior, the date, and duration of physical exercise.

In the non-intervention group, patients did not receive an 8-week vitamin D loading with cholecalciferol drops before the surgery, nor any of the previously mentioned supplementation or a physical exercise program before or after the surgery (Fig. 1). Findings on the non-interventional female population were recently reported elsewhere.[10]

At each study visit after bariatric surgery, the patients received specific instructions regarding food intake behavior,

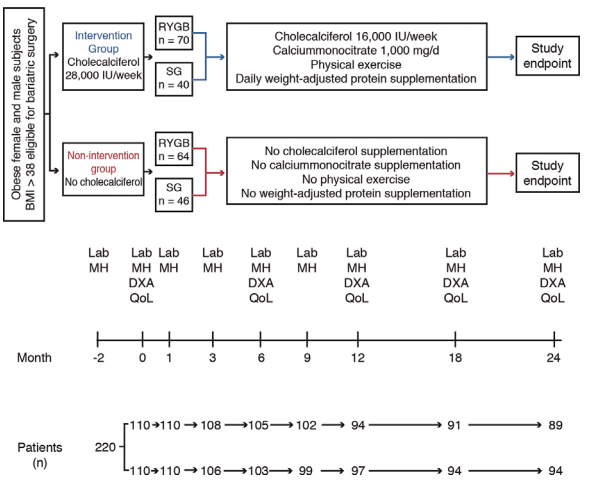

Fig. 1. Study flowchart. Lab = fasting serum laboratory examinations; MH = medical history; BMD = bone mineral density (BMD L_1 to L_4, BMD total hip, BMD total body). Patients (*n*) indicates the number of patients included in the analyses at each study visit.

the recommended exercises, and the necessary components of nutrition (35% protein, 30% carbohydrates, 35% high-quality fat; Supplemental Table S1) after bariatric surgery at each study visit. Each patient, regardless of grouping, also received a daily supplemental tablet containing essential vitamins and micronutrients (Supradyn forte: vitamin D 5 μg (200 IU), calcium carbonate 120 mg, vitamin A 800 μg, vitamin B1 1.1 mg, vitamin B2 1.4 mg, vitamin B6 1.4 mg, vitamin B12 2.5 μg, vitamin C 80 mg, vitamin E 12 mg, vitamin K 25 μg, folic acid 200 μg, niacin 16 mg, iron 14 mg, magnesium 80 mg, copper 1000 μg, selenium 50 μg, and zinc 10 mg). No patients were switched between the two study groups. Monthly telephone interviews were conducted; satisfactory treatment compliance in the intervention group was defined as ≥80% of all study-related procedures.

Initial laboratory assessments and the randomization were performed during the required examinations for determining the eligibility of each patient for bariatric surgery in line with the regulations of the Austrian health system (minimum 8 weeks before surgery). All other study-related laboratory and medical history procedures were performed within 3 days before RYGB or SG, after 1 month, after 3 months, and quarterly in the ongoing first year. During the second year of the study period, all patients had two additional visits (months 18 and 24). Dual-energy X-ray absorptiometry (DXA) was performed before the surgery (<3 days) and at months 6, 12, 18, and 24. The time frame for each visit was ±30 days (with the exception of the first visit 1 month after surgery: ±5 days) (Fig. 1).

Laboratory analyses

Blood sampling was performed between 8 a.m. and 10 a.m. after an overnight fast. Samples were immediately centrifuged, cooled, and stored at −70°C for later analysis. The sclerostin and Dickkopf-1 (DKK-1) levels from serum were quantitatively determined using an established enzyme immunoassay (EIA) kit (intra-assay coefficient of variation [CV] is 5% to 6% for sclerostin and 4% to 7% for DKK-1; Biomedica, Vienna, Austria). Cross-linked C-telopeptide (CTX), procollagen type 1 amino-terminal propeptide (P1NP), intact parathyroid hormone (PTH), and 25-hydroxyvitamin D (25-OH vitamin D) were measured via chemoluminescence on the IDS-iSYS microparticle immunoassay system (Immunodiagnostic Systems Ltd, Boldon, UK). The intra-/interassay coefficients of variation for CTX are 2.1% to 4.9%, for P1NP 2.6% to 3.0%, for PTH 1.1% to 3.7%, and for 25-OH vitamin D 5.5% to 7.1%. Total serum calcium levels were photometrically determined on the Architect ci8200 platform (Abbott Laboratories, Abbott Park, IL, USA).

DXA measurements

BMD was measured after daily cross-calibrations with a standardized control phantom using DXA (GE Lunar [Madison, WI, USA] iDXA scanner, software version Encore 13, 50,040). The CV for the spine was 0.41% and 0.53% for total hip. Body composition including total skeletal BMD, total body weight (kg), and lean body mass (kg) were also measured with this DXA scanner; CV for fat distribution was 2.3%. IOF-ISCD-certified technicians took all measurements. According to the manufacturer's recommendation, the upper weight limit for all DXA measurements was set to 160 kg and all measurements were performed with the "fat scan mode" at a BMI >28. The DXA files of the study population were digitally exported, and the raw data was extracted to a specific workstation for TBS calculation using the latest version of TBS iNsight software (Medimaps SA, Merignac, France). Unitless TBS values were calculated as the mean value of the measurement for vertebrae L_1 to L_4 exactly at the same ROI as spine aBMD. The TBS software is validated for BMI ranges from 15 to 37. In keeping with the manufacturer's recommendation, all TBS values measured at a BMI >37 were adjusted for tissue thickness. Reported in vivo precision for TBS ranges from 1.1% to 1.8%.[19]

Quality of life (QoL)

To evaluate changes of QoL, the Short-Form Health Survey (SF-36) questionnaire was used, which includes the following domains: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, emotional well-being, mental health, physical summary, and mental summary. All patients were invited to complete a questionnaire before the surgery and at months 6, 12, 18, and 24.

