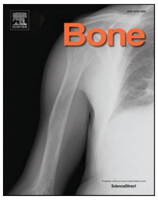
Bone 153 (2021) 116153

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone



The effect of bariatric surgery on gravitational loading and its impact on bone mass

Florêncio Diniz-Sousa [a,e,*], Lucas Veras [a,e], Giorjines Boppre [a,e], Vítor Devezas [b], Hugo Santos-Sousa [b], John Preto [b], Leandro Machado [c,d], João Paulo Vilas-Boas [c,d], José Oliveira [a,e], Hélder Fonseca [a,e]

[a] Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport, University of Porto, Porto, Portugal
[b] General Surgery Department, São João Medical Center, Porto, Portugal
[c] Centre of Research, Education, Innovation and Intervention in Sport (CIFI2D), Faculty of Sport, University of Porto, Porto, Portugal
[d] Biomechanics Laboratory (LABIOMEP-UP), University of Porto, Porto, Portugal
[e] Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal

ARTICLE INFO

Keywords:
Weight loss surgery
Bone mineral density
Mechanical loading
Physical exercise

ABSTRACT

Introduction: Mechanical unloading associated with weight loss might be one of the main causes for bariatric surgery (BS) induced bone loss. However, no study has tested this hypothesis through objectively measured accelerometry-derived gravitational loading. We aimed to assess how gravitational loading changes following BS and how this correlates with bone mass losses.
Methods: Twenty-one patients submitted to gastric bypass were assessed before, 1, 6 and 12 months after BS for areal bone mineral density (BMD), calciotropic hormones, sclerostin, body composition and daily physical activity. Gravitational loading was determined as the sum of ground reaction forces assessed by accelerometer which considered the interaction between weight and daily ambulation.
Results: Mechanical stimuli promoted through the significant increase in steps number counterbalanced the gravitational loading decreases derived from the significant weight loss after BS. Gravitational loading volume decreased between pre-BS and 1 month post-BS (-2215 kN·d^{-1}; $p = .023$), but remained stable between 6 and 12 months post-BS, despite decreases on hip (-7.0%; $p < .001$), femoral neck (-8.8%; $p < .001$) and lumbar spine (-5.2%; $p < .001$) BMD. Serum sclerostin increased from pre-BS to 1 month post-BS ($+0.118$ ng·mL^{-1}; $p = .021$), returning to pre-BS levels 6 months after surgery. Neither vitamin D nor parathyroid hormone were affected by BS. Weight variation was a predictor of BMD decreases at total hip ($R^2 = 0.06$; $p = .026$) and femoral neck ($R^2 = 0.12$; $p = .022$), whereas daily gravitational loading volume was not. Fat and lean mass changes were also predictors of BMD decrease at total hip ($R^2 = 0.05$; $p = .031$) and femoral neck ($R^2 = 0.14$; $p = .010$), respectively.
Conclusion: Our findings suggest that gravitational loading only decreased during the first month after surgery remaining stable thereafter, and these changes do not seem to explain BS-induced bone loss. The association between weight and bone loss seems to result from other physiological aspects, fat and lean mass loss, rather than from gravitational loading decrease.

1. Introduction

Obesity is an extremely prevalent disease with major health and economic consequences. So far, bariatric surgery (BS) is the best available treatment for severe obesity, but has some drawbacks, as bone mass loss and fracture risk increase [1]. Bone mass losses are more expressive during the initial massive weight loss phase, which occurs in the first 1–2 years post-BS [2].

Post-BS bone loss determinants are still not well established and seem to result from multiple factors [3]. Progressive mechanical unloading, prompted by weight loss, is hypothesized as one main factor to explain bone loss [4]. The aforementioned hypothesis is supported by

---

* Corresponding author at: Faculty of Sport, University of Porto, Rua Dr. Plácido Costa, 91, 4200-450 Porto, Portugal.
E-mail address: joseflorenciosousa@gmail.com (F. Diniz-Sousa).

https://doi.org/10.1016/j.bone.2021.116153
Received 2 April 2021; Received in revised form 23 July 2021; Accepted 10 August 2021

the fact that i) weight-bearing skeletal sites are mostly affected after BS [5], ii) serum sclerostin increases are frequently observed after BS [6] and iii) bone losses may occur despite adequate levels of calcium, vitamin D and parathyroid hormone (PTH) [7–9]. Nevertheless, several studies that analyzed post-BS weight loss effect on bone mass revealed contradictory findings, with some showing strong associations [10,11,8,12] whereas others did not [13,9,7].

Bone tissue is responsive to dynamic but not to static loading [14]. The gravitational loading influence on bone mass should not be based solely on weight, but instead should have in consideration the interaction between weight and the dynamic loads resulting from daily physical activity, as this combination modulates the gravitational loads imposed to bone tissue [15]. Even though post-BS patients have a substantial weight reduction, it is conceivable that if their physical activity levels increase, this might counterbalance the reduction in daily mechanical loading attributed to the weight loss effect *per se* [4]. Variations in the interaction between weight loss and daily physical activity could have the potential to more truly reflect the actual contribution of gravitational loading changes after BS on bone mass, rather than each one of the isolated parameters.

The possible association between post-BS weight and bone loss might also be explained by changes in the two main weight determinants — fat and lean mass. It is known that both fat and skeletal muscle influence bone metabolism independently of the direct gravitational loading effect [16,17]. No study has collectively investigated how changes in each one of these factors influence post-BS bone loss. This would aid to determine the mechanisms of BS-induced bone loss and implement adequate strategies to tackle this clinically significant problem.

This study aimed to investigate how weight and daily physical activity vary following BS, how the conjugation of these two factors is reflected on bone mechanical loading, and how this variation associates with post-BS bone mass losses. Our hypothesis is that changes in daily physical activity following BS will, up to some extent, compensate for decreases in weight maintaining thereby the level of skeletal mechanical loading. To test this hypothesis, we objectively measured gravitational loading and daily physical activity through the use of accelerometers and also monitored serum sclerostin concentrations through the first post-BS year. Calciotropic hormones were also measured to control for any confounding effect of secondary hyperparathyroidism induced bone loss. Also, considering that weight changes result from changes in different body composition components, as a secondary aim, we also explored the influence that changes of lean and fat mass could have on post-BS bone mass losses.

2. Materials and methods

2.1. Study design

This is an ancillary study derived from the BaSEIB clinical trial (clinicaltrials.gov/NCT02843048) in which the treatment effect of a structured exercise program in comparison to standard post-BS medical care for the prevention of bone mass loss was tested. Further details about the primary analysis of the outcomes are available elsewhere [18]. This study comprised a subgroup of patients with class II-III obesity submitted to Roux-en-Y gastric bypass (RYGB) that did not participate in the exercise intervention. Participants gave their written informed consent and the research protocol was approved by the local Ethics Committee (CES 192-14).

2.2. Bariatric surgery

Participants performed laparoscopic RYGB, according to standard procedures at the São João Academic Hospital Center by the same surgical team, and all patients received usual medical care post-BS. Multivitamin and protein supplements (e.g., Centrum®, Protifar®, Fantomalt®) were prescribed. Calcium and vitamin D supplements were not routinely prescribed, although the intake was advised to patients with particular needs. All patients received recommendations to increase daily physical activity, but no structured exercise prescription was given.

2.3. Measurements and outcomes

Patients were assessed before, 1, 6 and 12 months after surgery and bone mineral density (BMD), bone metabolism regulators, anthropometry, body composition and daily physical activity were evaluated. All measurements were conducted at the Research Centre in Physical Activity, Health and Leisure (CIAFEL), University of Porto, Portugal.

