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Obesity Treatment/Etiology and Pathophysiology

Bariatric surgery, bone loss, obesity and possible mechanisms

M. M. Brzozowska[1], A. Sainsbury[2,8], J. A. Eisman[1,3,4,5,6], P. A. Baldock[3,4,7] and J. R. Center[1,3,4]

[1]Osteoporosis and Bone Biology Division, Garvan Institute of Medical Research, Sydney, Australia; [2]The Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, The University of Sydney, Australia; [3]Clinical School, St Vincent's Hospital, Sydney, Australia; [4]Faculty of Medicine, University of New South Wales, Sydney, Australia; [5]Clinical Translation and Advanced Education Department, Garvan Institute of Medical Research, Sydney, Australia; [6]Faculty of Medicine, University of Notre Dame, Sydney, Australia; [7]Neurological Disease Division, Garvan Institute of Medical Research, Sydney, Australia; [8]School of Medical Sciences, University of New South Wales, Sydney, Australia

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Address for correspondence: A/Prof JR Center, Osteoporosis and Bone Biology Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia.
E-mail: j.center@garvan.org.au

Summary

Bariatric surgery remains the most effective treatment for severely obese patients. However, the potential long-term effects of bariatric surgical procedures on health, including bone health, are only partially understood. The goal of this review was to present data on the impact of bariatric surgery on bone metabolism and to analyse possible reasons for the loss of bone mass that frequently occurs after bariatric surgery. Such factors include nutritional deficiencies, rapid weight loss *per se*, effects of fat-derived adipokines and gut-derived appetite-regulatory hormones. However, the relative roles of these factors in skeletal regulation and the mechanisms by which they work are not yet fully defined. Our review was focussed on the complex relationship between body weight, fat mass and bone mass, as well as peripheral and central mediators potentially involved in the dual regulation of both energy and bone homeostasis. We also review the data on the inverse relationship between central obesity, bone marrow fat and osteoporosis. As the number of bariatric operations increases, it is imperative to recognize mechanisms responsible for bariatric surgery-induced bone loss, with careful monitoring of bone health including long-term fracture incidence in patients undergoing these procedures.

Keywords: Adipokines, bariatric surgery, gut hormones, skeletal health.

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Obesity and bone – not as simple as previously thought

The prevalence of obesity worldwide has increased significantly in recent decades because of a complex range of environmental and genetic factors. Severe obesity is associated with a number of comorbid conditions and shortened life expectancy (1). One area of research that is beginning to attract greater attention is the effect of obesity on bone. The general view among healthcare providers is that osteoporosis is not of concern for obese patients because of the known bone-strengthening effects of long-term weight bearing (2). Indeed, epidemiological evidence suggests that obesity is correlated with increased bone mass, and that increased body weight protects against bone loss (2,3). However, despite such evidence of a protective effect of obesity on bone, more recent data point to a potential detrimental effect. Contrary to popular belief, osteoporosis and obesity have been shown to coexist, as evident in disorders involving fat redistribution such as

type 2 diabetes mellitus, Cushing's disease and drug-induced lipodystrophies (4). Indeed, in some cohorts, the percent of total fat mass is strongly and inversely associated with bone mineral density (BMD) (5). Obese women have lower rates of bone formation, as indicated by circulating type I collagen levels, suggesting that increased body fat suppresses new collagen formation (6).

Besides effects on osteoporosis, BMD or bone formation rates, obesity is also associated with fractures, not only in older people. Human data show increased prevalence of forearm fractures among obese young adults as well as reduced bone mass in obese children (7,8). Postmenopausal obesity appears to be a risk factor for fracture at selected sites such as the tibia and ankle (9). Moreover, there is increasing evidence to suggest that visceral obesity and the metabolic syndrome have potential detrimental effects on bone health, with a higher incidence of osteoporotic fractures and impaired bone structure and strength observed among younger and older adults with increased visceral adiposity (10,11).

While not the focus of this review, potential mechanisms for any harmful effects of obesity on bone include the metabolic syndrome and its causes or consequences, as well as via effects on bone marrow fat. Increased fat in the bone marrow compartment is linked with endplate depression and compression fractures in subjects with morphologic evidence of bone weakness (12,13). Recent literature points to a positive correlation between bone marrow fat and visceral fat – women with high visceral fat were found to have higher bone marrow fat than women with low visceral fat (14) – as well as to an inverse association between vertebral bone marrow fat and trabecular BMD in pre-menopausal obese women. These findings suggest that the detrimental effects of visceral fat on bone health may be mediated in part by accumulation of bone marrow fat, providing further evidence of the link between central obesity and the risk for osteoporosis.

As well as effects of obesity on bone health, new evidence suggests that weight loss interventions may also induce detrimental effects on bone. While the treatment of obesity is associated with improved health outcomes and a better quality of life, potential effects on bone have received little attention. Bone loss is a part of ageing and occurs in both genders after peak bone mass has been attained (15). Starting from the middle of the third decade, women lose 35% of their cortical bone and 50% of their trabecular bone (16), whereas men lose approximately two-thirds of this amount over their lifetimes (17). Studies show that a 10% weight loss is associated with a measured 1–2% bone loss at the various bone sites (18–22). In addition, the bone response to weight reduction also varies among different populations. Studies reported with mixed populations including pre-, peri- and postmenopausal women, and/or men showed a loss of total body BMD (0–2.5%) and bone mineral content (BMC; 3–4%) with weight loss, as well as variable losses at peripheral bone sites (1–13%) (21,23,24). There is a positive relationship between BMI and age-adjusted BMC (25) with a trend to normative reduction in total BMC with a significant fall in BMI.

At present, the most effective, long-term treatment for obesity remains bariatric surgery, with Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric banding (LAGB or gastric banding) and increasingly sleeve gastrectomy being the most commonly performed surgical procedures. One of the reported consequences of bariatric surgery, particularly following RYGB, is bone loss, yet this issue receives minimal attention. The trend towards surgical treatment options not only for very obese adults, but also for younger and less obese patients, makes a comprehensive understanding of any adverse consequences of bariatric surgery vitally important.