Statistical analysis

The study was designed to enroll approximately 82 completers per study arm. With this available sample size of each group, the two-sided 95% confidence interval for an underlying power to detect a mean within a group difference of 0.035 g/cm^2 in total body BMD, assuming a standard deviation (SD) of 0.082 g/cm^2, will have limits ranging from 0.81 to 0.94.

All continuous outcome variables are described by the median and interquartile ranges. Patients with at least one post baseline value were included in the analyses data set. For each analysis, all available and valid values were included. Arithmetic median and interquartile ranges were calculated when determining categorical baseline data, percentages, and continuous variables. Log transformation was used for non-normally distributed data, and the Wilcoxon test was implemented if the normalization failed. Relative percentage changes of aBMD and biochemical markers between baseline and month 24 are presented. A likelihood-based method of the mixed-effect model repeated measure (MMRM) for the study population was applied that included all patients with at least

one post-baseline visit. Data from patients that were excluded during the study were included until the last visit before the date of exclusion. To minimize any statistical bias, no statistical imputations of missing values were calculated. For the changes and for pairwise comparisons between the groups, two-sided p values are presented. When testing the hypothesis (relationship between covariates and dependent measures) general linear models (analysis of variance, ANOVA) with type I and type III testing with or without adjustments on serum markers and DXA values for the respective patient group, sex, and surgical method were performed. The level of statistical significance was set to 5%. The SAS software (version 9.4, 2002–2012, SAS Institute Inc., Cary, NC, USA) was used for data analyses.

Results

Baseline demographic data

A total of 261 obese premenopausal women and similarly aged men were invited to participate. Of these 261, 238 opted in by way of a signed written informed consent document. Eighteen patients who had sustained immediate and severe postoperative complications (anastomotic leaks or postoperative bleeding complications resulting in a second surgery and/or sepsis) were excluded from all analyses. A total of 110 patients participated in each group with a median age of 41 years in the intervention group and 40 years in the non-intervention group and a BMI of 44.3 and 44.2 kg/m^2, respectively. The proportion of male patients was 40% and 44.5%, respectively. In the intervention group, 58% were current smokers, with 62% in the non-intervention group. Levels of fasting glucose, cholesterol, intact parathyroid hormone, and DKK-1 were elevated above the upper limit of normal, whereas sclerostin, CTX, and P1NP were within the lower limit of normal. Adjustments for smoking status did not significantly alter the statistical results based on the objectives of this study. Baseline values and differences between the two groups are shown in Table 1.

Bone turnover marker (BTM)

An increase of sclerostin levels with a peak 6 months after surgery was observed in both groups but to a lesser extent in the intervention group. Differences between the two groups remained significant until study endpoint (12.1% versus 63.8%). DKK-1 declined to minimum values at month 6 and then increased to values of –3.9% in the intervention group and –8.9% ($p = 0.041$) in the non-intervention group at the study's endpoint. In the non-intervention group, increases of CTX were more pronounced with relative changes of 158.3% compared with 82.6% ($p < 0.001$). Changes of P1NP levels were virtually equal in both groups until month 9; thereafter, the increase in the non-intervention group was more pronounced (12.0% and 41.2%; $p = 0.003$). Although levels of iPTH decreased in the intervention group and remained within normal range from month 3 until month 24, they remained elevated above the upper limit of normal in the non-intervention group. After an 8-week cholecalciferol loading phase with 28,000 IU/wk before surgery, median comparable low vitamin D levels were significantly elevated in the intervention group (28.0 versus 17.9 ng/mL, $p < 0.001$). In the non-intervention group, median vitamin D levels tardily increased above levels $\geq$20 ng/mL, mostly in the second year, whereas in the intervention group, these levels clearly remained above the threshold $\geq$30 ng/mL. Serum and albumin-adjusted calcium decreased in the non-intervention group until month 6. After 24 months, values comparable to baseline were observed in both groups. Serum phosphate increased in both groups. Albumin levels declined in both groups but to a lesser extent in the intervention group (Table 1, Fig. 2*A*–*H*).