2.3.1. Anthropometry

Height and weight were assessed according to standard procedures [19] with a stadiometer and digital scale, respectively, and body mass index (BMI) calculated.

2.3.2. Bone mineral density and body composition

Areal BMD ($g\cdot cm^{-2}$) at total hip (TH), femoral neck (FN), lumbar spine (LS; average of L1–L4) and one-third distal radius (1/3 radius) were assessed by regional dual-energy X-ray absorptiometry (DXA; Hologic Explorer QDR, Hologic INC, Bedford, MA, USA) according to the manufacturer's recommendations. Body fat (kg) and lean mass (kg) were determined through a whole-body DXA-scan. For participants who exceeded the width of the scanning area limits in the whole-body composition assessment, the left upper-limb was not completely scanned and these missing values were replicated with values measured from the right upper-limb. All assessments were performed by the same experienced technician. Coefficients of variation (CV) were: 0.8% LS BMD, 1.0% TH BMD, 1.4% FN BMD, 1.4% 1/3 radius BMD, 1.6% total fat mass and 0.7% total lean mass.

2.3.3. Bone metabolism regulators

Fasting venous blood samples were collected between 8 and 9 am after overnight fasting, centrifuged at 5000 rpm at 4 °C for 10 min and serum stored at −80 °C. All samples were thawed for a maximum of 2 cycles and analyzed in batch. Commercially available Enzyme-Linked Immunosorbent Assays (ELISA) were used to determine the concentration of all biochemical parameters, namely: intact PTH concentration (PTH 1-84; ref. 8044, TECOmedical group, Sissach, Switzerland), total 25-hydroxyvitamin D (25-OHD; ref. AC-57SF1, IDS Ltd., Boldon, UK) and sclerostin (ref. TE1023-HS, TECOmedical group, Sissach, Switzerland). The inter- and intraassay CV were: PTH 6.3% and 6.2%; 25-OHD 3.3% and 2.7%; sclerostin 8.7% and 4.6%, respectively.

2.3.4. Gravitational loading

Daily physical activity was objectively recorded for 1 week during the awake period through a triaxial accelerometer (GT9X Link, ActiGraph, Pensacola, FL, USA) worn at the waist level, with data collected at 100 Hz sampling frequency. Raw accelerometry data, expressed through gravitational acceleration units (*g*) resulting from body movements, was processed using Python programming language (Python Software Foundation, version 3.8.2; Beaverton, OR, USA). Initially, data was filtered, resultant accelerations determined and peak accelerations identified as previously described [20]. After, processed accelerometry data was used to quantify the following daily gravitational load outcomes: i) number of steps, ii) high-impact gravitational loading and iii) gravitational loading volume. Steps, which represent the number of ambulatory gravitational stimuli, were recognized through the identification of acceleration peaks above 1.3 *g* separated by at least 0.4 s [21]. High gravitational loading impacts were determined by the number of body movements involving acceleration peaks above 4.9 *g*, which have been previously identified as an osteogenic load threshold [22]. Gravitational loading volume was determined as the sum of ambulatory ground reaction forces (GRF, N) throughout an entire day. For this, peak

GRF derived from each identified step was predicted based on a previously validated equation that considers the interaction between weight and ambulatory gravitational stimuli (that varies with the resultant acceleration magnitude) [20]. Further details about this analysis can be found at https://bit.ly/3piwmFJ.

2.3.5. *Sedentary behavior and physical activity intensity*

Accelerometry data was also used to characterize physical activity related to cardiorespiratory outcomes. These outcomes are associated with energy expenditure typically expressed as metabolic equivalents (MET's), which are used to classify different physical activity intensities according to established activity cut-points. Accelerometer manufacturer-supplied software (ActiLife, version 6.13.3; ActiGraph, Pensacola, FL, USA) was used to convert raw acceleration data into activity counts, that were analyzed in 60 s epochs. Non-wear time was defined as 90 min of consecutive zero counts. Physical activity was only valid if data had ≥10 h per day of wear time for ≥3 days. Sedentary behavior, light, and moderate-to-vigorous physical activity were defined as <200 counts per min (cpm), 200 to 2689 cpm and >2690 cpm, respectively [23,24].

2.4. *Data analyses*

The R statistical software (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analyses. The R code used as well as detailed information regarding the analyses performed can be found in a registered open platform at https://bit.ly/375SCwm [25]. Changes occurred in each outcome throughout the first year after BS were tested by linear mixed-models analysis. Time variable (4 levels; pre-BS, 1, 6 and 12 months post-BS) was used as a fixed factor and subjects as a random factor. Within-group differences among evaluations were used to test time effect. Estimated mean differences with 95% confidence interval (95% CI) and adjusted *p*-value (Bonferroni-Holm correction) were reported. The influence of potential predictors of BMD changes occurred during the first year after BS was also tested. Prediction models were performed through mixed-models analysis, in which predictors were setup as a fixed factor, while subjects and time were setup as random factors. A statistically significant value was set as $\alpha = 0.05$.

3. Results

3.1. *Study participants*

Study participants characteristics are shown on Table 1. Twenty-one subjects (16 females; 5 males) aged 46.2 ± 8.0 years and 46.2 ± 8.1 $\text{kg}\cdot\text{m}^{-2}$ BMI, were recruited 1–3 months before BS. Regarding associated comorbidities, 23.8% had type 2 diabetes, 19.0% were thiazide diuretic users and 14.3% active smokers. Post-menopausal women represented 25.0% of the females recruited. All participants had no self-reported neurological impairment and were free from orthopedic or musculoskeletal limitations.

Table 1
Participants' characteristics before bariatric surgery.

Parameters	*n* = 21
Age (years)	46.2 ± 8.0
Sex (female, %)	76.2%
Height (m)	1.58 ± 0.09
Weight (kg)	113.4 ± 17.2
Body mass index ($\text{kg}\cdot\text{m}^{-2}$)	46.2 ± 8.1
Waist circumference (cm)	127.2 ± 10.2
Hip circumference (cm)	133.4 ± 8.4
Waist to hip ratio	0.96 ± 0.08
Menopause (%)	25.0%
Diabetes (%)	23.8%
Thiazide intake (%)	19.0%
Current smoker (%)	14.3%

Data: mean ± standard deviation.

Detailed information regarding changes on BMD, body composition, vitamin D, PTH, sclerostin and daily physical activity throughout the first year after RYGB are presented on Supplemental Table S1. The main findings for each of these variables are further described in the text below.

3.2. *BMD changes throughout the first year after RYGB*

Fig. 1 shows BMD changes at different skeletal regions of interest assayed throughout the first year after RYGB. Distinct BMD pattern changes were observed, with significant decreases at the LS and, even more expressively, at the hip region, whereas at 1/3 radius, an appendicular and non-weight-bearing skeletal site, there were no changes over time. Compared to pre-BS, there was a significant and progressive BMD decrease at 6 and 12 months post-BS at central skeletal sites, namely −2.4% (95% CI −3.8, −0.9; $p = .005$) and −5.2% (95% CI −6.8, −3.7; $p < .001$) at LS, −2.6% (95% CI −4.2, −1.0; $p = .005$) and −7.0% (95% CI −8.6, −5.3; $p < .001$) at TH, and −4.4% (95% CI −6.5, −2.2; $p = .001$) and −8.8% (95% CI −11.0, −6.6; $p < .001$) at FN, respectively.

