

# **Bone health and quality in subjects with morbid obesity - impact of type 2 diabetes and Roux-en-Y gastric bypass.**

Ingvild Kristine Blom-Høgestøl



Institute of Clinical Medicine  
Faculty of Medicine  
University of Oslo

Department of Endocrinology, Morbid Obesity and  
Preventive Medicine, Oslo University Hospital

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# Abbreviations

**aBMD:** Areal bone mineral density

**AGEs:** Advanced glycation end-products

**BALP:** Bone-specific alkaline phosphatase

**BMAT:** Bone marrow adipose tissue

**BMSi:** Bone material strength index

**BMI:** Body mass index

**BTMs:** Bone turnover markers

**CTX-1:** Carboxyl terminal telopeptide of type 1 collagen

**MRS:** Magnetic resonance spectroscopy

**PINP:** Procollagen type I N-terminal propeptide

**PTH:** Parathyroid hormone

**RANK:** Nuclear factor- $\kappa$ B

**RYGB:** Roux-en-Y gastric bypass

**SHPT:** Secondary hyperparathyroidism

**T2D:** Type 2 diabetes

**vBMD:** Volumetric bone mineral density

# List of papers

**Paper I:** Blom-Høgestøl IK, Mala T, Kristinsson J, Brunborg C, Gulseth HL, Eriksen EF. Changes in bone quality after Roux-en-Y gastric bypass: a prospective cohort study in subjects with and without type 2 diabetes. *Bone*. 2019 Oct 5:115069.

**Paper II:** Blom-Høgestøl IK, Mala T, Kristinsson J, Hauge EM, Brunborg C, Gulseth HL, Eriksen EF. Changes in bone marrow adipose tissue one year after Roux-en-Y gastric bypass: a prospective cohort study. *J Bone Miner Res*. 2019 Oct;34:1815-1823.

**Paper III:** Blom-Høgestøl IK, Hewitt S, Chahal-Kummen M, Brunborg C, Gulseth HL, Kristinsson J, Eriksen EF, Mala T. Bone metabolism, bone mineral density and low-energy fractures 10 years after Roux-en-Y gastric bypass. *Bone*. 2019 Oct;127:436-445



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# 1 Introduction and background

A low areal bone mineral density (aBMD) is associated with increased fracture risk and estimation of aBMD is the most widely used method in fracture prediction<sup>1</sup>. Obesity and type 2 diabetes (T2D) are common and highly related disorders where, a site specific, increased fracture risk is observed despite a normal or high aBMD<sup>2-5</sup>. The two disorders also differ from other common states of skeletal fragility in that bone turnover is generally decreased, compared to controls<sup>6,7</sup>, whereas the general trend in postmenopausal osteoporosis is an increase in bone turnover. The discrepancy between the increase in fracture risk despite normal bone mass seen in obesity and T2D may be partly explained by an observed decrease in bone material strength and increased bone marrow adipose tissue (BMAT) fraction<sup>8-13</sup>.

Today the most effective treatment available for morbid obesity and T2D is bariatric surgery<sup>14</sup>. For years Roux-en-Y gastric bypass (RYGB) was the most commonly performed bariatric procedure worldwide<sup>15</sup>. Data have shown beneficial effect on cancer and cardiovascular disease reduction, and decreased mortality<sup>16,17</sup>. On the other hand, studies reporting increased fracture rates after RYGB have been a cause of concern<sup>18,19</sup>. Previous studies have consistently reported increased bone turnover and loss, in lumbar spine, hip and total body, within the first year after RYGB<sup>20-24</sup>. Radiological imaging studies have revealed varying findings of one year changes in estimated bone failure load and BMAT fraction<sup>22,25-27</sup>, with one study suggesting that changes in BMAT fraction depends on glucose metabolism<sup>27</sup>. Measurements of in-vivo bone material strength and biopsy estimated BMAT could therefore provide valuable information and improve the understanding of the effects of weight loss, remission of T2D, and RYGB on bone health and quality.

The effects of RYGB on the skeleton seem to differ over time. Initially non-weight bearing bones appear protected<sup>20,25</sup>, however with prolonged follow-up, these sites are increasingly affected<sup>25,28,29</sup> and five years after surgery, skeletal changes in the non-weight bearing radius has exceeded that of weight bearing bones<sup>29</sup>. Within the first year of RYGB, subjects experience a drastic weight loss, while calciotropic hormones are generally unaltered. Thus changes in body composition with associated hormonal changes and skeletal unloading are

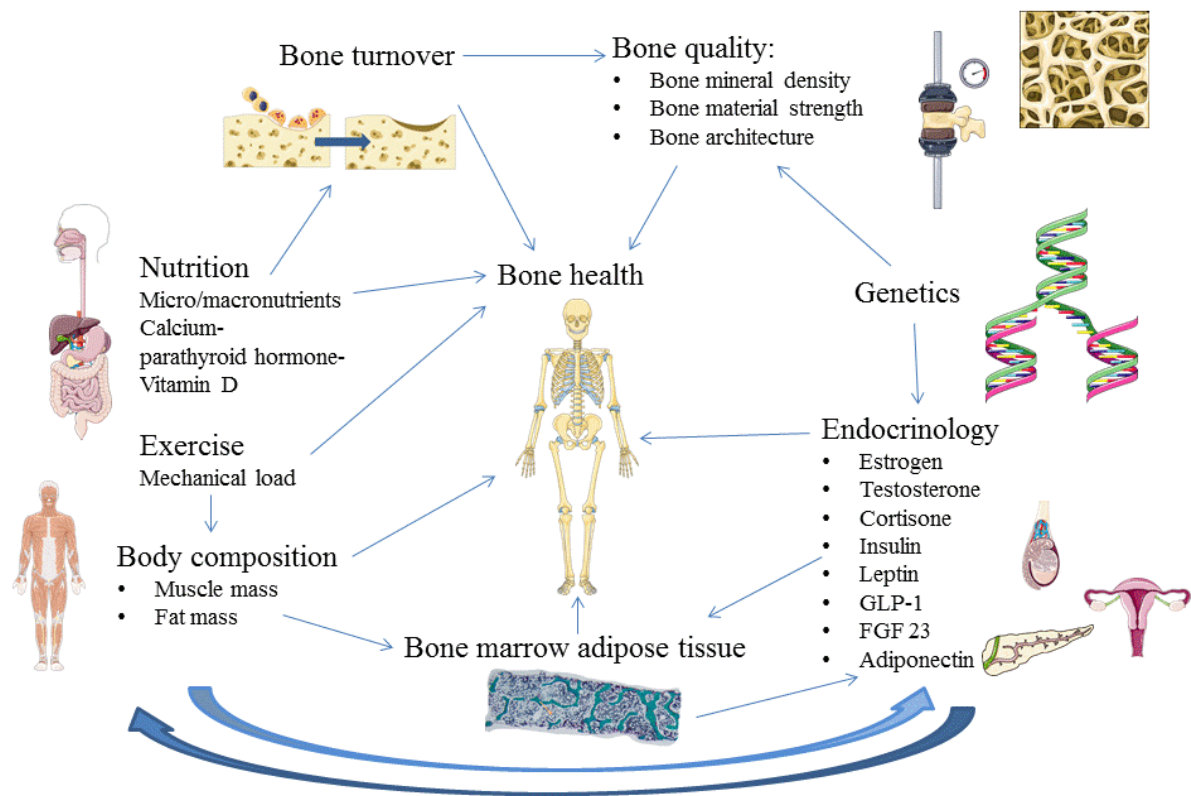
likely central to the observed skeletal changes<sup>23,24,26</sup>. Weight stabilization is usually achieved 12 to 18 months after RYGB<sup>25,28</sup>. From this time point the prevalence of secondary hyperparathyroidism (SHPT) increases<sup>30-32</sup>, with potential adverse effects on bone health<sup>33</sup>. So far, limited data exist regarding long-term bone health and associated risk factors, after RYGB.

The aim of this thesis was to increase the understanding of bone health and quality in morbidly obese subjects, and the impact of T2D and RYGB.

## **1.1 Bone health**

The skeleton maintains the integrity of the body in addition to being an active endocrine organ<sup>34</sup>. Bones are under constant remodeling, renewing cancellous bone surfaces every two years and cortical bone at a somewhat slower rate. This results in a more or less complete renewal of the skeleton over a 10 year period<sup>35</sup>. Bone health is influenced by a variety of intrinsic and extrinsic factors including diet, mechanical loading, hormonal status and genetics<sup>36</sup>. Central factors affecting bone health are illustrated in Figure 1.

**Figure 1:** Central factors affecting bone health.



When bone is exposed to a force that exceeds its strength, fracture occurs. Fractures are subdivided based on the magnitude of force into high and low energy fractures<sup>37</sup>. The most common bone disease predisposing to low energy fractures is osteoporosis. Osteoporosis is a complex and heterogeneous disorder that was first described by the French pathologist and surgeon Jean Lobstein in 1835. Later osteoporosis has been subdivided into primary osteoporosis; post-menopausal and senile osteoporosis, and secondary osteoporosis; idiopathic or caused by a known underlying medical condition<sup>38</sup>. In 2000, the National Institutes of Health Consensus Development Panel on Osteoporosis issued a consensus definition: Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures. Bone strength reflects the integration of two main features; bone density and quality<sup>38</sup>. Bone quality refers to architecture, turnover, damage accumulation (i.e., microfractures), and mineralization. Although the etiology of osteoporosis remains to be completely understood, major risk factors have been identified.

Examples include family history of osteoporosis, decreasing estrogen levels or hypogonadism, older age, immobility, hyperthyroidism, malabsorptive disorders, anorexia nervosa and glucocorticoid use <sup>39</sup>.

With the advances in modern medicine and science, life expectancy has drastically increased. As a result the proportion of the population with osteoporosis has increased and the Scandinavian countries have the highest prevalence of osteoporosis worldwide. In 2010, it was estimated that there were over 5.5 million men and 22 million women with osteoporosis in the European Union, representing 6.6 % and 22.1 % of the population over 50 years of age, respectively <sup>40</sup>. In 2004 to 2006 the United States incidence of osteoporotic fractures was 1420 000, four times the incidence rate of heart attacks <sup>39</sup>. Osteoporotic fractures are a major cause of morbidity and mortality. Increased mortality after hip fracture <sup>41</sup> was first described in the 1960s and the current five-year survival rate after hip or vertebral fracture is estimated to 80% <sup>39</sup>.

## 1.2 Bone mineral density

Bone mineral density is a quantification of bone mass expressing grams of mineral per area (areal bone mineral density) or volume (volume bone mineral density). Dual-energy X-ray absorptiometry (DXA) scanners were introduced in the late 1980s. In 1994 aBMD measurements were incorporated into the WHO diagnostic criteria for postmenopausal osteoporosis defined as a spine, femoral neck or total hip aBMD of 2.5 standard deviations or more below the population mean for healthy young women ( $t\text{-score} \leq -2.5$ ) <sup>37</sup>. Advantages of DXA scans include good measurement precision, low ionizing radiation dose (1-10  $\mu\text{Sv}$ ; comparable to the daily natural background radiation  $7\mu\text{Sv}$ ) and short scan times. Epidemiological studies have shown an exponential relationship between aBMD and risk of skeletal fracture, where the risk of fracture increases for each standard deviations decrease in aBMD <sup>1</sup>. The fracture prediction ability of aBMD is comparable to predictive strength of blood pressure on stroke and serum cholesterol on myocardial infarction <sup>42</sup>. Today DXA scans are the most widely used modality to estimate aBMD and predict fractures.



However, DXA scans are limited by the fact that they are 2D projection measurements which are affected by bone shape and size and measurement errors caused by heterogeneity in soft tissue composition<sup>43</sup> and findings in different measurement sites are discordant. DXA scans evaluate degree of mineralization, but the etiology of the mineralization defect is not specified, for example DXA scans does not discriminate between low bone calcium density due to osteomalacia or osteoporosis. As is the case with most other clinical risk factors, epidemiological studies have demonstrated a considerable overlap in aBMD values between fracture and fracture-free populations<sup>44</sup>. Bisphosphonate treatment induces a reduced fracture risk exceeding what would be expected from the observed increase in aBMD<sup>45</sup>. This discrepancy may be due to an effect on bone quality.

### 1.3 Bone quality

The human skeleton is composed of cortical and trabecular (cancellous) bone. Cortical bone predominates in the appendicular skeleton, while the axial skeleton has a larger fraction of trabecular bone. Bone matrix is composed of mineralized and non-mineralized components. The mineralized components, predominantly calcium hydroxyapatite crystals, are mainly responsible for the ability to resist deformation (stiffness). While non-mineralized components, collagen and non-collagen proteins, are primarily responsible for flexibility and ability to absorb energy by deformation (toughness)<sup>46</sup>.

Bone quality is described as the totality of features and characteristics that influence a bone's ability to resist fracture. Central factors affecting a bone's ability to resist fracture include<sup>46</sup>:

- *Overall composition (i.e., proportion of mineral, collagen, water and matrix proteins)*
- *Physical and biochemical characteristics of these components (i.e., nature of the collagen, degree and type of collagen cross-linking, size and structure of hydroxyapatite crystals and degree of mineralization)*
- *Morphology and architecture (i.e., bone size, cortical cross-sectional geometry, porosity, osteon size and density and trabecular microarchitecture)*

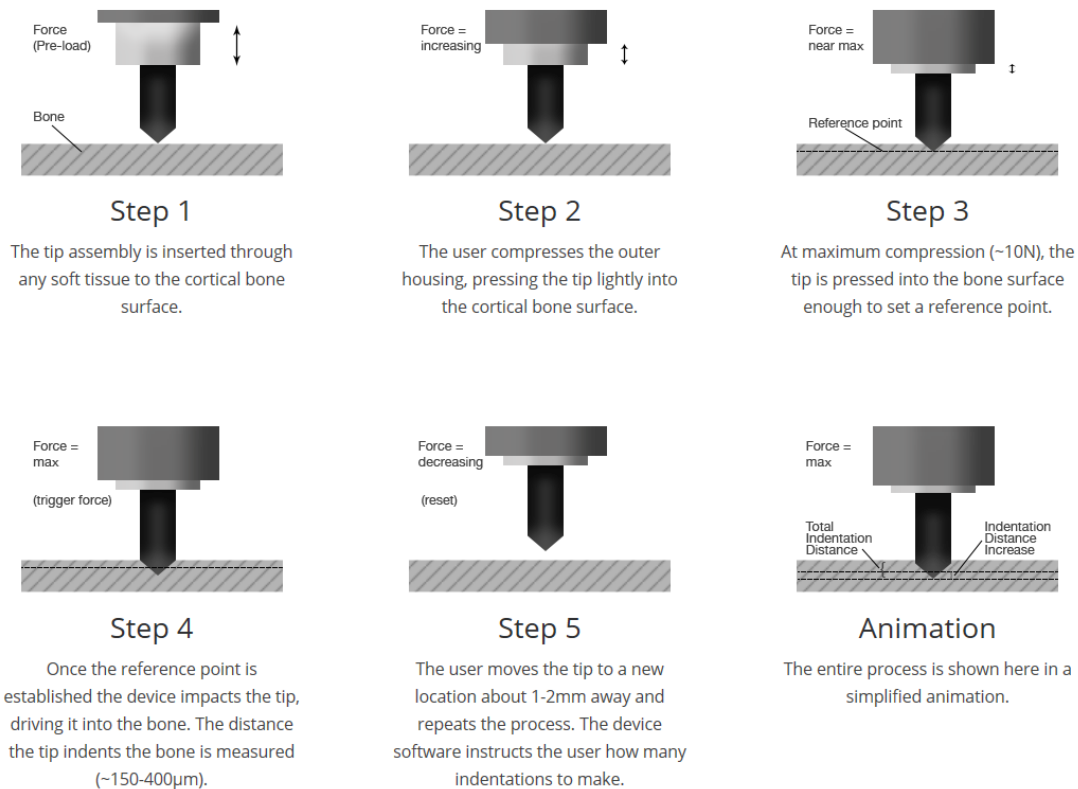
- *Amount and nature of preexisting microdamage (i.e. crack length, density and location).*

### **1.3.1 Evaluation of bone quality**

The gold standard for assessment of bone microstructure is histomorphometric analysis of a transiliac bone biopsy, however with advances in radiological imaging non-invasive methods providing X-ray based three-dimensional morphological assessment of bone microarchitecture are now available; high-resolution quantitative computed tomography and high-resolution peripheral quantitative computed tomography, for central and peripheral sites, respectively. They are able to assess volumetric bone mineral density (vBMD) and geometric properties of cortical and trabecular bone. Although they have yet to be incorporated into clinical practice, the methods are commonly utilized in research settings. These methods have been shown to be able to discriminate between women with osteopenia with and without a fragility fracture, where fractured subjects had lower trabecular density and more heterogeneous trabecular distribution than non-fractured subjects with the same aBMD at the spine and hip<sup>47</sup>. Furthermore, quantitative computed tomography-based finite element modeling of bone microarchitecture properties permits estimation of stiffness and failure load as surrogate measures for bone strength<sup>48</sup>.

Impact microindentation is a technique that utilizes a reference point indentation technique to measure tissue-level material properties of cortical bone<sup>49</sup>. A probe with a spheroconical tip (10 µm radius) is placed on the cortical periosteum in the mid diaphyseal region of the medial tibia. First a preload of 10N is applied, triggering additional 30N force at high speed, Figure 2. Eight to ten indents are performed with two mm distance before the measurements are calibrated against a phantom of poly-methyl methacrylate. Bone material strength index (BMSi) is the outcome variable for impact microindentation and is calculated as 100 times ratio of the indentation distance into the calibration material divided by the indentation distance increase into the bone. A low BMSi value indicates that the probe created a larger cavity, reflecting lower bone material strength.

**Figure 2:** Illustration of the impact microindentation procedure for measuring material properties of bone in vivo (<http://research.activelivescientific.com/how-does-osteoprobe-work/>).



The exact characteristics of cortical bone that determines bone material strength, and thus influences BMSi, are yet to be fully elucidated. However, a number of factors may theoretically affect the resistance of the tissue to impact microindentation <sup>50</sup>:

- The primary collagen fibril orientation relative to the indent direction (axial vs. transverse orientation)
- The crosslinking profile of collagen I (proportion of immature to mature crosslinks as well as the amount and type of glycation-mediated, nonenzymatic crosslinks)
- The relative amount of mineral to matrix (degree of mineralization)
- The number of interlamellar interfaces (potential for sliding as dictated by mineral-collagen interactions).

Studies exploring the relationship between BMSi and bone morphology have shown a positive association between tibial cortical vBMD, a negative association between tibial cortical porosity and total hip aBMD, but no association with cortical thickness or trabecular bone measurements <sup>8,51</sup>.

Clinically BMSi values have been shown to discriminate between subjects with and without fragility and osteoporotic fractures <sup>52,53</sup>, and to be lower in subjects with type T2D and obesity <sup>8-10</sup>. Obesity and T2D are conditions associated with elevated fracture risk out of proportion to aBMD and will be discussed in detail later.

Impact microindentation differs from preceding reference point indentation instruments in that it requires neither a reference probe nor removal of the periosteum that covers the bone. Additionally, it should be highlighted that the foregoing BioDent utilizes cyclic indentation with a lower force (2-10N), over longer time, with a sharp needle. Correlation have been observed between BioDent measurements and traditional mechanical tests (whole bone bending and compression test) <sup>54</sup>. Such comparative studies are yet to be performed for impact microindentation. In a comparative cadaver study only limited, or no, correlation was observed between the outcome measures of the two indentation techniques <sup>51</sup>. The two indentation techniques have been extensively reviewed by Allen et al. in 2015 <sup>55</sup>.

## **1.4 Bone remodeling and bone turnover markers**

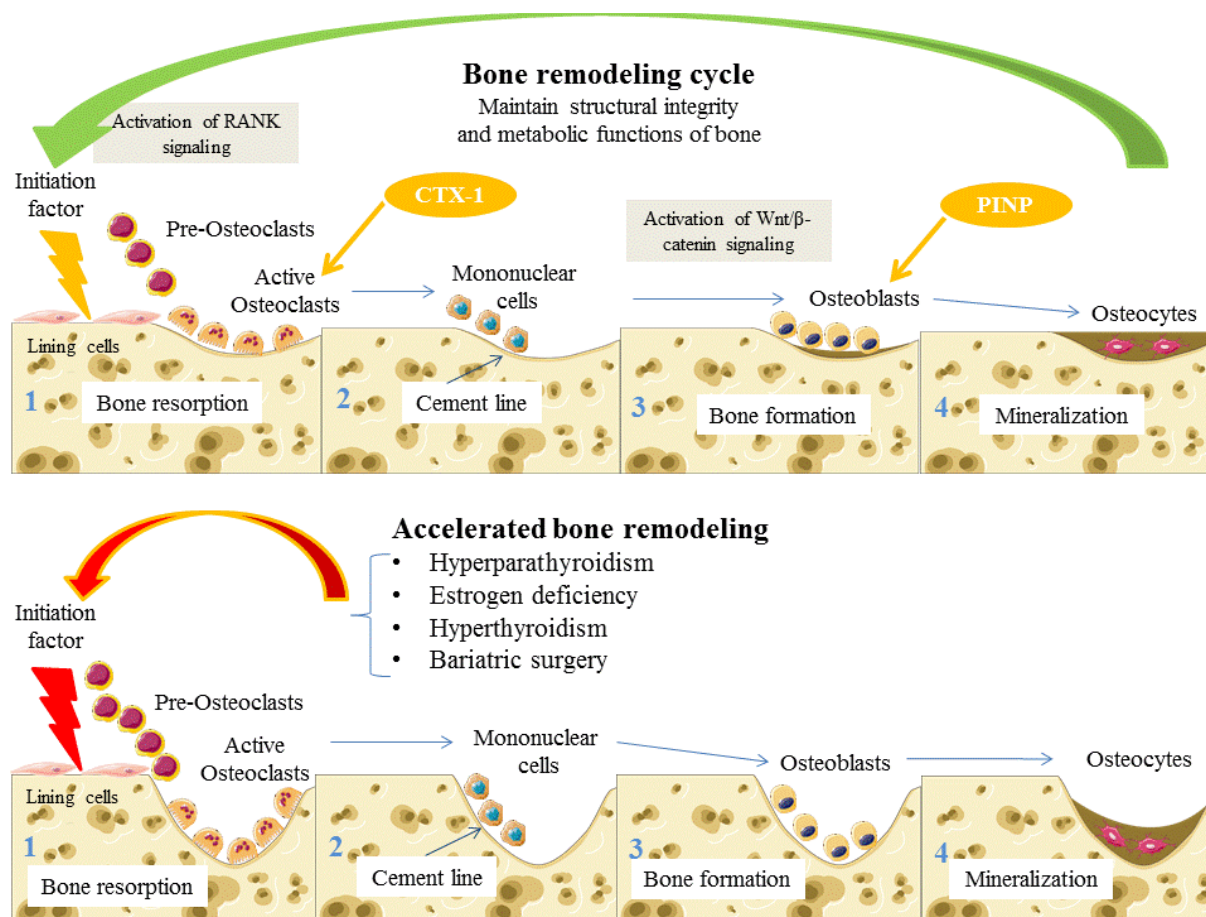
The skeleton is a dynamic organ that is modified through life, so that old bone is continuously replaced with new bone. This process was first described by H. M. Frost, in 1963 and termed bone remodeling <sup>56</sup>. Bone remodeling is characterized by two opposite activities; bone formation and resorption that are coupled at the bone remodeling unit, Figure 3. Osteoclasts are the bone resorptive cells. Through acidification the osteoclast mobilizes the mineralized

components of bone before it enzymatically degrades the organic bone matrix, creating a resorption cavity that is later filled with bone matrix synthesized by osteoblasts. Bone matrix is composed of collagen and non-collagen proteins, where collagen type I is the most predominant. Osteocytes derived from osteoblast differentiation and become embedded in the bone matrix where they are found inside small lacunae. The cytoplasmic processes of osteocytes form an interconnected network, with extensions through the living bone, creating a sensing system ready to respond to local strain and microdamage <sup>57</sup>.

Central to our understanding of bone remodeling are the activating and inhibitory signaling pathways. Osteoclasts are activated through stimulation of the nuclear factor- $\kappa$ B (RANK) ligand system and the macrophage-colony stimulation factor system. RANK ligand is produced by several bone cells, however secretion by osteocytes seems to be the most central formation pathway. RANK ligand binds the RANK receptor on osteoclast precursors to stimulate osteoclastogenesis. Osteoprotegerin plays an important role in regulation of bone resorption through binding to the RANK ligand and preventing binding to RANK receptor <sup>58</sup>. Wnt/ $\beta$ -catenin signaling pathway is central for osteoblastogenesis. In quiescent bone inhibitors of Wnt/ $\beta$ -catenin signaling, sclerostin and dickkopf-1, prevent bone formation <sup>59</sup>.

Bone histomorphometry is the gold standard for assessment of bone remodeling. This is, however, an invasive method that requires specialized personnel and techniques, limiting its use in clinical practice. Advances in laboratory medicine have yielded several specific protein- or peptide markers of bone remodeling, termed bone turnover markers (BTMs). Today several BTMs are widely available including; amino terminal telopeptide, osteocalcin, bone-specific alkaline phosphatase (BALP), procollagen type I N-terminal propeptide (PINP) and Carboxyl terminal telopeptide of type 1 collagen (CTX-1). The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommend that PINP and CTX-1 are used as reference analytes for bone remodeling in clinical studies <sup>60</sup>. Studies have shown that higher BTMs levels are associated with faster bone loss, of both trabecular and cortical bone, in males and females <sup>61</sup> and that BTMs predict fractures <sup>62</sup>, also after adjustment for aBMD <sup>63</sup>.

**Figure 3:** Bone remodeling in physiology and pathophysiology, including the origin of the bone turnover markers.



- i. Bone lining cells are mature osteoblasts that cover the quiescent bone surface. An initiation factor activates the RANK signaling system to initiate osteoclast differentiation from monocytic precursors and subsequent osteoclastic bone resorption. During bone resorption, type I collagen is degraded. One of the bone turnover markers (BTMs), Carboxyl terminal telopeptide of type I collagen (CTX-1), is a serum marker of bone resorption that is derived from this degradation.
- ii. Mononuclear cells remove the unmineralized matrix and create a cement line to enhance osteoblast adherence.
- iii. Osteoblast fills the resorptive cavity with collagen type I rich osteoid matrix. Procollagen type I N-terminal propeptide (PINP) is a serum marker of bone formations reflecting osteoblastic type I collagen production.
- iv. The osteoid matrix is mineralized by hydroxyapatite deposition between the collagen fibrils. In this process some osteoblasts become embedded in the osteoid matrix and undergo terminal differentiation as osteocytes. Through their cytoplasmic processes these osteocytes connect in a cellular network, which constitutes a sensing system linking mechanical stress and microdamage in bone to modeling and remodeling based adaptation throughout the skeleton. Upon sensing changes in load and microdamage osteoclasts produce RANK ligand to initiate the bone remodeling cycle via osteoclast differentiation.

Several circumstances accelerate bone remodeling; hyperparathyroidism, estrogen deficiency and bariatric surgery are examples. In these circumstances the bone resorption exceeds the bone formation leading to bone loss and increased fracture rate.

### 1.4.1 Endocrine regulation of bone remodeling cycle

Several endocrine and paracrine mechanisms are involved in regulation of the bone remodeling cycle. In the next paragraphs endocrine mechanisms significant for the understanding of this thesis are elaborated. These endocrine mechanisms are all associated with the state of accelerated bone remodeling and result in a negative remodeling balance at the bone remodeling unit (Figure 3).