DXA measurements

At the lumbar spine, median aBMD values did not significantly change in the intervention group but decreased in the non-intervention group (–1.2% versus –7.9%, $p > 0.001$). At the total hip, the decrease in aBMD was lower in the intervention group (–3.9% versus –9.9%, $p < 0.001$). Total body aBMD values continuously declined in both groups (–2.0% versus –4.1%, $p < 0.001$). In the early phase of the study, TBS values decreased in both groups but then remained stable in the intervention group (–3.4% versus –10.5%, $p < 0.001$). In both groups, changes in BMI were continuous but less so for the intervention group (–5.5% versus –7.3%, $p = 0.002$). Total body fat diminished in both groups without any statistical difference. The decline in lean body mass was significantly lower in the intervention group (–3.5% versus –12.4%, $p < 0.001$) (Table 1, Fig. 3*A*–*F*).

General linear model (GLM)—ANOVA

In the unadjusted GLM models, changes of DXA values expressed as least squares means (LSC) were tested on the effects of the covariates intervention/non-intervention group, surgical method (RYGB/SG), or sex. For aBMD spine, hip, total body, and for TBS, the changes at study endpoint in the intervention group significantly differed from the non-intervention group, whereas this was not observed for sex or—with the exception of BMD spine—the type of surgery. Changes of serum levels of sclerostin, CTX, and P1NP, but not DKK-1, had significant independent effects on the changes of aBMD or TBS values. After adjustment for sclerostin, the respective changes of aBMD and TBS values between the intervention group and partly between sexes were still significant. Similar associations were observed after adjustments for CTX and P1NP, although this was not noticed for DKK-1 (Table 2).

Fractures

Two atraumatic fractures occurred in the non-intervention group (radius, humerus), and one traumatic fracture was observed in the intervention group (rib).

Quality of life

During the entire study period, within the intervention group, significant improvements with regard to social functioning, emotional role, physical summary, and mental summary and mental health were observed from month 6 until study endpoint. The components of physical functioning, general health, and bodily pain improved from study month 6 and remained significant ($p < 0.05$) until study end. In the non-intervention group, significant improvements in social functioning, mental rapidity, and mental health (emotional role) were reported from month 9. Additionally, significant improvements in physical summary from month 12 ongoing were reported.

Dropout rate

The overall dropout rate was 16.4% (13.2% in the first year and 4.2% in the second year). In the intervention group, 13 patients were—according the threshold of 80%—noncompliant with

Table 1. Baseline Characteristics of the Study Population (1 to 3 Days Before Bariatric Surgery) and Relative Percentage Changes Between Month 0 and Month 24

	Baseline					Month 24 relative percentage changes from baseline to study endpoint				
	Intervention group Median/IQR $n = 110$		Non-intervention group Median/IQR $n = 110$		p Value	Intervention group Median/IQR $n = 89$		Non-intervention group Median/IQR $n = 94$		p Value
Age (years)	41.0	34.0, 45.0	40.0	35.0, 45.8	0.636	–	–	–	–	
Body mass index (kg/m^2)	44.3	41.1, 47.9	44.2	40.7, 47.7	0.748	−5.5%	−9.4, −3–2	−7.3%	−9.4, −1.7	**0.002**
Height (cm)	165	160, 170	1165	160, 171	0.754	0.3%	−0.4, 0.1	0.3%	−0.5, 0	0.817
Weight (kg)	119.6	110.0, 129.0	120.6	110.4, 131.8	0.632	−43.7%	−47.6, −39.1	−47.4%	−49.1, −40.0	**0.030**
Total body fat (kg)	59.0	51.1, 71.5	62.4	72.1, 53.6	0.191	−54.6%	−60.7, −51.6	−59.7%	−61.0, −57.8	0.119
Proportion fat (%)	53.4	52.1, 60.4	54.7	50.4, 58.9	0.583	−32.7%	−40.8, −44.3	−33.5%	–41.4, –42.6	0.316
Lean body mass (kg)	56.8	61.5, 52.8	54.5	60.4, 52.4	0.393	−3.5%	−2.4, −4.9	−12.4%	−16.1, −8.1	**<0.001**
Albumin (g/dL)	4.9	4.5, 5.2	4.8	4.4, 5.2	0.141	−16.8%	−13.2, −16.8	−27.3%	−31.5, −23.4	**0.017**
Sclerostin (pmol/L)	33.9	26.1, 50.7	32.2	24.8, 44.2	0.434	12.1%	8.5, 24.5	63.8%	34.0, 95.3	**<0.001**
Dickkopf-1 (pmol/L)	29.5	15.5, 45.0	29.0	17.3, 47.0	0.588	−3.9%	−28.9, 10.6	−8.9%	−21.3, −12.7	**0.041**
CTX (ng/mL)	0.27	0.20, 0.37	0.26	0.20, 0.34	0.561	82.6%	29.9, 122.3	158.3%	78.0, 268.3	**<0.001**
P1NP (μg/L)	36.0	30.7, 44.4	37.6	32.5, 46.2	0.563	12.0%	−2.2, 30.3	41.2%	0.1, 81.5	**0.003**
iPTH (pg/mL)	71.9	65.7, 86.4	78.4	63.7, 96.3	0.092	−17.3%	−28.9, −12.7	−7.6%	−17.2, 4.0	**0.040**
25-OH vitamin D	17.4[a]	13.4, 22.61[a]	17.7[a]	13.0, 21.9[a]	0.821[a]	44.6%[a]	34.9, 52.8[a]	18.0%[a]	15.0, 22.1[a]	**<0.001a**
(ng/mL)	28.0	25.1, 31.3	17.9	13.9, 22.2	**<0.001**	13.4%	5.7, 27.0	18.2%	15.1, 22.7	**<0.001**
Calcium (mmol/L)	2.39	2.33, 2.44	2.38	2.30, 2.45	0.405	−1.3%	−5.0, 5.1	−0.3%	−4.9, 5.3	0.648
Phosphate (mmol/L)	1.03	0.95, 1.13	1.03	0.95, 1.12	0.836	23.7%	18.1, 25.1	32.0%	24.9, 40.7	**<0.001**
BMD L_1 to L_4 (g/cm^2)	1.289	1.210, 1.402	1.263	1.188, 1.387	0.613	−1.2%	−1.7, −0.7	−7.9%	−10.0, −7.1	**<0.001**
BMD total hip (g/cm^2)	1.196	1.112, 1.286	1.222	1.100, 1.296	0.762	−3.9%	−4.4, 1.0	−9.9%	−12.4, −7.7	**0.014**
BMD femoral neck (g/cm^2)	1.127	0.935, 1.188	1.113	0.953, 1.190	0.848	−3.2%	−4.1, −0.9	−6.7%	−10.5, −6.4	**0.011**
BMD total body (g/cm^2)	1.275	1.177, 1.364	1.297	1.211, 1.371	0.252	−2.2%	−2.5, −1.5	−4.1%	−5.0, −3.0	**0.016**
TBS (unitless)	1.265	1.213, 1.319	1.271	1.208, 1.366	0.860	−3.4%	−8.1, 0.7	−10.5%	−13.6, −1.3	**0.034**