3.3. *Weight changes throughout the first year after RYGB*

During the first year after RYGB, in which BMD of weight-bearing skeletal sites decreased, there was also a consistent weight reduction throughout all follow-up moments (Fig. 2, panel a). Just one month after surgery a significant decrease on weight was observed (−11.9 kg [95% CI −14.9, −8.9]; $\Delta = -10.5\%$; $p < .001$), and this pattern of decrease continued until the end of the follow-up period, when the highest difference compared to pre-surgery weight was achieved, with an average reduction of −41.4 kg (95% CI −44.5, −38.4; $\Delta = -36.5\%$; $p < .001$).

3.4. *Physical activity changes throughout the first year after RYGB*

Physical activity also changed after RYGB with bariatric patients becoming more active (Fig. 2, panel b and Supplemental Fig. S1). Between pre-BS and 12 months post-BS, patients spent less time in sedentary behaviors (−0.9 $\text{h}\cdot\text{d}^{-1}$ [95% CI −1.6, −0.2]; $\Delta = -11.7\%$; $p = .028$), and more time performing light (0.8 $\text{h}\cdot\text{d}^{-1}$ [95% CI 0.2, 1.5]; $\Delta = 14.1\%$; $p = .046$) and moderate-to-vigorous physical activities (15.3 $\text{min}\cdot\text{d}^{-1}$ [95% CI 6.8, 23.8]; $\Delta = 64.3\%$; $p = .006$) (Supplemental Fig. S1). The number of daily steps performed also showed a similar pattern change after surgery, with a non-significant decrease from pre- to the first month, followed by a consistent increase until the end of the first year (2379 $\text{steps}\cdot\text{d}^{-1}$ [95% CI 1229, 3529]; $\Delta = 37.5\%$; $p = .001$) (Fig. 2, panel b).

3.5. *Changes on gravitational loading and sclerostin concentration throughout the first year after RYGB*

Daily ambulatory gravitational loading to which patients were exposed throughout the follow-up, which accounted for the variations in both weight and daily physical activity, showed that the mechanical stimuli promoted through the increase in the number of steps counterbalanced the gravitational loading decrease derived from the massive weight loss after RYGB (Fig. 2, panel c). The gravitational loading volume significantly decreased between pre-BS and 1 month post-BS (−2215 $\text{kN}\cdot\text{d}^{-1}$ [95% CI −3712, −717]; $\Delta = -19.5\%$; $p = .023$), but then remained stable with no more significant changes at 6 and 12 months post-BS. In the opposite direction, serum sclerostin concentration (Fig. 2, panel d) significantly increased from pre to 1 month after RYGB (0.118 $\text{ng}\cdot\text{mL}^{-1}$ [95% CI 0.041, 0.195]; $\Delta = 19.8\%$; $p = .021$) returning to pre-surgery levels 6 months after surgery. Interestingly, changes on sclerostin levels were neither associated with changes on daily gravitational loading volume ($\beta = -0.084$ $\text{kN}\cdot\text{d}^{-1}$ [95% CI −0.318,

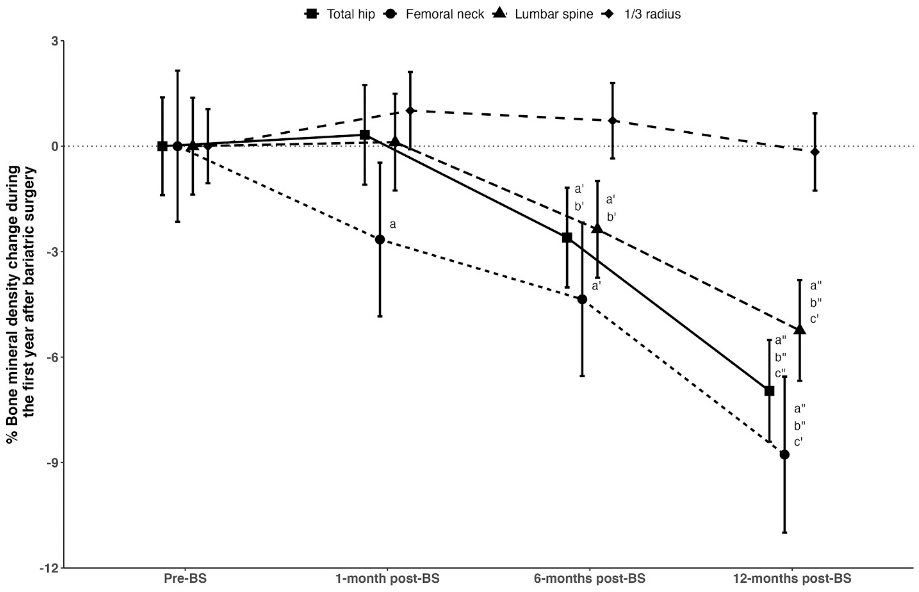


Fig. 1. Bone mineral density changes throughout the first year after Roux-en-Y gastric bypass. BS = bariatric surgery.
Data: estimated mean (confidence interval 95%).
Versus pre-BS: $^{a}p < .05$; $^{a'}p < .01$; $^{a''}p < .001$.
Versus 1-month post-BS: $^{b'}p < .01$; $^{b''}p < .001$.
Versus 6 months post-BS: $^{c'}p < .01$; $^{c''}p < .001$.

0.150]; $p = .486$; $R^2 = 0.01$) nor with weight loss (β = −0.036 kg [95% CI −0.310, 0.274]; $p = .828$; $R^2 = 0.00$).

Remarkably, the observed increase on physical activity levels, namely in moderate-to-vigorous intensity physical activity, did not reflect on a higher number of body movements involving high gravitational loading impacts throughout the follow-up (Fig. 3). While the time spent in moderate-to-vigorous physical activity increased from 23.8 min·d^{-1} (95% CI 13.7, 33.9) to 39.1 min·d^{-1} (95% CI 28.7, 49.5) from pre-BS to 12 months post-BS, the number of high-impact gravitational loads performed remained essentially unaltered and low through all the follow-up.

3.6. *Changes on calciotropic hormones concentration throughout the first year after RYGB*

Calciotropic hormones concentration (PTH and vitamin D) assessed throughout the first year after RYGB are presented on Table 2. There were no significant changes on vitamin D between pre-BS and 12 months post-BS, although a significant increase occurred at the end of the first semester after RYGB compared to pre-surgery levels (8.2 ng·mL^{-1} [95% CI 2.9, 13.6]; Δ = 30.3%; $p = .025$). A non-significant trend in PTH concentration decrease was also observed during follow-up.

3.7. *Changes on fat and lean mass throughout the first year after RYGB*

Weight loss occurred during the first year after RYGB resulted from a substantial decrease on both fat and lean mass (Fig. 4). Fat mass significantly decreased through all the follow-up, attaining a loss of −31.4 kg (95% CI −33.9, −29.0; Δ = −56.8%; $p < .001$) at 12 months post-BS. Lean mass also showed a significant decrease between pre-BS and 1 month post-BS (−6.4 kg [95% CI −7.9, −5.0]; Δ = −11.5%; $p < .001$), and between 1 and 6 months post-BS (−2.4 kg [95% CI −3.9, −1.0]; Δ = −4.9%; $p = .003$), remaining stable during the second semester after surgery.