In this review, we will highlight studies showing the impact of obesity surgery on bone metabolism, focussing on the three most commonly performed procedures: RYGB, gastric banding and sleeve gastrectomy. We will then examine potential underlying mechanisms for these observations of heightened bone turnover or bone loss after bariatric surgery. While reductions in weight bearing (26) and delivery of vital nutrients such as vitamin D and calcium (27,28) are likely important contributors to bariatric surgery-induced bone loss, in this review, we focus on novel potential mechanisms emanating from the adipose tissue–gut–brain axis. These include adipose tissue-derived adipokines such as leptin and adiponectin, the gut-derived appetite-regulatory hormones, namely peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and ghrelin, all of which are known to be influenced by bariatric surgery, and all of which have been shown to have significant effects on bone homeostasis, and hypothalamic regulators of energy balance, namely neuropeptide Y (NPY). Finally, we call for consideration of long-term bone health in further studies and registries examining the benefit, risk and cost profile of obesity surgery.

Literature cited in this review was published until March 2012 and was located via Pub Med using the search terms 'bariatric surgery', 'weight loss', 'bone loss', 'bone metabolism', 'gut hormones', 'adipokines', 'neuropeptides' and 'bone markers'.

Overview of the effects of bariatric surgery on body weight and bone

Weight loss (bariatric) surgery provides the best long-term results for patients with moderate obesity (BMI between 35 and 39.9 kg/m^2) or severe obesity (BMI of at least 40 kg/m^2) and who have not responded to more conservative approaches to weight management. Bariatric surgery is generally reserved for patients with a BMI greater than

40 kg/m^2, or for those with a BMI greater than 35 kg/m^2 whose obesity is complicated by one or more major diseases such as type 2 diabetes mellitus or sleep apnoea. There are two major surgical categories: gastrointestinal diversionary procedures such as RYGB and biliopancreatic diversion, as well as gastric interventions including laparoscopic adjustable gastric banding (LAGB or gastric banding), vertical gastroplasty and sleeve gastrectomy.

Bariatric surgical procedures induce much more substantial weight loss than can be produced by conventional medical and lifestyle management of obesity. The efficacy of different types of bariatric surgery varies in terms of weight loss achieved and improvement in obesity comorbidities. Weight loss post bariatric surgery is frequently expressed as percent loss of excess body weight, defined as the difference between the preoperative weight and the ideal body weight (i.e. BMI 25). After RYGB, patients lose up to approximately 35% of their initial weight, equivalent to a loss of 62–75% of excess body weight, with this loss being maintained at 10–14 years following surgery (29–32). Gastric banding results in an average loss of 20–30% of an initial body weight (33), equivalent to a loss of 41–54% of excess body weight loss (29). Sleeve gastrectomy causes an average body weight loss of 20–30%, equivalent to a loss of 45–64% of excess body weight (33,34).

As well as effects on weight loss, and in some cases even before significant loss of excess body weight, bariatric surgical procedures have been shown to resolve or ameliorate type 2 diabetes mellitus in 57–86% of cases, hyperlipidaemia in approximately 71% of cases, hypertension in 68% of cases, and sleep apnoea in 80–85% of cases, with RYGB having a greater beneficial effect on these comorbidities than other bariatric procedures (29,35).

As the use of bariatric surgery for the treatment of morbid obesity steadily increases, there is growing evidence that these procedures result in changes in bone and mineral metabolism, and in some studies, a decline in BMD has been observed. This evidence will be detailed for RYGB, gastric banding and sleeve gastrectomy, below. It is important to point out that interpretation of the data is limited at this stage because of small sample sizes, inconsistent study measures, varying degrees of bone loss as well as potential difficulties in the accuracy of measurement of BMD in morbid obesity and during weight loss.

For instance, the majority of studies have used areal BMD as the primary outcome for bone health in post-bariatric patients, as determined using dual-energy X-ray absorptiometry (DXA). Major changes in fat mass and its distribution induced by significant weight loss may affect the precision of BMD measurement in this group of patients, especially when assessing bone area and thus when estimating areal BMD (36–38). Variability in areal BMD increases significantly with tissue depths greater than 25 cm (39), and excess fat around bone can result in overestimation of areal BMD in obese subjects (40). Additionally, spuriously increased bone area compared with measures of BMC that are unaffected by obesity, results in a potential spurious decrease of BMD in obese subjects (41,42). Thus measurement of BMC, which is more closely related to fat-free mass than to fat mass (43), could increase an accuracy of addressing changes in bone mass by DXA in obese subjects undergoing weight loss interventions.

In addition to these technical limitations in obesity, effects of obesity on DXA-based measurements of BMD also vary according to the measurement technique, including the type of DXA system used, the distribution of body fat, the software used, and scan mode (44). Nonetheless, the emerging data are generally consistent with accelerated bone loss in response to bariatric surgery, and – as outlined in subsequent sections of this review – are backed by plausible hypotheses that could potentially explain such bone loss.

Effects of Roux-en-Y gastric bypass on bone

Several studies (45–57) have examined the effects of RYGB for morbid obesity on BMD and/or bone turnover. In brief, these studies show evidence of an early increase in bone remodelling, as indicated by an increase over baseline values of circulating or urinary concentrations of bone turnover markers such as breakdown products of type I collagen (a major organic component of bone matrix that is synthesized primarily in bone) as well as osteocalcin, a non-collagenous protein secreted specifically by osteoblasts. These changes have been noted as early as 3 months post surgery (45,50,51), and occurred in spite of patients taking routinely recommended calcium and vitamin D supplements (45–47,49,54,55,57).