#### Calcitropic hormones

Parathyroid hormone (PTH) is the primary regulator of calcium and phosphate homeostasis. It is secreted in response to decreasing calcium concentrations and exerts its primary effect in bone and kidney. PTH also increases calcium absorption from the gut by stimulating production of active vitamin D (1,25(OH) vitamin D) in the kidney. PTH exerts a dual effect on bone dependent on the mode of secretion. Continuous PTH secretion liberates calcium to the circulation by increasing RANK ligand and inhibiting osteoprotegerin leading to a net stimulation of osteoclastogenesis and bone resorption. Excessive continuous PTH secretion, the hallmark of primary hyperparathyroidism, exerts a catabolic effect on bone, increasing bone turnover, bone loss and fracture risk. Histomorphometric studies, of iliac crest bone biopsies, have indicated that primary hyperparathyroidism accelerates bone turnover in trabecular bone to a larger extent than in the cortical bone<sup>64</sup>. However, the accelerated bone turnover in trabecular bone seems to be more balanced, causing the main catabolic effect of excess PTH being exerted in the cortical bone<sup>65</sup>. Correspondingly, DXA changes observed in hyperparathyroidism have been more pronounced in the distal radius (predominantly cortical bone), rather than in the lumbar spine (predominantly trabecular bone) and hip (combination of trabecular and cortical bone)<sup>66</sup>.

Intermittent PTH secretion, on the other hand, increases Wnt signaling and reduces the expression of sclerostin and Dickkopf-1 (Wnt/ $\beta$ -catenin signaling inhibitors). This causes increased osteoblastogenesis and a net anabolic bone effect, resulting from combined modeling and remodeling bone formation<sup>33</sup>. Despite the detection of vitamin D receptors within several bone cells, a direct effect of vitamin D on bone remodeling is still debated.

Regardless, as serum calcium levels are the most potent stimulator of PTH secretion, vitamin D plays an unquestionable and important indirect role in regulating the bone remodeling cycle<sup>57</sup>.

### **Sex hormones**

Estrogens are essential for bone mass in both males and females. Estrogen increases osteoprotegerin activity and promotes osteoclast apoptosis, thus reducing osteoclast activation and bone resorption. During estrogen deficiency (e.g. in menopause) the lack of this moderator of bone remodeling induces a state of accelerated bone remodeling and negative bone balance at the individual bone remodeling units<sup>58</sup>.

### **Thyroid hormone**

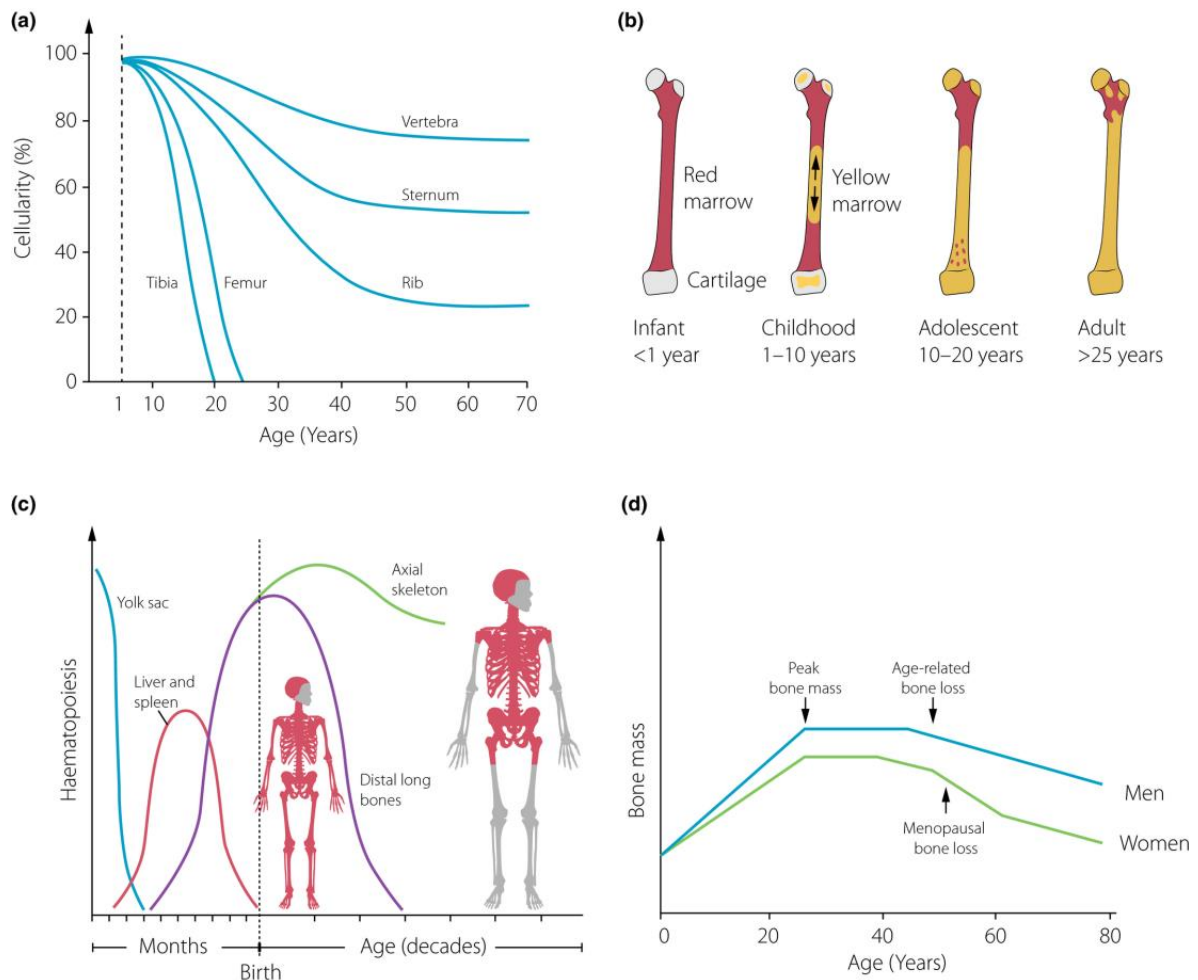
During adulthood, thyroid hormone is important for bone mass maintenance and strength<sup>67</sup>. Untreated hypothyroidism has been associated with prolonged bone remodeling, reduced bone turnover and a net positive effect on bone balance<sup>68</sup>. Thyroid hormone in excess is associated with uncoupling of osteoclast and osteoblast activity and increased bone turnover. Collectively, this can exert a catabolic effect on bone that may lead to secondary osteoporosis<sup>67</sup>.

## **1.5 Bone marrow adipose tissue**

The gradual replacement of the hematopoietic bone marrow with BMAT starts in the appendicular skeleton during childhood and is considered to be a physiological part of bone growth<sup>69</sup>. In young adults the remaining hematopoietic sites remain in the axial skeleton, pelvis and proximal metaphysis of the femur and humerus, Figure 4.



**Figure 4:** Changes in bone marrow haematopoietic cellularity per bone (a), conversion of haematopoietic to adipose marrow in the femur (b), development of haematopoiesis (c) and bone mass (d) during human life <sup>69</sup>. Reprinted with permission from the Copyright Clearance Center’s RightsLink® service.



With age the BMAT fraction at these sites steadily increases <sup>70</sup>. Younger adult males are reported to have more BMAT compared to females. This gender difference seems, however, to disappear after menopause <sup>71,72</sup>.

Osteoblasts and adipocytes both originate from the same mesenchymal precursor cell in the bone marrow <sup>73</sup>. In late adolescence and adulthood BMAT seems closely related to skeletal fragility. Conditions associated with decreased bone mass and increased fracture risk; anorexia nervosa, postmenopausal and idiopathic osteoporosis exhibit increased BMAT fractions <sup>74-76</sup>. A decrease in Wnt-signaling, causing a switch in differentiation of marrow precursor cells towards adipogenesis, is considered central in this process <sup>77</sup>.

BMAT has been considered to be an inactive fat depot, but was recently recognized as an endocrine organ with local and systemic effects <sup>78</sup>. The production of adiponectin <sup>78,79</sup> links BMAT to energy homeostasis <sup>80</sup>. Furthermore, it has been postulated that BMAT plays a role in lipid storage, hematopoietic regulation, bone turnover and thermogenesis <sup>69,81</sup>. However, despite increased research over the last decade the complete function of BMAT remains poorly elucidated.

### **1.5.1 Evaluation of bone marrow adipose tissue**

BMAT may be quantified histologically based on examinations of bone marrow biopsies or by imaging modalities like magnetic resonance spectroscopy (MRS). Acceptable correlations between lumbar spine BMAT, evaluated by MRS, and posterior superior iliac spine BMAT, estimated from bone marrow biopsy, have been published, though MRS is noted to report an approximately 10% higher BMAT fraction <sup>82</sup>.

## **1.6 Obesity**

The World Health Organization defines obesity as abnormal or excessive fat accumulation that presents a risk to health, and subjects with a BMI equal to or higher than 30 kg/m<sup>2</sup> are classified as obese. The prevalence of obesity has nearly tripled over the last 40 years, an increase commonly referred to as the obesity epidemic <sup>83</sup>. In 2016 it was estimated that 13% of the adult population worldwide are obese <sup>84</sup>. Obesity increases the risk of several diseases including cardiovascular disease, T2D, chronic kidney disease, and cancer <sup>85</sup>.

### 1.6.1 Obesity and bone health and quality

Traditionally, obesity is thought to protect bone. Body mass index (BMI) is positively associated with aBMD<sup>4</sup> and greater soft-tissue thickness is believed to reduce fall impact on bone and therefore reduce fracture risk<sup>86</sup>. Increased mechanical loading and strain, stimulating osteocyte mechanoreceptors and increasing Wnt signaling, is believed to be a central mechanism behind the enhanced bone mass in this population<sup>57</sup>. Generally, low bone turnover is observed in subjects with obesity<sup>6</sup>. Aromatization of androgen precursors in fat tissue is a large contributor to extra-gonadal estrogen production. In the lack of ovarian function, e.g. in postmenopausal females, this is the major source of estrogen production. Studies have indicated that obesity protects against postmenopausal bone loss<sup>87,88</sup>. Adipose tissue is an active endocrine organ that secretes adipokines, such as leptin and adiponectin, hormones shown to exert direct effects on bone. In obesity the high levels of leptin and low levels of adiponectin are believed to increase bone formation by stimulation of osteoblasts and inhibition of osteoclasts<sup>89,90</sup>. Gastrointestinal hormones upregulated in obesity such as insulin, amylin and preptin, are also thought to exert similar positive effects on bone<sup>91</sup>.

In recent years, these underlying assumptions of this hypothesis have been questioned. Newer studies on the association between BMI and aBMD have revealed the positive association between lean mass and aBMD to be stronger than that of fat mass with aBMD<sup>92</sup>. Furthermore, the higher aBMD and improved architectural and bone biomechanical properties observed in obesity seem to be out of proportion to the excess body weight<sup>92-94</sup>. Finally, subjects with obesity have been observed to have reduced bone material strength, measured with impact microindentation, when compared to lean controls<sup>8</sup>. Extra adipose fat accumulation may also affect the fat-bone interaction. Positive associations have been described between different fat depots (visceral/subcutaneous/total fat) and BMAT<sup>11,95</sup>. However, studies diverge as to whether subjects with obesity have relatively more BMAT than controls<sup>79,96,97</sup>. High BMAT may contribute to skeletal fragility in obesity. Increased intramuscular fat may translate to poor muscle function<sup>98,99</sup> and contribute to the increased fall risk observed in obesity<sup>93</sup>. The decreased levels of vitamin D and elevated levels of PTH observed in obesity constitute causes of concern for bone health<sup>100,101</sup>. Lastly, excess adipose tissue induces a chronic low-grade inflammation with increased levels of IL-6 and TNF $\alpha$ , known stimulators of osteoclastogenesis, promoting bone resorption<sup>102,103</sup>.

Studies assessing whether fracture risk is increased or decreased in obesity have shown diverging results and the risk of fracture seems to be dependent on fracture site, population examined and obesity measure. The Global Longitudinal Study of Osteoporosis in Women, including over 60 000 women from 10 different countries, revealed comparable fracture incidence between obese and non-obese women, but the fractures occurred at a younger age in the obese subjects <sup>104</sup>. A meta-analysis of cohort studies including close to 400 000 women revealed increased risk of elbow and humerus fractures in subjects with BMI > 30 kg/m<sup>2</sup>, and after adjustment for BMD the risk of all osteoporotic fractures was also increased. On the other hand, they observed that obesity protected against hip fractures <sup>3</sup>. In contrast, studies exploring the relationship between waist-hip ratio and risk of hip fracture have shown a 3% increased risk for hip fracture for every 0.1 unit increase in waist-hip ratio <sup>105</sup>. In orthopedic patients, obesity seems to increase the risk for ankle fractures in addition to humerus fractures <sup>106,107</sup>.

## 1.7 Type 2 diabetes

Diabetes mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose combined with disturbances of carbohydrate-, protein-, and fat metabolism<sup>108</sup>. The prevalence of diabetes is rising; in 1980 4.7% of the adult population worldwide had diabetes, in 2014 this prevalence had increased to 8.5% <sup>109</sup>. There are several forms of diabetes mellitus; of which T2D is the most common. T2D is characterized by a state of relative insulin deficiency due to insulin resistance and insufficient insulin production <sup>108</sup>. Obesity is a leading cause of insulin resistance and T2D <sup>110</sup>. Subjects with T2D have a two to threefold increased risk of heart attacks and strokes and increased risk of neuropathy, retinopathy and kidney failure <sup>109,111</sup>. In 2016 diabetes was estimated to be the direct cause of death for 1.6 million individuals globally <sup>112</sup>.

### 1.7.1 Type 2 diabetes and bone health and quality

T2D is associated with insulin resistance and increased levels of circulating insulin levels. Insulin is thought to exert direct and indirect effects on bone cells. Firstly, insulin stimulates osteoblast growth and thus is viewed as being anabolic for bone<sup>128</sup>. Secondly, hyperinsulinemia promotes sex hormone production in ovaries and inhibits sex hormone binding globulin in the liver, collectively enhancing bioactive sex steroids levels<sup>129</sup>. Lastly, elevated levels of saturated fatty acids are believed to inhibit osteoclastogenesis<sup>91</sup>.

Hyperglycemia is likewise central in T2D pathophysiology, and the accumulation of advanced glycation end-products (AGEs) underlies many well-known diabetic complications. AGEs are a heterogeneous group of compounds that are generated in response to hyperglycemia as a function of time and glucose concentration. Pentosidine is one of the best-studied AGEs. In vitro studies have revealed incubating human bone specimens in a ribosome solution, to mimic diabetes associated hyperglycemia, and increase AGE content and non-enzymatic cross-linking resulting in increased fracture propensity of bone<sup>130</sup>. Serum pentosidine is elevated in subjects with T2D, and has been identified as a risk factor for vertebral fracture independent of BMD, risk factors for osteoporosis, presence of diabetes complications, and renal function<sup>131</sup>. Thus AGE mediated enhanced non-enzymatic cross-linking has been believed to be a mediator of skeletal fragility in diabetic bone. This notion was supported by the finding of elevated bone pentosidine levels in patients with T2D undergoing arthroplasty<sup>132</sup>. This concept was recently questioned by Karim et al. who reported comparable levels of bone (femoral neck biopsies) and serum AGEs in patients with T2D and controls. Furthermore they did not find AGE accumulation to be related to biomechanical properties of femoral neck<sup>120</sup>.

A large number of studies have explored fracture risk in subjects with T2D and the results are often discordant and fracture site-dependent. Table 1 summarizes meta-analyses of fracture risk in subjects with T2D. When available data are viewed together it seems that findings of a 30% increase in hip and upper arm fractures are consistently observed, and possibly also an increase in total fracture rate.

**Table 1:** Meta-analyses of fracture risk in subjects with type 2 diabetes (T2D). Studies indicating significant increased fracture risk compared to controls without T2D are marked in red and non-significant studies in blue. The table is modified from Compston J et al. <sup>113</sup>.

Fracture site	Year	Number of studies	Summary relative risk	95% confidence interval
<b>Hip</b>	2007 <sup>5</sup>	8	1.38	1.25-1.53
	2007 <sup>114</sup>	12	1.7	1.3-2.2
	2016 <sup>115</sup>		1.34	1.19-1.15
	2017 <sup>116</sup>	12	1.30	1.07-1.57
	2017 <sup>117</sup>	15	1.20	1.17-1.23
	2019 <sup>2</sup>		1.27	1.16-1.39
<b>Spine</b>	2007 <sup>5</sup>	3	0.93	0.63-1.37
	2007 <sup>114</sup>		1.2	0.7-1.2
	2017 <sup>116</sup>	7	1.13	0.94-1.37
	2017 <sup>117</sup>	9	1.16	1.05-1.28
	2019 <sup>2</sup>		1.74	0.96-3.16
<b>Upper arm</b>	2007 <sup>114</sup>		1.3	0.8-2.2
	2017 <sup>117</sup>	5	1.09	0.86-1.31
	2019 <sup>2</sup>		1.54	1.19-1.99
<b>Wrist</b>	2007 <sup>5</sup>	3	1.19	1.10-1.41
	2007 <sup>114</sup>		0.98	0.8-1.2
	2017 <sup>117</sup>	10	0.98	0.88-1.07
	2019 <sup>2</sup>		0.97	0.66-1.09
<b>Ankle</b>	2007 <sup>114</sup>		1.13	0.9-2.0
	2017 <sup>117</sup>	3	1.13	0.95-1.32
	2019 <sup>2</sup>		1.15	1.01 -1.31
<b>All fractures</b>	2007 <sup>5</sup>	5	0.96	0.57-1.61
	2007 <sup>114</sup>	7	1.3	1.1-1.51
	2017 <sup>117</sup>	27	1.05	1.04-1.06

In contrast to most other disorders associated with increased fracture rates, bone turnover is decreased and aBMD increased in T2D <sup>5,7</sup>. Decreased bone turnover seems to be a consistent feature of T2D, be it measured with BTMs <sup>7</sup>, level of circulating osteoprogenitor cells <sup>122</sup> or bone histomorphometry <sup>122,123</sup>. Findings of elevated levels of sclerostin and Dickkopf-1, antagonists of the Wnt pathway, have been linked to decreased bone formation reflected in decreased PINP levels <sup>124,125</sup>. The inconsistency between decreased bone formation markers (PINP) and bone resorption markers (CTX-1) and comparable levels of the bone

mineralization marker; BALP has been related to a state of osteoid hypermineralization which may contribute to the high aBMD<sup>126</sup>. Additionally, rodent animal models have revealed decreased enzymatic cross-linking of collagen in diabetic bone<sup>127</sup>. Correspondingly the release of cross-linked teleopeptides during bone resorption would be reduced and thus CTX-1 levels could be underestimating bone resorption activity in diabetic bone.

Microarchitectural studies, utilizing high-resolution peripheral quantitative computed tomography, have revealed ambiguous results. Burghardt et al. were the first to utilize this modality in 19 patients with T2D, and they showed lower cortical density and higher cortical porosity in patients with T2D compared to controls<sup>118</sup>. In the following years several studies of similar size showed conflicting results. However, in recent years two larger studies have been published. A study of the Framingham offspring cohort revealed decreased cortical bone mass and enhanced porosity of the tibia in subjects with T2D, while measurements in the radius were comparable to controls<sup>119</sup>. In a similar sized Swedish population based study, Nilsson et al. revealed higher cortical bone mineral density and lower cortical porosity in both distal radius and tibia in patients with T2D<sup>9</sup>. In addition, evaluations of proximal cortical porosity have revealed comparable or decreased porosity in subjects with T2D<sup>120,121</sup>, in line with a decreased bone turnover. However, further investigations of bone quality have revealed that subjects with T2D have decreased bone material strength<sup>9,10</sup>, in line with increased fracture risk.

### **Type 2 diabetes and bone marrow adipose tissue**

Positive associations have been described between BMAT and glycosylated hemoglobin (HbA<sub>1c</sub>) and homeostasis model assessment of insulin resistance<sup>12,95,133</sup>. However, studies diverge with regard to whether subjects with T2D have relatively more BMAT than controls<sup>12,13,95,97,133</sup>. Elevated BMAT in T2D could be part of the explanation of the increased skeletal fragility.

## Complications and treatment of type 2 diabetes

Poor metabolic control, hypoglycemia, micro and macrovascular and neuropathic complications are associated with increased fracture risk in T2D. This association seems to persist also after adjustment for increased fall risk <sup>134</sup>. Today several treatment options exist for T2D. All improve metabolic control and reduce the risk of complications, however potential effect on bone, bone mineral density and fracture risk differ <sup>135</sup>. In Table 2 potential bone effects of different treatment options in T2D are summarized.

**Table 2** Effect of anti-diabetic treatments on bone, bone mineral density (BMD) and fracture risk in type 2 diabetes. The table is modified from Picke et al<sup>135</sup>.

Agent	Mechanism of action	Effect on BMD	Effect on fractures
Metformin	Cell and animal models suggest osteogenic effect	Neutral or increased	Neutral or decreased
Sulfonylureas	Animal studies indicate stimulation of bone formation. Increase insulin secretion. May induce hypoglycemia	Neutral	Increased
Thiazolidinediones	Promotes mesenchymal differentiation to adipocytes rather than osteoblasts	Decreased	Increased
Insulin	Anabolic effect on bone in rodent models. May induce hypoglycemia	Neutral	Neutral, decreased or increased
Incretins (GLP-1 analogs DPP-4 inhibitors)	Increase bone formation and induce weight loss	Neutral or increased	Neutral or decreased
SGLT-2 inhibitors	Increased tubular phosphate resorption, increasing serum PTH levels and induce weight loss	Neutral	Neutral or increased

Glucagon-like peptide 1 (GLP-1), dipeptidyl peptidase-4 enzyme (DPP-4), sodium glucose co-transporter 2 (SGLT-2)



## 1.8 Morbid obesity

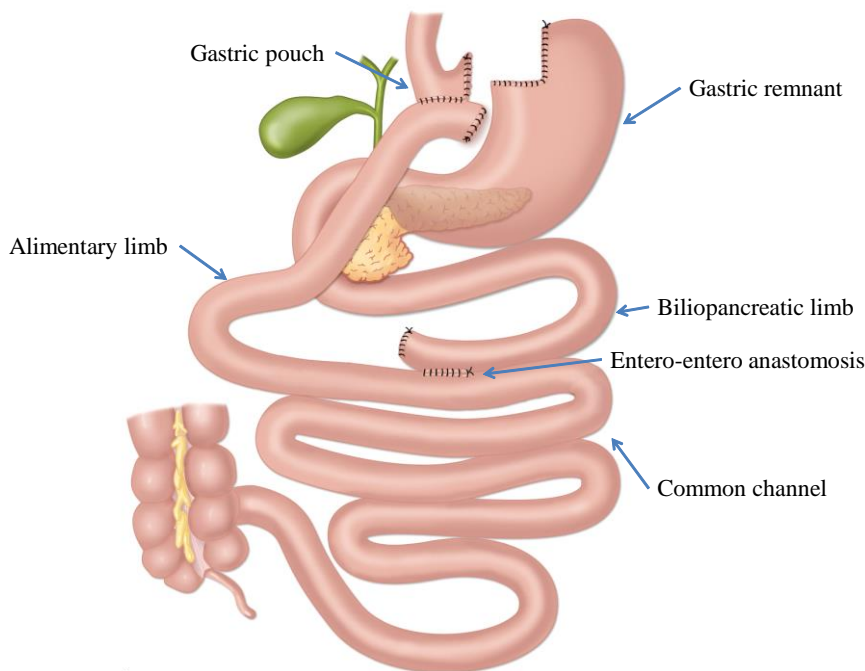
The term morbid obesity was defined by the National Institute of Health (1991) in a consensus report for identifying indications for bariatric surgery. Subjects with a BMI  $\geq 40$  kg/m<sup>2</sup>, or BMI  $\geq 35$  kg/m<sup>2</sup> with one or more obesity related disorders, have morbid obesity <sup>136</sup>. This definition has since then been incorporated into treatment guidelines in several countries, including Norway <sup>137</sup>. Norway has no national figures for obesity prevalence and estimates are based on population based cohort studies. The Norwegian Institute of Public Health estimates that 10-13% of the adult population has a BMI  $\geq 35$  kg/m<sup>2</sup> and that half of them have morbid obesity <sup>138</sup>. Treatment options for morbid obesity include lifestyle intervention, pharmacotherapy and bariatric surgery. Intensive lifestyle intervention generally leads to a weight loss of 5-8 %, in 60-65% of participants <sup>139</sup>. Pharmacotherapy may be prescribed in addition to lifestyle interventions and may facilitate additional 2-8 kg weight loss <sup>139</sup>. A weight loss of 5-10% is associated with several important health benefits <sup>140</sup>. Unfortunately, weight loss achieved by lifestyle interventions with or without pharmacotherapy may be viewed insufficient, and weight regain is a common problem <sup>141</sup>. Comparably, bariatric surgery leads to a larger weight loss and higher remission of obesity related disorders <sup>142</sup>. In recent years metabolic effects of bariatric surgery have gained increasing attention, and Norwegian and international diabetes recommendations currently advice that bariatric surgery can be considered as a treatment option for patients with BMI  $\geq 30$  kg/m<sup>2</sup> and inadequately controlled T2D despite optimal medical treatment <sup>143,144</sup>.

### 1.8.1 Bariatric surgery - Roux-en-Y gastric bypass

RYGB was introduced by Edward Manson in 1967 <sup>145</sup>. The procedure was based on observations of sustained weight loss following partial gastrectomy (Billroth II). Since then the procedure has been modified, with implementation of laparoscopic or minimally invasive approach <sup>146</sup>, Figure 5. In 2016 close to 700 000 bariatric procedures were performed worldwide. For years RYGB was the leading procedure, however, in recent years sleeve gastrectomy has surpassed RYGB as the most commonly performed bariatric procedure globally and in Europe <sup>147</sup>. About 3000 bariatric procedures are currently performed in

Norway annually, of these 57% were sleeve gastrectomies and 43% RYGB <sup>148</sup>. In Norway and Sweden about 55/100 000 inhabitants receive bariatric surgery <sup>149</sup>.

**Figure 5:** Roux-en-Y gastric bypass. A gastric pouch of about 25 ml is created and connected to a 150 cm antecolic alimentary limb. The gastric remnant is connected to the jejunum through a 50 cm biliopancreatic limb at the entero-entero anastomosis <sup>150</sup>. The illustration is printed with permission from Kari C.Toverud ©.



### Health benefits and adverse effects after Roux-en-Y gastric bypass

RYGB enables a substantial and persistent weight loss and resolution of obesity related comorbidities such as T2D, dyslipidemia, hypertension and obstructive sleep apnea. Two years after surgery the total mean weight loss is 35%, decreasing to 27% after 12 years. Remission rates of comorbidities follow the same pattern, with remission rates of T2D decreasing from 75% to 55% <sup>14</sup>. Correspondingly, decreased incidences of myocardial infarction, HR (95%CI), 0.51 (0.36-0.73), stroke 0.41 (0.21-0.79) and cancer 0.76 (0.65-0.89) are observed for patients operated with RYGB compared to obese controls <sup>16,17</sup>. Large improvements in obesity related quality of life and health related quality of life, both in physical and psychosocial components, are seen within the first two years after RYGB. This

effect diminishes somewhat over time, but subjects operated with RYGB still have significantly better quality of life six years after surgery, when compared to obese controls <sup>151</sup>.

All-cause mortality is observed to decrease by 40%, due to a decrease of disease specific mortality; with 92% reduction in diabetes related mortality, 59% reduction in death due to coronary artery disease and 60% reduction in cancer mortality. Notably, the rate of non-disease related deaths (violent deaths or suicide) was increased by 58% <sup>17</sup>.

Data from the Swedish Scandinavian Obesity Registry showed that laparoscopic RYGB is associated with a 8.3% rate of early complications ( $\leq 30$  days after surgery), with serious complications (Clavien Dindo IIIb or higher) occurring in 3.4% of subjects and a 90-day mortality rate of 0.04% <sup>152</sup>. Long-term complications include internal herniation, strictures, gallstone disease, gastric ulcer and abdominal pain. In a Swiss cohort of more than 600 patients 10 years after RYGB 21.3% reported one or more long-term complications, with 14.6% requiring surgical intervention <sup>153</sup>. Following RYGB 67.6% of patients report to have sought medical advice for a symptom related complaint. Of these symptoms abdominal pain (34.2%), fatigue (34.1%), and anemia (27.7%) were the most prevalent <sup>154</sup>. Six years after the procedure patients operated with RYGB were close to three times more likely to be admitted to hospital than controls <sup>155</sup>.