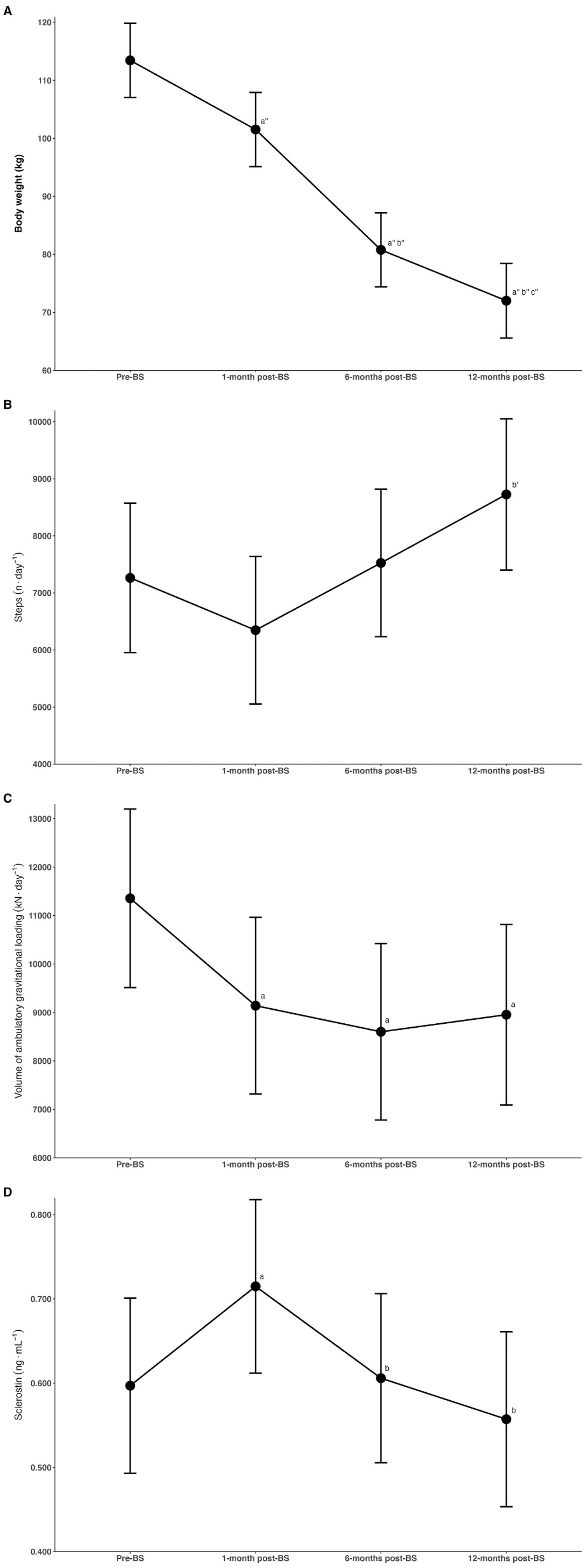
3.8. *BMD change predictors*

The influence of potential predictors of BMD loss was tested (Table 3). Weight loss was a significant predictor of BMD changes at TH (β = 0.238 [95% CI 0.075, 0.401]; $p = .026$; $R^2 = 0.06$) and FN (β = 0.321 [95% CI 0.174, 0.469]; $p = .022$; $R^2 = 0.12$), but not at LS. Fat mass loss was also a significant predictor of BMD changes at TH (β = 0.213 [95% CI 0.078, 0.347]; $p = .031$; $R^2 = 0.05$), but not at FN, while lean mass change was a significant predictor of BMD loss at FN (β = 0.342 [95% CI 0.094, 0.590]; $p = .010$; $R^2 = 0.14$), but not at TH. Neither fat mass nor lean mass were significant predictors of LS BMD changes. Changes on daily gravitational loading volume to which bariatric patients were exposed after RYGB were not associated with BMD loss at any of the weight-bearing skeletal sites analyzed. Therefore, predictors of TH BMD changes were weight and fat mass, while predictors of FN BMD changes were weight and lean mass (Supplemental Fig. S2). These predictors were not tested together in a final model because they presented moderate to high collinearity, with a variance inflation factor of 2.4 between weight and lean mass changes, and 8.5 between weight and lean mass changes.

4. Discussion

This study aimed to investigate how weight and daily physical activity vary following BS, how the conjugation of these two factors reflect on bone mechanical loading, and how this variation explains bone mass losses. Additionally, we analyzed if changes on mechanical loading were reflected on serum sclerostin concentration and explored the influence that post-BS changes on fat and lean mass could have on these patients bone mass loss. Our results showed that despite a substantial weight decrease, bariatric patients became physically more active after surgery compensating thereby the gravitational loading decrease derived from the massive weight loss. Thus, mechanical loading remained globally stable from the first post-BS month onwards, while bone mass loss continued throughout the 12 months of the follow-up. Moreover, our results revealed that BMD decreases observed at the hip region were associated with changes on weight, fat and lean mass, but not with daily ambulatory gravitational loading volume to which these patients were exposed after RYGB.

The negative effect of weight loss interventions on bone health has been consistently described in different populations, from non-obese young subjects [26] to older adults with obesity [27]. Calorie-restricted diet interventions achieving moderate weight loss (≈−10%) have been associated with significant bone mass reduction (≈−2%) especially at the hip region [27,26]. Similar detrimental effects on skeletal health were observed in post-BS patients, although to a much higher extent [5]. Our results showed that at the end of the first year after surgery, bariatric patients had a mean weight loss of −36.5% and a substantial BMD decrease at axial skeletal sites, namely −7.0% at total



(caption on next column)

Fig. 2. Weight, daily steps, daily gravitational loading volume and sclerostin levels throughout first year after Roux-en-Y gastric bypass.
BS = bariatric surgery.
Data: estimated mean (confidence interval 95%).
Versus pre-BS: $^{a}p < .05$; $^{a''}p < .001$.
Versus 1-month post-BS: $^{b}p < .05$; $^{b'}p < .01$; $^{b''}p < .001$.
Versus 6 months post-BS: $^{c''}p < .001$.

hip, −8.8% at femoral neck and −5.2% at lumbar spine, but no changes at 1/3 radius. These findings are in line with previous studies [8,12,10] showing that bone mass changes following BS might be substantially different across different skeletal sites, with weight-bearing bones being the most affected.

The influence of mechanical loading on bone mass is well established and has been described by the Wolff's law and Frost's mechanostat theory [28]. Bone tissue is highly responsive to mechanical strain or the lack of thereof, adapting itself by increasing or decreasing its mass to accommodate the loads it bears in the most energetically cost-efficient way [15]. The higher bone mass losses observed at central skeletal sites in our study, mainly at the hip region seems, at first sight, to support the hypothesis that bone loss after BS reflects a physiological adaptation to lower gravitational load demands resulting from a massive weight reduction. A similar site-specific bone response has been described in subjects involved in prolonged bed rest [29] and microgravity [30]. Although our results have shown that bone mass loss was partially explained by weight loss magnitude, the mechanisms underlying this association seem not to be related to the gravitational loading changes after RYGB.

Our findings showed that, although weight constantly decreased over time, the daily ambulatory gravitational loading volume only had a significant decrease during the first month after surgery, but then remained stable for the next 11 months. This occurred because after surgery, bariatric patients became physically more active, which counterbalanced the negative effect of weight loss on bone mechanical loading. These changes on physical behavior are not surprising inasmuch as regular clinical practice guidelines have already included recommendations to encourage the increase of regular physical activity after surgery [31]. Moreover, bariatric patients' physical function perception substantially improves at the same time that weight loss occurs, which may promote greater involvement in labor or recreational daily physical activities [32]. Indeed, several studies have shown an average increase ranging from 1271 to 2749 additional daily steps throughout the first year after BS, which is in agreement with the results observed in our study [33,34].