Indices of increased bone resorption have been demonstrated to persist long after the RYGB procedure. A recent study documented elevated serum osteocalcin and serum N-terminal telopeptide (NTX) concentrations relative to baseline values at 18 months post RYGB surgery (54), indicating a prolonged increase in bone turnover. This observation is consistent with a previous report describing raised serum osteocalcin levels in patients examined 10 years after RYGB (49). It remains unclear whether the reported long-term increase in bone turnover markers represents an adverse effect of surgery with the development of metabolic bone disease, or if it reflects a physiological adjustment by the skeleton to large changes in body weight and thus skeletal loading. In either case, an increase in bone turnover is known to have a catabolic effect on bone, with resorption exceeding formation (58). Moreover, accelerated rates of bone remodelling increase bone fragility by creation of a greater number of unfilled resorptive excavation sites and impaired cross-linking between collagen

fibres (59). Surgery-induced bone remodelling that continues despite achievement of normal weight could lead to long-term bone disease as suggested by some case studies (60).

However a strong association between bariatric surgery and osteoporosis is not supported by the literature (61). As both bone mass and bone turnover rate predict fracture risk (62), changes in these parameters during weight maintenance are key to determining the future fracture risk associated with weight reduction. A recent retrospective study found no significantly increased risk of fracture in patients a mean of 2.2 years after bariatric surgery, compared with controls. However, it did show a trend towards an increased fracture risk 3–5 years post bariatric surgery and in patients who had a greater decrease in body mass index (63).

Despite these demonstrations of increased bone turnover, there are inconsistent findings of changes in bone mass in subjects post RYGB surgery (45,46). Most (45,50–52) but not all (46,49) reports suggest a decline in BMD and BMC during the first year post surgery, predominantly at the hip. Decreases in whole-body BMC have been reported to vary from 3 to 12% at 9–24 months after RYGB surgery (45–52,55). The magnitude of bone lost in adults after RYGB is associated with the amount of weight lost in several studies (50–52,56). Some of these studies will now be outlined in greater detail.

A prospective study (45) compared bone turnover in 25 patients post RYGB with that of 30 obese control participants. Compared with controls, significant elevations in bone turnover markers such as serum osteocalcin and urinary NTX were observed at 3 and 9 months post-operatively, in association with prominent and significant decreases in BMD of the total hip ($7.8 \pm 4.8\%$, $P < 0.001$), trochanter ($9.3 \pm 5.7\%$, $P < 0.001$), and total body ($1.6 \pm 2.0\%$, $P < 0.05$), as well as significantly reduced BMC at all of these sites by 9 months post surgery. Other assessments of bone and mineral metabolism (i.e. serum concentrations of parathyroid hormone and calcium as well as 24-h urinary calcium excretion) were not different between controls and post-surgical patients. The post-surgical group self-reported significantly higher dietary vitamin D and calcium intakes, suggesting that vitamin and mineral supplementation may protect against secondary hyperparathyroidism after RYGB surgery.

In a recent prospective analysis comparing pre- and post-surgery values, a similar BMD loss of 8% was reported in the hip of 23 patients investigated at 12 months post RYGB (51). In this study, a linear relationship was observed between weight loss and the decline in BMD, which was associated with raised bone turnover markers relative to baseline ($P < 0.01$) for both urinary NTX and serum osteocalcin). There was evidence of calcium and vitamin D malabsorption with reduced urinary calcium excretion after RYGB, as well as raised serum concentrations of parathyroid hormone and unchanged serum 25-hydroxyvitamin D concentrations. Self-reported oral vitamin D intake increased by 260% by 12 months post surgery. The results from this study inferred that RYGB related bone loss is associated with alterations in the calcium-vitamin D-parathyroid hormone axis, through calcium and vitamin D malabsorption and secondary hyperparathyroidism.

Perhaps more troubling than the above reports of RYGB-induced bone loss in adults are reports on the potential effect of bariatric surgery on BMD in younger subjects. RYGB remains the most commonly performed bariatric surgical procedure for morbidly obese teens in most US centres. A retrospective study (53) of 61 adolescents reported significant post-operative bone loss, with a 7.4% decrease in BMC and a decline in the *Z*-score for BMD from 1.5 to 0.1, remaining within expected value for gender and age, likely due to the high BMC and density before surgery in this extremely obese population. Longer follow-up is required to determine whether bone loss in this group of young patients continues, stabilizes or reverses.

Contrary to the above findings of surgery-induced bone loss, a study (46) in 44 pre- and postmenopausal women reported unchanged bone mass after RYGB in premenopausal patients compared with un-operated age- and weight-matched control women. By contrast, postmenopausal women had significantly higher lumbar spine BMD ($P < 0.05$) and BMC ($P < 0.05$) and significantly lower femoral neck BMC ($P < 0.001$) at up to 3 years after RYGB surgery, compared with corresponding values in control postmenopausal women. In addition, postmenopausal women showed evidence of secondary hyperparathyroidism and elevated bone resorption markers after RYGB surgery in spite of dietary calcium and vitamin D supplementation. This pattern of bone loss may relate to elevated levels of serum parathyroid hormone in postmenopausal women which cause cortical (i.e. femoral neck or 33% radius) but not trabecular bone loss (i.e. lumbar spine) (64–66). This study inferred that postmenopausal status is associated with accelerated patterns of bone loss after RYGB as compared with premenopausal status and non-surgical control participants. This link between hypoestrogenism and post-surgical loss of BMD in postmenopausal females has not been clearly explored in the literature.

Taken together, these studies show that RYGB surgery induces changes in bone remodelling as indicated by consistent increase in bone turnover markers. Nutritional deficiencies that accompany RYGB surgery may result in metabolic bone disease, with associated secondary hyperparathyroidism and rapid loss in BMD. The clinical significance of the observed biological and radiological changes is unclear. More studies are needed to assess the impact of

RYGB surgery on skeletal health, notably to determine the degree of post-operative bone loss and the clinical consequences of these changes.