### **1.8.2 Roux-en-Y gastric bypass and bone health and quality**

RYGB alters physiology and thus may induce changes central to bone quality and health. Altered micronutrients, hormones, body weight and composition, T2D status and BMAT, are likely to be central and will be described in the sections below.

## **Micronutrients**

RYGB reduces the functional gastric volume allowing intake of less food volumes per meal and may reduce peptic acid availability for optimized calcium absorption<sup>156</sup>. Calcium absorption occurs mostly in the duodenum and proximal jejunum, which is bypassed after RYGB<sup>146</sup>. A delayed intestinal mixture of bile acids and pancreatic enzymes with the ingested nutrients may affect absorption of fat soluble vitamins such as vitamin D. Patients operated with RYGB are thus advised lifelong calcium and vitamin D supplements<sup>150,157,158</sup>. Despite this, disrupted calcium homeostasis and high prevalence of SHPT have been shown<sup>30,159</sup>. Observational studies have reported SHPT prevalences of 34%, 40% and 51% at two, five and six years after RYGB, respectively<sup>30-32</sup>, indicating an increase over time. Higher PTH levels have been associated with lower aBMD in lumbar spine five years after RYGB<sup>160</sup>.

## **Hormones**

Mechanisms by which the adipose tissue and gut communicate with bone have been revealed over the last decade, and thus changes in these hormones may further explain the changes in bone after RYGB surgery. Of the adipokines the decrease in leptin and increase in adiponectin are thought to contribute to the postoperative bone loss<sup>21,161</sup>. The gastrointestinal hormones Glucose-dependent insulintropic polypeptide (GIP) and Ghrelin exert a stimulatory effect on bone formation<sup>128,162</sup>. Following RYGB surgery they both decrease possibly causing reduced bone formation<sup>163,164</sup>. Insulin is believed to be anabolic for bone and is reduced after RYGB<sup>165</sup>. No effect between decreased fasting insulin levels after RYGB and aBMD has, however, been demonstrated<sup>25</sup>. Adipose tissue is the primary source of estrogen in postmenopausal women. RYGB surgery leads to depletion of fat stores leading to reduced impact of estrogen on bone, and may explain why more severe bone deterioration after RYGB surgery is observed in postmenopausal women compared to premenopausal women<sup>22</sup>.

On the other hand exogenous administration of glucagon-like peptide 1 (GLP-1) has shown to improve bone mineral density<sup>166</sup>. Thus the GLP-1 increase seen after RYGB surgery<sup>165</sup> may be hypothesized to exert a positive effect on bone health. Likely the increase in testosterone

observed in males following RYGB may mediate positive effects <sup>167</sup>. The decrease in Dickkopf-1 observed three to nine months following RYGB is in theory positive for bone formation, however no direct association with aBMD has been observed <sup>168</sup>.

## **Body weight and composition**

The drastic weight loss after RYGB leads to skeletal unloading. Several studies have noted an association between weight loss the first year after RYGB and decline in aBMD <sup>23,26,169</sup>. This effect is thought to be mediated through reduced stimulation of osteocyte mechanoreceptors and increase in the load-responsive hormone sclerostin <sup>168</sup>. Muscle mass is known to be anabolic for bone. In line with this notion, the observed decrease in lean mass has been shown to be the most important change in body composition associated with decreased bone mass <sup>25,170</sup>. Intervention studies have shown that exercise programs after RYGB ameliorates the decrease in lean and bone mass <sup>171,172</sup>. However, mechanical unloading cannot account for the observed decrease in bone mineral density of non-weight bearing bones like the radius <sup>28</sup>. The observed continued bone loss following weight stabilization - indicates effects of RYGB on bone beyond adaptation to a reduced weight <sup>25,28,29,160,170</sup>. This notion is further supported by the lack of association between degree of weight loss and fracture rate after RYGB <sup>18</sup>.

Despite a decrease in lean mass the relative reduction in fat mass is the most pronounced body composition change after RYGB <sup>21</sup>. Decreased intramuscular fat may be the explanation for the improved physical function observed after RYGB <sup>173</sup>, and contribute to decreased fall propensity. Reduction in the metabolically active visceral adipose tissue may directly and indirectly be beneficial for bone health, and a concomitant reversal of the pro-inflammatory state of obesity after RYGB <sup>174</sup> is also potentially beneficial for bone health.

## **Remission of type 2 diabetes**

High remission rates of T2D are likely to decrease glucose toxicity on bones additionally decreasing risk of vascular and neurological complications possibly exerting a negative effect on bones.

## **Bone marrow adipose tissue**

Studies indicate that a diet-induced weight loss is accompanied by a 1.1-3.5% reduction in MRI/MRS estimated BMAT<sup>175,176</sup>. Unexpectedly, studies evaluating BMAT with MRS six to twelve months after RYGB did not note any change<sup>26,27</sup>. However, one of the studies reported that the subpopulation of subjects with preoperative T2D experienced a 6.5% decline in BMAT<sup>27</sup>. Histological quantification of BMAT is yet to be performed morbidly obese subjects and after RYGB.

### **1.8.3 Bone and bone turnover changes after Roux-en-Y gastric bypass**

In 2004 Coates et al. noted that in 15 patients nine months after RYGB urinary N-telopeptide cross-linked collagen type 1 was increased by more than 300% and aBMD at the lumbar spine (L1-L4), femoral neck, total hip and total body had decreased with 3.3%, 9.3%, 7.8% and 1.6%, respectively. No significant changes in aBMD of the distal 1/3 of the radius were observed and serum vitamin D and PTH remained stable<sup>20</sup>. Subsequent studies with one year follow-up have generally yielded consistent findings<sup>21-24</sup>, but some studies show non-significant changes<sup>22,23</sup> and one study reported a greater decline in lumbar spine aBMD<sup>21</sup>.

Quantitative computed tomography measurements of vBMD have revealed comparable or higher reductions at the lumbar spine (3.4-8.1% decrease)<sup>22,24,28</sup>, but in contradiction to the DXA findings significant changes in femoral neck and total hip vBMD have not been observed<sup>24,28</sup> one year after RYGB. Trabecular vBMD, however, decreased at all sites (lumbar spine, femoral neck and total hip)<sup>24</sup>.

HR-pQCT derived measurements of the radius and tibia have enabled evaluation of microarchitecture of peripheral skeletal sites. One year after RYGB Shanbhogue et al. revealed site specific changes of total and cortical vBMD, where a decrease was observed in the tibia but not in radius. Cortical porosity, trabecular vBMD and estimated failure load was unchanged one year after surgery at both sites<sup>25</sup>. In a second study including older patients with higher ratio of females, however, decreased vBMD, estimated failure load and stiffness, and increase in cortical porosity were observed in both radius and tibia<sup>22</sup>. In-vivo bone material properties remain to be explored after RYGB.

RYGB induces a state of high bone turnover. One year increases in serum CTX-1 and PINP of 159-278% and 82-117%, respectively, have been noted<sup>22,24,26</sup>. The rate of postoperative BTM increase have been shown to predict several long-term bone related parameters including; decrease in lumbar spine and hip aBMD, cortical and trabecular vBMD, increase in cortical porosity and estimated failure load<sup>25,28</sup>. Furthermore BTMs remain elevated long after weight stabilization 12 to 18 months following RYGB<sup>29</sup>. In line with the high turnover state increased fracture rates have been reported both when compared to controls with morbid obesity and after restrictive bariatric surgery<sup>18,19</sup>.

Bone mass and microarchitecture seem to continue to deteriorate, also after body weight loss has stagnated; with lumbar spine and total hip aBMD decreases of 1.5-2.2% and 2.1-2.6%, respectively<sup>25,28</sup>. Moreover, accelerated decreases in total, trabecular and cortical vBMD and trabecular number at radius and tibia, increase in cortical porosity and trabecular heterogeneity have been observed. Corresponding to a decrease in estimated failure load<sup>25,28</sup>.

Lindeman et al. studied change in aBMD over time. They noted that spine and hip aBMD steadily decreased through the five-year observation after RYGB, however the rate of decrease declined over time. At peripheral sites, cortical and trabecular microarchitecture also decreased, however the changes were relatively less in tibia as compared to radius in the later

years. Five years after RYGB the estimated failure load was reduced by 20% and 13% in the radius and tibia, respectively<sup>29</sup>. Correspondingly, a study of subjects with preoperative T2D also showed larger vBMD and microarchitectural defects in radius compared to tibia six years after RYGB. Notably, 51% of the subjects six years after RYGB had SHPT<sup>32</sup>.

Taken together, current research indicates that RYGB affects bone differently at early compared to later stages; early changes predominate in weight-bearing bones and long-term changes seems to have a larger effect on non-weight bearing bones. Unfortunately studies describing bone health beyond six years are limited, characterized by small samples of females only, and with low or undefined rates of follow-up<sup>177,178</sup>.

### **Recommended bone specific follow-up after Roux-en-Y gastric bypass**

Effects of RYGB on the skeleton are widely recognized and preventive measures are incorporated into guidelines. In the Obesity Task force of the European Association for the Study of obesity for the Post-Bariatric Surgery Medical management recommendations from 2017 regular consumption of elemental calcium (1200-2000 mg/day) and vitamin D (400-800 U) are recommended. After surgery, regular follow-up of bone mineral metabolism is advised and supplementation is considered adequate when levels of serum calcium, bone specific alkaline phosphatase or osteocalcin, vitamin D, PTH and 24-hours urinary calcium excretion rates are within normal range. DXA scans are recommended before surgery and bi-annually thereafter<sup>157</sup>. The Norwegian and Nordic recommendations, published in 2011 and 2018, advice similar supplement regimen, however modifications of supplement doses are recommended according to serum measurements, and DXA scans are not included in the recommendations<sup>150,158</sup>. Correspondingly, it is our clinical experience that DXA scans, BTMs, and 24h-urine collection are not a part of routine follow-up regimens after RYGB in Norway. In post-bariatric patients with established osteoporosis European guidelines recommend to consider pharmacologic treatment with bisphosphonates<sup>157</sup>.



## 2 Aims

### 2.1 General aim

To increase the understanding of bone health and quality in morbidly obese subjects, and the impact of T2D and RYGB.

### 2.2 The specific project aims

#### **4B study**

To explore potential factors associated with bone material strength and BMAT fraction in morbid obesity (Paper I and Paper II)

To explore potential changes in factors influencing bone health and quality (bone material strength, aBMD, BMAT fraction, BTMs and calciotropic hormones) after RYGB (Paper I and II).

To explore whether changes in factors influencing bone health and quality after RYGB, differed between participants with and without T2D (Paper I and II).

To explore potential factors associated with changes in bone material strength, aBMD and BMAT fraction one year after RYGB (Paper I and II).

#### **10 year follow-up study**

To estimate the prevalence of aBMD below the expected range for age and the prevalence's of osteopenia, osteoporosis and low-energy fractures 10 years after RYGB (Paper III).

To describe aBMD, BTMs and calciotropic hormones 10 years after RYGB (Paper III)

To explore potential factors associated with increased risk of aBMD z-score or t-score of -1.1 or lower 10 years after RYGB (Paper III).

# 3 Hypotheses

## **Paper I**

Bone material strength and aBMD will deteriorate and bone turnover increase one year after RYGB, and collectively pose negative effects on bone quality.

## **Paper II**

The BMAT fraction will decrease after RYGB and participants with T2D will have a larger decrease in BMAT fraction than participants without T2D.

## **Paper III**

10 years after RYGB SHPT is associated with an increased risk of z-score or t-score of  $-1.1$  or lower.

# 4 Methods

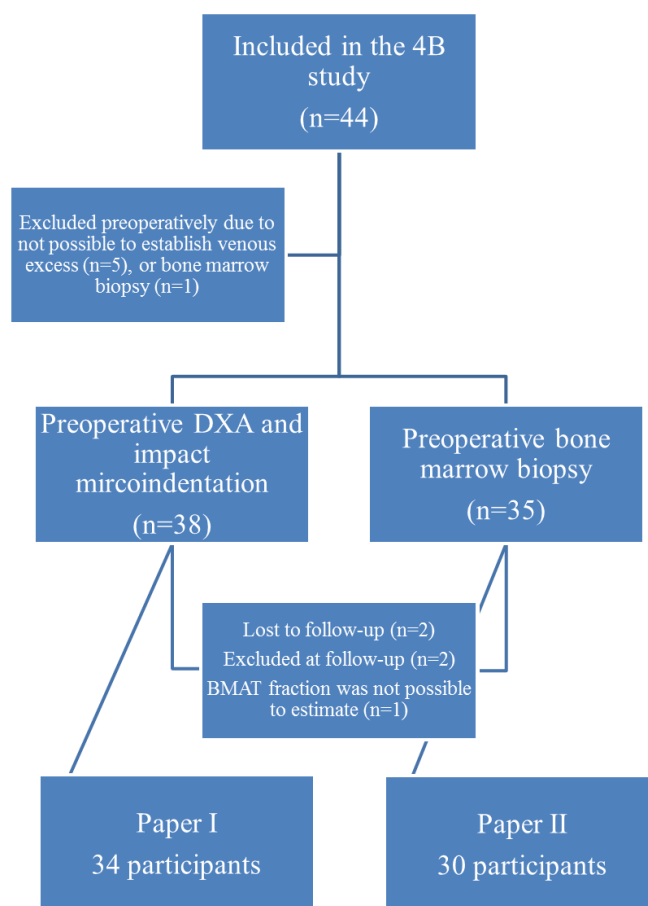
## 4.1 Trial design, participants and settings

This thesis is based on two cohort studies conducted at the Department of Endocrinology, Morbid obesity and Preventive Medicine at Oslo University Hospital. All participants were operated and assessed preoperatively at the Section for Morbid Obesity and Bariatric Surgery. The centre was established in 2004 and annually operates 250-300 patients for morbid obesity. Patients are referred to the centre from the specialist health services following failed attempts of sustained weight loss by non-surgical measures. All study examinations were performed at the Diabetes and Metabolic Research laboratory and at the Endocrinology outpatient clinic.

### Paper I and II

The 4B study (**B**one, **B**ariatric surgery, **B**one marrow adipose tissue, **B**lood glucose) included patients with morbid obesity scheduled for RYGB. Patients with T2D were encouraged to participate. Participants were excluded if they were unable to read Norwegian language or if they had severe psychiatric comorbidity, connective tissue disorders or other hormonal diseases, kidney failure (glomerular filtration rate  $< 30 \text{ mL/min/1.73m}^2$ ), type 1 diabetes, BMI  $> 47 \text{ kg/m}^2$ , history of treatment with bone active substances (bisphosphonates, denosumab, hormone replacement or PTH), or if they were currently receiving anticoagulation or steroid treatment (estrogen, testosterone or glucocorticoids), Figure 6. To avoid heterogeneity in our study population non-Caucasians were excluded.

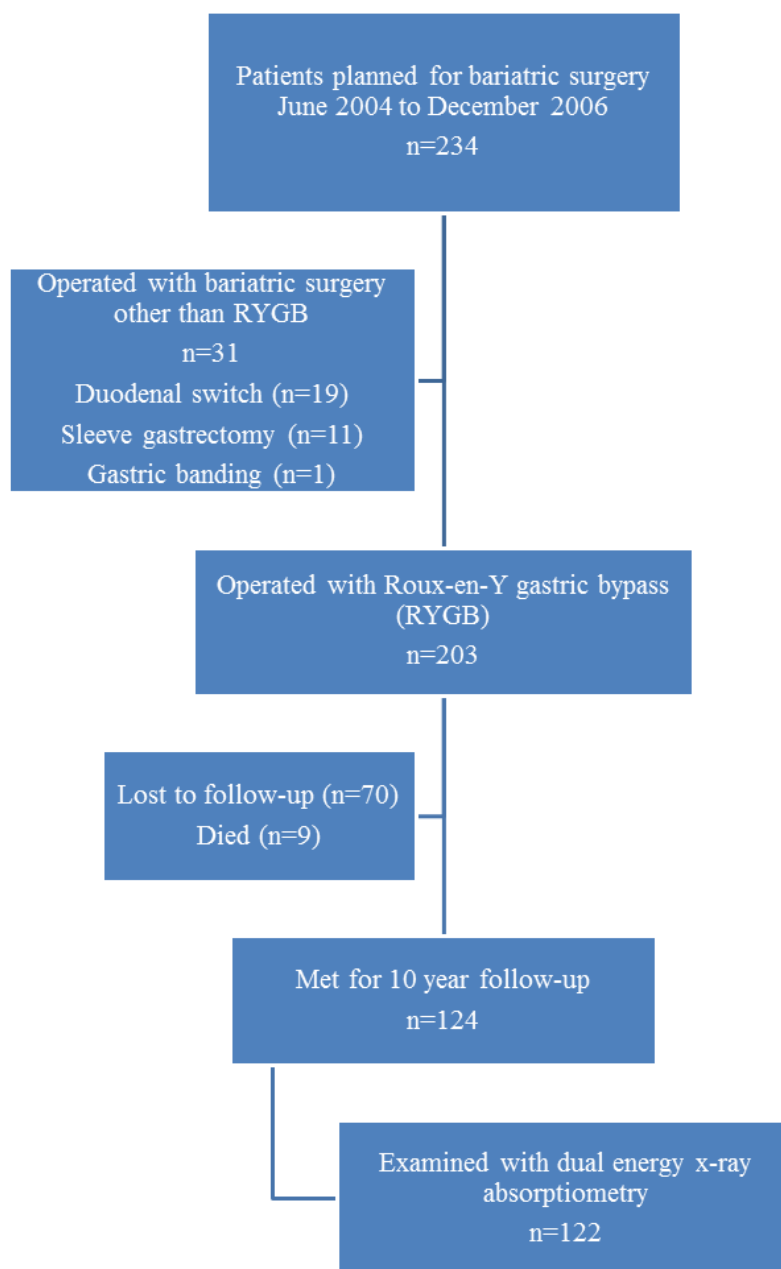
**Figure 6:** Flowchart describing the 4B study inclusion and follow-up.



### Paper III

The 10 year follow-up study is based on a cohort of patients with morbid obesity operated with RYGB from June 2004 to December 2006. Nine out of 203 patients died prior to the 10 year follow-up, thus 194 were eligible for study inclusion. Of these 124 met for 10 year follow-up and 122 (63%) were examined with DXA and are presented in this study, Figure 7.

**Figure 7:** Flowchart describing 10 year follow-up study inclusion and follow-up.



#### **4.1.1 Surgical intervention**

Prior to surgery all patients had one or more individual consultations with a nurse, a dietician and a surgeon and if indicated consultations with an internist or a psychologist. For the last three weeks before surgery the patients were advised a mandatory low-caloric diet (1000 kcal

daily). All patients underwent a laparoscopic RYGB creating a gastric pouch of about 25 ml, a 150 cm antecolic alimentary limb and a 50 cm biliopancreatic limb <sup>146</sup>.

#### **4.1.2 Supplementation and follow-up**

Postoperative routine clinical follow-up visits were conducted six-eight weeks, six months, one, two and five years after surgery. After surgery all patients were prescribed oral nutritional supplementation consisting of daily doses of calcium carbonate (500mg x two daily), vitamin D3 (400IU x two daily), Nycoplus multivitamin – Nycomed® (one tablet), iron (100-200 mg) as well as vitamin B12 injections (1mg) every three months. At routine clinical visits, vitamin levels were monitored and additional supplements were recommended if appropriate.

#### **4.1.3 Study visits**

##### **Paper I and Paper II**

In the 4B study the study visits were performed preoperatively and one year after RYGB and included morning fasting blood samples, anthropometric measures, intravenous glucose tolerance test, euglycemic hyperinsulinemic clamp, indirect calorimetry, DXA scan, impact microindentation, and bone marrow biopsy. Data concerning comorbidity, medications, nutritional supplements, menstrual status and fracture history were recorded in a predefined case report form. Preoperative study visits were performed prior to initiation of the low-calorie diet. In this thesis the results of the blood samples, anthropometric measures, DXA, impact microindentation, and bone marrow biopsy are presented.

##### **Paper III**

The 10 year follow-up study is based on the 10 year follow-up visit that included morning fasting blood samples, clinical examination, anthropometric measures and DXA scan. Data of

comorbidities, medications, nutritional supplements, menstrual status and fracture history were recorded in a predefined case report form. All 10 year follow-up visits, but four, were performed by one clinician (Stephen Hewitt, Internist). Preoperative data were registered in a database from predefined forms.

## **4.2 Dual energy x-ray absorptiometry**

DXA scans were performed for assessment of lumbar spine (L<sub>1</sub>-L<sub>4</sub>), hip, proximal femur and total body aBMD, and total body composition (including percent body fat). Lateral X-rays were recorded for vertebral fracture assessment. All DXA scans were performed by the same nurse. GE Lunar Prodigy was used until August 26<sup>th</sup> 2016 when it was replaced by GE Lunar iDXA (Lunar Corporation, Madison, WI, USA). Body composition performed with GE Lunar Prodigy was re-analyzed with iDXA software to optimize comparability. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine (L1-L4) intra-class correlation coefficient (ICC) (95% CI) of 0.989 (0.968 to 0.996), and ICC (95% CI) for femoral neck was 0.994 (0.982 to 0.998) and 0.996 (0.988 to 0.999) for total hip. ICC values of 0.75 or higher were considered excellent<sup>179</sup>. Furthermore, studies have shown excellent in vivo precision of the GE Lunar iDXA for the measurement of lumbar spine, hip and total body aBMD in normal weight and obese adults<sup>180,181</sup>. The DXA machine was calibrated daily against the standard calibration phantom, supplied by the manufacturer, with estimated short-term precision errors of < 1.0 % for aBMD at the lumbar spine and at the femoral neck.

## **4.3 Bone marrow biopsies**

Preoperative and follow-up bone marrow biopsies were taken from the right posterior superior iliac spine (except for in one participant where both biopsies were taken from the left) after injection of local anaesthesia. The posterior superior iliac spine was identified by palpation. Bone marrow biopsies were obtained using an 8G T-Lok™ Jamshidi crista biopsy needle from Argon Medical Devices (Stenløse, Denmark), fixed in 70% ethanol directly, and stored at 4°C. For histological analysis the biopsies were embedded undecalcified in

methylmetacrylate<sup>182</sup>. After embedding 7 µm sections were cut using a Jung microtome model K (R. Jung GmbH, Heidelberg, Germany) equipped with a tungsten knife. To achieve a largest possible area the biopsies were cut through the middle. Then two levels were cut with a distance of 100 µm. These sections were stained with Masson Goldner Trichrome. BMAT fraction was quantified as adipocyte volume (AdV) relative to marrow volume (MarV) using grid based point-counting. Grid size were 0,03 mm<sup>2</sup> and 0,06 mm<sup>2</sup>, where the smaller grid was used for lower BMAT fractions and the larger for higher BMAT fractions. We used a light microscope (Nikon Eclipse 80I, Tokyo, Japan) equipped with a motorized specimen stage (Prior Proscan 11 TM, Rockland, MA, USA), and a digital video camera (Olympus DP72, Tokyo, Japan) connected to a PC running the NewCast interactive stereology software (Visiopharm, Hørsholm, Denmark). The estimates were performed at x 230 magnification. The presented BMAT fraction is the mean of the estimated AdV/MarV from two levels of the biopsy. Biopsies obtained preoperatively and one year after RYGB were processed and analysed in batches by one lab technician blinded for all clinical data. Coefficient of variation was calculated by recounting five randomly selected biopsies and the mean value was 2.8% and 3.3% preoperatively and one year after RYGB, respectively.

## 4.4 Impact microindentation

Tissue level bone material strength of cortical bone was assessed by impact microindentation using a commercially handheld device (OsteoProbe<sup>®</sup>, Active Life Scientific, Santa Barbara, California). The impact microindentation was performed on the anterior surface of the mid-shaft of the right tibia (with the exception of one participant where the examinations were performed on the left) 10 cm below the inferior margin of the patella after injection of local anesthetics. To avoid overlying skin and subcutaneous tissue opposing the measurement, an insertion channel was made with a sharp needle (BD Microlance<sup>™</sup> 3, 21G) prior to indenting the cortical bone surface. To ensure sufficient width of the insertion channel the needle was moved in circular movements expanding the insertion channel. The first eight indentations were made in vivo, with two mm separating two measurements. Subsequently, five indentations against a phantom of poly-methyl methacrylate were performed for calibration of participant measurements. Indentations with obvious operator errors were removed. The output for OsteoProbe<sup>®</sup> is BMSi, which is a normalized measure of indentation depth<sup>50</sup>. To



minimize inter-observer variations, all measurements were made by the same investigator (I.K.B.H).

## **4.5 Blood samples**

Blood samples were taken before 10 am after an overnight fast. In the 4B study (Paper I and Paper II) serum for BTMs was centrifuged and stored at -80°C and analyzed after study follow-up was completed to avoid inter-assay variation. All other study blood sample analyses were made shortly after retrieval. Analyses were performed at the Hormone Laboratory and the Central Laboratory of Oslo University Hospital.

## **4.6 Clinical outcomes**

### **4.6.1 Osteopenia, osteoporosis and aBMD below expected range for age**

In paper I and III t-scores and z-scores of the lumbar spine L1-L4 were calculated after exclusion of vertebrae with osteoarthritic changes (spondylosis) or compression fractures, in Paper II the lumbar vertebra with the lowest aBMD was used for this analysis. Areal BMD t-scores represent the number of standard deviations an actual aBMD deviates from the peak bone mass of young women. The aBMD z-score represents the number of standard deviations an actual BMD deviates from the expected aBMD of age, gender and ethnicity. Both scores are based on the reference population from the NHANES and Lunar studies given by the manufacturer. In paper III the percent estimates of aBMD z-score were referred to as percent of expected aBMD.

In order to explore aBMD outcomes in paper III the population was split according to gender, age and menopausal status:

### **Premenopausal females and males 49 years or younger**

- i. aBMD in the higher range of normal: 1.0 standard deviations lower than the expected aBMD of age, gender and ethnicity or higher (z-score  $> -1.0$ )
- ii. aBMD in the lower range of normal: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score  $< -1.0$  to  $> -2.0$ )
- iii. aBMD below expected range for age: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score  $\leq -2.0$ )<sup>183</sup>.

### **Postmenopausal females and males 50 years or older**<sup>37</sup>

- i. Normal aBMD: 1.0 standard deviations lower than the peak bone mass of young women or higher (t-score  $> -1.0$ )
- ii. Osteopenia: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score  $< -1.0$  to  $> -2.5$ )
- iii. Osteoporosis: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score  $\leq -2.5$ )

## **4.6.2 Fractures**

Vertebral fractures were assessed on lateral X-rays recorded on the DXA scanner and by visual semiquantitative technique (VFA). Moderate (reduction of vertebral height of  $> 25$ - $40\%$ ) and severe (reduction of vertebral height of  $> 40\%$ ) vertebral fractures are reported<sup>184</sup>. Vertebral fractures revealed by DXA are referred to as morphometric vertebral fractures.

Clinical fractures were self-reported and registered in predefined case report forms. A low energy fracture was defined as a fracture resulting from minimal trauma quantified as forces equivalent to a fall from standing height or less<sup>37</sup>. In paper I and II all fractures except digital fractures were reported and in paper III all low energy fractures, except digital fractures, acquired during the 10 years after RYGB were reported.

### 4.6.3 Calcitropic hormones and supplements

Vitamin D deficiency was defined as serum 25(OH) vitamin D levels below 50 nmol/L and insufficiency as levels between 50 and 74 nmol/L<sup>185,186</sup>. Seasons were defined as; Summer: June to August, fall: September to November, winter: December to February and spring: March to May. SHPT was defined as a serum concentration of PTH above 7.0 pmol/L in the absence of serum ionized calcium above 1.33 mmol/L. Intake of vitamin supplements was self-reported. Patients were classified as taking calcium and vitamin D supplements if they reported intake of 1000 mg and 800 IU, respectively, or more, at least five days a week.