CTX = serum type 1 collagen cross-linked C-telopeptide; P1NP = intact amino terminal propeptide of type 1 procollagen; iPTH = intact parathyroid hormone; BMD = areal bone mineral density; TBS = trabecular bone score.

Values are presented as median and interquartile ranges (IQR). Significant findings are bold.

[a]Serum values obtained 8 weeks before surgery.

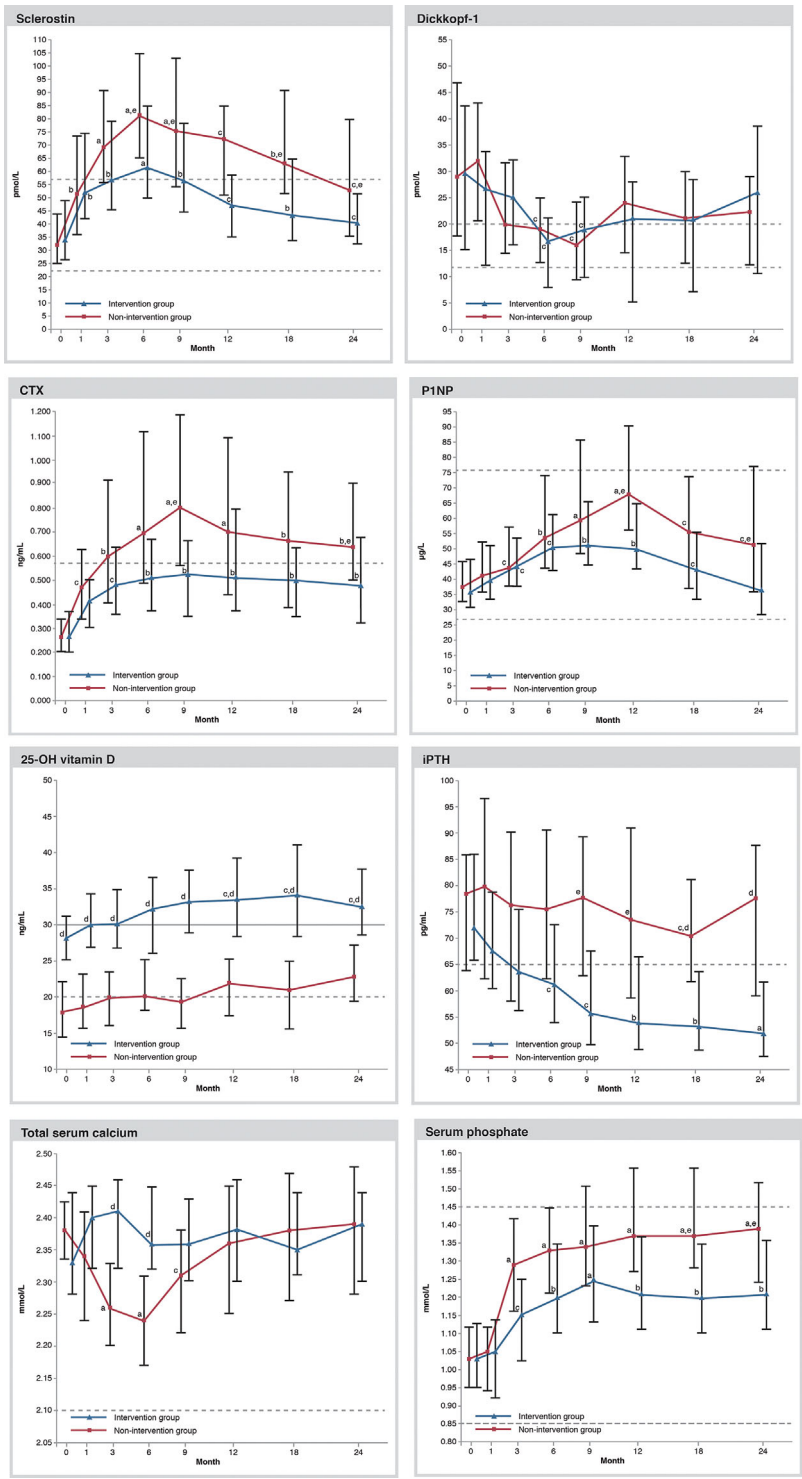

Fig. 2. Median and interquartile fasting serum parameters: (*A*) sclerostin; (*B*) Dickkopf-1; (*C*) CTX; (*D*) P1NP; (*E*) 25-OH vitamin D; (*F*) iPTH; (*G*) total serum calcium; and (*H*) serum phosphate. The *p* values indicate absolute median changes between time points. a = $p < 0.001$ versus baseline; b = $p < 0.01$ versus baseline; c = $p < 0.05$ versus baseline; d = $p < 0.001$ between intervention and non-intervention group; e = $p < 0.05$ between intervention and non-intervention group. Dotted horizontal lines indicate the age-adjusted reference range of sclerostin, Dickkopf-1, P1NP, and serum phosphate, respectively; single dotted horizontal lines indicate the lower limit of normal for vitamin D, CTX, and total serum calcium, respectively, and the upper limit of normal for iPTH. Normal values for sclerostin and Dickkopf-1 are given in Dovjak and colleagues.[36]