Sclerostin is a protein expressed by osteocytes in response to mechanical unloading that inhibits the Wnt/β-catenin signaling pathway, which decreases osteoblast differentiation, proliferation and activity [35]. A relevant finding from our study, which corroborates the hypothesis that bone loss was not predominantly triggered by the decrease of mechanical loading, was that serum sclerostin concentration only increased from pre-surgery to 1 month post-BS, when patients were still recovering and therefore mostly inactive, and then returned to baseline concentrations at 6 months post-BS. Although there are conflicting findings [6,12], several studies have shown a similar sclerostin pattern change throughout the first 12 months post-BS [36,37]. Our findings suggest that the transient sclerostin changes observed after BS occurred as a physiological response to the observed gravitational loading changes, increasing with the reduction in gravitational loading during the first month after surgery and then returning to baseline concentration accompanying the gravitational loading stabilization that occurred in the following months. Nevertheless, no significant association between sclerostin concentration and gravitational loading changes was identified, which might result from the fact that sclerostin expression is not only determined by mechanical loading per se, but also by other factors concomitantly modified by BS [35], namely glucose metabolism [37].

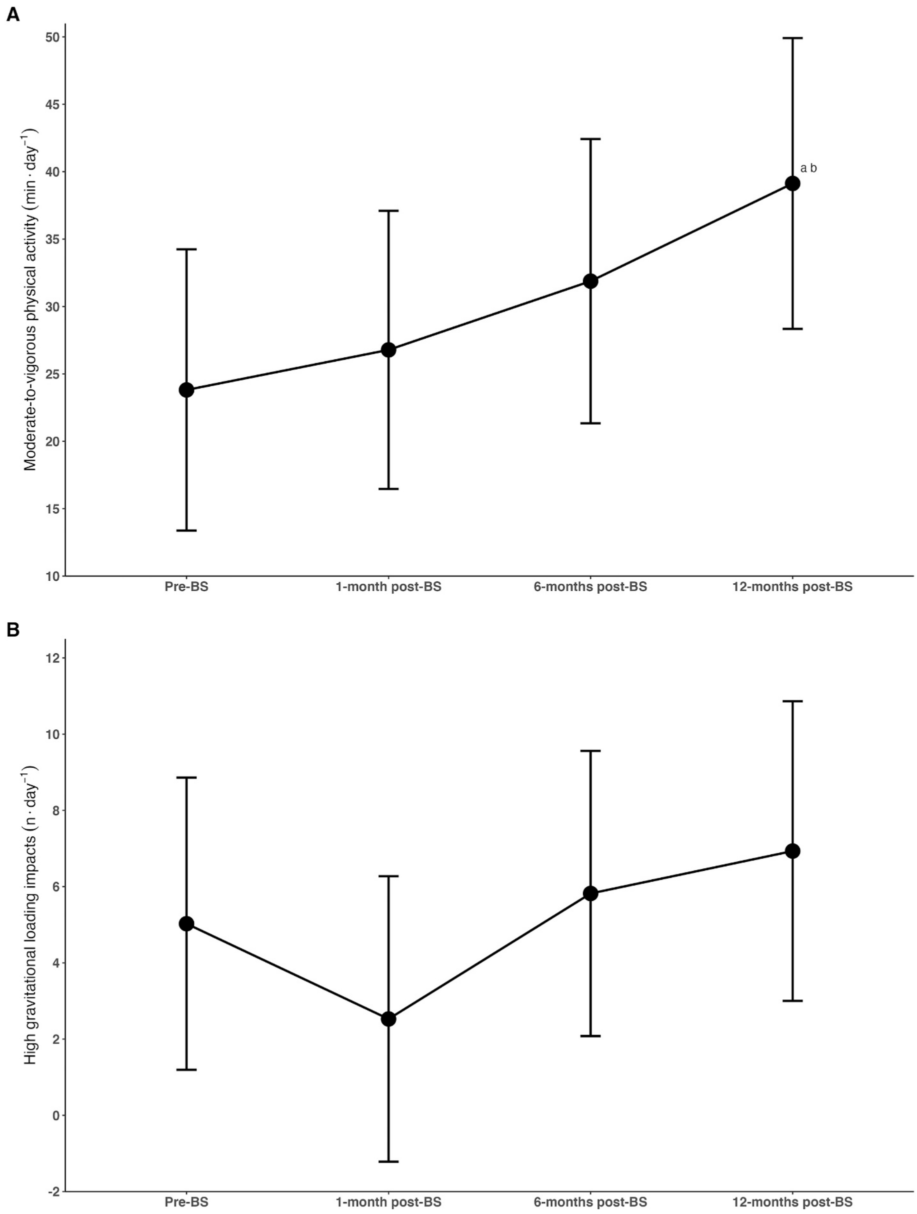


Fig. 3. Changes occurred throughout first year after Roux-en-Y gastric bypass on moderate-to-vigorous physical activity and high gravitational loading impacts.
BS = bariatric surgery.
Data: estimated mean (confidence interval 95%).
Versus pre-BS: $^{a}p < .05$.
Versus 1-month post-BS: $^{b}p < .05$.

Table 2
Changes on calciotropic hormones throughout the first year after Roux-en-Y gastric bypass.

Parameters	Pre-BS	1-Month post-BS	6 Months post-BS	12 Months post-BS
PTH (pg·mL^{-1})	35.8 (30.2, 41.5)	34.5 (29.1, 40.0)	31.9 (26.6, 37.1)	30.5 (25.0, 36.1)
Vitamin D (ng·mL^{-1})	27.1 (20.2, 34.0)	29.7 (22.9, 36.5)	35.3 (28.7, 41.9)*	34.4 (27.5, 41.2)

BS = bariatric surgery; PTH = parathyroid hormone.
Data: estimated mean (confidence interval 95%).
* $p < .05$ compared to pre-surgery moment.

Our findings suggest that the association between weight and bone loss after BS might be more related with changes on fat and lean mass, since these two weight components have shown to be significant predictors of BMD changes at the hip region. These results seem to be supported by findings from other studies, in which bone loss was also associated with changes on fat and lean mass [12,38].

Besides gravitational loading, massive fat mass reduction observed after BS might induce bone loss through changes on adipokines levels, although our knowledge about the mechanisms by which they interfere with bone metabolism is still incomplete [17]. For instance, the adiponectin concentration increase, usually observed after BS, has been described as negatively correlated with BMD [9], while BS-induced leptin decrease seems to be associated with BMD decrease [11]. Fat mass reduction may also prompt bone mass loss by lowering estrogen synthesis derived from adipose tissue aromatase activity, although this hypothesis has not been supported by current evidence [12]. It should be noted that although only fat mass changes were significantly and positively associated with BMD loss at TH, the same trend was observed at FN and LS, although not reaching statistical significance.

The influence of lean mass changes on the observed bone loss after BS may be due to the substantial reduction in skeletal muscle mass, because of the skeletal muscle effect on bone mechanical strain and local paracrine myokine signaling [16]. Hue and colleagues [39] showed that one year after BS maximal muscle strength decreased on upper and lower limbs. However, this decrease seemed to be more expressive in antigravitational muscles, more specifically at the knee extensors [39].