### 4.6.4 Menopausal status

Hormonal intrauterine devices made clinical evaluation of menstrual cycle challenging. For this reason a postmenopausal status was defined as a serum follicle stimulating hormone (FSH)  $\geq 25$  IU/L<sup>187</sup>.

### 4.6.5 Comorbidities

- Morbid obesity was defined as BMI  $\geq 40$  kg/m<sup>2</sup> or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity-related co-morbidity<sup>136</sup>.
- T2D was defined as HbA<sub>1c</sub>  $\geq 6.5\%$  and/or the use of one or more oral glucose lowering drugs with or without the use of insulin. Diabetes remission was defined as HbA<sub>1c</sub>  $< 6.5\%$  without the use of glucose lowering drugs in participant with T2D preoperatively.
- Hypercholesterolemia was defined as low density lipoprotein cholesterol  $\geq 3$  mmol/L or use of statins.
- Preoperative hypothyroidism was based on information given by the participant preoperatively, stated in referrals or based on use of levothyroxine substitution treatment.

## **4.7 Power calculation and statistical analysis**

### **4.7.1 Power calculations**

#### **Paper I and paper II**

The 4B study was an explorative study. It was the first to use impact microindentation and to evaluate BMAT fraction in bone marrow biopsy in a population with morbid obesity and after bariatric surgery. However, a sample size estimation was performed using BMAT as the primary endpoint based on data from a previous study evaluating change in BMAT following teraparatide treatment <sup>188</sup>. Given a mean change in BMAT of 5.5% between baseline and follow-up with an estimated standard deviation of 9.0%, type I error of 5% and power of 90%, a total of 31 participants should be included. An additional 10% was added to account for possible technical difficulties with bone marrow biopsies and participants lost to follow-up.

#### **Paper III**

The 10 year follow-up study is a descriptive study where we aimed to describe factors associated with bone health in a predefined cohort and no power calculation was performed.

### **4.7.2 Statistical analysis**

The statistical evaluations were performed in collaboration with Oslo Centre for Biostatistics and Epidemiology, Research Support Services at Oslo University Hospital. All statistical analyses were made using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY: IBM Corp). Two tailed p-values < 0.05 were considered statistically significant. Participant characteristics were presented as mean values  $\pm$  standard deviation, median (range), or as proportions (percentage). When comparing characteristics of subgroups independent sample t-test or Mann-Whitney U test were used for continuous variables, and Pearson Chi-square or Fisher's exact test for categorical variables as appropriate. When exploring changes between preoperative and one year after RYGB, paired-sample t-tests or

Wilcoxon signed-rank test were used for continuous variables and McNemar's test for paired proportions for categorical variables.

In Paper I and Paper II correlations were assessed with Pearson ( $r$ ) or Spearman ( $r_s$ ) correlation coefficients as appropriate. Adjustments for confounding factors were performed using multiple linear regression analyses. Only variables with significant relationships with both the exposure and the outcome variables were considered as possible confounders in addition to variables of known clinical importance. Confounders that correlated,  $r > 0.7$ , were not adjusted for in order to avoid multicollinearity. The results from the linear regression analyses are presented as regression coefficients ( $\beta$ ) with 95% confidence intervals (CI). In linear regression analysis all continuous variables were checked for deviation from normality, non-linear effects, multicollinearity, and homoscedasticity.

In Paper III logistic regression analyses were performed to identify factors associated with aBMD z-score or t-score of -1.1 or lower 10 years after RYGB. Variables associated with  $p < 0.25$  from the univariable analysis were entered into a multivariable logistic regression model using a manual backward stepwise elimination procedure. Multivariable analyses were preceded by estimation of correlation between risk factors. To avoid multicollinearity predictors that correlated,  $r > 0.7$ , were not included in the model. The associations between factors and aBMD z-score or t-score of -1.1 or lower 10 years after RYGB at 10 year follow-up visit were quantified by calculating odds ratios (OR) with 95% CI. For logistic regression the analysis were checked for deviation of linearity of the logit and multicollinearity. Evaluation of the models' predictive accuracy was assessed by calibration (Hosmer and Lemeshow goodness-of-fit test) and by the discriminatory capability (area under the ROC curve).

## **4.8 Ethics and funding**

The studies were conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics North Norway; 2015/604 and 2015/142. Written informed consent was obtained from all included participants.

Preoperative data were registered in a database licensed by The Norwegian Data Inspectorate (Paper III). Funding was obtained from the South Eastern Norway Regional Health Authority, project nr. 2014073.

# 5 Main results – summary of papers

## 5.1 Paper I

*This paper reports factors associated bone material strength in subjects with morbid obesity and changes in factors associated with bone quality (aBMD, BMSi, serum markers of bone turnover and calcitropic hormones) one year after RYGB.*

In subjects with morbid obesity BMSi was inversely associated with BMI ( $\beta$  -1.1 (-1.9 to -0.28),  $p$  0.010). No association was noted between BMSi and aBMD and participants with and without T2D had comparable levels of factors associated with bone quality. After RYGB the participants had lost a mean of 33.9 kg  $\pm$ 10.9 kg, had increased physical activity and all but one of the 13 participants with T2D were in diabetes remission. One year after RYGB BMSi increased by 6%. aBMD decreased by four to 12%, BTMs (CTX-1, PINP and osteocalcin) increased by 52 to 195%, and PTH levels also increased,  $p < 0.05$  for all. However, 25(OH) vitamin D and calcium levels remained unchanged. Higher age was associated with a greater decrease of the lumbar spine aBMD  $\beta$  -0.003 (-0.005 to -0.001),  $p$  0.002, and this remained significant when adjusting for gender and preoperative aBMD. After adjustments for relevant confounders participants with and without preoperative T2D had comparable changes in factors associated with bone quality. However, in participants with preoperative T2D a larger decrease in HbA<sub>1c</sub> was associated with a larger increase in BMSi  $\beta$  -9.2 (-16.5 to -1.9),  $p$  0.019 and this association remained significant after adjusting for change in BMI and age. We observed no association between age, gender and menopausal status and change in BMSi.

## 5.2 Paper II

*This paper reports change in BMAT fraction one year after RYGB and explores factors associated with BMAT fraction in morbidly obese subjects and with change in BMAT fraction after RYGB. Specifically, we investigated whether changes in BMAT fraction differed in participants with and without T2D*

One year after RYGB BMAT fraction decreased from  $40.4 \pm 1.7\%$  at baseline to  $35.6 \pm 12.8\%$  at follow-up,  $p=0.042$ , or with mean decrease of 10.7% of preoperative BMAT fraction. After adjustments for relevant covariates preoperative BMAT fraction was positively associated with HbA<sub>1c</sub> and negatively associated with lumbar spine aBMD. Females and participants who lost more BMI units or decreased more in total body fat mass decreased more in BMAT fraction after RYGB. Participants with lower preoperative BMAT fraction had a smaller reduction of BMAT fraction. These associations remained significant after adjustments for relevant covariates. In males we noted an association between changes in serum estradiol levels and change in BMAT fraction. Changes in BMAT fraction after RYGB were similar in participants with and without T2D. No associations were observed between changes in BMAT and changes in HbA<sub>1c</sub>, aBMD or BTMs.



### 5.3 Paper III

*This paper reports factors associated with bone health and quality (aBMD, BTMs and calciotropic hormones), the prevalence of aBMD below the expected range for age, osteopenia, osteoporosis and low-energy fractures 10 years after RYGB. Finally pre- and postoperative factors associated with aBMD z-score or t-score of -1.1 or lower 10 years after RYGB were assessed.*

Ten years after RYGB more than one in three had an aBMD of less than 90% of expected for age, gender, and ethnicity. Among premenopausal females and males 49 years or younger the prevalence of aBMD below expected range for age was 8% and among the postmenopausal females and males 50 years or older the prevalence of osteoporosis was 27%. Participants with hypothyroidism preoperatively, older age, postmenopausal status, higher PINP levels, or SHPT at 10 year follow-up, had higher odds of having an aBMD z-score or t-score of -1.1 or lower. At 10 year follow-up 33% had vitamin D deficiency and 75% insufficiency. The prevalence of SHPT was 31%. An inverse association between PTH and aBMD z-scores of femoral neck ( $\beta -0.051$  95% CI (-0.099 to -0.002),  $p = 0.040$ ), but not at total hip or lumbar spine, was noted. Levels of serum markers of bone turnover were high, and the BTMs were higher in participants with aBMD z-score or t-score of -1.1 or lower. Eighteen participants (15%) reported a clinical low-energy fracture after RYGB, with a mean duration from RYGB to first low-energy fracture was 8.4 years  $\pm$  1.8. In addition morphometric vertebral fracture assessments on lateral spine x-rays from the DXA scanner revealed that three male (11%) and seven female (7%) participants had experienced one or more moderate to severe vertebral fractures.



# 6 Discussion

## 6.1 Methodological considerations

Cohort studies may have inherent flaws leading to potential bias and error, potentially affecting the internal and external validity, which will be discussed in the following sections.

### 6.1.1 Selection and attrition bias

Both cohort studies described in this thesis include a bariatric population operated with RYGB at one center. Thus the same bariatric team treated all subjects, improving the internal validity, but limiting external validity. For the last 15 to 20 years RYGB has been routinely used, for years it was the predominant procedure and is still considered by many as the gold standard. However, despite relatively stable numbers of RYGB surgeries during the last decade it is no longer the dominant procedure due to a large increase in number of sleeve gastrectomies<sup>147</sup>. The RYGB technique and its follow-up regimen are quite standardized in Norway and the other Scandinavian countries. However, globally variations exist that could impact the external validity of our findings, including variation in preoperative regimens, limb lengths, supplementation advice and follow-up. Lastly, it should be noted that the bariatric population is a selected part of the morbidly obese population. There may be differences between the subjects who seek or undergo a bariatric surgery and those who do not. For example Rousseau et al. showed that subjects seeking bariatric surgery in Canada had a higher risk of fracture than BMI matched controls already prior to the surgery<sup>189</sup>.

### 4B study (Paper I and II)

This study had a number of inclusion and exclusion criteria limiting the number of eligible subjects and thus the external validity. Firstly, the study included only subjects planned for RYGB. At inclusion no decisive recommendations for choice of bariatric procedure existed, and selection of bariatric procedure for each individual patient was based on the clinical evaluation of the surgeon, thus this selection may differ between bariatric centers. Including

only patients planned for RYGB meant excluding more than half of our center's bariatric population at the time. Furthermore, the inclusion was further limited by including only patients with a BMI  $\leq 47$  kg/m<sup>2</sup> and of Caucasian ethnicity. The prevalence of subjects with  $\geq 50$  kg/m<sup>2</sup> prior to RYGB surgery has been reported between 21 and 32%<sup>190,191</sup>, thus an additional one third of the RYGB population was potentially excluded. Lastly, subjects with comorbidities potentially affecting the feasibility and security of the study examinations (severe psychiatric or currently treated with anticoagulation) and the main outcomes (connective tissue diseases or other hormonal diseases, kidney failure, current steroid treatment or previous treatment with bone active substances) were excluded. In a bariatric population these conditions are less common, however should be taken into account when interpreting the results of the study. On the other hand, patients with diabetes were encouraged to participate in the 4B study, thus the fraction of participants with diabetes in this study (38%) exceeds the fraction in patients scheduled for RYGB (25-30%) at our institution.

The study involved several study visits, only possible on specific days (due to limited staff availability), with potentially painful examinations, all factors probably affecting the participation rate.

To what extent patient characteristic of the non-participants differed from the participants are unknown. Participants lost to follow-up is a major source of bias. The 4B study had a low attrition rate, follow-up bias was less of a concern and we did not use imputation methods for missing data.

### **10 year follow-up study (Paper III)**

In this study all patients operated with RYGB between June 2004 and December 2006 were considered eligible. At the time of inclusion 86% of all bariatric procedures at our institution were RYGB<sup>192</sup>, thus the cohort is representative for the bariatric population at Oslo University Hospital at the time of inclusion. However, at the 10 year visit 37% were lost to follow-up. This may have biased the population. The letter inviting patients to participate in the study contained information that the 10 year follow-up would include evaluation of bone

health and DXA scan. This might have been an incitement for subjects suspecting bone disease to attend the 10 year follow-up and be included in the study.

### 6.1.2 Observation bias

Both studies reported mainly objective measures (BMAT, BMSi, aBMD, result of blood samples etc.), which do not exclude, but reduce the likelihood of reporting bias. DXA was utilized to evaluate aBMD and body composition. The introduction of a new DXA machine during the study could have affected the results. However, cross calibration revealed high intra-class correlation coefficient indicating good consistency between the measurements of the two machines. After bariatric surgery DXA measurements may be affected by technical reproducibility issues that in part may be related to imaging artifacts in the setting of morbid obesity and large weight loss<sup>22,24,26</sup>. Fracture data were self-reported and can thus be subject to recall bias. The classification of low or high energy fracture was based on information provided by the participant.

The papers report different outcomes. Below possible biases of observation are discussed for the separate papers;

**Paper I:** The use of the impact microindentation to evaluate bone material strength in humans is a relatively new method and our study was the first to use this technique in a bariatric population. Potential biases of obesity and weight loss on BMSi measurements remain to be explored. In a study of more than 200 elderly women Sundh et al. observed a negative correlation between tibial subcutaneous fat and BMSi<sup>8</sup>. Whether this finding represents a bias due to larger amounts of pretibial subcutaneous fat on the BMSi measurements or a direct negative effect of local adipose tissue on bone material strength remains unknown. In this context we analyzed the relation between the lower extremity fat percent assessed by DXA and BMSi prior to and one year after RYGB. We found no correlation, be it at baseline ( $r^2=0,015$ ) or one year postoperatively ( $r^2 =0,002$ ).

Regardless of these calculations, a subject of discussion, is whether a larger amount of subcutaneous tissue overlying the pretibial fossa might influence OsteoProbe measurements.

In the 4B study we detected our point of measurement on the anterior surface of the tibia and injected a local anesthetic. The needle of the OsteoProbe is dull; therefore in order to place in on the pretibial surface a sharp needle must first penetrate overlying skin and subcutaneous tissue. This was done with a sharp needle, creating an insertion channel, prior to measurement initiation. Nonetheless, the evaluation of unopposed penetration of overlying tissue was based on tactile sensations of the investigator, in other words a subjective measure. BMSi value is calculated as 100 times the ratio of indentation distance into PMMA divided by the indentation distance into bone. Thus we imagine that if indeed larger amounts of subcutaneous tissue affect the OsteoProbe measurements, we believe that more pretibial tissue would be associated with falsely high BMSi levels rather than falsely low BMSi levels. The reason we suspect this is that a larger amount of pretibial tissue surrounding the probe would slow bone penetration and thus shorten the indentation depth resulting in a falsely high BMSi value. Though, in shortage of mechanistic studies this remains speculative.

**Paper II:** The area of bone marrow investigated histologically is smaller than the area sampled using MRS. Our intra-observer variation was lower than the detected difference between preoperative and postoperative BMAT fraction and the lab technician performing the BMAT estimation was blinded for all clinical data. However, we did not have data on BMAT fraction variation between two repeated bone marrow biopsies taken from the same participant at same point in time. In lack of a control group the natural change of bone marrow biopsy assessed BMAT fraction could not be determined. However, previous cross-sectional studies have indicated a positive association between higher BMAT fractions and increasing age<sup>76,193</sup> and prospective cohort studies have quantified the increase to be 3-20% per year in postmenopausal osteoporotic women<sup>194,195</sup>. In light of this research it seems less likely that BMAT fraction would spontaneously decrease. Regardless, potential causality of RYGB induced weight loss on the observed BMAT fraction decrease cannot be determined from the 4B study.

### 6.1.3 Random errors

#### Biological

Several blood samples may be subject to circadian variation, e.g. CTX-1<sup>60</sup>. To avoid effect of circadian variation all blood samples were obtained in the morning (before 10 am) in both studies. To avoid possible influence of food intake all blood samples were drawn and all weight measures were performed at fasting state. Lastly, the gonadal hormones were taken at random times of the menstrual cycle, limiting its value in premenopausal females (Paper II).

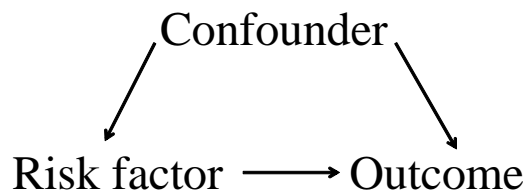
Norway has distinct seasons. Possible seasonal variations of the main outcomes (BMSi, BMAT and aBMD) remain to be explored. SHPT associated with vitamin D and calcium levels and vitamin D is known to be influenced by season<sup>30</sup>. Not correcting the vitamin D and PTH levels for season may have induced a bias in this thesis. However, we did not notice a statistical difference between the 25(OH) vitamin D levels of the different seasons in the 10 year follow-up study (paper III).

### 6.1.4 Confounding

Confounding may be considered as confusion of effects. A confounding factor affects both the risk factor and the outcome, but the confounder cannot be affected by the risk factor or outcome or be a causal pathway for the effect of the risk factor on the outcome<sup>196</sup>.

Confounders are illustrated in Figure 8. In this thesis adjustment for known confounding factors was performed in the analysis phase using multiple regression analyses. The selection of confounders was based on prior knowledge, supported by a directed acyclic graph. Any errors in this step cannot be ruled out. An advantage of multiple regression analyses is that it allows adjustment for several confounding factors. However, the number of participants in the 4B study limited the possible number of confounders adjusted for, thus increasing the risk of residual confounding. The results of the 4B study should thus be viewed as exploratory and hypothesis generating. Also, in the 10 year follow-up study there is a risk of residual confounding, the bias that remains after adjustment for confounders, which can never be ruled out in observational studies<sup>196</sup>.

**Figure 8:** Illustration of a confounder.



### **6.1.5 Sample size**

In the 4B study relatively few participants were included inducing a concern for type II error. Especially the negative finding of no differences in change in BMAT fraction and BMSi between participants with and without T2D should be interpreted with care. In addition several statistical analyses were performed on secondary endpoints increasing the risk of type I error. The sample size calculation in the 4B study was performed for change in BMAT and not for change in BMSi thus the findings in Paper I should be considered exploratory. The 10 year follow-up study was a descriptive study based on a predefined study and no sample size calculation was performed.



## 6.2 Discussion of results

### 6.2.1 Short term effects of Roux-en-Y gastric bypass on bone health and quality

The 4B study (Paper I and II) explored several factors influencing bone health and quality. It is the first study to describe in-vivo bone material strength and biopsy measured BMAT fraction in a population with morbid obesity, and the change one year after RYGB. The observed inverse relationship between bone material strength and BMI in morbidly obese subjects and the improvement in bone material strength one year after RYGB support the idea that higher morbid obesity is associated with decreased bone material strength, and can implicate that surgically induced weight loss improves bone material strength. Studies comparing patients with and without fragility fractures have described 4-4.5% lower bone material strength in the fracture population<sup>52,197</sup>. Our observed increase of 6.3% after RYGB therefore seems clinically relevant. The 4B study also revealed a 10.7% decrease in BMAT one year after RYGB. Higher levels of BMAT has been linked to skeletal fragility<sup>75</sup> and BMAT fraction has been shown to predict future aBMD loss<sup>198</sup>. In light of these findings, we believe the observed BMAT reduction to be positive for bone health one year after RYGB. The relative preservation of lean mass, and the increased physical activity are other features, which may exert positive effects on skeletal health. That selected short term changes after RYGB might be positive for bone health and quality is supported by a large study revealing decreased risk of fracture within the first year after RYGB<sup>18</sup>. On the other hand, changes believed to pose negative effects on bone health and quality was also observed in the 4B study; an increase in PTH and BTMs, and a reduction in aBMD at all measured sites were found. The aBMD and BTM findings are in line with previous studies<sup>22,24-28,199</sup>, however the observed elevation of PTH levels are in contradiction to several other studies one year after RYGB, where PTH has been noted to be unchanged<sup>20,23-26,28</sup>.

The finding of improved bone material strength after RYGB goes against our hypothesis and challenges preceding studies describing unchanged or decreased estimated failure load one year after RYGB<sup>22,25,28</sup>. Also, our results of decreased BMAT challenges findings of other studies, describing lumbar spine BMAT assessed by MRS six and 12 months after RYGB, reporting non-significant BMAT decrease<sup>26,27</sup>. BMAT is a distinct fat tissue that appears to

respond differently compared to white, subcutaneous and visceral, adipose tissue. A larger degree of caloric restriction has been associated with higher BMAT fraction, in parallel to depletion of white adipose tissue storage, in subjects with anorexia nervosa<sup>200</sup>. This appears to contradict our findings of reduction in BMAT fraction after loss of 30% of total weight and 45% of total fat mass. However, this could support a hypothesis of a U-shaped association between total body fat and BMAT, where BMAT is elevated in circumstances of high or low total body fat and normalizes with normalization of total body fat. The observed positive association between a decrease in fat mass and BMAT, where participants who had a larger total fat mass reduction decreased more in BMAT may further emphasize this.

Preoperative BMAT fraction was inversely associated with aBMD, in line with previous studies of subjects with morbid obesity<sup>12</sup> and subjects with increased fracture rates<sup>74-76</sup>. Interventional studies in osteoporotic pre- and postmenopausal women have shown an inverse association between aBMD and BMAT fraction, where increased aBMD was associated with decreased BMAT fraction<sup>188,195,201</sup>. This is opposed to the trends observed in the 4B study where aBMD decreased in parallel to a decrease in BMAT fraction. Our findings are at the same time, in line with the subpopulation with T2D in the study by Kim et al. who reported reductions of 6.5% in BMAT (L3 and L4), estimated with MRS, and 4.5% in volumetric BMD of the lumbar spine six months after RYGB<sup>27</sup>. We believe further studies are needed to understand the interaction between aBMD and BMAT.

Studies exploring the effect of BMI > 35 kg/m<sup>2</sup> on bone health are sparse, thus possible relations between degree of morbid obesity and bone health and quality remains largely unexplored. If morbid obesity poses a net negative effect on bone health and quality, changes in factors influencing bone health and quality one year after RYGB, must be viewed in the light of the extensive weight loss during this year.

## 6.2.2 Type 2 diabetes and bone health and quality

We did not observe any difference between preoperative BMSi values or changes of bone material strength after RYGB in participants with and without T2D. Opposing previous studies indicating lower bone material strength in subjects with T2D compared to controls<sup>9,10</sup>. Nevertheless, the association between the decrease in HbA<sub>1c</sub> and the improvement in bone material strength, observed in participants with T2D, supports the notion that improved glucose control influences bone health (Paper I). Participants with preoperative T2D had comparable BMAT fraction to participants without diabetes; however, we observed a significant association between preoperative BMAT and HbA<sub>1c</sub>. This supports a potential association between glycemic control and BMAT in subjects with morbid obesity. Despite a high diabetes remission rate we did not observe additional reductions in BMAT fraction in participants with preoperative T2D, as we had hypothesized (Paper II). This observation is in contrast to the results described by Kim et al., who report a significant difference in BMAT change, after RYGB between participants with and without T2D and only observed a reduction in BMAT in participants with preoperative T2D<sup>27</sup>. Notably, the study by Kim et al. differed from the 4B study in several key elements including using MRS to quantify BMAT, included only women and a mix of ethnicities, rendering the studies difficult to compare directly.

The 4B study was the first to compare aBMD changes in participants with and without T2D one year after RYGB. We did not detect any difference in aBMD decrease in the participants with or without T2D. In agreement with this no different prevalence of aBMD z-score or t-score of -1.1 or lower were noted in respect to preoperative T2D status in the 10 year follow-up study. In the 10 year follow-up study we observed a diabetes remission rate of 57%<sup>202</sup>. Madsen et al. has further explored the potential effect of diabetes remission on bone health after RYGB. Ninety-six subjects with history of preoperative T2D were examined six years after RYGB. No difference was noted between the 50 subjects in diabetes remission and subjects with current T2D in respect to aBMD, vBMD, cortical and trabecular measurements and estimated failure load<sup>32</sup>. In light of the fact that the group of subjects in diabetes remission were younger and had a lower prevalence of postmenopausal subjects this might be viewed as a surprising finding. Possibly these factors were balanced out by the accelerated

bone turnover (CTX-1 and PINP) and larger weight loss noted in participants in diabetes remission. However, this remains a speculation.

### **6.2.3 Long-term effects of Roux-en-Y gastric bypass on bone health**

In paper III we found that among premenopausal females and males 49 years or younger the prevalence of aBMD below expected range for age was 8% and among the postmenopausal females and males 50 years or older the prevalence of osteoporosis was 27%. Duran et al. observed an osteoporosis prevalence (t-score  $\leq -2.5$ ) of 13% in a study of 30 females, with a median age of 46 years, eight years after RYGB<sup>177</sup>. However, without knowing the occurrence of postmenopausal status it is difficult to directly compare this study to ours. The Tromsø study, a Norwegian population based study, reported a prevalence of osteoporosis of the femoral neck of 4.8% in males and 6.1% in females for subjects aged 50–69 years in contrast to our findings of 13% and 33%, respectively<sup>203</sup>. Furthermore, we noted that more than one in three of the participants in the 10 year follow-up study had aBMD measurement below 90% of that expected for age, gender and ethnicity. Collectively, our findings suggest that RYGB may be a risk factor for aBMD below expected range for age and osteoporosis.

Ten years after RYGB we observed higher levels of BTMs (CTX-1 and PINP) than those reported in publications of preoperative cohorts of similar age<sup>22,26,28</sup> indicative of elevated bone turnover 10 years after RYGB. Participants with aBMD z-score or t-score of  $-1.1$  or lower had higher levels of both CTX-1 and PINP, suggesting that longstanding accelerated bone turnover is a central mechanism underlying the decreased aBMD after RYGB. During the 10 years of follow-up 15% of our participants experienced clinical low-energy fractures, similar to reports for subjects exposed to long-term glucocorticoid treatment<sup>204</sup>. In addition 11% of male participants and 7.4% of females had a moderate or severe morphometric vertebral fracture detected by vertebral fracture assessment. These rates seem high compared to those reported in the general Norwegian population (60 years or younger) where the prevalence is reported to be 7.5% in males and 3% in females<sup>205</sup>. Median duration to low energy fracture post RYGB was more than eight years. Collectively, our findings indicate that subjects 10 years after RYGB are at increased risk of low-energy fracture, in line with studies

exploring other high turnover states <sup>206</sup>. However, to what extent the RYGB surgery itself contributed to this increased fracture rate cannot be answered by this thesis. Interestingly, a study has shown higher fracture rates in subjects seeking bariatric surgery when compared to BMI matched controls <sup>189</sup>. Yu et al. observed that patients operated with RYGB have been noted to have a 43% higher risk of skeletal fractures compared to subjects operated with adjustable gastric banding surgery, supporting the notion that RYGB leads to increased fracture rates <sup>19</sup>.

#### **6.2.4 Factors associated with aBMD z-score or t-score of -1.1 or lower 10 years after Roux-en-Y gastric bypass**

In the 4B study the study population maintained unchanged calcium and vitamin D levels, however we noted a significant increase in PTH levels. Although our two study populations are not directly comparable, the 31% SHPT prevalence in the 10 year follow-up study seems higher compared to the 12% and 18% prevalence noted preoperatively and one year after RYGB in the 4B study (Paper I). Other studies have shown higher SHPT prevalence in the years following weight stabilization <sup>31,32</sup>. However, the observed 10 year prevalence of SHPT is comparable to a large cross-sectional study from our center two years after RYGB where the prevalence rate was 34% <sup>30</sup>. Regardless, the clinical relevance of SHPT is elaborated in our 10 year follow-up study where the participants with SHPT had close to three times higher odds for aBMD z-score or t-score of -1.1 or lower 10 years after RYGB, and with the inverse association between PTH and aBMD z-scores of the femoral neck.