Effects of laparoscopic adjustable gastric banding (gastric banding) on bone

With a paucity of available data, it is currently uncertain whether bone loss is a consequence of gastric banding, and whether or not any such effects are greater or lesser than those induced by RYGB. Two studies (67,68) of obese premenopausal women reported an early increase above baseline in markers of bone resorption (urinary and serum concentrations of type I collagen breakdown products) and a decrease in femoral and trochanter BMD with no significant change in total body BMD at 12 (67,68) and 24 months after gastric banding (67). Total body BMC showed progressive decreases relative to baseline in the majority of patients at 12 and 24 months after gastric banding (67), which correlated with the changes in body composition and the decrease in fat mass. In both of these studies, there was an absence of secondary hyperparathyroidism, indicating that other factors besides lack of calcium and vitamin D were involved in post-surgical bone remodelling.

We know of two other gastric studies (50,69) investigating effects of gastric banding or vertical banded gastroplasty on bone; both of these studies included an RYGB arm and both produced conflicting results. A small Swiss pilot study (50) compared the skeletal effects of gastric banding ($n = 9$) with those of RYGB ($n = 4$) over 24 months, with morbidly obese men and women in the control arm ($n = 6$). No significant changes from baseline in BMC were reported at 24 months in the gastric banding and control groups. However, there was a significant decrease from baseline in BMC in the RYGB group ($P = 0.005$), accompanied by significant increases in bone turnover markers (serum osteocalcin and urinary deoxypyridinoline). In the other study (69), by contrast, men and women who underwent RYGB surgery ($n = 29$) showed less loss of BMC and BMD in the first year after surgery than people who had undergone vertical banded gastroplasty ($n = 31$), these difference reaching statistical significance in female, but not in male subjects. These changes are all the more noteworthy given that RYGB patients lost significantly more weight and total body fat than those who underwent gastric procedures, indicating that the underlying mechanism for any bone loss after gastric banding or gastroplasty – if confirmed in future studies – is more involved than a simple decrease in adiposity or weight bearing.

Effects of sleeve gastrectomy on bone

There is also little available data on changes in bone mass or metabolism following sleeve gastrectomy, an increasingly popular form of bariatric surgery. A single prospective, comparative study (70) that examined the impact of sleeve gastrectomy and RYGB on bone mass and remodelling found significant loss of bone mass in the lumbar spine and hip at 12 months post-operatively in all surgical patients, relative to baseline values. Bone loss was less pronounced in the spine and femur after sleeve gastrectomy than after RYGB, albeit this difference among surgeries was not statistically significant and the bone resorption marker NTX was significantly elevated over baseline in both surgical groups. Clearly, further work is required to assess the effect of sleeve gastrectomy, if any, on bone mass.

Potential mechanisms for bone loss after bariatric surgery

Several mechanisms may explain the changes in bone metabolism observed after bariatric surgery. Reduced delivery of essential nutrients, including calcium and vitamin D, are consequence of diversionary bariatric procedures such as RYGB, as well as of the energy restriction that follows all successful bariatric operations. On top of this, pre-existing alterations in calcium homeostasis (71) and vitamin D deficiencies, which are common in obese patients (28), may further contribute to inadequate delivery of these essential nutrients, thereby contributing to secondary hyperparathyroidism and metabolic and skeletal abnormalities. However, although some studies implicate decreased calcium and vitamin D intake or absorption (60,72) in bone loss after bariatric surgery, several of the studies reported in the previous section (45,67,72) demonstrated heightened bone turnover or bone loss after bariatric surgery despite apparently adequate calcium and vitamin D supplementation, as indicated by reported intake and/or the absence of any secondary increase in circulating parathyroid hormone levels.

The direct impact of micronutrients and vitamin deficiencies on bone health in post bariatric RYGB patients has not been well documented, partly because of lack of standardized guidelines for the nutritional care and assessment of these individuals. The described studies relate to different post-operative calcium and vitamin D dietary and supplementation protocols. Prescribed supplements have varied in dose, and although sufficient for a general population, they might not have prevented nutritional deficiencies in bariatric surgery patients. Lack of rigorous post-operative follow-up, and varying recommended dosages of calcium and vitamin D supplements across studies, might also have contributed to differences in estimated bone loss across studies. Only a single study (51), which reported evidence of calcium and vitamin D malabsorption after RYGB surgery, monitored vitamin D and calcium compliance through standardized questionnaires of intake from food sources and supplements.

Besides nutritional deficiencies, another major mechanism that is thought to contribute to increased bone turnover and reduced bone mass after bariatric surgery is weight loss *per se*, resulting in reduced mechanic load on the skeleton. However, the study reported earlier (69), demonstrating that bone loss was less pronounced after RYGB than after gastroplasty – despite significantly greater weight and fat loss – suggests that other factors besides mechanical loading are at play. Indeed, it has been reported in adolescents that weight loss accounted for as little as 14% of the loss of BMC after bariatric surgery, indicating a significant contribution by additional factors (73). While studies to date have not enabled definitive dissection of the relative contribution of changes in nutrient status and/or weight loss to changes in bone after bariatric surgery, these studies point to other mediators of change.

Hormonal responses to bariatric surgery could conceivably contribute to the associated bone loss. Dietary energy restriction (without bariatric surgery) has been shown to induce neuroendocrine changes that would be expected to accelerate bone loss (74). Such changes include reduced circulating concentrations of thyroid hormones, sex hormones and insulin-like growth factor-1, with concomitant increases in circulating cortisol levels (74). However, it is not known whether bariatric surgery – like weight loss elicited by dietary intervention alone – also induces such hormonal changes that could impair bone health. On the other hand, there is a growing body of literature showing that bariatric surgery alters the secretion of hormones from adipose tissue (notably the adipokines leptin and adiponectin) and the gastrointestinal tract (notably the appetite-regulating gut hormones PYY, GLP-1 and ghrelin), all of which have recently been shown to have significant effects on bone homeostasis. Current knowledge about the extent of their involvement in any bariatric surgery-induced bone loss and the mechanisms underlying such effects is limited, but we hereby outline current insights as a framework for future research.