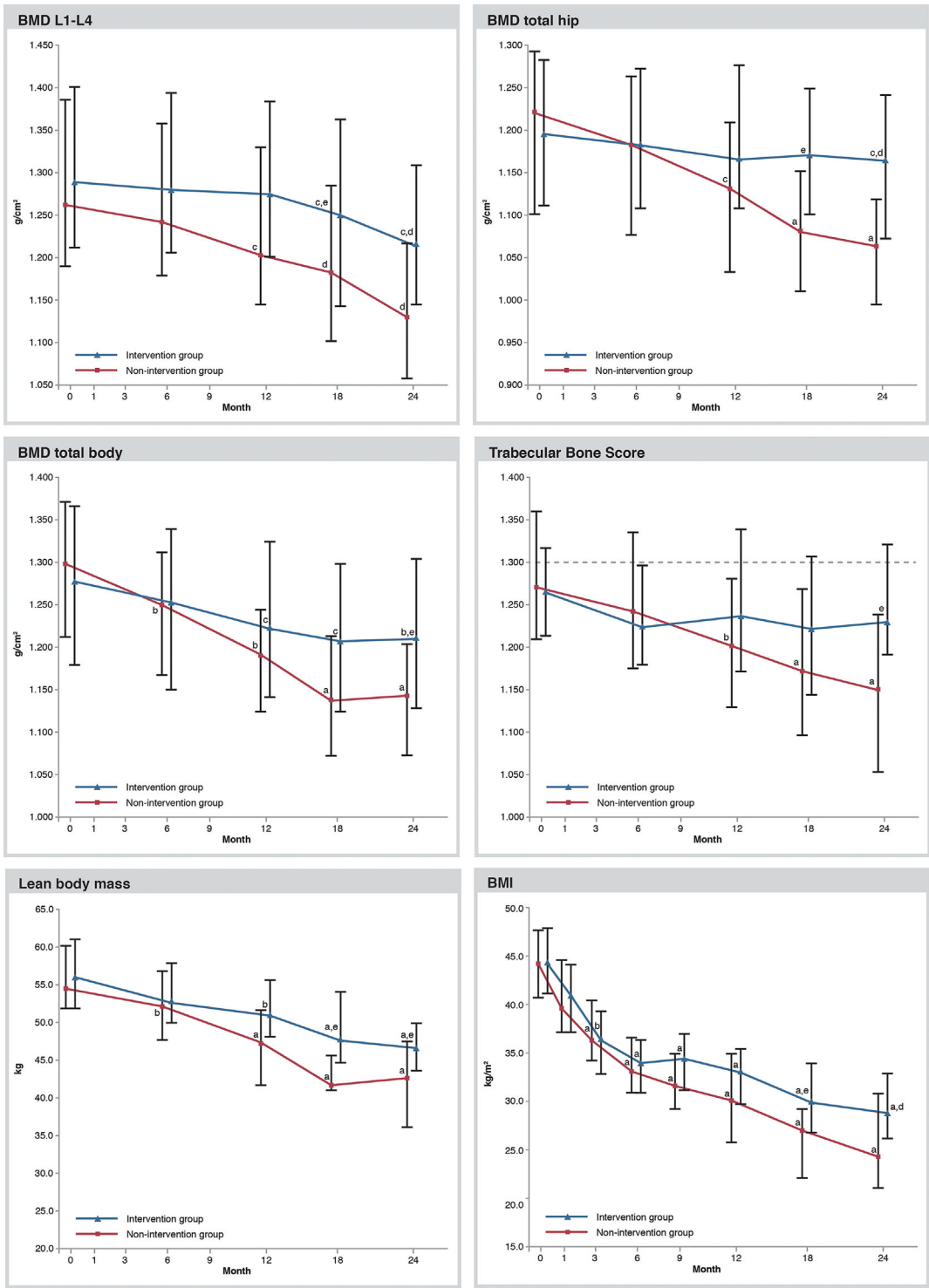

Fig. 3. Median and interquartile values of areal bone mineral density and body composition parameters: (*A*) bone mineral density (BMD) lumbar spine L_1 to L_4; (*B*) BMD total hip; (*C*) BMD total body; (*D*) trabecular bone score (TBS); (*E*) lean body mass; and (*F*) body mass index (BMI). The *p* values indicate absolute median changes between time points. a = $p < 0.001$ versus baseline; b = $p < 0.01$ versus baseline; c = $p < 0.05$ versus baseline; d = $p < 0.001$ between intervention and non-intervention group; e = $p < 0.05$ between intervention and non-intervention group. Single dotted horizontal lines indicate the lower limit of normal TBS values.

Table 2. General Linear Model (ANOVA)[a]

BMD spine	Unadjusted			Adjusted for sclerostin			Adjusted for DKK-1			Adjusted for CTX			Adjusted for P1NP		
				Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value
Serum marker				0.273		**0.039**	0.0031		0.832	0.943		**<0.001**	0.035		**0.014**
	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value
Intervention group	−1.09			−1.18			−2.23			−2.39			−2.02		
Non-intervention group	−7.74	6.65	**<0.001**	−8.06	6.88	**<0.001**	−2.81	0.58	0.593	−6.36	3.97	**<0.001**	−5.34	3.32	**<0.001**
RYGB	−5.52			−5.31			−3.62			−5.15			−4.52		
SG	−6.03	0.51	**0.031**	−5.90	0.59	0.152	−2.96	−0.66	0.694	−4.26	0.89	**0.022**	−2.45	2.07	**0.003**
Female	−5.12			−4.52			−2.61			−4.62			−3.52		
Male	−5.67	0.55	0.068	−5.05	0.53	**0.042**	−3.05	0.44	0.731	−4.56	−0.06	0.539	−3.65	−0.17	0.734

BMD total hip	Unadjusted			Adjusted for sclerostin			Adjusted for DKK-1			Adjusted for CTX			Adjusted for P1NP		
				Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value
Serum marker				0.052		**0.044**	0.0049		0.756	1.048		**<0.001**	0.476		**0.035**
	LSM	Δ	*p* Value	*LSM*	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value
Intervention group	−1.35			−1.77			−1.54	0.09	0.593	−2.04			−3.54		
Non-intervention group	−10.4	9.03	**<0.001**	−10.7	8.94	**<0.001**	−1.63			−9.45	7.41	**<0.001**	−7.31	3.77	**<0.001**