To what extent regular intake of calcium and vitamin D supplements could have prevented SHPT in our studies was not explored. Notably, the standard recommendation of calcium supplementation was in the form of calcium carbonate, in contradiction to recent European and American recommendations of calcium citrate. Furthermore, the standard doses of 800 IU vitamin D3 daily is in line with Norwegian and European guidelines for post-bariatric management <sup>150,157</sup>, but are considerably lower than the 3000 IU daily recommended by the American guidelines <sup>186</sup>. Additionally, we found that only one of three participants reported regular intake of the recommended dose of calcium and vitamin D supplements. Interestingly, an intervention study has showed effect of pre- and postoperative high dose vitamin D combined with postoperative calcium citrate supplements, enhanced protein intake and

physical activity program on reducing PTH levels and ameliorating the negative impact of RYGB on bone mass <sup>171</sup>.

The pathophysiology of aBMD z-score or t-score of  $-1.1$  or lower, and high rate of osteoporosis 10 years after RYGB, are likely multifactorial. Participants with preoperative hypothyroidism had more than four times higher odds for aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. Studies of subjects receiving substitution treatment for hypothyroidism have revealed discordant results in regard to whether or not it is associated with lower aBMD <sup>207,208</sup>. Supraphysiologic doses, however, have been noted to exert negative effect on bone in hypothyroid patients <sup>209</sup>. In addition, a hyperthyroid state preceding hypothyroidism could have negatively affected bone health in this group. We therefore favor the notion that excessive doses of thyroid hormone or possible hyperthyroidism preceding hypothyroidism, after thyroiditis or surgical/radioiodine treatment of hyperthyroidism, constitute plausible reasons for our findings. Mechanical loading of bone plays a key role in determining bone mass, strength and size <sup>210</sup>. In line with this we noted that participants with a BMI of less than  $35 \text{ kg/m}^2$  10 years after RYGB had a doubled risk of aBMD z-score or t-score of  $-1.1$  or lower, however this association did not remain significant in the multivariable analysis, indicative of limited importance in the long-term after RYGB. In agreement, a recent study, exploring fracture rate after RYGB, failed to observe a negative effect of larger weight loss on fracture risk <sup>18</sup>. As for the general population, and in line with the aBMD observations in the 4B study, older age and postmenopausal status was associated with higher odds for aBMD z-score or t-score of  $-1.1$  or lower and osteoporosis development <sup>37</sup>.

# 7 General conclusion

In a population with morbid obesity, higher BMI was inversely associated with bone material strength. One year after RYGB surgery, bone material strength was improved and BMAT fraction decreased on one hand, and bone turnover and PTH increased, and aBMD decreased, on the other. These changes did not appear to differ between subjects with and without T2D. Ten years after RYGB surgery, the prevalence of osteoporosis and low-energy fractures was high.

## 7.1 Specific conclusions

- Higher BMAT fraction was associated with poorer glycemic control in subjects with morbid obesity.
- Improved glucose control after RYGB was associated with improved bone material strength in participants with T2D.
- Changes in BMAT fraction after RYGB appeared to be gender specific.
- In males changes in BMAT fraction was inversely associated with changes in serum estrogen levels.
- Subjects with hypothyroidism prior to RYGB, or subjects with SHPT, older age, postmenopausal status or higher PINP levels 10 years after RYGB had higher odds of aBMD z-score or t-score of  $-1.1$  or lower.

## 8 Clinical aspects and future perspectives

The complex role of BMAT in physiology and pathology remains to be fully elaborated, and is key to understand the observed decrease in BMAT fractions impact on bone health. Impact microindentation enabled quick and easy measurement of bone material strength, but the impact of potential biases due to morbid obesity and weight loss should be further investigated. Both the improvement in bone material strength and decrease in BMAT fraction, observed one year after RYGB, are believed to pose positive effects on bone health and quality. How these factors will evolve over time should be a focus of further studies.

At a group level, bone material strength improved and BMAT fraction decreased, but the study revealed large individual differences in response to RYGB. Interestingly, change in BMAT fraction after RYGB appeared gender specific and an association between changes in endogenous sex steroids and change in BMAT fractions were also noted, but in males only. In the 4B study females had comparable sex steroid levels preoperatively and one year after RYGB. We believe the lack of significant changes to be a likely explanation of why we did not observe any association between a change in BMAT fraction and sex steroids in females. One might speculate that endogenous estradiol regulates BMAT in a gender-specific manner, a thought to be further explored.

The excessive metabolic changes induced by bariatric surgery lead us to believe that it can serve as a good model to learn more about the pathophysiology of T2D. Our thesis did not reveal different responses between subjects with and without T2D in the evaluated factors influencing bone health and quality. The restricted number of study participants may pose a limiting factor on the interpretation of these findings, and future studies are needed.

RYGB induces several changes to the neuro and hormonal axis and the gut microbiome, which might affect bone health and quality. In this thesis potential effects of such changes were not assessed. Studies exploring these would be expected to gain important knowledge of the bone-fat connection and the effects of RYGB on bone health.



Our 10 year follow-up study revealed a high prevalence of osteoporosis and low-energy fractures. This information should be a part of the preoperative information given to patients opting for RYGB. The findings are also relevant for evaluations of and recommendations for clinical long term follow-up protocols. Several of the identified risk factors for lower bone mineral density 10 years after RYGB identified in our study are non-modifiable (age, postmenopausal status). SHPT, however, is potentially modifiable. Lifelong nutritional supplements are recommended, however to what extent full compliance of the recommended doses are sufficient to avoid SHPT, and if so potentially neutralize the negative effects and increased fracture risk after RYGB is beyond the scope of the study. The association between SHPT and lower bone mineral density demonstrated in this thesis highlights the potential positive impact of avoiding SHPT in this population and should be a focus of future research. Effective anti-osteoporotic treatment exists, but no study to date has evaluated the effects of this treatment on osteoporosis after RYGB. Furthermore, the optimal treatment intervention criteria for fracture reduction in this population remain to be explored.

This thesis describes changes factors influencing bone health and quality one year after, and status 10 years after, RYGB. Several surgical treatment options for morbid obesity exist. How different bariatric surgeries affect bone health and quality is not elaborated by this thesis. Fracture rate and aBMD change are examples of bone health measures that differ between available procedures<sup>19,26,189</sup>. Yet, more knowledge is needed to determine which bariatric procedure balances optimal weight and metabolic effect at the lowest costs of the skeleton.

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# Appendix

Paper I

Paper II

Paper II



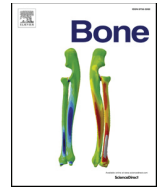






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Full length article

## Changes in bone quality after Roux-en-Y gastric bypass: A prospective cohort study in subjects with and without type 2 diabetes

Blom-Høgestøl I.K.<sup>a,b,\*</sup>, Mala T<sup>a,c</sup>, Kristinsson J.A.<sup>a,c</sup>, Brunborg C<sup>d</sup>, Gulseth H.L.<sup>a,e</sup>, Eriksen E.F.<sup>a,b</sup><sup>a</sup> Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway<sup>b</sup> Institute of Clinical Medicine, University of Oslo, Oslo, Norway<sup>c</sup> Department of Gastrointestinal Surgery and Paediatric Surgery, Oslo University, Norway<sup>d</sup> Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway<sup>e</sup> Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health, Norway

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## ABSTRACT

**Background:** Obesity and type 2 diabetes (T2D) are associated with an increased risk of skeletal fractures despite a normal areal bone mineral density (aBMD) and low bone turnover, possibly due to reduced bone material strength. Roux-en-Y gastric bypass (RYGB) enables a substantial and persistent weight loss and resolution of obesity related comorbidities such as T2D. However, the procedure induces a decrease in aBMD and increased bone turnover and fracture rate. To our knowledge, changes in bone material strength after RYGB have not been explored. This study aimed to evaluate changes in factors influencing bone quality; bone material strength, aBMD and bone turnover markers, in a population with morbid obesity undergoing RYGB and whether these changes differed in participants with and without T2D. We also sought to assess factors associated with bone material strength and bone mineral density in obese subjects before and after RYGB.

**Methods:** We examined 34 participants before and one year after RYGB, of whom 13 had T2D. Bone material strength index (BMSi) was evaluated by impact microindentation, aBMD and body composition by Dual energy X-ray absorptiometry, levels of bone turnover markers and calciotropic hormones were estimated from fasting serum samples. Participants with and without T2D were comparable before surgery, with the exception of glycosylated hemoglobin (HbA<sub>1c</sub>).

**Results:** Preoperatively, BMSi was inversely associated with BMI,  $\beta_{\text{unadjusted}} -1.1$  (-1.9 to -0.28),  $R^2 = 0.19$ ,  $p = 0.010$ , and this association remained significant after adjusting for age and gender. After RYGB the participants had lost a mean  $\pm$  SD of  $33.9 \pm 10.9$  kg,  $48.7 \pm 14.2$  % of total body fat, increased physical activity, unchanged vitamin D levels, and all but one of the 13 participants with T2D were in diabetes remission. BMSi increased from  $78.1 \pm 8.5$  preoperatively to  $82.0 \pm 6.4$  one year after RYGB, corresponding to an increase of  $4.0 \pm 9.8$  in absolute units or  $6.3 \pm 14.0$  %,  $p = 0.037$ . The increase was comparable in participants with and without T2D. In subjects with T2D, a larger decrease in HbA<sub>1c</sub> was associated with a larger increase in BMSi  $\beta_{\text{unadjusted}} -9.2$  (-16.5 to -1.9),  $R^2 = 0.47$ ,  $p = 0.019$ . Bone turnover markers (CTX-1 and PINP) increased by  $195.1 \pm 133.5$  % and  $109.5 \pm 70.6$  %, respectively. aBMD decreased by  $3.9 \pm 5.5$  % in the lumbar spine,  $8.2 \pm 4.6$  % in the femoral neck,  $11.6 \pm 4.9$  % in total hip and  $9.4 \pm 3.8$  % in total body.

**Conclusion:** Our findings indicate that bone material strength improves despite an increase in bone turnover and a decrease in aBMD one year after RYGB. Trends were statistically comparable in participants with and without T2D. However, improved glucose control was associated with improved bone material strength in participants with T2D.

**Abbreviations:** aBMD, Areal bone mineral density; BMI, Body mass index; BMSi, Bone material strength index; CTX-1, Carboxyl terminal telopeptide of type 1 collagen; FSH, Follicular stimulating hormone; DXA, Dual energy X-ray absorptiometry; HRpQCT, High-resolution peripheral quantitative computed tomography; PINP, Procollagen type 1 N-terminal propeptide; RYGB, Roux-en-Y gastric bypass; SHPT, Secondary hyperparathyroidism; T2D, Type 2 diabetes; 25-hydroxyvitamin D, 25(OH) vitamin D

\* Corresponding author at: Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway.

E-mail address: [inkhoe@ous-hf.no](mailto:inkhoe@ous-hf.no) (I.K. Blom-Høgestøl).

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## 1. Introduction

Fracture risk is most commonly evaluated based on areal bone mineral density (aBMD), despite aBMD being only a modest risk factor for fracture [1]. Epidemiological studies demonstrate a considerable overlap in aBMD values between fracture and fracture-free populations [2]. Bisphosphonate treatment induces a reduced fracture risk out of proportion to the observed increase in aBMD [3]. This discrepancy may be due to a difference or change in bone quality. Bone quality is described as the totality of features and characteristics that influence a bone's ability to resist fracture. Collectively, aBMD, bone architecture, and bone material properties interact to define bone quality; and all three are affected by bone turnover [4]. Obesity and type 2 diabetes (T2D) are associated with site specific increased incidence of fracture [5–7], despite a normal aBMD [8] and low bone turnover [9,10]. An important factor proposed to explain this discrepancy is reduced bone material strength and thus compromised bone quality [11–13].

Roux-en-Y gastric bypass (RYGB) is among the most commonly performed surgical procedures for weight loss offered to patients with morbid obesity. RYGB enables a substantial and persistent weight loss and resolution of obesity related comorbidities such as T2D [14–16]. The procedure is incorporated in the American Diabetes Associations treatment guidelines for selected patients with T2D and Class I-III obesity [17].

After RYGB, a reduction in aBMD and an increase in bone turnover and fracture rate are observed [18–27]. Possible mechanisms include weight loss mediated reduced stimulation of osteocyte mechanoreceptors, disturbances of the calcium and vitamin D, balance and changes in gastrointestinal- and sex hormone levels. Following weight stabilization a continued aBMD decrease and persistent elevated serum bone turnover markers has also been observed, indicating possible effects of RYGB on bone beyond adaptation to a reduced weight [28]. However, studies utilizing high-resolution peripheral quantitative computed tomography (HRpQCT) to estimate failure load have revealed diverging results one year after RYGB [22,24] and, to our knowledge, changes in tissue level bone material strength have not been previously explored.

This study aimed to evaluate changes in factors influencing bone quality; bone material strength, aBMD and bone turnover markers, in a population with morbid obesity undergoing RYGB, and whether these changes differed in participants with and without T2D. We also sought to assess factors associated with bone material strength and aBMD in subjects with obesity and after RYGB. Our hypothesis was that bone material strength would deteriorate, aBMD would decrease and bone turnover increase after RYGB and collectively pose a negative effect on bone quality.

## 2. Methods

### 2.1. Study population

We recruited patients referred for RYGB at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral center for treatment of morbid obesity. Eligibility criteria for RYGB were body mass index (BMI)  $\geq 40$  kg/m<sup>2</sup> or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity-related co-morbidity, age between 18 to 65 years, and previously failed attempts of sustained weight loss. Patients with T2D were encouraged to participate in the study. The inclusion period was from October 8th, 2015, to January 27th, 2017. Participants were excluded if they were unable to read Norwegian language, if they had severe psychiatric comorbidity, connective tissue disorders or other hormonal diseases, kidney failure (glomerular filtration rate  $< 30$  mL/min/1.73 m<sup>2</sup>), type 1 diabetes, BMI  $> 47$  kg/m<sup>2</sup>, history of treatment with bone active substances (bisphosphonates, denosumab, hormone replacement or parathyroid hormone), or if they were currently receiving anticoagulation or steroid treatment (estrogen, testosterone or

glucocorticoids). To avoid heterogeneity in our study population, non-Caucasians were excluded.

### 2.2. Surgery and study visits

All participants had a laparoscopic RYGB with a gastric pouch of about 25 ml, a 150 cm antecolic alimentary and 50 cm biliopancreatic limb [29]. Participants attended study visits preoperatively and one year after RYGB. Study visits included blood samples, anthropometric measures, dual-energy absorptiometry scan (DXA), impact micro-indentation, intravenous glucose tolerance test, euglycemic hyperinsulinemic clamp, indirect calorimetry, and bone marrow biopsy. In this paper results of the blood samples, anthropometric measures, DXA measurements and impact micro-indentation are presented. Results of bone marrow biopsy measurements of bone marrow adipose tissue in this population has been previously published [30].

In addition to study visits, the participants also attended regular clinical follow-up visits six to eight weeks, six months, and one year after surgery. After surgery, all participants were advised oral supplementation with 1000 mg of calcium, 800 IU vitamin D, one multi-vitamin, 200 mg of iron daily and B12 injections 1 mg every three months. At clinical visits, vitamin levels were monitored and additional supplements were advised as appropriate.

### 2.3. Clinical parameters

T2D was defined as glycosylated hemoglobin (HbA<sub>1c</sub>)  $\geq 6.5$  %, or use of one or more oral glucose lowering drug (GLD). Diabetes remission was defined as HbA<sub>1c</sub>  $< 6.5$  % without GLD in participant with T2D preoperatively. Vitamin D deficiency was defined as serum 25(OH) vitamin D levels below 50 nmol/L. Secondary hyperparathyroidism (SHPT) was defined as a serum concentration of PTH above 7.0 pmol/L in the absence of serum ionized calcium above 1.33 mmol/L. All previous fractures are reported, except digit fractures. Hormonal intrauterine devices made clinical evaluation of menstrual cycle difficult. For this reason a postmenopausal status was defined as a serum follicle stimulating hormone (FSH)  $\geq 25$  IU/l [31]. Physical activity was reported by the participants on a predefined non-validated form and categorized as (i) 0–1 hour; (ii) 1–2 hours or (iii)  $\geq 3$  h per week.

### 2.4. Blood samples

Blood samples were taken before 10 a.m. after an overnight fast, centrifuged, and stored in refrigerator or freezer. Serum samples for evaluation of bone turnover markers were stored at -80 C and analyzed at the end of the study to avoid inter-assay variation. All other study blood sample analyses were made shortly after retrieval.

The Hormone Laboratory, Oslo University Hospital, analyzed carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP) using Roche® electrochemiluminescence immunoassay (ECLIA), and osteocalcin using LIAISON® chemiluminescence immunoassay (CLIA). Serum 25(OH) vitamin D levels were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method, serum parathyroid hormone (PTH) by Immulite 2000 XPI, Siemens Healthineers a chemiluminometric assay, serum ionized calcium using Roche® Cobas b221, and C-peptide using Modular E170 Roche® ECLIA. FSH was analyzed using Immulite 2000 XPI, Siemens Healthineers, a non-competitive immunoluminometric assay.

The Central Laboratory of Oslo University Hospital analyzed HbA<sub>1c</sub> using Tosoh G8 high-performance liquid chromatography. Phosphate and magnesium were analyzed using Cobas 6000 Roche® photometry.

The reference range for the ten variables reported were: CTX-1  $\mu\text{g/L}$ : females 25–49 years:  $\leq 0.57$ ,  $\geq 50$  years:  $\leq 1.01$ , males 30–50 years:  $\leq 0.58$ , 51–70 years:  $\leq 0.7$ ; PINP  $\mu\text{g/L}$ : females  $> 25$  years: 11–94, males  $> 25$  years: 20–91; osteocalcin nmol/L: females  $\geq 21$

years: 1.5–5.4, males  $\geq$  21 years 1.6–4.3; 25(OH) vitamin D nmol/L: 37–132; PTH pmol/L: 1.5–7.0; ionized calcium mmol/L: 1.15–1.33; C-peptide pmol/L: 300–1480; HbA<sub>1c</sub> %:  $<$  6; phosphate mmol/L: females  $\geq$  16 years: 0.9–1.7, males 16–49 years: 0.8–1.7; magnesium mmol/L: 0.71–0.94. The coefficient of variance was 5 % for CTX-1, 5 % for PINP and 6 % for osteocalcin.

### 2.5. Impact microindentation

Tissue level bone material strength of cortical bone was assessed by impact microindentation using a commercially handheld device (OsteoProbe®, Active Life Scientific, Santa Barbara, California). The impact microindentation was performed on the anterior surface of the mid-shaft of the right tibia (with the exception of one participant where the examinations were performed on the left) 10 cm below the inferior margin of the patella after injection of local anesthetics. To avoid overlying skin and subcutaneous tissue opposing the measurement, an insertion channel was made with a sharp needle (BD Microlance™ 3, 21 G) prior to indenting the cortical bone surface. To ensure sufficient width of the insertion channel the needle was moved in circular movements expanding the insertion channel. The first eight indentations were made in vivo, with 2 mm separating two measurements. Subsequently, five indentations against a phantom of poly-methyl methacrylate were performed for calibration of participant measurements. Indentations with obvious operator errors were removed. The output for OsteoProbe® is the Bone Material Strength index (BMSi), which is a normalized measure of indentation depth [32]. To minimize inter-observer variations, all measurements were made by the same investigator (I.K.B.H).

### 2.6. Areal bone mineral density

DXA scan including whole body scans for assessment of body composition, including whole body fat and lean mass, was performed. aBMD, g/cm<sup>2</sup> of the lumbar spine (L<sub>1</sub>–L<sub>4</sub>), total hip, proximal femur, and total body was assessed. T-scores and Z-scores of the lumbar spine L1–L4 were calculated after exclusion of vertebrae with osteoarthritic changes (spondylosis) or compression fractures. All scans were performed by the same nurse. GE Lunar Prodigy was used until August 26th 2016, when it was replaced by GE Lunar iDXA (Lunar Corporation, Madison, WI, USA). Body composition performed with GE Lunar Prodigy was reanalyzed with iDXA software, to optimize comparability. Cross calibration between the measurements of two DXA machines has been published previously [30]. The DXA machine was calibrated daily against the standard calibration phantom supplied by the manufacturer, and the estimated short-term precision errors for aBMD at the lumbar spine and at the femoral neck is  $<$  1.0 %.

### 2.7. Statistical analysis

Participant characteristics are presented as mean values  $\pm$  standard deviation (SD), median (range), or as proportions (percentage). When comparing preoperative characteristics of participants with and without T2D, independent sample *t*-test or Mann-Whitney U test were used for continuous variables, and Pearson Chi-square or Fisher's exact test for categorical variables. Correlations were assessed with Pearson (*r*) or Spearman (*rsp*) correlation coefficients, as appropriate. When exploring changes between preoperative and one year after RYGB, paired-sample *t*-tests or Wilcoxon signed-rank test were used for continuous variables and McNemar's test for paired proportions was used for evaluation of changes in categorical variables. To explore difference in changes from preoperative to one year after RYGB between participants with and without T2D, delta values were compared with independent sample *t*-test or Mann-Whitney U test. Adjustments for confounding factors were performed using multiple linear regression analyses. Only variables with significant relationships with both

the exposure and the outcome variables were considered as possible confounders in addition to variables of known clinical importance. Possible confounding variables were age, gender, BMI change, and preoperative value. In order to avoid multicollinearity, confounders that correlated,  $r > 0.7$ , were not adjusted for. The results from the regression analyses are presented as regression coefficients ( $\beta$ ) with 95 % confidence intervals (CI) and R square (R<sup>2</sup>). Two tailed *p*-values  $<$  0.05 were considered statistically significant. All statistical analyses were made using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY: IBM Corp).

### 2.8. Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Northern Norway Regional Committee for Medical and Health Research Ethics; 2015/604. Written informed consent was obtained from all participants.

## 3. Results

A total of 44 participants were included. Preoperative study examinations were possible in 38, and 36 (92%) of these met for follow-up one year after RYGB. Two of the 36 participants were excluded from the study at follow-up due to sex reassignment and glucocorticoid treatment, respectively. Thus the study population ultimately consisted of 34 participants. Of these BMSi estimation was not possible in one participant preoperatively; due to edema of the lower extremity, and in three participants postoperatively; as the OsteoProbe apparatus was not available.

### 3.1. Preoperative characteristics

Preoperative characteristics are presented in Table 1. Nineteen participants had undergone one or more skeletal fracture, fracture site, time, and energy is presented in supplementary Table 1. Thirteen participants (38 %) had T2D at study inclusion, eight (62 %) of these were treated with one or more oral GLD, and one with both insulin and oral GLD. Median (range) duration of T2D was 4 years (5 months to 18 years). Except for HbA<sub>1c</sub>, preoperative characteristics including body composition, aBMD, BMSi values, calcitropic hormones, and bone turnover markers did not differ statistically between participants with and without T2D (Table 1).

### 3.2. Changes in body composition and physical activity

All participants had lost weight one year after RYGB. Mean  $\pm$  SD weight loss was 33.9  $\pm$  10.9 kg or 28.3  $\pm$  8.9 % of total weight. BMI decreased with 11.6  $\pm$  4.3 points, fat mass decreased with 48.8  $\pm$  14.2 % or 27.4  $\pm$  9.5 kg, and lean mass decreased with 10.5  $\pm$  4.1 % or 6.3  $\pm$  2.5 kg, all *p*  $<$  0.001. The mean tissue fat of the lower limb decreased from 17.0  $\pm$  5.1 kg preoperatively to 8.7  $\pm$  2.9 kg postoperatively, *p*  $<$  0.001. Participants with and without T2D had comparable changes in body composition. The proportion of participants reporting less than one hour of hard physical activity a week decreased from 55.8% preoperatively to 23.5% one year after RYGB. Participants reporting 1–2 hours or more than 3 h a week increased from 17.6% to 32.4% and 26.5 % to 38.2 %, respectively.

### 3.3. Changes in T2D status and related parameters

All but one of the thirteen participants with T2D was in diabetes remission at study follow-up. One year after RYGB, we observed a decrease in C-peptide levels for all participants combined, and an HbA<sub>1c</sub> reduction for all but two participants. For participants with T2D, HbA<sub>1c</sub> decreased from 6.7 % to 5.6 % one year after RYGB. For participants without T2D, HbA<sub>1c</sub> decreased from 5.5 % to 5.2 %. C-peptide levels

**Table 1**

Baseline characteristics of 34 participants with morbid obesity awaiting Roux-en-Y gastric bypass surgery.

Characteristics	All participants	With type 2 diabetes (n = 13)	Without type 2 diabetes (n = 21)	p-value T2D vs. non T2D
Age, years	45.2 ± 9.7	48.3 ± 7.9	43.2 ± 10.4	0.14
Female gender	21 (63%)	8 (62%)	13 (62%)	0.98
Postmenopausal*	8 (36%)	5 (62%)	3 (23%)	0.12
Smoking, current or previous	21 (60%)	6 (43%)	15 (71%)	0.20
Previous fracture	19 (56%)	9 (64%)	10 (48%)	0.33
Weight, kg	120.0 ± 14.9	117.5 ± 13.3	121.5 ± 15.9	0.47
BMI, kg/m <sup>2</sup>	40.9 ± 3.5	40.3 ± 4.0	41.2 ± 3.1	0.47
Fat mass, kg	55.2 ± 8.6	52.6 ± 9.1	56.8 ± 8.0	0.17
Lean mass, kg	61.1 ± 11.2	61.0 ± 11.4	60.6 ± 10.2	0.90
Impact microindentation, BMSi	78.1 ± 8.4	78.6 ± 7.4	77.7 ± 9.1	0.78
Lumbar spine aBMD, g/cm <sup>2</sup>	1.18 ± 0.16	1.15 ± 0.14	1.20 ± 0.17	0.41
Lumbar spine t-score	0.1 ± 1.3	-0.1 ± 1.1	0.2 ± 1.4	0.49
Femoral neck aBMD, g/cm <sup>2</sup>	1.09 (0.78-1.3)	1.04 (0.78-1.3)	1.11 (0.88-1.3)	0.12
Femoral neck t-score	0.6 ± 1.1	0.2 ± 1.1	0.8 ± 1.0	0.09
Total hip aBMD, g/cm <sup>2</sup>	1.17 ± 0.1	1.14 ± 0.13	1.19 ± 0.18	0.22
Total hip t-score	1.2 ± 1.1	0.9 ± 1.1	1.3 ± 1.1	0.33
Whole body BMD, g/cm <sup>2</sup>	1.35 ± 0.10	1.31 ± 0.10	1.36 ± 0.10	0.16
Whole body t-score	2.2 ± 1.1	1.8 ± 0.70	2.4 ± 1.2	0.16
HbA <sub>1c</sub> , %	5.8 (5.0-8.0)	6.7 (5.9-8.0)	5.5 (5.0-6.2)	< 0.001
C-peptide, pmol/L	1394 (680-2436)	1379 (837-2436)	1402.0 (680-1994)	0.70
Serum ionized calcium, mmol/L	1.2 ± 0.13	1.2 ± 0.04	1.2 ± 0.16	0.47
Parathyroid hormone, pmol/L	4.2 (1.7-9.8)	4.1 (1.7-8.9)	4.3 (1.9-9.8)	0.53
25(OH)Vitamin D, nmol/L	61.0 ± 22.0	65.2 ± 16.5	55.6 ± 25.0	0.23
CTX-1, µg/L	0.33 ± 0.14	0.37 ± 0.13	0.30 ± 0.14	0.19
PINP, µg/L	47.1 ± 20.1	53.8 ± 23.7	43.0 ± 16.7	0.13
Osteocalcin, nmol/L	3.1 ± 0.66	3.4 ± 0.71	3.0 ± 0.60	0.11

Abbreviations: body mass index (BMI), bone mineral density (BMD), bone material strength index (BMSi), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), type 2 diabetes (T2D).

Continuous variables with normal distribution are presented as mean ± SD and analyzed with independent t-variables with normal distribution are presented as mean ± SD and analyzed with independent t-test. Other continuous variables are presented as median (range) and analyzed with Mann-Whitney U test.

Categorical variables are presented as n (%) and analyzed with Person Chi-squared variables are presented as n (%) and analyzed with Person Chi-square or Fisher exact test if less than 5 expected frequencies.