Adipokines, bariatric surgery and bone

Adipose tissue releases a wide variety of proteins, called adipokines, including leptin, adiponectin, visfatin and resistin, among others, which are known to not only regulate adipose tissue, but to also be involved in the complex regulation of bone physiology (75–81). More recently, the skeleton has also emerged as an endocrine organ, with bone cells – osteoblasts and osteoclasts – secreting a variety of proteins, called osteokines. Osteokines not only influence bone homeostasis, they also influence energy and glucose homeostasis (82–89). The cross-talk between adipose tissue and the skeleton thus constitutes a homeostatic feedback system, with adipokines and osteokines linking these tissues in an active adipose–bone axis. For the purposes of this review, we will focus on the possibility that bariatric surgery can affect bone metabolism and bone mass via effects on adipokine secretion.

Leptin

Leptin is a cytokine-like hormone secreted by adipocytes (90). One of the major determinants of leptin secretion is fat mass, with circulating leptin levels increasing with increasing fat mass (91). Leptin is well known for its role in the regulation of food intake, energy expenditure and energy balance (92), but it has also emerged as a regulator of bone mass via direct effects on bone cells as well as via indirect effects involving the hypothalamus (93), as will be outlined later. However, before examining leptin-induced regulation of bone, we will focus on what is known about the effects of bariatric surgery on leptin.

Several studies have shown that serum leptin concentration decreases after the massive weight reduction induced by bariatric surgery. These studies point to strong correlations between changes in serum leptin concentration with changes in BMI (94–96) as well as fat mass, serum insulin concentration, and the insulin resistance index (96). Emerging clinical evidence is consistent with the possibility that this post-surgical reduction in circulating leptin levels could contribute to any associated bone catabolism. For instance, a prospective study of 20 individuals (54) examined serum concentrations of bone turnover markers as well as leptin at 6 and 18 months after RYGB surgery. In this study, the increase in serum NTX relative to baseline levels significantly correlated with the decrease in serum leptin levels ($r = 0.45$; $P = 0.04$) as well as the reduction in BMI ($r = 0.58$; $P = 0.009$) and the increase in serum 25-hydroxy vitamin D ($r = 0.43$; $P = 0.05$). In multiple regression analysis, however, only the reduction in circulating leptin levels was a significant predictor of the increase in NTX ($P = 0.016$), indicating that the RYGB-induced decrease in leptin may be causally related to increased bone turnover.

In keeping with the possibility of reduced circulating leptin levels inducing bone catabolism after gastric surgery, several reports have shown that leptin directly stimulates bone growth *in vitro* and increases bone density in leptin-deficient animals (97–99) likely via direct effects on bone. For instance, peripherally administered leptin had a stimulatory effect on bone mass in leptin-deficient *ob/ob* mice (97). This effect was thought to be due to a direct anabolic effect within the bone, driving the differentiation of bone marrow stem cells into the osteoblastic (bone forming) cell lineage, while simultaneously inhibiting the differentiation of osteoclasts (bone-resorbing cells) (100,101).

In contrast to the anabolic effects of leptin acting directly on bone, leptin has been reported to have centrally mediated antiosteogenic actions on trabecular (spongy) bone. The central antiosteogenic effect of leptin was first revealed

as an increased trabecular bone mass observed in leptin-deficient *ob/ob* mice and leptin receptor-deficient *db/db* mice, as well as the effect of hypothalamic leptin administration to reduce trabecular bone mass in leptin-deficient and wild-type mice (93). However, more recent work shows that the antiosteogenic actions of leptin are limited to trabecular bone, with leptin-deficient *ob/ob* mice exhibiting decreases in cortical bone mass (102) in conjunction with consistently elevated trabecular bone mass (103). Given the greater contribution of cortical bone mass to total BMD and content than trabecular bone (104), this differential effect of leptin on trabecular and cortical bone probably accounts for the observation of decreased weight-corrected BMC and BMD in *ob/ob* mice (102), as well as independent reports of overall decreases in BMD in leptin-deficient animals (97,99,105,106).

In light of these findings, we hypothesize that the reduction in circulating leptin levels consistently observed after bariatric surgery would lead to a reduction in the direct stimulatory effects of leptin on bone cells. Reduced leptin signalling in the hypothalamus, would predict a decrease in cortical bone mass and overall BMD or content, albeit with a potential increase in trabecular bone formation. However, leptin insensitivity is likely to modulate aspects of leptin signalling in the chronically obese subjects (107). More work would be required to test this working hypothesis.

Adiponectin

Adiponectin is an adipokine, the circulating concentrations of which correlate negatively with obesity in general and central adiposity in particular (108,109). Adiponectin increases insulin sensitivity, and its circulating levels are reduced in obesity and diabetes (110,111). Osteoblasts express both adiponectin and its receptors (112), and show increased differentiation in response to the peptide (113). Oshima *et al*. (113) also showed that adiponectin could conceivably increase bone mass by suppressing osteoclastogenesis as well as the bone resorption activity of osteoclasts *in vivo* (in mice) and *in vitro*. In contrast to these stimulatory effects on bone, circulating adiponectin has been shown to have a negative effect on bone formation due to indirect induction of formation of bone-resorbing osteoclasts via stimulation of receptor activator of nuclear factor kappa-B ligand (RANKL) and inhibition of osteoprotegerin production by osteoblasts (114). Further evidence for potential antiosteogenic actions of adiponectin comes from observations of its ability to bind some growth factors (115) and to reduce circulating insulin concentrations (116), which would tend to oppose any anabolic effects of this cytokine on bone and other tissues.