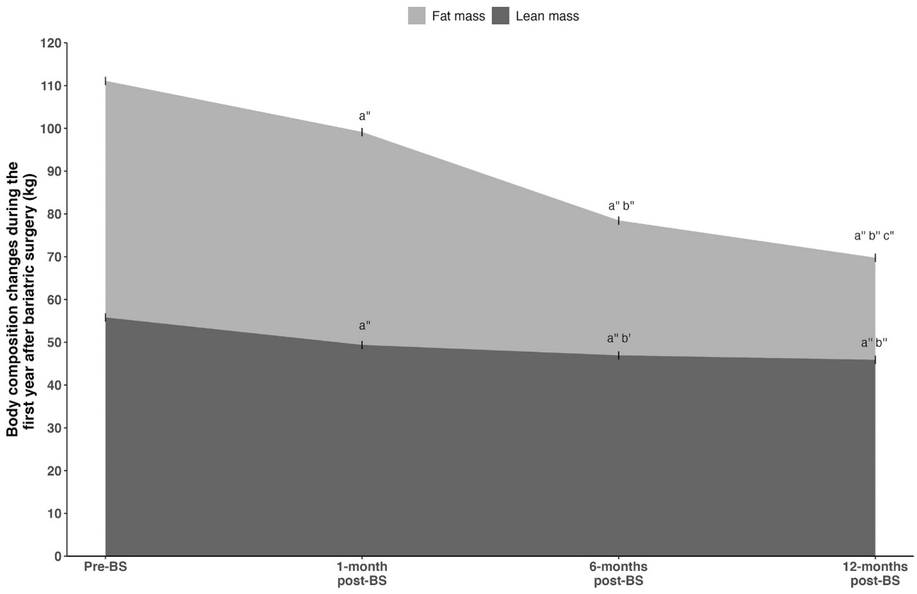


Fig. 4. Fat and lean mass changes throughout first year after Roux-en-Y gastric bypass.
BS = bariatric surgery.
Data: estimated mean.
Versus pre-BS: $^{a''}p < .001$.
Versus 1-month post-BS: $^{b'}p < .01$; $^{b''}p < .001$.
Versus 6 months post-BS: $^{c''}p < .001$.

Table 3
The influence of potential predictors of bone mineral density loss from weight bearing skeletal sites during the first year after Roux-en-Y gastric bypass.

	Total hip BMD (g·cm^{-2})			Femoral neck BMD (g·cm^{-2})			Lumbar spine BMD (g·cm^{-2})		
	β (95% CI)	*p* value	R^2	B β (95% CI)	*p* value	R^2	B β (95% CI)	*p* value	R^2
Weight (kg)	**0.238** **(0.075, 0.401)**	**.026**	**0.06**	**0.321** **(0.174, 0.469)**	**.022**	**0.12**	0.149 (0.004, 0.294)	.144	0.02
Fat mass (kg)	**0.213** **(0.078, 0.347)**	**.031**	**0.05**	0.233 (−1.298, 1.764)	.093	0.05	0.177 (0.079, 0.275)	.057	0.03
Lean mass (kg)	0.148 (−0.076, 0.372)	.200	0.02	**0.342** **(0.094, 0.590)**	**.010**	**0.14**	−0.038 (−0.256, 0.181)	.736	0.00
Daily volume of gravitational loading (kN)	−0.350 (−0.226, 0.474)	.583	0.00	0.043 (−0.143, 0.229)	.654	0.00	−0.022 (−0.135, 0.092)	.709	0.00

β = standardized beta coefficient; BMD = bone mineral density; CI = confidence interval; R^2 = coefficient of determination.
Significant values are marked in bold.

Theoretically, the lower strength required to move a lighter body, and therefore with lower inertia, combined with a reduced muscle mass and lower muscle strength might lead to substantial decreases of muscle-derived forces acting on the skeleton during daily activities and, consequently, induce lower bone strains [28]. This might be especially relevant, since an important portion of the forces generated at the femur during walking derive from muscle contractions and not only through gravitational loading [40].

Although our findings seem to indicate that the association between weight and bone mass losses might be more related to changes on fat and lean mass than with mechanical (un)loading changes, the hypothesis that the initial reduction in gravitational loading after surgery was enough to drive the observed bone loss throughout the follow-up period cannot be fully excluded, as these changes could reflect the slow readjustment of bone mass to a new level of mechanical stimulation. However, considering the findings on sclerostin changes, this hypothesis seems less likely.

Interestingly, our findings revealed that, contrarily to what could be expected, the increase in physical activity levels observed after BS, particularly the time spent on moderate-to-vigorous intensity activities, was not paralleled by an increase in the number of high gravitational loading impacts. These findings highlight two points. First, physical activity measurements related to cardiorespiratory outcomes may not be a good indicator to reflect gravitational loading. Second, the positive effect that the raise of physical activity might have had on counterbalancing the gravitational loading decrease due to weight reduction occurred almost exclusively through low mechanical load stimuli. These findings suggest that future therapeutic approach that aim to minimize BS-induced bone loss should not only focus on increasing the daily ambulatory gravitational loading volume, but also on favoring other important parameters associated with mechanical loading induced bone formation, such as load magnitude [41]. Vainionpää and colleagues [22] reported that fewer than 60 high gravitational loading impacts per day were associated with an increase in proximal femur BMD in premenopausal women.

Another relevant finding was that calciotropic hormones were not negatively affected by RYGB. Several studies [7–9] have shown that the malabsorption typically associated with the intestinal bypass might not necessarily result in a decrease in blood calcium and vitamin D or an increase in PTH during the first year after surgery. Some of the most frequent justifications for this observation might be: i) calcium and vitamin D supplements prescribed after surgery [42], ii) adipose tissue stored vitamin D released as a consequence of adipocyte shrinkage [43], iii) higher synthesis of vitamin D through sun exposure due to more time spent in outdoor physical activities [44] and iv) higher hepatic vitamin D hydroxylation due to reduced non-alcoholic fatty liver disease [45]. These findings are also corroborated by some studies on animal models [45,46].

4.1. *Strength and weakness*

The main strength of this study was the proposal of a new perspective on the possible role that mechanical loading changes might have on BS-induced bone loss, as well as testing this hypothesis through an innovative approach that allowed us to objectively assess daily ambulatory gravitational loading throughout the first year after BS. However, there are some limitations. Although the prediction models developed to estimate gravitational loading have been shown to be accurate for walking [20], their validity was not tested in other activities such as running and jumping. Nevertheless, our results suggest that the potential impact that this source of error might have on our findings is residual, since post-BS patients daily physical activity was almost exclusively composed of activities involving low mechanical load stimuli as those that characterize walking-related activities. It also should be highlighted that findings reported here could be specific for post-RYGB patients and may not reflect the underlying mechanisms of bone loss associated with other BS techniques, such as the sleeve gastrectomy [6,7]. Moreover, the magnitude of the influence of different factors contributing to bone loss may depend on age and could vary over time after surgery [2]. Therefore, additional research with larger sample sizes, longer follow-up periods and the inclusion of patients submitted to other surgical procedures are needed to confirm these findings and elucidate the mechanisms of BS-induced bone loss.

5. Conclusions

Our findings showed that ambulatory gravitational loading only decreased during the first month after surgery remaining stable thereafter, and that these changes do not seem to explain BS-induced bone loss. This occurred because after surgery patients became physically more active, which counterbalanced the negative effect of weight loss on bone mechanical loading. Our findings seem to suggest that the association between weight and bone loss after BS might be more related to changes on fat and lean mass per se since these two weight components showed to be significant predictors of BMD changes.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2021.116153.

CRediT authorship contribution statement

Florêncio Diniz-Sousa: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Lucas Veras:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Giorjines Boppre:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Vítor Devezas:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Hugo Santos-Sousa:** Conceptualization, Methodology, Investigation, Writing – review & editing. **John Preto:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Leandro Machado:** Conceptualization, Methodology, Investigation, Writing – review & editing. **João Paulo Vilas-Boas:** Conceptualization, Methodology, Investigation, Writing – review & editing. **José Oliveira:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Hélder Fonseca:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Florêncio Diniz-Sousa, Lucas Veras, Giorjines Boppre, Vítor Devezas, Hugo Santos-Sousa, John Preto, Leandro Machado, João Paulo Vilas-Boas, José Oliveira and Hélder Fonseca declare that they have no conflict of interest.