Missing values: BMSi n = 1, Serum ionized calcium: n = 2.

\* Proportion of postmenopausal females is given as proportion of total number of females.

decreased from median (range) 1394 (680–2436) pmol/L to 747 (426–1115) pmol/L after RYGB. The decrease was comparable for participants with and without T2D.

### 3.4. Changes in calciotropic hormones and bone turnover markers

Mean 25(OH) vitamin D levels remained unchanged for both participants with and without T2D (Table 2). However, the number of participants with vitamin D deficiency decreased from 11 preoperatively to seven one year after RYGB,  $p = 0.34$ . PTH levels increased from a median (range) 4.2 pmol/L (1.7–9.8) preoperatively to 4.7 pmol/L (2.1–15.2),  $p = 0.024$ , and the number of participants with SHPT increased from four to six,  $p = 0.69$ . The increase in PTH levels for the total study population seemed to be driven by a significant increase in PTH for participants with T2D, whereas the increase was non-significant for participants without T2D. A similar pattern was noted for ionized calcium levels (Table 2). CTX-1 increased by 195.1 ± 133.6 %, PINP by 109.5 ± 70.6 % and Osteocalcin by 52.2 ± 28.0 %. The observed increase in bone turnover markers was comparable between participants with and without T2D (Table 2).

### 3.5. Changes in bone material strength of cortical bone

BMSi (preoperative and one year after RYGB) did not correlate with aBMD (all measured locations), or tissue fat of the lower limb,  $r < 0.30$ ,  $p > 0.05$  for all. Preoperatively, BMSi was inversely associated with BMI ( $\beta_{\text{unadjusted}} -1.1$  (-1.9 to -0.28),  $R^2 = 0.19$ ,  $p = 0.010$ ), and this association remained significant after adjusting for age and gender ( $\beta_{\text{adjusted}} -1.5$  (-2.3 to -0.65),  $R^2 = 0.35$ ,  $p = 0.001$ ). One year after RYGB, BMI did not correlate with BMSi ( $r = 0.12$ ,  $p = 0.51$ ), also changes in BMSi did not correlate with weight loss after RYGB

( $r = 0.15$ ,  $p = 0.44$ ). BMSi increased from 78.1 ± 8.4 preoperatively to 82.0 ± 6.4 one year after RYGB, corresponding to a mean difference of 4.0 ± 9.8 in absolute units, or 6.3 ± 14.0% increase,  $p = 0.037$ . The change was not statistically different in participants with and without T2D (Fig. 1).

For all participants combined, the change in HbA<sub>1c</sub> was not associated with change in BMSi,  $\beta_{\text{unadjusted}} -2.1$  (-8.4–4.2),  $R^2 = 0.017$ ,  $p = 0.50$ . When the cohort was divided based on presence or absence of preoperative T2D we observed that for participants without T2D, a larger decrease in HbA<sub>1c</sub> was associated with a smaller improvement or deterioration of BMSi levels ( $\beta_{\text{unadjusted}} 16.7$  (0.26–33.2),  $R^2 = 0.23$ ,  $p = 0.047$ ). This association remained significant after adjustment for BMI change and age ( $\beta_{\text{adjusted}} 18.4$  (1.7–35.0),  $R^2 = 0.33$ ,  $p = 0.033$ ). In participants with T2D, however, a larger decrease in HbA<sub>1c</sub> was associated with a larger increase in BMSi ( $\beta_{\text{unadjusted}} -9.2$  (-16.5 to -1.9),  $R^2 = 0.47$ ,  $p = 0.019$  (Fig. 2), this association remained significant after adjusting for change in BMI and age ( $\beta_{\text{adjusted}} -7.8$  (-15.2 to -0.38),  $R^2 = 0.68$ ,  $p = 0.042$ ).

Preoperative BMSi values were not associated with postoperative BMSi values ( $r = 0.15$ ,  $p = 0.43$ ). However, preoperative BMSi values were negatively associated with delta BMSi  $\beta_{\text{unadjusted}} -0.89$  (-1.2 to -0.59),  $R^2 = 0.60$ ,  $p < 0.001$ . This association remained significant after adjustment for age, gender and BMI change ( $\beta_{\text{adjusted}} -0.90$  (-1.2 to -0.60),  $R^2 = 0.63$ ,  $p < 0.001$ ). We observed no association between age, gender, and menopausal status and change in BMSi.

### 3.6. Changes in areal bone mineral density

One year after RYGB, aBMD decreased with 3.9 ± 5.5% in the lumbar spine, 8.2 ± 4.6 % in the femoral neck, 11.6 ± 4.9 % in total hip, and 9.4 ± 3.8 % in total body. This corresponded to a decrease in

**Table 2**

Changes in bone mineral density, markers of bone turnover and calcium metabolism one year after Roux-en-Y gastric bypass surgery, in participants with and without type 2 diabetes.

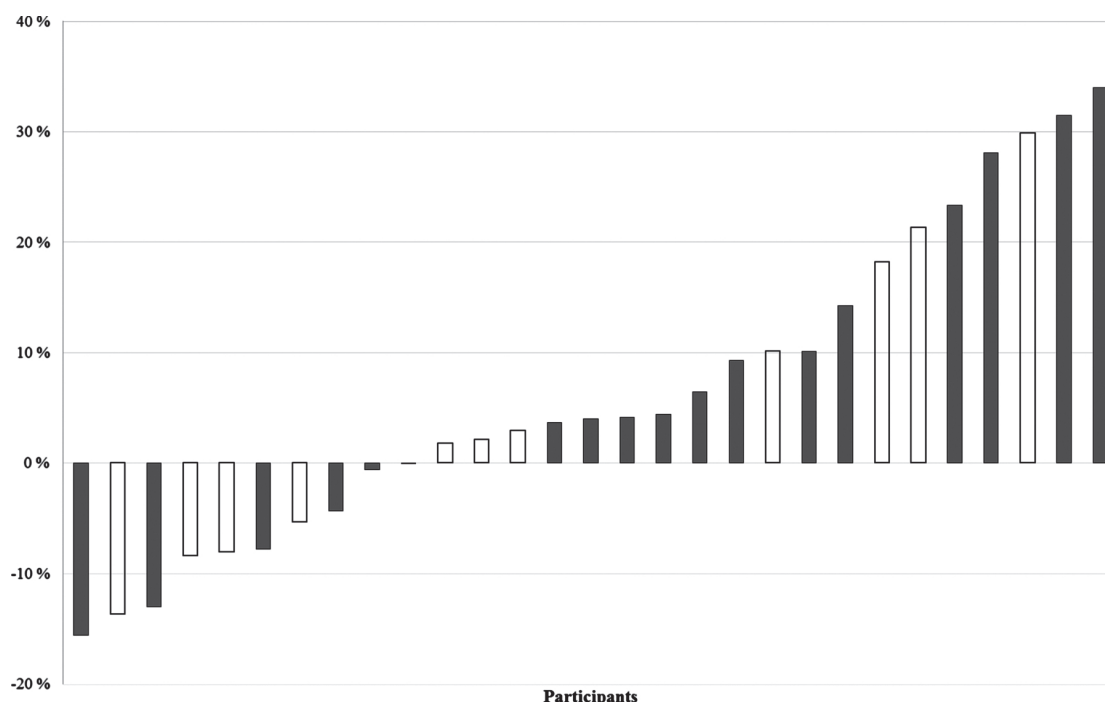
	Participants with type 2 diabetes (n = 13)				Participants without type 2 diabetes (n = 21)				
	Preoperatively	One year after RYGB	Difference	p-value within group	Preoperatively	One year after RYGB	Difference	p-value within group	p-value between groups
Impact microindentation, BMSi	78.6 ± 7.4	81.5 ± 5.6	2.8 ± 10.0	0.38	77.7 ± 9.1	82.2 ± 7.0	4.7 ± 9.8	0.059	0.63
Lumbar spine, aBMD	1.15 ± 0.14	1.09 ± 0.16	-0.063 ± 0.052	0.001	1.20 ± 0.17	1.16 ± 0.17	-0.033 ± 0.060	0.021	0.15
Lumbar spine, t-score	-0.1 ± 1.1	-0.5 ± 1.3	-0.42 ± 0.50	0.010	0.2 ± 1.4	0.02 ± 1.3	-0.21 ± 0.60	0.12	0.30
Femoral neck, aBMD	1.0 (0.78-1.3)	0.93 (0.71-1.3)	-0.11 (-0.16 to -0.02)	0.001	1.1 (0.88-1.3)	1.0 (0.84-1.2)	-0.073 (-0.18 to 0.01)	< 0.001	0.16
Femoral neck, -score	0.23 ± 1.1	-0.65 ± 1.1	-0.88 ± 0.43	< 0.001	0.81 ± 1.0	-0.048 ± 0.76	-0.86 ± 0.42	< 0.001	0.92
Total hip, aBMD	1.1 ± 0.13	1.0 ± 0.13	-0.12 ± 0.047	< 0.001	1.2 ± 0.12	1.1 ± 0.11	-0.12 ± 0.047	< 0.001	0.77
Total hip, t-score	0.93 ± 1.0	0.062 ± 1.0	-0.87 ± 0.49	< 0.001	1.3 ± 1.1	0.51 ± 0.89	-0.82 ± 0.40	< 0.001	0.74
Total body, aBMD	1.3 ± 0.10	1.2 ± 0.10	-0.12 ± 0.036	< 0.001	1.4 ± 0.10	1.3 ± 0.090	-0.11 ± 0.049	< 0.001	0.67
Total body, t-score	1.8 ± 0.70	0.66 ± 0.64	-1.2 ± 0.35	< 0.001	2.4 ± 1.2	1.2 ± 1.2	-1.1 ± 0.48	< 0.001	0.77
CTX-1	0.37 ± 0.13	0.91 ± 0.26	0.54 ± 0.19	< 0.001	0.30 ± 0.14	0.82 ± 0.31	0.52 ± 0.29	< 0.001	0.81
PINP	54.8 ± 23.7	93.5 ± 19.6	39.7 ± 24.9	< 0.001	43.0 ± 16.7	86.4 ± 26.3	43.4 ± 20.1	< 0.001	0.64
Osteocalcin	3.4 ± 0.71	4.9 ± 0.99	1.6 ± 0.79	< 0.001	3.0 ± 0.60	4.5 ± 0.68	1.49 ± 0.60	< 0.001	0.80
Parathyroid hormone	4.1 (1.7-8.9)	5.1 (2.1-13.8)	1.3 (-0.3-5.5)	0.011	4.3 (1.9-9.8)	4.4 (2.1-15.2)	0.50 (-4.4-9.3)	0.34	0.58
25(OH) vitamin D	65.2 ± 16.5	66.3 ± 16.4	1.2 ± 24.6	0.87	55.6 ± 25.0	66.7 ± 23.4	11.0 ± 27.7	0.083	0.32
Ionized calcium	1.2 ± 0.041	1.2 ± 0.032	0.022 ± 0.020	0.002	1.2 ± 0.16	1.2 ± 0.032	-0.031 ± 0.15	0.39	0.22
Phosphate	0.99 ± 0.14	1.2 ± 0.16	0.17 ± 0.17	0.004	0.95 ± 0.14	1.2 ± 0.14	0.23 ± 0.17	< 0.001	0.30
Magnesium	0.83 ± 0.057	0.86 ± 0.050	0.032 ± 0.085	0.20	0.84 ± 0.044	0.84 ± 0.045	0.0019 ± 0.044	0.84	0.18

Abbreviations; areal bone mineral density (aBMD), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP). Units of measurement: Impact microindentation; bone material strength index (BMSi), aBMD; g/cm<sup>3</sup>, CTX-1; µg/L, PINP; µg/L, osteocalcin; nmol/L, 25(OH) vitamin D; nmol/L, ionized calcium; mmol/L, phosphate; mmol/L, magnesium; mmol/L.

All measures are normally distributed parameters are presented as mean ± SD and analyzed with paired t-test. Parameters with non-linear distribution are presented as median (range), and analyzed with Wilcoxon Signed Rank test. Analyses between groups were made with independent sample t-test (normal distribution) or Mann Whitney U test (not normally distributed). Missing values preoperatively: BMSi; n = 1, Serum ionized calcium; n = 2. One year after RYGB: BMSi; n = 4.

t-score at all evaluated skeletal locations (Table 2). Higher age was associated with a greater decrease of the lumbar spine aBMD  $\beta_{unadjusted}$  -0.004 (-0.005 to -0.002), R<sup>2</sup> = 0.37, p < 0.001, and this remained

significant when adjusted for gender and preoperative aBMD ( $\beta_{adjusted}$  -0.004 (-0.006 to -0.003), R<sup>2</sup> = 0.48, p < 0.001). Postmenopausal women exhibited a higher lumbar spine aBMD loss compared to



**Fig. 1.** Percent change in bone material strength index (BMSi) one year after RYGB in 30 participants with and without type 2 diabetes (T2D). Participants with T2D are marked with white columns. Overall mean (SD) BMSi increased from 78.0 (8.4) at baseline to 82.2 (6.3) after RYGB, corresponding to a mean difference of 4.23 (9.7) or 6.6% increase, p = 0.024. Mean changes in BMSi were comparable in participants with and without T2D. BMSi was measured with impact microindentation on the anterior surface of the mid-shaft of the tibia using an OsteoProbe®.

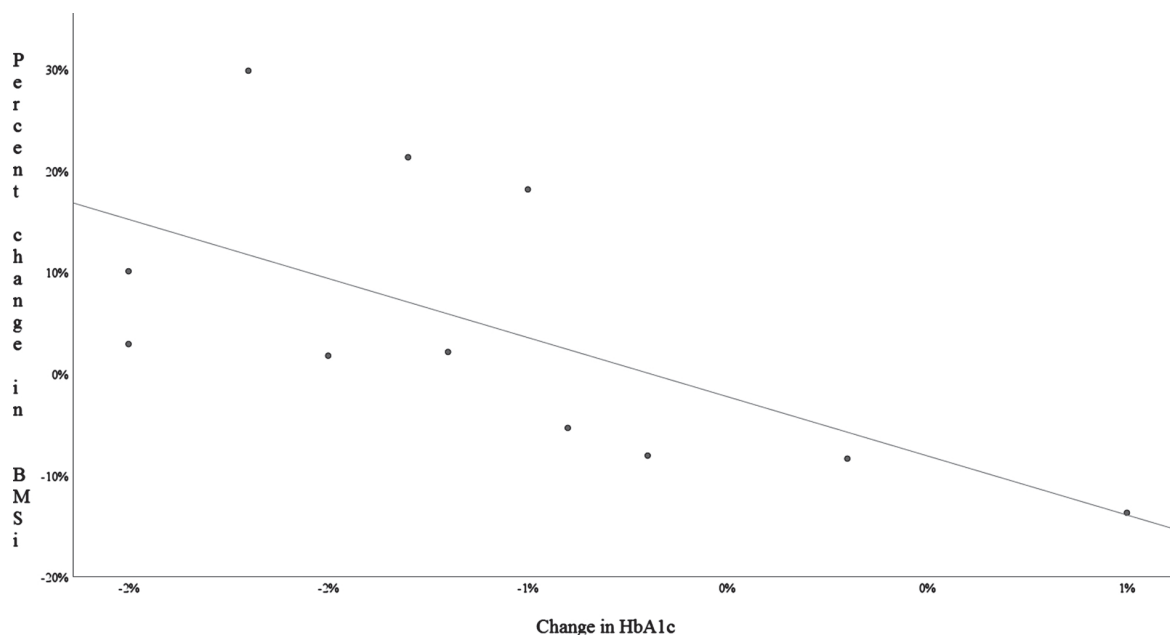


Fig. 2. Association between change in HbA<sub>1c</sub> and percent change in bone material strength index (BMSi) one year after Roux-en-Y gastric bypass (RYGB) in 11 participants with type 2 diabetes (T2D). A larger decrease in HbA<sub>1c</sub> was associated with a higher increase in bone material strength measured by impact micro-indentation (OsteoProbe®);  $\beta_{\text{unadjusted}}$  -9.2 (-16.5 to -1.9),  $R^2 = 0.47$ ,  $p = 0.019$ .

premenopausal women  $\beta_{\text{unadjusted}}$  -0.073 (-0.11 to -0.032),  $R^2 = 0.42$ ,  $p = 0.001$ . However, this was no longer significant when adjusted for age and preoperative aBMD ( $\beta_{\text{adjusted}}$  -0.031 (-0.78 to 0.017),  $R^2 = 0.66$ ,  $p = 0.19$ ). The aBMD loss in the lumbar spine, femoral neck, total hip, and total body did not differ statistically between participants with and without T2D (Table 2).

#### 4. Discussion

##### 4.1. Bone material strength after RYGB

We conducted a prospective cohort study evaluating skeletal health after RYGB. This is the first study to describe *in vivo* measurements of cortical bone material strength in a bariatric surgery population, and changes induced after RYGB. We observed that for participants with morbid obesity, BMSi was inversely associated to BMI, and the mean BMSi increased one year after RYGB. Our findings support the hypothesis that higher BMI is associated with decreased bone material strength, and implicate that surgically induced weight loss has a positive effect on bone quality. Studies comparing patients with and without fragility fractures have described 4–4.5% lower bone material strength in the fracture population [33,34]. Our observed increase of 6.3% therefore seems clinically important.

The relative preservation of lean mass, maintenance of calcium, vitamin D homeostasis, and the increased physical activity noted in our population might contribute to the observed improvement in bone material strength. Our findings are in line with a study reporting that high-intensity loading leads to increased bone material strength [35]. The presented finding of improved bone material strength after RYGB challenges preceding studies describing failure load to decrease or remain unchanged one year after RYGB [20,22,24]. Studies reporting failure load, however, are based on microfinite element analysis of microstructure images (HRpQCT) and not *in vivo* bone material strength measurements, and are thus not directly comparable.

##### 4.2. Bone material strength measurements in a bariatric population

The use of the impact microindentation to evaluate bone material

strength in humans is a relatively new method, and our study the first to use this technique in a bariatric population. Potential biases of obesity and weight loss on BMSi measurements remains to be explored. In a study of more than 200 elderly women, Sundh et al observed a negative correlation between tibial subcutaneous fat and BMSi [11]. Whether this finding represents a bias due to larger amounts of pretibial subcutaneous fat on the BMSi measurements, or a direct negative effect of local adipose tissue on bone material strength, remains unknown. In this context, we analyzed the relation between the lower extremity fat assessed by DXA and BMSi prior to and one year after RYGB. We found no correlation, be it at baseline ( $r^2 = 0.006$ ) or one year post-operatively ( $r^2 = 0.020$ ). Notably, these results should be interpreted with caution as they are based on measurements of entire lower extremity fat and not solely pretibial fat.

Although preoperative BMSi values were not correlated with post-operative BMSi values, preoperative BMSi were negatively associated with delta BMSi values, implying that participants with higher preoperative BMSi values experienced smaller increase or decrease in BMSi values after RYGB. Individual difference in BMSi response to intervention should be emphasized in future studies.

##### 4.3. Bone material strength and type 2 diabetes

Studies have shown that subjects with T2D have lower bone material strength compared to controls [12,13]. We did not observe any difference between preoperative BMSi values, or changes of bone material strength after RYGB in participants with and without T2D. Nevertheless, the association between the decrease in HbA<sub>1c</sub> and the improvement in bone material strength, observed in participants with T2D supports the notion that glucose control influences bone health. A larger sample size might have identified relevant differences not identified in our series.

##### 4.4. Bone mineral density changes after RYGB

RYGB induces a large and rapid weight loss that is accompanied by an increased bone turnover and reduction in bone mineral density. Our findings are in line with other evaluations of bone turnover and aBMD



after RYGB [18–24]. Notably, studies comparing changes in volumetric BMD (QCT) and aBMD (DXA) after bariatric surgery may be affected by technical reproducibility issues that in part may be related to imaging artifacts in the setting of morbid obesity and large weight loss [18,21,22]. Earlier studies comparing aBMD changes after RYGB in participants with and without T2D have been conflicting. In their pilot study, Schafer et al showed a non-significantly larger aBMD decrease in total hip in participants with T2D than controls six months after RYGB [19]. However, in the final study, with a 2 fold higher number of participants, this finding was not reproduced. Actually, they noted that T2D participants had a smaller femoral neck aBMD loss than participants without T2D, with similar trends in the lumbar spine and total hip [23]. Our study is the first to compare aBMD changes in participants with and without T2D one year after RYGB, and we observed comparable changes in aBMD. Our study diverges from previous studies as both men and women are included.

#### 4.5. Long-term bone quality changes after RYGB

The presented improvements in bone material strength are based on early findings post RYGB. Studies evaluating participants in the years following weight stabilization have noted persistent elevated bone turnover markers, continued bone loss, and estimated failure load decrease [20,24], corresponding to an increased fracture rate observed by recent studies [25–27]. In light of this knowledge, it is unlikely that bone material strength continues to increase in the years following weight stabilization. However, this is beyond the scope of our study and remains to be further explored.

#### 4.6. Strengths and limitations

Strengths of our study include the use of in vivo measurement of tissue level bone material strength of cortical bone, and a low rate of participants lost to follow-up. However, the study is limited by the restricted duration of follow-up, limited number of participants, and lack of a control group. The evaluation of unopposed penetration of tissue overlying the bone surface was based on tactile sensations of the investigator. The introduction of a new DXA machine during the study could affect the aBMD results, albeit probably insignificantly, as proper cross calibration was performed. Twenty-three of the participants had their preoperative examination on the GE Lunar Prodigy and the follow-up with the GE Lunar iDXA, and eleven had both with the iDXA. Patients with diabetes were encouraged to participate in the study, thus the fraction of participants with diabetes in this study (38%) exceeds the fraction in patients seeking RYGB (25–30 %) at our institution [36].

#### 5. Conclusion

In conclusion, our study shows that a higher BMI is associated with a lower bone material strength in a morbid obese population before RYGB. One year after RYGB, we observed improved bone material strength despite, an increase in bone turnover and decrease in aBMD. Bone changes were comparable in participants with and without T2D, however, improved glucose control was associated with improved bone material strength in participants with T2D.

#### Declaration of Competing Interest

Blom-Høgestøl IK: Nothing to declare  
Mala T: Nothing to declare  
Kristinsson JA: Nothing to declare  
Brunborg C: Nothing to declare  
Gulseth HL: Nothing to declare

Eriksen EF: Consults for Shire, Amgen, Ascendis, Lilly, Novartis and Merck, has received grant support from Shire and lectures for Lilly, Novartis, Amgen and Takeda.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.bone.2019.115069>.

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# Changes in Bone Marrow Adipose Tissue One Year After Roux-en-Y Gastric Bypass: A Prospective Cohort Study

Ingvild Kristine Blom-Høgestøl,<sup>1,2</sup> Tom Mala,<sup>1,3</sup> Jon A Kristinsson,<sup>1,3</sup> Ellen-Margrethe Hauge,<sup>4</sup> Cathrine Brunborg,<sup>5</sup> Hanne Løvdal Gulseth,<sup>1,6</sup> and Erik Fink Eriksen<sup>1,2</sup>

<sup>1</sup>Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway

<sup>2</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway

<sup>3</sup>Department of Gastrointestinal Surgery and Paediatric Surgery, Oslo University Hospital, Oslo, Norway

<sup>4</sup>Department of Rheumatology, Aarhus University Hospital, and Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

<sup>5</sup>Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway

<sup>6</sup>Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health, Oslo, Norway

## ABSTRACT

Bone marrow adipose tissue (BMAT) has been postulated to mediate skeletal fragility in type 2 diabetes (T2D) and obesity. Roux-en-Y gastric bypass (RYGB) induces a substantial weight loss and resolution of comorbidities. However, the procedure induces increased bone turnover and fracture rates. No previous study has evaluated biopsy-measured BMAT fraction preoperatively and after RYGB. In this study, we aimed to investigate BMAT fraction of the hip in participants with and without T2D preoperatively and 1 year after RYGB and explore factors associated with BMAT change. Patients with morbid obesity scheduled for RYGB were examined preoperatively and 1 year after RYGB. Forty-four participants were included and preoperative examinations were possible in 35. Of these, 33 (94%) met for follow-up, 2 were excluded, and BMAT estimation was not possible in 1. Eighteen (60%) of the participants were females and 11 (37%) had T2D. Preoperative BMAT fraction was positively associated with glycosylated hemoglobin and negatively associated with areal bone mineral density (aBMD). After RYGB, BMAT fraction decreased from  $40.4 \pm 1.7\%$  to  $35.6 \pm 12.8\%$ ,  $p = 0.042$ , or with mean percent change of 10.7% of preoperative BMAT fraction. Change in BMAT fraction was positively associated with change in body mass index (BMI) and total body fat. In females, we observed a mean percent reduction of  $22.4 \pm 19.6\%$ , whereas in males BMAT increased with a mean percent of  $6.8 \pm 37.5\%$ ,  $p = 0.009$ . For males, changes in estradiol were associated with BMAT change; this was not observed for females. In participants with and without T2D, the mean percent BMAT reduction was  $5.8 \pm 36.9\%$  and  $13.5 \pm 28.0\%$ , respectively,  $p = 0.52$ . We conclude that a high BMAT seems to be associated with lower aBMD and poorer glycemic control in obese subjects. After RYGB, we observed a significant decrease in BMAT. The reduction in BMAT did not differ between participants with and without T2D, but appeared sex specific. © 2019 The Authors. *Journal of Bone and Mineral Research* Published by Wiley Periodicals, Inc.

**KEY WORDS:** MORBID OBESITY; ROUX-EN-Y GASTRIC BYPASS; WEIGHT LOSS; BONE MARROW ADIPOSE TISSUE; BONE MARROW FAT; BONE MINERAL DENSITY

## Introduction

The gradual replacement of the hematopoietic bone marrow with bone marrow adipose tissue (BMAT) starts in the appendicular skeleton during childhood and continues to increase with age.<sup>(1)</sup> In early adulthood, males seem to have a higher BMAT fraction; however, after menopause, this sex difference appears reversed.<sup>(2,3)</sup> BMAT has been considered an inactive fat depot but was recently recognized as an endocrine organ with local and systemic effects.<sup>(4)</sup> Increased BMAT fraction has been associated with increased fracture rates in conditions like anorexia nervosa and postmenopausal and idiopathic osteoporosis.<sup>(5–7)</sup> Although the function of BMAT remains to be fully understood, it has been postulated that BMAT plays a role in lipid storage, metabolic

homeostasis, hematopoietic regulation, mechanical function, thermogenesis, skeletal remodeling, and fragility.<sup>(8)</sup> BMAT may be quantified histologically based on examinations of bone marrow biopsies or by imaging modalities like magnetic resonance spectroscopy (MRS). One study has published acceptable correlations between lumbar spine BMAT, evaluated by MRS, and posterior superior iliac spine BMAT, estimated from bone marrow biopsy. However, this study was performed in lean premenopausal women and MRS was noted to report approximately 10% higher BMAT fraction.<sup>(9)</sup> Thus potential artifacts of obesity and weight loss are yet to be explored for noninvasive radiological methods of BMAT estimation.

Obesity and type 2 diabetes (T2D) are associated with increased fracture risk despite a normal areal bone mineral density

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Address correspondence to: Ingvild K Blom-Høgestøl, MD, Lilleåsen 15C, 1396 Billingstad, Norway. E-mail: ingvild.hogestol@gmail.com

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(aBMD).<sup>(10–12)</sup> Increased BMAT may be a contributing mediator of this skeletal fragility. Positive associations have been described between glycosylated hemoglobin (HbA<sub>1c</sub>) and BMAT<sup>(13,14)</sup> and visceral/subcutaneous/total fat and BMAT.<sup>(14,15)</sup> However, studies diverge with regard to whether subjects with T2D<sup>(13,14,16)</sup> or obesity<sup>(17)</sup> have relatively more BMAT than controls.