Despite uncertainty from basic research as to the role of adiponectin in the regulation of bone, with potential osteogenic and antiosteogenic actions reported, most of the published clinical studies indicate that adiponectin is a negative regulator of bone mass in women and men (76,77,117–119). Thus, given the negative correlation between body weight and adiponectin levels, it might be expected that bariatric surgery-induced weight loss would result in increased circulating adiponectin levels and, consequently, an increase in its antiosteogenic actions. Support for this notion comes from a prospective study of 42 women who were investigated at 12 months after gastric bypass surgery (52). In this study, circulating adiponectin levels had a significant and positive correlation with the reduction in BMD relative to baseline, and this effect was unrelated to baseline parameters of body weight or body composition, or to the gastric bypass-induced changes in these parameters.

Other adipokines

Adipokines such as visfatin and resistin have been reported to have a variety of effects on bone, predominantly of the type that would be expected to inhibit bone mass. In keeping with a generally negative effect of these adipokines on bone, resistin may play a role in bone remodelling as it stimulates osteoclastogenesis as well as the proliferation of osteoblasts in mice (80), and circulating resistin was found to be a negative determinant of lumbar BMD in middle-aged men (76). By contrast visfatin is hypothesized to have an insulin-like effect as it stimulates glucose uptake as well as the proliferation and production of type 1 collagen by human osteoblasts (81), which may be expected to contribute to increased bone mass. Clearly, further research is required to define the precise role of these adipokines in bone homeostasis, and whether their expression and secretion are affected by bariatric surgical procedures.

Gut hormones, bariatric surgery and bone

Besides effects on adipokine output from adipose tissue, another potential mechanism by which bariatric surgery could influence bone homeostasis is via alterations in the secretion of gut hormones, several of which have been shown to not only influence appetite and thereby possibly contribute to reduced hunger post bariatric surgery, but to also have significant effects on bone. There are several appetite-regulating hormones secreted by the gut, the circulating concentrations of which have been shown to be altered by bariatric surgery. Examples include PYY (120,121), GLP-1 (121), ghrelin (122,123), oxyntomodulin (124), glucose-dependent insulinotropic polypeptide (121), cholecystokinin (121) and pancreatic polypeptide (PP) (121,125). For the purposes of this review, however, we will focus on three particular gut peptides: PYY, GLP-1 and ghrelin. The reason for this selection is that these are the three gut hormones for which the greatest body of literature exists both on effects of different forms of bari-

atric surgery on their circulating concentrations, as well as effects of these gut hormones on bone.

Peptide YY

PYY, a 36-amino-acid peptide, is a member of the NPY family of peptides that also includes NPY and PP. Like other members of the NPY family of peptides, PYY binds to and induces effects via Y receptors (Y1, Y2, Y4, Y5 and – in some species, but not humans – y6). PYY is secreted primarily from L-cells residing in the intestinal mucosa of the ileum and large intestine (126). It is released into the circulation as the full-length version, $PYY_{1–36}$, as well as the truncated version, $PYY_{3–36}$, the latter being the major form of PYY found in the circulation in the postprandial state (126). NPY and $PYY_{1–36}$ have similar affinities for Y1, Y2, and Y5 receptors, whereas $PYY_{3–36}$ has selective affinity for Y2 receptors (126,127).

PYY is recognized as a critical regulator of food intake and energy homeostasis. Circulating PYY concentrations are increased in response to acute food intake as well as short-term energy excess in lean, overweight and obese people (128). Increased PYY output likely contributes to restoration of energy homeostasis during periods of energy excess, mediated by inhibition of food intake (129), most likely via activation of Y2 receptors in the arcuate nucleus of the hypothalamus (130). Besides inhibiting appetite, PYY has also been shown to increase energy expenditure in lean and obese men (131) and to decrease energy efficiency and increase core body temperature in rodents (132), thereby further contributing to restoration of energy balance.

In light of the above-mentioned weight-reducing effects of PYY, as well as observations of increased circulating PYY levels in response to at least some forms of bariatric surgery (120,121,133), PYY is thought to contribute to the success of surgery-induced weight loss. For instance, several studies have shown an exaggerated postprandial increase in plasma PYY levels following RYGB surgery, which may contribute to the ability of an individual to maintain weight loss after this procedure (121,134). Additionally, two studies have reported increases over baseline in fasting and postprandial circulating PYY concentrations at 3, 6 and 12 months post sleeve gastrectomy (135,136). It remains unclear whether gastric banding has any stimulatory effect on fasting or postprandial circulating PYY levels (137). In contrast to effects of RYGB, a recent study reported that fasting PYY concentrations in the circulation decreased in proportion to weight loss following LAGB, compared with BMI-matched controls (138). These findings suggest that – in contrast to RYGB, which seems to have the opposite effect – compensatory changes in circulating mediators of appetite could encourage weight regain after LAGB-induced weight loss (138).

While an increase in circulating PYY levels may reduce hunger and promote weight loss after RYGB or sleeve gastrectomy, it may also promote bone loss, with human data suggesting a negative correlation between PYY levels and bone mass under a variety of conditions. Obese subjects have been reported to have significantly reduced circulating fasting and postprandial levels of PYY (139) and greater BMD (140). Moreover, in lean premenopausal women, circulating PYY levels are significantly and negatively correlated with total body and hip bone mass, with PYY contributing to 9% of the variance in BMD of the hip (141). Circulating PYY concentrations are markedly elevated in anorexia nervosa (142), and this change is related to the significant bone loss in this illness, particularly in the spine (143). Moreover, circulating PYY levels were found to be a negative predictor of serum concentrations of aminoterminal propeptide of type 1 collagen (PINP), a bone formation marker, and lumbar BMD *Z*-scores in amenorrhoeic athletes, albeit their PYY levels were comparable with that of eumenorrheic athletes (144). While these significant associations are consistent with the hypothesis that enhanced PYY action post RYGB or sleeve gastrectomy could contribute to the associated alterations in bone homeostasis and bone loss, whether these data are due to an effect of PYY on bone metabolism or are a result of numerous effects of the response to altered energy metabolism, remains to be determined. Animal studies, however, support the former scenario.