Acknowledgements

This study was funded by the Foundation for Science and Technology of Portugal (FCT) (grant PTDC/DTP-DES/0968/2014) and by the European Regional Development Fund (ERDF) through the Operational Competitiveness Programme (COMPETE) (grant POCI-01-0145-FEDER-016707). Florêncio Diniz-Sousa was supported by the FCT (grant SFRH/BD/117622/2016), Giorjines Boppre was supported by the FCT (grant SFRH/BD/146976/2019) and Lucas Veras was supported by the FCT (grant UI/BD/150673/2020). The study was developed in the Research Centre in Physical Activity, Health and Leisure (CIAFEL) funded by ERDF through the COMPETE and by the FCT (grant UIDB/00617/2020).

References

[1] S. Ahlin, M. Peltonen, K. Sjöholm, Å. Anveden, P. Jacobson, J.C. Andersson-Assarsson, et al., Fracture risk after three bariatric surgery procedures in Swedish obese subjects: up to 26 years follow-up of a controlled intervention study, J. Intern. Med. 287 (5) (2020) 546–557, https://doi.org/10.1111/joim.13020.

[2] K.G. Lindeman, L.B. Greenblatt, C. Rourke, M.L. Bouxsein, J.S. Finkelstein, E. W. Yu, Longitudinal 5-year evaluation of bone density and microarchitecture after Roux-en-Y gastric bypass surgery, J. Clin. Endocrinol. Metab. 103 (11) (2018) 4104–4112, https://doi.org/10.1210/jc.2018-01496.

[3] C. Gagnon, A.L. Schafer, Bone health after bariatric surgery, JBMR Plus. 2 (3) (2018) 121–133, https://doi.org/10.1002/jbm4.10048.

[4] L.M. Scibora, S. Ikramuddin, H. Buchwald, M.A. Petit, Examining the link between bariatric surgery, bone loss, and osteoporosis: a review of bone density studies, Obes. Surg. 22 (4) (2012) 654–667, https://doi.org/10.1007/s11695-012-0596-1.

[5] Y. Rodriguez-Carmona, F.J. Lopez-Alavez, A.G. Gonzalez-Garay, C. Solis-Galicia, G. Melendez, A.E. Serralde-Zuniga, Bone mineral density after bariatric surgery. A systematic review, Int. J. Surg. 12 (9) (2014) 976–982, https://doi.org/10.1016/j.ijsu.2014.08.002.

[6] C. Muschitz, R. Kocijan, C. Marterer, A.R. Nia, G.K. Muschitz, H. Resch, et al., Sclerostin levels and changes in bone metabolism after bariatric surgery, J. Clin. Endocrinol. Metab. 100 (3) (2015) 891–901, https://doi.org/10.1210/jc.2014-3367.

[7] M.A. Bredella, L.B. Greenblatt, A. Eajazi, M. Torriani, E.W. Yu, Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on bone mineral density and marrow adipose tissue, Bone. 95 (2017) 85–90, https://doi.org/10.1016/j.bone.2016.11.014.

[8] J. Fleischer, E.M. Stein, M. Bessler, M. Della Badia, N. Restuccia, L. Olivero-Rivera, et al., The decline in hip bone density after gastric bypass surgery is associated with extent of weight loss, J. Clin. Endocrinol. Metab. 93 (10) (2008) 3735–3740, https://doi.org/10.1210/jc.2008-0481.

[9] V.V. Shanbhogue, R.K. Støving, K.H. Frederiksen, S. Hanson, K. Brixen, J. Gram, et al., Bone structural changes after gastric bypass surgery evaluated by HR-pQCT: a two-year longitudinal study, Eur. J. Endocrinol. 176 (6) (2017) 685–693, https://doi.org/10.1530/eje-17-0014.

[10] E.M. Stein, A. Carrelli, P. Young, M. Bucovsky, C. Zhang, B. Schrope, et al., Bariatric surgery results in cortical bone loss, J. Clin. Endocrinol. Metab. 98 (2) (2013) 541–549, https://doi.org/10.1210/jc.2012-2394.

[11] A.H. Maghrabi, K. Wolski, B. Abood, A. Licata, C. Pothier, D.L. Bhatt, et al., Two-year outcomes on bone density and fracture incidence in patients with T2DM randomized to bariatric surgery versus intensive medical therapy, Obesity (Silver Spring) 23 (12) (2015) 2344–2348, https://doi.org/10.1002/oby.21150.

[12] A.L. Schafer, G.J. Kazakia, E. Vittinghoff, L. Stewart, S.J. Rogers, T.Y. Kim, et al., Effects of gastric bypass surgery on bone mass and microarchitecture occur early and particularly impact postmenopausal women, J. Bone Miner. Res. 33 (6) (2018) 975–986, https://doi.org/10.1002/jbmr.3371.

[13] E.W. Yu, M.L. Bouxsein, M.S. Putman, E.L. Monis, A.E. Roy, J.S. Pratt, et al., Two-year changes in bone density after Roux-en-Y gastric bypass surgery, J. Clin. Endocrinol. Metab. 100 (4) (2015) 1452–1459, https://doi.org/10.1210/jc.2014-4341.

[14] A.G. Robling, K.M. Duijvelaar, J.V. Geevers, N. Ohashi, C.H. Turner, Modulation of appositional and longitudinal bone growth in the rat ulna by applied static and dynamic force, Bone. 29 (2) (2001) 105–113, https://doi.org/10.1016/s8756-3282(01)00488-4.

[15] N. Rosa, R. Simoes, F.D. Magalhães, A.T. Marques, From mechanical stimulus to bone formation: a review, Med. Eng. Phys. 37 (8) (2015) 719–728, https://doi.org/10.1016/j.medengphy.2015.05.015.

[16] T. Bettis, B.J. Kim, M.W. Hamrick, Impact of muscle atrophy on bone metabolism and bone strength: implications for muscle-bone crosstalk with aging and disuse, Osteoporos. Int. 29 (8) (2018) 1713–1720, https://doi.org/10.1007/s00198-018-4570-1.

[17] K. Gkastaris, D.G. Goulis, M. Potoupnis, A.D. Anastasilakis, G. Kapetanos, Obesity, osteoporosis and bone metabolism, J. Musculoskelet. Neuronal Interact. 20 (3) (2020) 372–381.

[18] F. Diniz-Sousa, L. Veras, G. Boppre, P. Sa-Couto, V. Devezas, H. Santos-Sousa, et al., The effect of an exercise intervention program on bone health after bariatric surgery: a randomized controlled trial, J. Bone Miner. Res. (2020), https://doi.org/10.1002/jbmr.4213.

[19] National Health and Nutrition Examination Survey (NHANES) – Anthropometry Procedures Manual, in: https://wwwn.cdc.gov/nchs/data/nhanes/2019-2020/manuals/2020-Anthropometry-Procedures-Manual-508.pdf, 2020.

[20] L. Veras, F. Diniz-Sousa, G. Boppre, V. Devezas, H. Santos-Sousa, J. Preto, et al., Accelerometer-based prediction of skeletal mechanical loading during walking in normal weight to severely obese subjects, Osteoporos. Int. 31 (7) (2020) 1239–1250, https://doi.org/10.1007/s00198-020-05295-2.

[21] D. John, A. Morton, D. Arguello, K. Lyden, D. Bassett, "What is a step?" Differences in how a step is detected among three popular activity monitors that have impacted physical activity research, Sensors (Basel) 18 (4) (2018), https://doi.org/10.3390/s18041206.

[22] A. Vainionpää, R. Korpelainen, E. Vihriälä, A. Rinta-Paavola, J. Leppäluoto, T. Jämsä, Intensity of exercise is associated with bone density change in premenopausal women, Osteoporos. Int. 17 (3) (2006) 455–463, https://doi.org/10.1007/s00198-005-0005-x.