Roux-en-Y gastric bypass (RYGB) is offered to patients with morbid obesity.<sup>(18)</sup> RYGB induces a large and persistent weight loss and remission of obesity-related comorbidities, most notably T2D.<sup>(19,20)</sup> On the other hand RYGB appears to induce bone loss and increase bone turnover and fracture rates.<sup>(21–24)</sup>

Studies indicate that a diet-induced weight loss is accompanied by a 1.1% to 3.5% reduction in MRI/MRS-estimated BMAT.<sup>(25,26)</sup> Unexpectedly, studies evaluating BMAT with MRS 6 to 12 months after RYGB did not note any change.<sup>(27,28)</sup> However, one of the studies reported that the subpopulation of subjects with preoperative T2D experienced a 6.5% decline in BMAT.<sup>(27)</sup> To our knowledge, no previous study has evaluated bone marrow biopsy-measured BMAT preoperatively and 1 year after RYGB.

We aimed to explore potential changes in BMAT after RYGB and search for possible associated factors. Specifically, we wanted to investigate if such changes in BMAT fraction differed in participants with and without T2D. Secondly, we wanted to explore factors associated with BMAT, including age, sex, sex steroids, menopausal status, metabolic homeostasis (glycemic control, blood lipid levels, and T2D), body mass index (BMI) and body composition, bone mineral density, and bone turnover, in a morbidly obese population. We hypothesized that the BMAT fraction would decrease after RYGB and that participants with T2D would have a larger decrease in BMAT fraction than participants without T2D.

## Materials and Methods

### Study population

Patients scheduled for RYGB at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral center for treatment of morbid obesity, were recruited. Eligibility criteria for RYGB were BMI  $\geq 40$  kg/m<sup>2</sup> or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity-related comorbidity, aged 18 to 65 years, and failed attempts of sustained weight loss.<sup>(29)</sup> Patients with T2D were encouraged to participate. Participants were included from October 8, 2015 to January 27, 2017. Participants were excluded if they were unable to read Norwegian language or if they had severe psychiatric comorbidity, connective tissue disorders, or other hormonal diseases, kidney failure (glomerular filtration rate  $< 30$  mL/min/1.73 m<sup>2</sup>), type 1 diabetes, BMI  $> 47$  kg/m<sup>2</sup>, history of treatment with bone active substances (bisphosphonates, denosumab, hormone replacement, or parathyroid hormone), or if they were currently receiving anticoagulation or steroid treatment (estrogen, testosterone, or glucocorticoids). To avoid heterogeneity in our study population, non-whites were excluded.

### Surgery, study visits, and follow-up

A laparoscopic RYGB with a gastric pouch of about 25 mL, a 150-cm antecolic alimentary, and a 50-cm biliopancreatic limb was performed in all participants.<sup>(30)</sup> Participants attended study visits preoperatively and 1 year after RYGB. Study visits included morning fasting blood samples, anthropometric measures, dual-energy absorptiometry (DXA) scan, impact microindentation, intravenous glucose tolerance test, euglycemic hyperinsulinemic

clamp, indirect calorimetry, and bone marrow biopsy. In this article, the results of the blood samples, anthropometric measures, DXA, and bone marrow biopsy are presented. Baseline characteristics with comparison of participants with and without T2D and changes in impact microindentation, aBMD, and bone turnover markers will be published separately. The sex distribution and mean preoperative BMI was comparable among participants with and without T2D.

The participants also attended routine clinical follow-up with visits 6 weeks, 6 months, and 1 year after surgery. After surgery, all participants were advised and prescribed oral supplementation with 1000 mg calcium, 800 IU vitamin D, one multivitamin, 200 mg iron daily, and B12 injections 1 mg every third month. At routine clinical visits, vitamin levels were monitored and additional supplements were advised on demand.

### Bone marrow biopsies

Preoperative and follow-up bone marrow biopsies were taken from the right posterior superior iliac spine (except for in one participant where both biopsies were taken from the left) after injection of local anesthesia. The posterior superior iliac spine was identified by palpation. Bone marrow biopsies were obtained using an 8G T-LokTM Jamshidi crista biopsy needle from Argon Medical Devices (Stenløse, Denmark), fixed in 70% ethanol directly, and stored at 4 °C. For histological analysis, the biopsies were embedded undecalcified in methylmetacrylate.<sup>(31)</sup> After embedding, 7- $\mu$ m sections were cut using a Jung microtome model K (R Jung GmbH, Heidelberg, Germany) equipped with a tungsten knife. To achieve a largest possible area, the biopsies were cut through the middle. Then two levels were cut with a distance of 100  $\mu$ m. These sections were stained with Masson Goldner Trichrome. BMAT fraction was quantified as adipocyte volume (AdV) relative to marrow volume (MarV) using grid-based point-counting. Grid sizes were 0.03 mm<sup>2</sup> and 0.06 mm<sup>2</sup>, where the smaller grid was used for lower BMAT fractions and the larger for higher BMAT fractions. We used a light microscope (Nikon Eclipse 80I, Tokyo, Japan) equipped with a motorized specimen stage (Prior Proscan 11, Rockland, MA, USA) and a digital video camera (Olympus DP72, Tokyo, Japan) connected to a PC running the NewCast interactive stereology software (Visiopharm, Hørsholm, Denmark). The estimates were performed at  $\times 230$  magnification. The presented BMAT fraction is the mean of the estimated AdV/MarV from two levels of the biopsy. Biopsies obtained preoperatively and 1 year after RYGB were processed and analyzed in batches by one lab technician blinded for all clinical data. Coefficient of variation was calculated by recounting 5 randomly selected biopsies, and the mean value was 2.8% and 3.3% preoperatively and 1 year after RYGB, respectively.

### Bone mineral density

DXA scans, including whole-body scans, were performed for assessment of body composition, including whole-body fat and lean mass. aBMD, g/cm<sup>2</sup>, of the lumbar spine (L<sub>1</sub> to L<sub>4</sub>), hip, proximal femur, and total body were assessed. The lumbar vertebra with the lowest aBMD was used in the analysis. All scans were performed by the same nurse. GE Lunar Prodigy was used until August 26, 2016; from then on, GE Lunar iDXA was used. Body composition performed with GE Lunar Prodigy was reanalyzed with iDXA software to optimize comparability. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine

(L<sub>1</sub> to L<sub>4</sub>) intra-class correlation coefficient (ICC) (95% confidence interval [CI]) of 0.989 (0.968 to 0.996), and for femoral neck and total hip, ICC (95% CI) was 0.994 (0.982 to 0.998) and 0.996 (0.988 to 0.999), respectively. The DXA machine was calibrated daily against the standard calibration phantom, supplied by the manufacturer, and the estimated short-term precision errors for aBMD at the lumbar spine and at the femoral neck was <1.0%.

### Blood samples

All blood samples were taken before 10 a.m. after an overnight fast. Serum for bone turnover markers (CTX-1 and PINP) was centrifuged and stored at -80 °C and analyzed after study follow-up was completed to avoid interassay variation. All other study blood sample analyses were made shortly after retrieval.

The Hormone Laboratory, Oslo University Hospital, analyzed carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP) using Roche (Mannheim, Germany) electrochemiluminescence immunoassay (ECLIA). Serum 25(OH) vitamin D and testosterone levels were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method; PTH by Immulite 2000 XPI (Siemens Healthineers, Erlangen, Germany), a noncompetitive chemiluminometric assay; FSH using Immulite 2000 XPI (Siemens Healthineers), a noncompetitive immunoluminometric assay; and estradiol with a competitive immunoluminometric assay Liaison XL kit from DiaSorin Inc. (Stillwater, MN, USA). The Central Laboratory of Oslo University Hospital analyzed HbA<sub>1c</sub> using Tosoh G8 high-performance liquid chromatography, and total cholesterol, low-density lipoprotein cholesterol, and triglycerides were analyzed with a Cobas 6000 from Roche using an enzymatic colorimetric method.

### Clinical parameters

Morbid obesity was defined as BMI  $\geq 40$  kg/m<sup>2</sup> or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity-related comorbidity.<sup>(29)</sup> T2D was defined as HbA<sub>1c</sub>  $\geq 6.5\%$  or use of one or more oral glucose-lowering drug with or without insulin treatment. Diabetes remission was defined as HbA<sub>1c</sub>  $< 6.5\%$  without the use of glucose-lowering drugs in participants with T2D preoperatively. Hypercholesterolemia was defined as low-density lipoprotein cholesterol  $\geq 3$  mmol/L or use of statins. All fractures except digit fractures are reported. Hormonal intrauterine devices made clinical evaluation of menstrual cycle difficult. For this reason, a postmenopausal status was defined as a serum follicle-stimulating hormone (FSH)  $\geq 25$  IU/L.<sup>(32)</sup>

### Study size

This was an explorative study and the first to evaluate BMAT fraction in bone marrow biopsy preoperatively and 1 year after RYGB. However, sample size estimation was performed using BMAT as the primary endpoint based on data from a previous study evaluating change in BMAT after teraparotide treatment.<sup>(33)</sup> Given a mean change in BMAT of 5.5% between baseline and follow-up with an estimated standard deviation of 9.0%, type I error of 5%, and power of 90%, a total of 31 participants should be included. Additional 10% was added to account for possible technical difficulties with bone marrow biopsies and loss to follow-up.

### Statistical analysis

Normally distributed continuous variables are presented as mean  $\pm$  SD; others are presented as median (range).

Categorical data are presented as proportions (percentage). When comparing preoperative sex characteristics, independent sample *t* test or Mann-Whitney *U* test was used for continuous variables. Pearson chi-square or Fisher's exact test was used for categorical variables as appropriate. Intraclass correlation coefficient (ICC) with 95% CI was used to assess concordance between the two DXA scanners (GE Lunar Prodigy and GE Lunar iDXA). ICC values of 0.75 or higher were considered excellent.<sup>(34)</sup> For evaluation of changes from pre- to post-RYGB, paired-sample *t* tests or Wilcoxon signed-rank tests were used. To explore differences in changes from pre- to post-RYGB between subgroups, delta values were compared with independent sample *t* test or Mann-Whitney *U* test. Adjustments for confounding factors were performed using multiple linear regression analyses. Only variables with significant relationships with both the exposure and the outcome variables were considered as possible confounders in addition to variables of known clinical importance. Possible confounding variables were age, sex, BMI, and preoperative BMAT fraction. Confounders that correlated,  $r > 0.7$ , were not adjusted for in order to avoid multicollinearity. The results from the regression analyses are presented as regression coefficients ( $\beta$ ) with 95% CI. Two-tailed *p* values  $< 0.05$  were considered statistically significant. All statistical analyses were made using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY, USA).

### Ethics

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Northern Norway Regional Committee for Medical and Health Research Ethics 2015/604. Written informed consent was obtained from all participants.

## Results

A total of 44 participants were included. Preoperative bone marrow biopsies were possible in 35, and 33 of these (94%) met for the 1-year follow-up visit. Two participants were excluded at follow-up due to sex reassignment and glucocorticoid treatment, respectively. BMAT fraction was not possible to estimate in one biopsy at 1-year follow-up. Thus the study population ultimately consisted of 30 participants.

### Preoperative participant characteristics

Preoperative characteristics are presented in Table 1. Males had a higher body weight, more lean mass, and a higher fraction reported previous fractures compared with females. Eleven participants had T2D, median duration since diagnosis of T2D was 5 years (range 1 to 18 years), and a mean preoperative HbA<sub>1c</sub> of  $6.9 \pm 0.70\%$ . Eight (73%) were treated with oral glucose-lowering drugs and one with oral glucose-lowering drugs and insulin. BMAT fractions in participants with and without T2D were  $43.3 \pm 10.8\%$  and  $38.7 \pm 8.1\%$ , respectively,  $p = 0.20$ . Preoperative BMAT fraction was positively associated with HbA<sub>1c</sub>; this association remained significant after adjustment for sex. Both lumbar spine and femoral neck aBMD were negatively associated with BMAT fraction, but only the association between lumbar spine aBMD and BMAT fraction remained significant after adjustment for age and sex (Table 2).

**Table 1.** Preoperative Characteristics in 30 Participants With Morbid Obesity Scheduled for Roux-en-Y Gastric Bypass

	All subjects N = 30	Females n = 18	Males n = 12
Age (years)	46.3 ± 9.6	44.8 ± 8.5	48.5 ± 11.1
Postmenopausal		8 (44%)	
Smoking, current or previous	18 (60%)	11 (61%)	7 (58%)
Any previous fracture	17 (57%)	7 (39%)	10 (83%)*
Type 2 diabetes	11 (37%)	7 (39%)	4 (33%)
Hypercholesterolemia	10 (33%)	5 (28%)	5 (42%)
Weight (kg)	120.1 ± 15.3	113.2 ± 11.7	130.4 ± 14.5*
Body mass index (kg/m <sup>2</sup> )	40.7 ± 3.6	41.6 ± 3.3	39.4 ± 3.6
Total body fat (kg)	54.5 ± 8.5	56.2 ± 8.0	54.4 ± 9.4
Total body lean mass (kg)	61.8 ± 11.5	54.3 ± 4.4	71.8 ± 9.0*
Systolic blood pressure (mmHg)	126.0 ± 11.8	125.4 ± 10.0	126.9 ± 13.5
Diastolic blood pressure (mmHg)	82.0 ± 7.8	82.3 ± 6.4	81.4 ± 9.8
HbA <sub>1c</sub> (%)	6.0 ± 0.83	5.9 ± 0.66	6.1 ± 1.1
Total cholesterol (mmol/L)	4.3 ± 0.73	4.5 ± 0.63	4.0 ± 0.79
LDL cholesterol (mmol/L)	2.8 ± 0.69	2.9 ± 0.64	2.6 ± 0.78
Triglyceride (mmol/L)	1.5 ± 0.64	1.4 ± 0.63	1.6 ± 0.64
25(OH) vitamin D (nmol/L)	56.1 ± 20.2	55.4 ± 19.8	57.2 ± 21.7
Parathyroid hormone (pmol/L)	4.7 ± 2.0	4.6 ± 1.8	4.8 ± 2.3
Areal bone mineral density (g/cm <sup>2</sup> )			
Lumbar spine	1.11 ± 0.13	1.13 ± 0.12	1.09 ± 0.15
Femoral neck	1.08 ± 0.12	1.09 ± 0.12	1.07 ± 0.12
Total hip	1.16 ± 0.12	1.18 ± 0.13	1.13 ± 0.11
Total body	1.33 ± 0.087	1.31 ± 0.090	1.35 ± 0.081
Bone marrow adipose tissue (%)	40.4 ± 9.3	39.4 ± 9.9	41.9 ± 8.4
CTX-1 (µg/L)	0.34 ± 0.14	0.31 ± 0.13	0.39 ± 0.15
P1NP (µg/L)	47.3 ± 20.7	44.0 ± 18.4	52.2 ± 23.7
Testosterone (nmol/L)		0.59 ± 0.35	13.8 ± 4.1*
Estradiol (nmol/L)		0.11 ± 0.14	0.078 ± 0.030

HbA<sub>1c</sub> = glycosylated hemoglobin; LDL = low-density lipoprotein; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Normally distributed continuous variables are presented as mean ± SD; others are presented as median (range). Categorical data are presented as proportions (percentage).

Any fracture except digit fractures are reported.

\*Significant difference between sexes,  $p < 0.05$ .

### Participant characteristics after RYGB

All participants lost weight after RYGB. Mean weight loss was  $32.6 \pm 10.8$  kg or  $27.2 \pm 8.7\%$  of total preoperative weight. Fat mass decreased with  $26.2 \pm 9.4$  kg, and lean mass decreased with  $6.2 \pm 2.6$  kg, all  $p < 0.001$ . Females lost  $12.4 \pm 4.4$  units of BMI (kg/m<sup>2</sup>), whereas males lost  $9.1 \pm 3.0$  units of BMI (kg/m<sup>2</sup>),  $p = 0.032$ ; however, this sex difference was no longer significant when adjusting for preoperative BMI. Weight loss in kg and decrease in lean mass and fat mass were not different between males and females. One year after RYGB, aBMD decreased with  $4.3 \pm 5.9\%$  in the lumbar spine,  $8.2 \pm 4.8\%$  in the femoral neck,  $11.8 \pm 4.9\%$  in total hip, and  $9.4 \pm 3.9\%$  in total body. Of the 11 participants with T2D preoperatively, all except 1 were in diabetes remission 1 year after RYGB and the mean HbA<sub>1c</sub> decrease was  $1.1 \pm 0.76\%$  1 year after RYGB. BMAT fraction decreased from  $40.4 \pm 1.7\%$  preoperatively to  $35.6 \pm 12.8\%$  at follow-up,  $p = 0.042$ , or with mean percent change of 10.7% of preoperative BMAT fraction (Fig. 1). Example of a bone marrow biopsy taken preoperatively and 1 year after RYGB from the same participant is shown in Fig. 2.

Serum testosterone levels increased in males with a mean  $4.7 \pm 3.4$  nmol/L,  $p < 0.001$ , and serum estradiol levels decreased by  $0.025 \pm 0.036$  nmol/L,  $p = 0.035$ . In postmenopausal females,

similar trends, although nonsignificant, were observed; serum testosterone increased  $0.14 \pm 0.20$  nmol/L,  $p = \text{n.s.}$ , and serum estradiol decreased  $0.070 \pm 0.097$  nmol/L,  $p = \text{n.s.}$  In premenopausal females, we observed nonsignificant decreases in both serum testosterone and serum estradiol;  $0.078 \pm 0.19$  nmol/L and  $0.024 \pm 0.12$  nmol/L,  $p = \text{n.s.}$  for both.

### Factors associated with changes in BMAT fraction after RYGB

In females, BMAT fraction changed from  $39.4 \pm 9.9\%$  preoperatively to  $30.1 \pm 9.0\%$  at follow-up,  $p < 0.001$ , or with mean percent change of  $22.4 \pm 19.6\%$  of the preoperative BMAT fraction. In males, BMAT fraction changed from  $41.9 \pm 8.4\%$  preoperatively to  $43.7 \pm 13.8\%$  at follow-up corresponding to a mean percent change of  $6.8 \pm 37.5\%$  of the preoperative BMAT,  $p = \text{n.s.}$  The mean between-group difference (95% CI) was  $-11.1$  ( $-19.8$  to  $-2.4$ ),  $p = 0.014$ . This difference remained significant after adjusting for age and preoperative BMAT fraction and BMI. Five of the 12 males demonstrated an increase in BMAT fraction after RYGB and 7 a decreased or unchanged BMAT fraction (Fig. 1). Males who increased in BMAT fraction had a



**Table 2.** Factors Associated With Bone Marrow Adipose Tissue Fraction in 30 Subjects With Morbid Obesity Scheduled for Roux-en-Y Gastric Bypass

	Unadjusted			Adjusted		
	$\beta$	95 % CI for $\beta$	<i>p</i> Value	$\beta$	95 % CI for $\beta$	<i>p</i> Value
Female sex	-2.5	-9.6 to 4.7	0.48			
Postmenopausal	6.0	0.21 to 15.8	0.21			
Age	0.286	-0.072 to 0.64	0.11			
Type 2 diabetes	4.6	-2.5 to 11.7	0.20			
Hypercholesterolemia	4.7	-2.6 to 12.0	0.20			
<b>HbA<sub>1c</sub></b>	<b>4.4</b>	<b>0.41 to 8.3</b>	<b>0.032</b>	<b>4.3</b>	<b>0.22 to 8.3</b>	<b>0.039</b>
Body mass index	-0.93	-1.87 to 0.013	0.053			
Total body fat mass	-0.44	-0.83 to -0.055	0.078			
Total body lean mass	0.025	-0.29 to 0.34	0.83			
<b>Lumbar spine aBMD</b>	<b>-31.1</b>	<b>-55.1 to -7.1</b>	<b>0.013</b>	<b>-27.5</b>	<b>-52.8 to -2.3</b>	<b>0.034</b>
<b>Femoral neck aBMD</b>	<b>-30.8</b>	<b>-58.4 to -3.2</b>	<b>0.030</b>	<b>-25.7</b>	<b>-58.7 to 7.2</b>	0.12
Total hip aBMD	-23.2	-51.3 to 4.9	0.10			
Total body aBMD	-18.3	-58.9 to 22.3	0.36			
aBMD <i>T</i> -score <-1.0	8.0	-0.23 to 16.3	0.056			
CTX-1	18.0	-6.4 to 42.5	0.14			
PINP	0.025	-0.15 to 0.20	0.77			

HbA<sub>1c</sub> = glycosylated hemoglobin; aBMD = areal bone mineral density; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Linear regression; HbA<sub>1c</sub> was adjusted for sex. Lumbar spine and femoral neck aBMD were adjusted for age and sex. The results from the regression analysis are presented as regression coefficients ( $\beta$ ) with 95 % confidence intervals (CI).

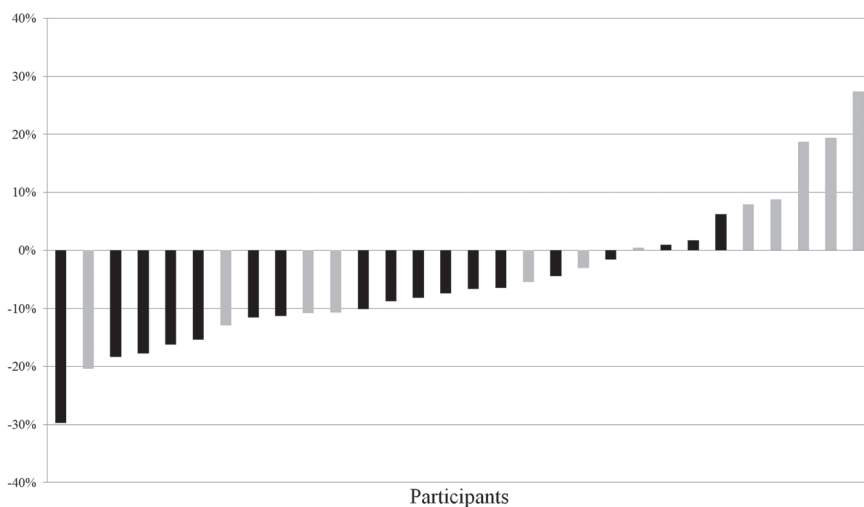
mean preoperative BMAT fraction of  $36.4 \pm 5.5\%$ , compared with  $45.8 \pm 8.2\%$  for the remaining males,  $p = 0.051$ .

The mean BMAT fraction decreased from  $43.3 \pm 10.9\%$  to  $40.3 \pm 15.3\%$  in participants with preoperative T2D and from  $38.7 \pm 8.1\%$  to  $32.8 \pm 10.7\%$  in participants without T2D; the changes in BMAT fraction were comparable between the two groups. No associations were observed between changes in BMAT and changes in HbA<sub>1c</sub>, aBMD (all measured regions) or bone turnover markers (Table 3).

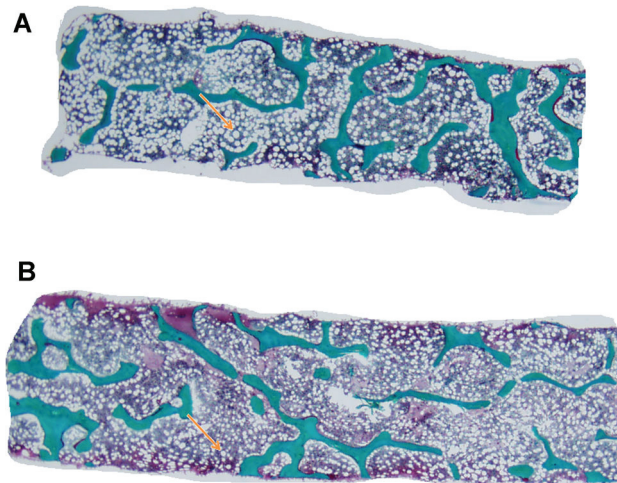
Participants who lost more BMI units or decreased more in total body fat mass decreased more in BMAT fraction, and this

remained significant after adjusting for sex, preoperative BMI, and BMAT fraction. Lower preoperative BMAT fraction was associated with smaller changes in BMAT after RYGB, and this remained significant after adjusting for age, sex, and preoperative BMI (Table 3).

In males, we noted an association between changes in serum estradiol levels and change in BMAT fraction (Fig. 3). This association remained significant after adjustment for age. In females, no association between change in serum estradiol levels and BMAT fraction was noted. Postmenopausal females revealed a mean percent decrease in BMAT comparable to that



**Fig. 1.** Individual percent changes in bone marrow adipose tissue (BMAT) fraction in 30 participants after Roux-en-Y gastric bypass (RYGB). Female participants are marked with black bars and male participants are marked with gray bars.



**Fig. 2.** Bone marrow biopsy from one participant preoperatively (A) and 1 year after Roux-en-Y gastric bypass (RYGB) (B). Body mass index preoperative and 1 year after RYGB was 39.8 kg/m<sup>2</sup> and 25.1 kg/m<sup>2</sup>, respectively. Bone marrow adipose tissue fraction preoperatively was 41.1 % and 1 year after RYGB was 20.7 %. Arrows indicate bone marrow adipose tissue.

of premenopausal females, 18.8 ± 18.0% and 25.3 ± 21.3%, respectively,  $p = 0.50$ .

## Discussion

This is the first study to describe BMAT fraction preoperatively and after RYGB assessed by bone marrow biopsies. We observed that BMAT decreased with a mean percent of 10.7% 1 year after RYGB, but there was no statistical difference in BMAT reduction in participants with and without preoperative T2D.

## Reductions in BMAT fraction

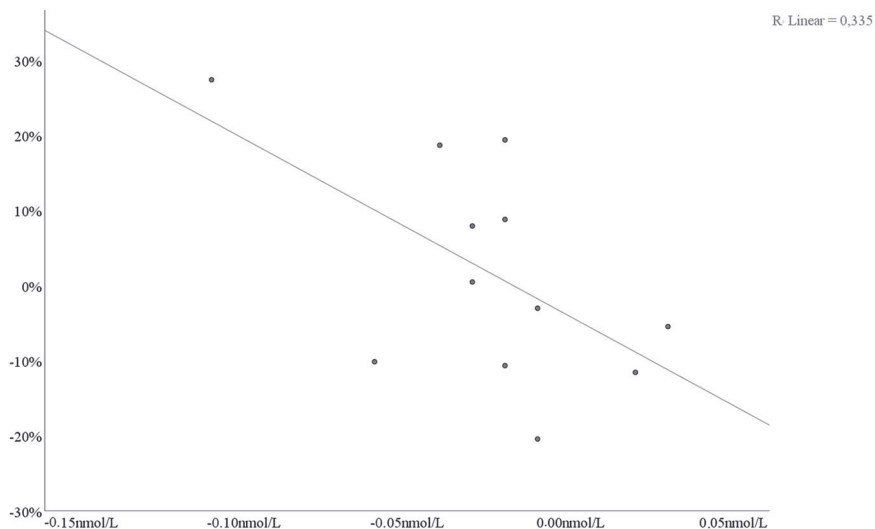
Studies have shown 15% to 26% higher BMAT fraction in osteoporotic subjects compared with controls.<sup>(6,7)</sup> Other studies indicate a 5.9% to 24% decrease in BMAT after osteoporosis treatment<sup>(33,35–37)</sup> and a 4% increase in BMAT after growth hormone treatment.<sup>(38)</sup> Although not directly comparable, our 10.7% decrease in BMAT fraction could be clinically relevant. Griffith and colleagues have previously reported that BMAT fraction may predict future aBMD loss,<sup>(39)</sup> giving the results possible clinical importance. Our results differ from the study from Bredella and colleagues, who observed a nonsignificant

**Table 3.** Factors Associated With Change in Bone Marrow Adipose Tissue (BMAT) Fraction 1 Year After Roux-en-Y Gastric Bypass

	Unadjusted			Adjusted		
	$\beta$	95 % CI for $\beta$	$p$ Value	$\beta$	95 % CI for $\beta$	$p$ Value
<b>Female sex</b>	<b>-11.1</b>	<b>-19.8 to -2.4</b>	<b>0.014</b>	<b>-8.4</b>	<b>-16.1 to -0.65</b>	<b>0.035</b>
Postmenopausal	2.2	-6.6 to 11.0	0.60			
Age	0.20	-0.30 to 0.70	0.42			
Type 2 diabetes (preoperative)	2.9	-6.8 to 12.7	0.54			
Hypercholesterolemia (preoperative)	0.078	-10.0 to 10.1	0.99			
<b>BMAT (preoperative)</b>	<b>-0.45</b>	<b>-0.94 to 0.045</b>	<b>0.074</b>	<b>-0.60</b>	<b>-1.1 to -0.12</b>	<b>0.017</b>
$\Delta$ HbA <sub>1c</sub>	-0.78	-8.5 to 6.9	0.84			
<b><math>\Delta</math> Body mass index</b>	<b>1.3</b>	<b>0.31 to 2.4</b>	<b>0.013</b>	<b>1.7</b>	<b>0.72 to 2.7</b>	<b>0.002</b>
<b><math>\Delta</math> Total body fat mass</b>	<b>0.50</b>	<b>0.027 to 0.98</b>	<b>0.039</b>	<b>0.59</b>	<b>0.14 to 1.0</b>	<b>0.013</b>
$\Delta$ Total body lean mass	0.77	-1.1 to 2.6	0.40			
$\Delta$ Lumbar spine aBMD	-3.9	-82.9 to 75.0	0.92			
$\Delta$ Femoral neck aBMD	-7.6	-103.6 to 88.4	0.87			
$\Delta$ Total hip aBMD	41.5	-58.7 to 141.6	0.40			
$\Delta$ Total body aBMD	-18.0	-113.7 to 77.8	0.70			
$\Delta$ CTX-1	3.4	-22.4 to 29.3	0.79			
$\Delta$ P1NP	-0.16	-0.41 to 0.098	0.22			

HbA<sub>1c</sub> = glycosylated hemoglobin; aBMD = areal bone mineral density; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Linear regression; female sex was adjusted for age and preoperative BMAT. BMAT (preoperative) was adjusted for age, sex, preoperative body mass index (BMI).  $\Delta$  body mass index was adjusted for age, sex, preoperative BMI, and preoperative BMAT.  $\Delta$  total body fat mass was adjusted for age, sex, and preoperative total body fat and BMAT. The results from the regression analysis are presented as regression coefficients ( $\beta$ ) with 95 % confidence intervals (CI).