RYGB	−6.43			−6.41			−1.87	0.14	0.196	−4.58			−3.58		
SG	−6.04	−0.39	0.475	−6.09	−0.32	0.488	−2.01			−3.96	−0.62	0.079	−4.01	0.43	0.649
Female	−5.48			−5.39			−3.41	−0.12	0.423	−6.38			−6.27		
Male	−6.99	1.51	0.059	−7.02	1.63	**0.014**	−3.29			−5.97	−0.41	0.385	−6.02	−0.25	0.539

BMD total body	Unadjusted			Adjusted for sclerostin			Adjusted for DKK-1			Adjusted for CTX			Adjusted for P1NP		
				Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value
Serum marker				0.248			0.079		0.089	0.785			0.384		
	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value
Intervention group	−1.96			−1.99			−0.87			−2.93			−2.43		
Non-intervention group	−3.74	1.78	**<0.001**	−3.71	1.72	**<0.001**	−0.77	−0.10	0.864	−6.42	3.49	**<0.001**	−4.62	2.19	**<0.001**
RYGB	−2.79			−2.82			−1.48			−4.23			−3.22		
SG	−2.88	0.09	0.612	−2.91	0.09	0.512	−1.74	0.26	0.245	−4.01	−0.22	0.283	−3.93	0.71	0.087
Female	−2.87			−2.86			−2.43			−3.57			−1.96		
Male	−3.41	0.54	0.079	−2.84	−0.02	0.941	−2.58	0.15	0.102	−2.89	−0.68	0.129	−2.95	0.99	0.064

TBS	Unadjusted			Adjusted for sclerostin			Adjusted for DKK-1			Adjusted for CTX			Adjusted for P1NP		
				Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value	Intercept		*p* Value
Serum marker				−0.33		**0.003**	0.085		0.634	0.265		**0.042**	0.383		**0.004**
	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value	LSM	Δ	*p* Value
Intervention group	−3.04			−3.54			−2.46			−3.66			−3.27		
Non-intervention group	−7.61	4.57	**<0.001**	−4.93	1.39	**0.008**	−5.34	2.88	0.069	−8.24	4.58	**<0.001**	−6.72	3.45	**0.008**
RYGB	−5.48			−5.02			−6.32			−4.12			−5.87		
SG	−6.11	0.63	0.074	−5.78	0.76	**0.028**	−5.78	−0.54	0.275	−5.02	0.90	**0.042**	−4.98	−0.89	0.072
Female	−5.02			−4.55			−6.31			−4.39			−5.77		
Male	−5.43	0.41	0.211	−4.87	0.32	0.185	−6.22	−0.09	0.799	−4.87	0.48	0.175	−5.23	−0.54	0.353

BMD = bone mineral density; RYGB = Roux-en-Y gastric bypass; SG = sleeve gastrectomy; DKK-1 = Dickkopf-1; CTX = serum type 1 collagen cross-linked C-telopeptide; P1NP = intact amino terminal propeptide of type 1 procollagen; TBS = trabecular bone score.