An effect of PYY *per se* on bone has been confirmed by a recent analysis of PYY knockout and PYY overexpressing transgenic mouse models, with male and female knockouts demonstrating enhanced osteoblast activity and greater trabecular bone mass, and transgenic mice exhibiting reduced osteoblast activity, reduced femoral BMD as well as increased bone resorption (145). The contrasting effects of the PYY knockout and transgenic models on bone strongly indicate that PYY reduces bone mass by inhibiting osteoblast activity and/or stimulating bone resorption through osteoclasts (145). While these data fit the hypothesis that increases in circulating PYY levels seen post RYGB or sleeve gastrectomy may contribute to any associated increase in bone turnover or decrease in bone mass, it is important to point out that another study of an independently-generated PYY knockout mouse line reported an osteopenic phenotype, with reductions in vertebral cancellous bone mass and bone strength (146). Further work is required to clarify the role of PYY in the regulation of bone mass and metabolism.

Glucagon-like peptide-1

GLP-1 is a key incretin which, along with PYY, is released from the lower intestinal endocrine L-cells in response to

ingested nutrients (147). GLP-1 exerts glucoregulatory actions by stimulating insulin secretion, slowing gastric emptying and attenuating glucose-dependent glucagon secretion (148). GLP-1 promotes satiety (149), and sustained GLP-1-receptor activation via treatment with agents such as exenatide (marketed as Byetta or Bydureon) is associated with weight loss in both preclinical and clinical studies (150).

Relatively few studies have examined changes in circulating GLP-1 concentrations in obese patients after gastric bariatric procedures. There have been reports of either a reduction or no change in fasting GLP-1 levels following gastric banding (137,151,152). Conversely, increases in fasting and postprandial circulating GLP-1 levels have been reported in a single study following sleeve gastrectomy (153). Similarly, post-surgical increases in plasma postprandial GLP-1 levels have been documented following diversionary operations, and augmented levels of circulating GLP-1 may account for the antidiabetic effect of the RYGB procedure, evident before any weight loss has occurred (121). On balance, the available studies to date suggest that fasting and/or postprandial circulating GLP-1 levels are increased following RYGB and sleeve gastrectomy, with either no change or a decrease in fasting GLP-1 following the less invasive procedure of gastric banding.

It is conceivable that any differences in GLP-1 actions post bariatric surgery could influence bone, because recent rodent studies have indicated that changes in incretins such as GLP-1 may play a role in bone metabolism (154). However, the directionality of any such effect is not yet clear. GLP-1 receptor knockout mice exhibit cortical osteopenia and bone fragility as assessed by bone densitometry, as well as increased numbers of osteoclasts (bone-resorbing cells) and heightened bone resorption (154). In contrast to these indications of antiosteogenic GLP-1 actions from knockout mice, rats treated with GLP-1 for 3 days via a subcutaneously implanted osmotic pump displayed elevated expression of osteoblastic genes in bone tissue (155). This effect of exogenous GLP-1 administration on bone was apparent in both normal rats, as well as in glucose intolerant rats, and it occurred without any change in plasma glucose and insulin after treatment (155). These findings demonstrate an insulin-independent anabolic effect of GLP-1 on bone, and suggest that GLP-1 could be a useful therapeutic agent for improving the deficient bone formation and bone structure associated with glucose intolerance (155). However, information about the role of GLP-1 in the regulation of bone is scanty and – moreover – has not been investigated in human studies. More work will be required to determine whether and how potential changes in GLP-1 action post bariatric surgery contribute to any observed changes in bone metabolism and mass.

Ghrelin

Ghrelin is a potent appetite-stimulating hormone, synthesized in the gastric antrum and fundus (156). The circulating concentrations of ghrelin increase under pre-prandial and fasting (157) conditions, and this change likely contributes to the drive to eat after periods without food (157). Ghrelin is thought to play a role in long-term maintenance of energy stores, because as well as stimulating appetite, it also decreases energy expenditure (158). Ghrelin is thought to act, at least in part, via increasing expression of the orexigenic hypothalamic NPY system (159), which also stimulates appetite and reduces metabolic rate (160).

Reports of circulating ghrelin levels after bariatric surgery vary, with either no change or increases in fasting plasma ghrelin relative to baseline after gastric banding (152,161,162). By contrast, a reduction in fasting plasma ghrelin relative to baseline levels was found when measured up to 5 years after sleeve gastrectomy (163). Inconsistent post-surgical changes in circulating ghrelin levels have been found after diversionary bariatric procedures such as RYGB, with studies demonstrating a reduction (164,165), no change (122,166) or increased ghrelin levels versus baseline (167–169). However, most studies demonstrate a decrease in fasting and/or postprandial circulating ghrelin levels in RYGB patients compared with control patients and to baseline readings, with this change possibly contributing to the greater weight loss associated with this procedure than with less invasive procedures such as gastric banding (120,123).

In addition to clear effects on energy homeostasis, ghrelin may play a role in the regulation of bone metabolism through its effects on growth hormone and – as a consequence – insulin-like growth factor-1 secretion via actions on the growth hormone secretagogue receptor, which binds ghrelin (170). The growth hormone secretagogue receptor is also expressed by osteoblastic cells, which have been reported to secrete ghrelin (171). Ghrelin stimulates osteoblast proliferation and differentiation *in vitro* (171–174), while also promoting osteoclastogenesis and the bone-resorbing activity of mature osteoclasts (175). At present, the relative contributions of gastric and osteoblastic ghrelin to the control of bone mass are yet to be defined. However, anabolic effects of ghrelin appear to predominate *in vivo*, because it increases BMD when administered to rats (171). Contrary to the increased BMD observed in ghrelin-infused rats (171), ghrelin knockout mice have unaltered BMD and BMC (176), and similar effects were observed in growth hormone secretagogue receptor knockout mice (177), suggestive of compensatory pathways that counteract effects of ghrelin deficiency

While animal studies suggest a predominantly anabolic effect of ghrelin on bone, clinical findings are not yet clear. Overnight, ghrelin secretion has been found to be positively

and significantly related to BMD in adolescent women (178), but no consistent relationship was seen between fasting ghrelin levels and BMD in older men and women (179).