[23] N. Aguilar-Farias, W.J. Brown, G.M. Peeters, ActiGraph GT3X+ cut-points for identifying sedentary behaviour in older adults in free-living environments, J. Sci. Med. Sport 17 (3) (2014) 293–299, https://doi.org/10.1016/j.jsams.2013.07.002.

[24] J.E. Sasaki, D. John, P.S. Freedson, Validation and comparison of ActiGraph activity monitors, J. Sci. Med. Sport 14 (5) (2011) 411–416, https://doi.org/10.1016/j.jsams.2011.04.003.

[25] L. Veras, verasls/BaSEIB_bariatric_surgery_bone_health: the effect of bariatric surgery on gravitational loading and its impact on bone mass (version v1.1.0), Zenodo. (2021, February 12), https://doi.org/10.5281/zenodo.4537251.

[26] D.T. Villareal, L. Fontana, S.K. Das, L. Redman, S.R. Smith, E. Saltzman, et al., Effect of two-year caloric restriction on bone metabolism and bone mineral density in non-obese younger adults: a randomized clinical trial, J. Bone Miner. Res. 31 (1) (2016) 40–51, https://doi.org/10.1002/jbmr.2701.

[27] K. Shah, R. Armamento-Villareal, N. Parimi, S. Chode, D.R. Sinacore, T.N. Hilton, et al., Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones, J. Bone Miner. Res. 26 (12) (2011) 2851–2859, https://doi.org/10.1002/jbmr.475.

[28] H.M. Frost, Muscle, bone, and the Utah paradigm: a 1999 overview, Med. Sci. Sports Exerc. 32 (5) (2000) 911–917, https://doi.org/10.1097/00005768-200005000-00006.

[29] G. Armbrecht, D.L. Belavý, M. Backström, G. Beller, C. Alexandre, R. Rizzoli, et al., Trabecular and cortical bone density and architecture in women after 60 days of bed rest using high-resolution pQCT: WISE 2005, J. Bone Miner. Res. 26 (10) (2011) 2399–2410, https://doi.org/10.1002/jbmr.482.

[30] L. Vico, B. van Rietbergen, N. Vilayphiou, M.T. Linossier, H. Locrelle, M. Normand, et al., Cortical and trabecular bone microstructure did not recover at weight-bearing skeletal sites and progressively deteriorated at non-weight-bearing sites during the year following International Space Station missions, J. Bone Miner. Res. 32 (10) (2017) 2010–2021, https://doi.org/10.1002/jbmr.3188.

[31] L. Busetto, D. Dicker, C. Azran, R.L. Batterham, N. Farpour-Lambert, M. Fried, et al., Practical recommendations of the obesity management task force of the European Association for the study of obesity for the post-bariatric surgery medical management, Obes. Facts 10 (6) (2017) 597–632, https://doi.org/10.1159/000481825.

[32] L.Y. Herring, C. Stevinson, M.J. Davies, S.J. Biddle, C. Sutton, D. Bowrey, et al., Changes in physical activity behaviour and physical function after bariatric surgery: a systematic review and meta-analysis, Obes. Rev. 17 (3) (2016) 250–261, https://doi.org/10.1111/obr.12361.

[33] W.C. King, J.Y. Chen, D.S. Bond, S.H. Belle, A.P. Courcoulas, E.J. Patterson, et al., Objective assessment of changes in physical activity and sedentary behavior: pre-through 3 years post-bariatric surgery, Obesity (Silver Spring) 23 (6) (2015) 1143–1150, https://doi.org/10.1002/oby.21106.

[34] D.A. Josbeno, J.M. Jakicic, A. Hergenroeder, G.M. Eid, Physical activity and physical function changes in obese individuals after gastric bypass surgery, Surg. Obes. Relat. Dis. 6 (4) (2010) 361–366, https://doi.org/10.1016/j.soard.2008.08.003.

[35] M.T. Drake, S. Khosla, Hormonal and systemic regulation of sclerostin, Bone. 96 (2017) 8–17, https://doi.org/10.1016/j.bone.2016.12.004.

[36] M.F.G. Biagioni, A.L. Mendes, C.R. Nogueira, C.V. Leite, L. Gollino, G.M. Mazeto, Bariatric Roux-En-Y gastric bypass surgery: adipocyte proteins involved in increased bone remodeling in humans, Obes. Surg. 27 (7) (2017) 1789–1796, https://doi.org/10.1007/s11695-017-2546-4.

[37] A.F. Turcotte, T. Grenier-Larouche, R.V. Ung, D. Simonyan, A.M. Carreau, A. C. Carpentier, et al., Effects of biliopancreatic diversion on bone turnover markers and association with hormonal factors in patients with severe obesity, Obes. Surg. 29 (3) (2019) 990–998, https://doi.org/10.1007/s11695-018-3617-x.

[38] M. Geoffroy, I. Charlot-Lambrecht, J. Chrusciel, I. Gaubil-Kaladjian, A. Diaz-Cives, J.P. Eschard, et al., Impact of bariatric surgery on bone mineral density: observational study of 110 patients followed up in a specialized center for the treatment of obesity in France, Obes. Surg. 29 (6) (2019) 1765–1772, https://doi.org/10.1007/s11695-019-03719-5.

[39] O. Hue, F. Berrigan, M. Simoneau, J. Marcotte, P. Marceau, S. Marceau, et al., Muscle force and force control after weight loss in obese and morbidly obese men, Obes. Surg. 18 (9) (2008) 1112–1118, https://doi.org/10.1007/s11695-008-9597-5.

[40] T.W. Lu, S.J. Taylor, J.J. O'Connor, P.S. Walker, Influence of muscle activity on the forces in the femur: an in vivo study, J. Biomech. 30 (11–12) (1997) 1101–1106, https://doi.org/10.1016/s0021-9290(97)00090-0.

[41] C.H. Turner, A.G. Robling, Designing exercise regimens to increase bone strength, Exerc. Sport Sci. Rev. 31 (1) (2003) 45–50, https://doi.org/10.1097/00003677-200301000-00009.

[42] J.I. Mechanick, A. Youdim, D.B. Jones, W.T. Garvey, D.L. Hurley, M.M. McMahon, et al., Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient—2013 update: cosponsored by American Association of Clinical Endocrinologists, the Obesity Society, and American Society for Metabolic & Bariatric Surgery, Endocr. Pract. 19 (2) (2013) 337–372, https://doi.org/10.4158/ep12437.Gl.

[43] A. Carrelli, M. Bucovsky, R. Horst, S. Cremers, C. Zhang, M. Bessler, et al., Vitamin D storage in adipose tissue of obese and normal weight women, J. Bone Miner. Res. 32 (2) (2017) 237–242, https://doi.org/10.1002/jbmr.2979.

[44] M.R. Fernandes, W.D.R.J. Barreto, Association between physical activity and vitamin D: a narrative literature review, Rev. Assoc. Med. Bras. (1992) 63 (6) (2017) 550–556, https://doi.org/10.1590/1806-9282.63.06.550.

[45] K. Abegg, N. Gehring, C.A. Wagner, A. Liesegang, M. Schiesser, M. Bueter, et al., Roux-en-Y gastric bypass surgery reduces bone mineral density and induces metabolic acidosis in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 305 (9) (2013) R999–r1009, https://doi.org/10.1152/ajpregu.00038.2013.

[46] E.W. Yu, J.S. Carmody, D.J. Brooks, S. LaJoie, L.M. Kaplan, M.L. Bouxsein, Cortical and trabecular deterioration in mouse models of Roux-en-Y gastric bypass, Bone. 85 (2016) 23–28, https://doi.org/10.1016/j.bone.2016.01.017.