[a]Least squares means (LSM) and mean difference of percentage points (Δ) of dependent variables (bold) and covariates. Intercept = predicted value of the dependent variable when all the independent variables are 0 for testing the hypothesis. Significant *p* values are bold.

physical exercise requirements (months 6, 9, 12, 18, and 24) and 8 patients reported inadequate nutritional behavior (months 3, 12, and 18). In the non-intervention group, 12 patients stated that they were unwilling to stay within the study (months 3, 6, 9, and 18) and 4 patients (months 12 and 18) had to be excluded because of new employment outside the area of Vienna.

Discussion

This was an interventional open-label clinical trial in premenopausal women and similarly aged men with morbid obesity. We hypothesized that vitamin D loading before bariatric surgery and ongoing vitamin D, calcium, and protein supplementation, as well as physical exercise might have beneficial effects regarding bone metabolism and loss of lean body mass.

With this study, we report that the oral supplementation of vitamin D, calcium, and protein in patients after RYGB and SG, when compared with patients without supplementation, leads to smaller increases of sclerostin and CTX levels, lower decline of DKK-1, and a normalization of iPTH levels. Additionally, supplementation affected the loss of aBMD at the spine, hip, and total body less when compared with the non-intervention group. Although there was no difference in the loss of total body fat between the two groups, our multifactorial approach influenced the loss of lean body mass less than no supplementation.

Increases of serum sclerostin, CTX, and P1NP levels provide important information on the continuous decline of aBMD after bariatric surgery.[10] The Wnt pathway and its endogenous inhibitors sclerostin and DKK-1 are key regulators in bone formation. Although we observed a significant and ongoing increase of sclerostin with a peak after 6 months and a continuous decline in both groups, these changes were less pronounced in the intervention group. The lack of mechanical loading caused by the rapid and excessive weight loss might explain the increases of sclerostin serum levels via changes of SOST, RANKL, and OPG activity. In contrast, DKK-1 levels only transiently changed without difference between both groups. Changes of sclerostin, but not DKK-1, levels were found to be a significant discriminator for the nonfavorable changes in BMD and TBS in these patients in the ANOVA model.

In both groups, bone serum resorption and formation markers increased after surgery. In the non-intervention group, increases of sclerostin, CTX, and P1NP levels were more pronounced. CTX and P1NP changes were highly significant covariates on the changes of aBMD and TBS in the whole study population. This is in contrast to young patients with idiopathic osteoporosis and fragility, where P1NP and CTX (but not sclerostin levels) are significant values for trabecular number, which results in an improvement of the noninvasive predictability of bone microarchitecture.[20]

Obese patients are also known to have elevated levels of iPTH, mainly because of diminished levels of vitamin D. In the intervention group, we were able to increase low levels of vitamin D to recommended normal levels with a cumulative loading dose of 224,000 IU cholecalciferol before the bariatric surgery.[21] With a weekly sublingual dose of 16,000 IU cholecalciferol, vitamin D levels remained stable within the normal range, and iPTH levels continuously declined in the intervention group. Hyperparathyroidism shows a trend toward lower sclerostin levels and higher markers of bone resorption with negative effects on bone quality in postmenopausal and also in young obese patients. Bariatric surgery causes severe and irrecoverable changes in intestinal anatomy with diminished capability of calcium resorption. The upper intestine is the key target for the vitamin D receptor (VDR) because high calcium intake, or selective VDR rescue in the intestine, restores a normal bone.[22–24] Recent data strongly suggest adequate calcium and vitamin D supplementation after bariatric surgery.[8,9] In the non-intervention group, a slight but significant increase of median vitamin D levels without specific supplementation of cholecalciferol was observed. These levels clearly remained below recommended thresholds. The medical history of the patients showed no evidence of supplementation/enrichment with the exception of a daily tablet with micronutrients and slight doses of vitamin D (200 IU/d). In Austria, food is not supplemented with vitamin D and the use of supplements or OTC drugs is not common. The likeliest explanations for this increase are the changes of nutritional behavior after bariatric surgery from a high-fat/low-vitamin diet toward a structured, well-balanced diet of foods high in natural vitamins, beneficial exposure to the sun, and an overall improved QoL.

Although there is debate regarding aBMD imaging accuracy with DXA technology in obese patients, bone loss after bariatric surgery and changes in body composition is well documented.[10,25] Rapid and ongoing decreases of aBMD with increased BTM after bariatric surgery, which are not solely based on the adaptive process of the skeleton to the reduced body weight, negatively influence biomechanical properties of bone.