Taken together, while different forms of bariatric surgery may alter circulating ghrelin levels, and while ghrelin is emerging as a potentially positive regulator of bone, whether ghrelin contributes to changes in bone metabolism or mass in response to bariatric surgery is yet to be determined.

Central control of bone

In dissecting possible links between bariatric surgical procedures, the changes in adipokine or gut hormones concentrations they induce, as well as the associated changes in bone metabolism and mass, it is important to address central mechanisms controlling bone, because these central mechanisms may provide a link between these associations. The fields of neuroscience and bone biology have recently converged, following the discovery that bone remodelling is directly regulated by the brain (93,180). Orexigenic neurons, which express both NPY and agouti-related peptide (AgRP), and anorectic neurons expressing pro-opiomelanocortin – the precursor to the anorexigenic alpha melanocyte-stimulating hormone (α-MSH) – and cocaine- and amphetamine-regulated transcript (CART), are located in the arcuate nucleus of the hypothalamus, a major brain centre involved in the regulation of energy homeostasis (181). Activity of these anabolic and catabolic hypothalamic neurons is regulated by both leptin and insulin (182). Recent work has demonstrated that NPY, AgRP, α-MSH and CART likely play a role in the regulation of bone (93,183–185). Other pathways implicated in the central control of bone include nitric oxide signalling (186), neuromedin U signalling (187), and the cannabinoid system (188,189). For the purposes of highlighting potential mechanisms by which changes in adipokine or gut hormone concentrations associated with bariatric surgery may induce changes in bone metabolism or mass, however, in this review, we will focus uniquely on the NPY system, with which we have worked extensively. The reader is referred to other reviews (190–192) for a more detailed assessment of central control of bone.

Neuropeptide Y and bone

NPY is produced predominantly by neurons in both the central and peripheral nervous systems and is present in both sympathetic and parasympathetic nerve fibres, often co-secreted with noradrenaline (193). NPY is a critical downstream regulator of leptin signalling, with hypothalamic NPY expression inversely associated with serum leptin levels (194). The NPY system regulates energy homeostasis and is one of the most potent stimulators of appetite (195). It also has a marked effect on bone mass.

Experimental increases in central NPY expression in mice produce a marked (up to sevenfold) decrease in bone formation and bone mass (103). Consistent with a negative relationship between NPY and bone mass, ablation of NPY production or signalling in mice induces a generalized increase in bone mass and bone formation (102,196). These NPY effects on bone formation have been shown to involve signalling both within the hypothalamus (103,197) as well via direct effects on osteoblasts, through Y1 receptor pathways (196,198).

In light of the antiosteogenic effect of NPY, it has been postulated that NPY acts as a critical integrator of body weight and bone homeostatic signals; increasing bone mass during times of energy excess (and consequent obesity) when hypothalamic NPY expression levels are low, and reducing bone formation to conserve energy under 'starving' conditions, when hypothalamic NPY expression levels are high (197,199). As such, we postulate that negative energy balance after bariatric surgery, which is known to be associated with a reduction in serum leptin (94–96) may result in increased hypothalamic NPY expression, which in turn may contribute to associated increases in bone turnover or loss of bone mass (103).

Sleeve gastrectomy and gastric bypass surgery appear to attenuate the normal adaptive response to energy restriction and weight loss with increased circulating concentrations of the anorexigenic, gut-derived hormone, PYY (200). PYY inhibits NPY secretion by binding to hypothalamic Y2 receptors (201,202), which are known to regulate bone mass (180). As NPY null mice demonstrated a generalized bone anabolic phenotype (197), down-regulation of NPY in response to PYY could not explain how increases in PYY might lead to bone mass loss post bariatric surgery. However, osteoblasts express Y1 receptors, thus PYY could inhibit osteoblast activity and reduce bone mass through a direct pathway (203,204). Such a direct PYY signalling pathway in osteoblasts has been confirmed by a recent analysis of PYY knockout and PYY transgenic mouse models. The contrasting skeletal phenotypes of these two models indicated that PYY has a negative relationship with osteoblast activity in cancellous and cortical bone (145). Thus, PYY may directly reduce bone mass in those surgical procedures that result in increased PYY secretion.

Conclusion

Bariatric surgery remains the most effective treatment for severely obese patients. However, the potential long-term effects of bariatric surgical procedures on health, including bone health, in this unique group of people are only partially understood. As outlined in this review, there is a complex relationship between body weight, fat mass and

bone mass, with many peripheral and central mediators potentially involved in the dual regulation of both energy and bone homeostasis. Increased body weight may not be a simple protective factor for bone as previously thought, and thus bariatric surgical procedures that result in bone loss need careful consideration. Multiple factors are involved in post-surgical bone mass loss including nutritional deficiencies, rapid weight loss, as well as possible effects of changes in circulating concentrations of fat-derived adipokines and gut-derived appetite-regulatory hormones. However, the roles of adipokines and gut hormones in skeletal regulation, as well as the mechanisms by which they work, are not yet fully defined. As the number of bariatric operations increases, and the age and BMI range of patients on which they are performed broadens, it is imperative to recognize mechanisms responsible for bariatric surgery-induced bone loss, with careful monitoring of bone health and long-term fracture incidence in patients undergoing these procedures.

Conflict of interest statement

Authors declare no relevant conflict of interest.

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