In the treatment of osteoporosis, adequate supplementation of calcium and vitamin D is able to maintain aBMD at a certain level or to diminish aBMD decreases. In both groups, a loss of aBMD values at each investigated skeletal site was observed, but the decline was minor in the intervention group. In the intervention group at the hip, a bone site predominantly composed of cortical bone, a significant reduction of aBMD only occurred at study endpoint, suggesting that adequate supplementation and exercise is able to slow bone loss caused by bariatric surgery. Areal BMD values at the lumbar spine and total body aBMD values continuously decreased but were more pronounced in the non-intervention group. These findings on aBMD changes are in line with the changes in BTM. In the intervention group, the increase of bone remodeling was lower than the non-intervention group. This resulted in a lesser reduction of aBMD. A recent population-based, retrospective case-control cohort study with a mean follow-up time of 2.2 years after bariatric surgery stated no significant effect on fracture risk, but a trend toward an increased fracture risk after 3 to 5 years after surgery should be taken into account.[26] To date, no prospective studies have been performed with antiresorptive or anabolic clinical interventions in these patients who should be at their peak bone mass.

TBS is a feasible, noninvasive surrogate technique for the assessment of cancellous bone texture from DXA scans in clinical routine. TBS significantly differs between patients with and without fractures when considering vertebral and nonvertebral fractures and correlates to trabecular bone microarchitecture.[20,27] Although there was an ongoing decline in TBS values in the non-intervention group, our therapeutic approach seemed to restore these levels after the first 6 months of the study, resulting in a plateau for the additional 18 months of the study. This suggests that adverse changes of trabecular bone structures in the intervention group were less pronounced. At the lumbar spine, a skeletal site predominantly composed of trabecular bone, changes of sclerostin, CTX, and P1NP were also significant covariates on the dependent variables aBMD and TBS

in both study groups. TBS and/or aBMD measurements are easily obtainable and capable of reflecting trabecular changes in conjunction with BTM in this specific patient population, as was recently demonstrated with volumetric high-resolution peripheral quantitative computed tomography (HR-pQCT).[28]

The outcomes on body fat values were similar between RYGB and SG in each group, which is in line with other studies published on this topic.[29] Weight loss was comparable in both groups until month 18. During the last 6 months of the study, the ongoing decline of BMI in the non-intervention group was more pronounced. Regarding the loss of total body fat, no differences were observed between both groups, but the differences on lean body mass were highly significant. Severe loss of lean body mass after bariatric surgery without adequate protein intake and physical activity has been reported previously.[10,30] Consequently, the importance of protein supplementation during weight loss is emphasized regardless of surgery. In a recently published trial, a protein and vitamin D–supplemented diet combined with physical activity effectively preserved appendicular muscle mass compared with an isocaloric control group, whereas weight and fat loss was comparable in both groups.[31] Observational evidence indicates that the patients' physical functioning improves with physical exercise after bariatric surgery, likely owing to improved efficiency in performing activities caused by weight loss.[32] This approach in combination with adequate nutritional supplementation seems capable of reducing bariatric surgery–induced sarcopenia. Bariatric surgery furthermore leads to a reduction in carbohydrate oxidation with increased protein oxidation, lipolysis, and reduced muscle expenditure and consecutively less mechanical strain on bone.[33] Our approach in combination with adequate nutritional supplementation and exercise seems capable of reducing these unfavorable alterations. Moreover, the benefit of the combination of recommended protein intake and at least 800 mg daily calcium supplementation in regard to hip fracture prevention has been reported in the Framingham Offspring cohort study.[34] In contrast to protein supplementation, carbohydrate substitution before and after bariatric surgery did not have beneficial effects on lean body mass.[35]

The improvements on QoL scores at different stages were superior and evident at earlier stages in the intervention group. Mental and physical health is important for the prevention of diseases and contributes to the reduction of costs in health systems.

Limitations

Because of the decision of the department of visceral surgery on the respective surgical method, this study lacked structured randomization (and therefore has differing numbers of RYGB and SG patients within the two groups). Furthermore, this study was not designed to evaluate any potential clinical risks or benefits such as fracture outcome of the investigated population. We cannot conclusively determine whether the positive effects on bone metabolism are based mainly on the several types of oral supplementation and physical exercise or are a result of a multifactorial approach. Moreover, a significant limitation is the lack of data on patients in the non-intervention group on their dietary behavior and physical activities after surgery. The SF-36 questionnaire does not adequately address changes in activity levels. Specific diaries in this group would have been helpful to evaluate diverse effects of the intervention.

Based on our findings, the approach of vitamin D loading before RYGB or SG and an ongoing vitamin D, calcium, and BMI-adjusted protein supplementation in combination with aerobic physical exercise decelerates the loss of aBMD and lean body mass after bariatric surgery. Moreover, the increases of BTM are less pronounced because of vitamin D, calcium, and protein, regardless of the method of surgery.

We conclude that supplementation and exercise have a positive effect on the long-term outcome in bone protection after RYGB/SG and should, therefore, be recommended for all patients undergoing bariatric surgery.

Disclosures

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