**Fig. 3.** Changes in serum estradiol in males was negatively associated with changes in bone marrow adipose tissue (BMAT) fraction after Roux-en-Y gastric bypass (RYGB). Linear regression analysis shows a regression coefficient ( $\beta$ ) of  $-236.4$  with 95 % confidence intervals for  $\beta$   $-471.0$  to  $-1.8$ ,  $p = 0.049$ , indicating that for every 1 unit change in delta estradiol, the delta BMAT fraction decreased with 236.4. This finding remained significant after adjustment for age.

decrease in  $L_1$  to  $L_2$  BMAT, assessed by MRS, 1 year after RYGB in 11 patients.<sup>(28)</sup>

### BMAT and T2D

Participants with preoperative T2D had comparable BMAT fraction to participants without diabetes; however, we observed a significant association between preoperative BMAT and  $HbA_{1c}$ . This supports a potential association between glycemic control and BMAT in subjects with morbid obesity. One year after RYGB, all but one of the participants with preoperative T2D were in diabetes remission. Despite a high diabetes remission rate, we did not observe additional reductions in BMAT fraction in participants with preoperative T2D, as we had hypothesized. This observation is in contrast to the results described by Kim and colleagues, who report a significant difference in BMAT change after RYGB between participants with and without T2D and only observed a reduction in BMAT in participants with preoperative T2D.<sup>(27)</sup> Notably, Kim and colleagues included only women and a mix of ethnicities, whereas we included both sexes and only whites.

### BMAT and sex

Studies exploring groups with a large age span have not noted any sex differences in BMAT fraction.<sup>(7,40)</sup> However, a study limited to participants 30 to 60 years of age noted that males had 6% to 10% higher BMAT fraction compared with females.<sup>(2)</sup> Preoperatively, males and females in our study presented with comparable BMAT fractions. However, females lost more BMAT than males after RYGB. A sex-specific BMAT response to intervention has to our knowledge not been shown before. Notably, the majority of human studies evaluating BMAT response to interventions have been performed in females.

Our study is the first to show an association between change in endogenous serum estradiol and change in BMAT. Estrogen deficiency has been linked to higher BMAT fraction, and in

postmenopausal females, exogenous estradiol supplementation has been reported to reduce in BMAT.<sup>(36)</sup> Endogenous estradiol levels, however, have only been shown to be associated with BMAT in older males, where an inverse association was noted.<sup>(41)</sup> In line with this study, we observed an inverse association between change in estradiol and BMAT fraction in males only. In light of these findings, one might suspect that endogenous estradiol regulates BMAT in a sex-specific manner. However, pre- and postmenopausal females in our study had comparable serum estradiol values preoperatively and 1 year after RYGB. Study blood samples were drawn at random times during the menstrual cycle, confounding sex hormone interpretation in premenopausal subjects. Regardless, the lack of significant changes in serum estradiol levels in females is viewed a more likely explanation of why we did not observe any association between change in estradiol and change in BMAT fraction in these participants.

### BMAT fraction change, total body fat change, and preoperative BMAT fraction

An association between larger degree of caloric restriction in patients with anorexia nervosa and higher BMAT fraction has been reported.<sup>(42)</sup> This appears to contradict our findings of reduction in BMAT fraction after loss of 30% of total weight and 45% of total fat mass. This could support a hypothesis of a U-shaped association between total body fat and BMAT, where BMAT is elevated in circumstances of high or low total body fat and normalizes with normalization of total body fat. Participants with lower preoperative BMAT fraction experienced smaller decrease or increase in BMAT fraction after RYGB. Furthermore, male participants who increased in BMAT fraction after RYGB had a tendency of lower preoperative BMAT fraction when compared with those who decreased or experienced minimal changes. Individual differences in BMAT response to intervention should be the focus of future studies.

## BMAT and BMD

We observed that preoperative BMAT fraction was inversely associated with aBMD, in line with previous studies of subjects with morbid obesity<sup>(13)</sup> and subjects with increased fracture rates.<sup>(5–7)</sup> Interventional studies in osteoporotic pre- and postmenopausal women have shown an inverse association between change in aBMD and changes in BMAT fraction.<sup>(33,36,37)</sup> After RYGB, reductions in aBMD have been consistently observed,<sup>(21,22)</sup> in line with our findings. However, aBMD decreased in parallel to a decrease in BMAT fraction. This finding is in line with the subpopulation with T2D in the study by Kim and colleagues, who reported reductions of 6.5% in BMAT and 4.5% in volumetric BMD of the lumbar spine 6 months after RYGB,<sup>(27)</sup> but opposing the trends reported in studies evaluating treatment of osteoporosis.

## Strength and limitations

Strengths of our study include the use of bone marrow biopsies to evaluate BMAT fraction, inclusion of both sexes, and a low attrition rate. However, the study is limited by the restricted duration of follow-up. Because of limited sample size, the negative finding of no differences in BMAT fraction between participants with and without T2D should be interpreted with care. The area of bone marrow investigated histologically is smaller than the area sampled using MRS. Our intra-observer variation was lower than the detected difference between preoperative and postoperative BMAT fraction and the bone marrow biopsy technique standardized. However, we did not have data on BMAT fraction variation between two repeated bone marrow biopsies taken from the same participant at same point in time. In the setting of morbid obesity and large weight loss, DXA assessment of aBMD might be affected by imaging artifacts.<sup>(21)</sup> The introduction of a new DXA machine during the study could affect the aBMD results, albeit probably insignificantly, as proper cross-calibration was performed. For 23 participants, the preoperative DXA examination was performed using the GE Lunar Prodigy, whereas the follow-up examination was performed using the GE Lunar iDXA. In 11 participants, GE Lunar iDXA was used for both pre- and postoperative analyses. Patients with T2D were encouraged to participate in the study; thus, the fraction of participants with T2D (38%) exceeds the fraction in patients seeking RYGB (25% to 30%) at our institution in general.<sup>(43)</sup> For technical reasons, concerning the bone marrow biopsy, subjects with a preoperative BMI > 47 kg/m<sup>2</sup> were excluded. The prevalence of subjects with ≥ 50 kg/m<sup>2</sup> before RYGB surgery has been reported between 21% and 32%.<sup>(43,44)</sup> Thus, possibly one-third of the bariatric population was potentially excluded, perhaps affecting the generalizability of the study results.

## Conclusion

Our findings indicate that a high BMAT fraction seems to be associated with lower aBMD and poorer glycemic control in subjects with morbid obesity. One year after RYGB, we observed a 10.7% decrease in BMAT fraction. This reduction was comparable in participants with and without T2D but appeared to be sex specific.

## Disclosures

IKBH, TM, JAK, EMH, CB, and HLG have nothing to declare. EFE consults for Shire, Amgen, Ascendis, Eli Lilly, Novartis, and

Merck; has received grant support from Shire; and has received lecture fees from Eli Lilly, Novartis, Amgen, and Takeda.

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Authors' roles: Study design: IKBH, TM, CB, HLG, and EFE. Study conduct: IKBH, TM, JAK, HLG, and EFE. Data collection: IKBH. Data analysis: IKBH, CB, and EFE. Data interpretation: IKBH, TM, JAK, CB, EMH, HLG, and EFE. Drafting manuscript: IKBH. Revising manuscript content: IKBH, MT, JAK, CB, EMH, HLG, and EFE. Approving final version of manuscript: IKBH, MT, JAK, CB, EMH, HLG, and EFE. IKBH and CB take responsibility for the integrity of the data analysis.

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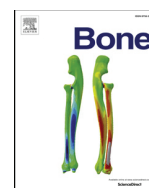






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## Full Length Article

## Bone metabolism, bone mineral density and low-energy fractures 10 years after Roux-en-Y gastric bypass



Ingvild Kristine Blom-Høgestøl<sup>a,b,\*</sup>, Stephen Hewitt<sup>a,b</sup>, Monica Chahal-Kummen<sup>a,b</sup>, Cathrine Brunborg<sup>c</sup>, Hanne Løvdal Gulseth<sup>a,d</sup>, Jon A. Kristinsson<sup>a,e</sup>, Erik Fink Eriksen<sup>a,b</sup>, Tom Mala<sup>a,e</sup>

<sup>a</sup> Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway

<sup>b</sup> Institute of Clinical Medicine, University of Oslo, Oslo, Norway

<sup>c</sup> Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway

<sup>d</sup> Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health, Norway

<sup>e</sup> Department of Gastrointestinal and Pediatric Surgery, Oslo University, Norway

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Weight loss

## ABSTRACT

**Background:** Roux-en-Y gastric bypass (RYGB) is a common surgical procedure for treatment of morbid obesity. RYGB induces considerable and sustained weight loss, and remission of obesity related-comorbidities. While studies have suggested negative effects of RYGB on bone health, long-term data are lacking. We aimed to evaluate the prevalence of aBMD below the expected range for age, osteopenia, osteoporosis and low-energy fractures in a defined patient cohort 10 years after RYGB. Secondly, we wanted to identify factors associated with increased risk of aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB.

**Methods:** Patients undergoing RYGB surgery from June 2004 to December 2006 at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral centre for treatment of morbid obesity, were invited to a 10 year follow-up. Follow-up visits included morning fasting blood samples, clinical examination, anthropometric measures and dual energy X-ray absorptiometry (DXA).

**Results:** Out of 194 patients eligible for the study, 124 attended the 10 year follow-up and 122 (63%) were examined with DXA. Mean (SD) age was 50.3 (9.0) years, 118 (97%) were of Caucasian ethnicity, 94 were females (77%), of whom 41 (44%) were postmenopausal.

Secondary hyperparathyroidism (SHPT) was noted in 37 participants (31%) and vitamin D deficiency (value below 50 nmol/L) and insufficiency (value below 75 nmol/L) in 40 (33%) and 91 (75%), respectively. Among the 63 participants who were premenopausal females or males 49 years or younger the prevalence of areal bone mineral density (aBMD) in the lower range of normal (z-score  $-1.1$  to  $-1.9$ ) was 30% (n = 19) and aBMD below the expected range for age (z-score  $\leq -2.0$ ) was noted in 8% (n = 5). Among the 59 participants who were postmenopausal females or males 50 years or older, the prevalence of osteopenia (t-score  $-1.1$  to  $-2.4$ ) was 51% (n = 30) and osteoporosis (t-score  $\leq -2.5$ ) was 27% (n = 16). The bone resorption markers CTX-1 and PINP were higher in participants with aBMD z-score or t-score of  $-1.1$  or lower compared to participants with aBMD z-score or t-score of  $-1.0$  or higher. Preoperative hypothyroidism, or higher age, postmenopausal status, BMI  $< 35$  kg/m<sup>2</sup>, SHPT or higher PINP levels at 10 year follow-up were independently associated with aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. Eighteen participants (15%) reported a clinical low-energy fracture after RYGB. In addition, vertebral fracture assessment by DXA revealed that 10 participants (8%) had experienced at least one moderate to severe morphometric vertebral fracture.

**Conclusion:** Ten years after RYGB 27% of postmenopausal females and males 50 years or older were osteoporotic, and 8% of premenopausal females and males 49 years or younger exhibited aBMD below the expected range for age. The prevalence of fragility fractures was high. SHPT, higher age, postmenopausal status or higher

**Abbreviations:** aBMD, Areal bone mineral density; BALP, Bone specific alkaline phosphatase; CKD, Chronic kidney disease; CTX-1, Carboxyl terminal telopeptide of type 1 collagen; eGFR, estimated glomerular filtration rate; FSH, Follicular stimulating hormone; DXA, Dual energy X-ray absorptiometry; PINP, Procollagen type 1 N-terminal propeptide; RYGB, Roux-en-Y gastric bypass; SHPT, Secondary hyperparathyroidism; T2D, Type 2 diabetes; TSH, Thyroid stimulating hormone; 25-hydroxyvitamin D, 25(OH) vitamin D

\* Corresponding author at: Lilleåsen 15C, 1396 Billingstad, Norway.

E-mail address: [ingvild.hogestol@gmail.com](mailto:ingvild.hogestol@gmail.com) (I.K. Blom-Høgestøl).

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PINP levels at 10 years and preoperative hypothyroidism were all independent risk factors for aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB.

## 1. Introduction

Laparoscopic Roux-en-Y gastric bypass (RYGB) surgery is a widely applied surgical technique for treatment of morbid obesity following failed attempts of sustained weight loss [1]. RYGB induces considerable and sustained weight loss, remission of obesity related comorbidities and reduces mortality [2–4]. However, changes to gastrointestinal anatomy and physiology may pose negative effects on bone health.

Shortly after RYGB, increased levels of bone turnover markers and reduced bone mineral density, assessed by dual energy X-ray absorptiometry (DXA) or quantitative computed tomography, have been noted [5–8]. Persistent elevation of serum bone turnover markers and continuous reductions in areal bone mineral density (aBMD) following weight stabilization indicate effects of RYGB on bone metabolism beyond that of adaptation to a reduced weight [9]. In line with the high turnover state increased fracture rates have been reported in several studies [10,11]. The high turnover state after RYGB is probably multifactorial. The high prevalence of secondary hyperparathyroidism (SHPT) [7,12], likely contributes to this state of high bone turnover.

These findings emphasize the need for long-term evaluations of bone health after RYGB. Unfortunately studies describing bone health five to 10 years after RYGB are few, characterised by small samples of females only, and with low or undefined rates of follow-up [13,14].

We aimed to evaluate the prevalence of aBMD below the expected range for age, osteopenia, osteoporosis and low-energy fractures in a defined patient cohort 10 years after RYGB. Secondly, we wanted to identify factors associated with an increased risk of aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. We hypothesized that SHPT would be associated with an increased risk of aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB.

## 2. Methods

### 2.1. Study population

Patients undergoing RYGB surgery between June 2004 and December 2006 at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral centre for treatment of morbid obesity, were invited to a 10 year follow-up. Eligibility criteria for RYGB were body mass index (BMI)  $\geq 40$  kg/m<sup>2</sup> or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity-related comorbidity, age between 20 and 60 years, and failed attempts of sustained weight loss. Results from the five year follow-up have been published previously [15]. Ten year follow-up visits were conducted from June 2016 to March 2018.

### 2.2. Surgery, follow-up and study visits

Prior to surgery all patients had one or more individual consultations with a nurse, a dietician and a surgeon. If indicated they also had consultations with an internist or a psychologist. Preoperative data were registered on predefined forms. Until December 2005 preoperative data were registered retrospectively, with subsequent prospective registration. Patients underwent a laparoscopic RYGB with a gastric pouch of about 25 mL, a 150 cm antecolic alimentary limb and a 50 cm biliopancreatic limb [16]. Postoperative routine clinical follow-up visits were conducted six-eight weeks, six months, one, two and five years after surgery. After surgery all patients were prescribed oral supplementation therapy consisting of daily doses of calcium carbonate (500 mg  $\times$  2 daily), vitamin D3 (400IU  $\times$  2 daily), Nycoplus multivitamin – Nycomed® (one tablet), iron (100–200 mg) as well as vitamin

B12 injections (1 mg) every three months. At routine clinical visits, vitamin levels were monitored and additional supplements were recommended if appropriate.

Ten year follow-up visit included morning fasting blood samples, clinical examination, anthropometric measures and DXA. All, but four, 10 year follow-up visits were performed by one clinician (SH). Data concerning comorbidity, medications, nutritional supplements, menstrual status and fracture history were recorded in a predefined case report form.

DXA scans were performed for assessment of lumbar spine (L<sub>1</sub>-L<sub>4</sub>), hip, proximal femur and total body aBMD and body composition (including percent body fat). Vertebral fracture assessment by DXA was evaluated for the presence of vertebral fractures. All DXA scans were performed by the same nurse. GE Lunar Prodigy was used until August 26, 2016 when it was replaced by GE Lunar iDXA. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine (L1-L4) intra-class correlation coefficient (ICC) (95% CI) of 0.989 (0.968 to 0.996), and for femoral neck and total hip, ICC (95% CI), was 0.994 (0.982 to 0.998) and 0.996 (0.988 to 0.999), respectively. Furthermore, studies have shown excellent *in vivo* precision of the GE Lunar iDXA for the measurement of lumbar spine, hip and total body aBMD in adults with normal and obese BMI [17,18]. Our DXA machine is calibrated daily against the standard calibration phantom, supplied by the manufacturer, with estimated short-term precision errors of  $< 1.0\%$  for aBMD at the lumbar spine and at the femoral neck.

Blood samples were drawn before 10 am after an overnight fast, centrifuged and analyzed shortly after retrieval. The Hormone Laboratory, Oslo University Hospital analyzed: thyroid stimulating hormone (TSH) using a non-competitive immunofluorometric method from DELFIA (reference range TSH mLU/L: 0.5–3.6). Carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP) using Roche® electrochemiluminescence immunoassay (ECLIA) (reference ranges based on information from the manufacturer; CTX-1  $\mu$ g/L: females 25–49 years:  $\leq 0.57$ ,  $\geq 50$  years:  $\leq 1.01$ , males 30–50 years:  $\leq 0.58$ , 51–70 years:  $\leq 0.7$  and PINP  $\mu$ g/L: females  $> 25$  years: 11–94, males  $> 25$  years: 20–91). Bone specific alkaline phosphatase (BALP) and osteocalcin using LIAISON® chemiluminescence immunoassay (CLIA) (reference range based on information from the manufacturer; BALP  $\mu$ g/L: 5.5–24.6, osteocalcin nmol/L: females  $\geq 21$  years: 1.5–5.4, males  $\geq 21$  years 1.6–4.3 [19]). Serum 25-hydroxyvitamin D (25(OH) vitamin D) levels were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method, Calcitriol (1.25(OH)<sub>2</sub>D<sub>3</sub>) vitamin D with enzymatic immunoassay from IDS Nordic (reference range 1.25(OH)<sub>2</sub>D<sub>3</sub> pmol/L: 39–193). Serum parathyroid hormone (PTH) by Immulite 2000 XPI, Siemens Healthineers a chemiluminoimmunometric assay (reference range PTH pmol/L: 1.5–7.0). Serum ionized calcium using Roche® Cobas b221 (reference range ionized calcium mmol/L: 1.15–1.33). FSH was analyzed using Immulite 2000 XPI, Siemens Healthineers, a non-competitive immunoluminometric assay. The coefficient of variance for CTX-1, PINP, osteocalcin, 25(OH) vitamin D and PTH is 5%, 5%, 6%, 11% and 7%, respectively.

The Central Laboratory of Oslo University Hospital analyzed HbA<sub>1c</sub> using Tosoh G8 high-performance liquid chromatography and calculated estimated glomerular filtration rate (eGFR) using CKD-EPI formula (mL/min/1.73m<sup>2</sup>); were categorized into the following stages of chronic kidney disease (CKD): CKD stage I:  $> 90$ , CKD stage II: 60–90, CKD stage III: 30–59, stage IV: 5–29, stage V:  $< 15$ .

### 2.3. Outcomes

#### 2.3.1. Osteopenia, osteoporosis and aBMD below expected range for age

Areal BMD t-scores represent the number of standard deviations an actual aBMD deviates from the peak bone mass of young women. The aBMD z-score represents the number of standard deviations an actual BMD deviates from the expected aBMD of age, gender and ethnicity. Both scores are based on the reference population from the NHANES and Lunar studies given by the manufacturer. T-scores and Z-scores of the lumbar spine L1-L4 were calculated after exclusion of vertebrae with osteoarthritic changes (spondylosis) or compression fractures. The percent estimates of aBMD z-score were referred to as percent of expected aBMD.

In order to explore aBMD outcomes the population was split according to gender, age and menopausal status:

#### i. Premenopausal females and males 49 years or younger

**aBMD in the higher range of normal:** 1.0 standard deviations lower than the expected aBMD of age, gender and ethnicity or higher (z-score > -1.0).

**aBMD in the lower range of normal:** 1.1 to 1.9 standard deviations

lower than the expected aBMD of age, gender and ethnicity (z-score < -1.0 to > -2.0).

**aBMD below expected range for age:** 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score ≤ -2.0) [20].

#### ii. Postmenopausal females and males 50 years or older [21]:

**Normal aBMD:** 1.0 standard deviations lower than the peak bone mass of young women or higher (t-score > -1.0).

**Osteopenia:** 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score < -1.0 to > -2.5).

**Osteoporosis:** aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score ≤ -2.5).

#### 2.3.2. Fractures

Vertebral fractures were assessed on lateral X-rays recorded on the DXA scanner and by visual semiquantitative technique (VFA). Moderate (reduction of vertebral height of > 25–40%) and severe (reduction of vertebral height of > 40%) vertebral fractures are reported [22]. Vertebral fractures revealed by DXA are referred to as morphometric vertebral fractures.

**Table 1**

Participant characteristics 10 years after Roux-en-Y gastric bypass (RYGB).

	All n = 122	Premenopausal females and males younger than 50 years of age n = 63	Postmenopausal females and males 50 years or older n = 59	p-Value
Caucasian, n	118 (97%)	62 (98%)	56 (95%)	0.47
Age, years	50.3 ± 9.0	44.2 ± 6.7	56.8 ± 6.1	< 0.001
Body mass index, kg/m <sup>2</sup>	35.6 ± 7.2	37.4 ± 7.0	33.6 ± 6.6	0.003
% fat mass	45.4 ± 7.8	46.4 ± 7.2	44.2 ± 7.1	0.043
% total weight loss after RYGB	24.6 ± 13.6	22.6 ± 11.5	26.8 ± 15.4	0.093
Loss of height, cm	1.5 ± 1.9	0.91 ± 1.6	2.1 ± 2.0	0.001
First degree relative with osteoporosis	22 (18%)	8 (13%)	14 (24%)	0.11
First degree relative with hip fracture	17 (14%)	6 (10%)	11 (19%)	0.15
TSH, mIU/L	1.7 (< 0.001–7.4)	1.5 (< 0.001–7.4)	1.9 (0.2–4.8)	0.026
eGFR, mL/min/1.73m <sup>2</sup>	98.4 ± 16.0	104.8 ± 12.8	91.5 ± 16.4	< 0.001
Ionized calcium, mmol/L	1.2 ± 0.039	1.2 ± 0.032	1.2 ± 0.045	0.70
25(OH) vitamin D, nmol/L	60.0 ± 22.9	56.1 ± 21.8	64.2 ± 23.5	0.052
1.25(OH) <sub>2</sub> D <sub>3</sub> , pmol/L	135.1 ± 41.9	139.0 ± 38.9	133.2 ± 41.6	0.44
PTH, pmol/L	5.9 ± 2.8	5.9 ± 2.4	5.9 ± 3.2	0.96
CTX-1, µg/L	0.48 ± 0.24	0.41 ± 0.18	0.54 ± 0.27	0.002
PINP, µg/L	55.4 ± 26.3	58.6 ± 28.5	52.1 ± 23.5	0.19
BALP, µg/L	14.3 ± 5.4	14.0 ± 5.5	14.6 ± 5.3	0.51
Osteocalcin, nmol/L	4.1 ± 1.2	3.9 ± 1.1	4.2 ± 1.4	0.22
Areal bone mineral density				
Lumbar spine (L1-L4), g/cm <sup>2</sup>	1.17 ± 0.16	1.21 ± 0.14	1.12 ± 0.17	0.001
Femoral neck (left), g/cm <sup>2</sup>	0.92 ± 0.13	0.97 ± 0.11	0.86 ± 0.13	< 0.001
Total hip, g/cm <sup>2</sup>	0.97 ± 0.16	1.02 ± 0.13	0.93 ± 0.18	0.002
Total body, g/cm <sup>2</sup>	1.17 ± 0.15	1.22 ± 0.12	1.11 ± 0.16	< 0.001
T-score				
Lumbar (L1-L4) <sup>a</sup>	-0.9 ± 1.3	-0.55 ± 1.17	-1.3 ± 1.34	0.001
Femoral neck (left)	-0.8 ± 0.98	-0.36 ± 0.85	-1.19 ± 0.96	< 0.001
Total hip	-0.4 ± 1.1	0.07 ± 1.01	-0.79 ± 1.09	< 0.001
Total body	0.64 ± 1.4	1.19 ± 1.2	0.016 ± 1.3	< 0.001

The results are given as number (proportion in per cent) for categorical variables, mean (SD) for continuous variables with normal distribution and median (range) for other variables ≥ .

Loss of height is the difference between the measured preoperative height and the measured height at 10 year follow-up.

Abbreviations; Thyroid stimulation hormone (TSH), estimated glomerular filtration rate (eGFR), 25-hydroxyvitamin D (25(OH) vitamin D), Calcitriol (1.25(OH)<sub>2</sub>D<sub>3</sub>), parathyroid hormone (PTH), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), bone specific alkaline phosphatase (BALP).

Missing (n): FSH (4), % total weight loss (3), TSH (2), eGFR (1), ionized calcium (2), 1.25(OH)<sub>2</sub> vitamin D (5), PTH (1), CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10), aBMD lumbar spine (1), aBMD left femoral neck (6), aBMD total hip (5), aBMD total body (3).

Reference ranges: TSH mIU/L: 0.5–3.6; eGFR (mL/min/1.73 m<sup>2</sup>): Stages of chronic kidney disease (CKD): CKD stage I: > 90, CKD stage II: 60–90, CKD stage III: 30–59, stage IV: 5–29, stage V: < 15; ionized calcium mmol/L: 1.15–1.33; 25(OH) vitamin D nmol/L: 37–132; 1.25(OH)<sub>2</sub>D<sub>3</sub> pmol/L: 39–193; PTH pmol/L: 1.5–7.0; CTX-1 µg/L: Females 25–49 years: ≤ 0.57, ≥ 50 years: ≤ 1.01, males 30–50 years: ≤ 0.58, 51–70 years: ≤ 0.7; PINP µg/L: Females > 25 years: 11–94, males > 25 years: 20–91; BALP µg/L: 5.5–24.6; osteocalcin nmol/L: Females ≥ 21 years: 1.5–5.4, males ≥ 21 years: 1.6–4.3.

<sup>a</sup> Vertebrae with osteoarthritic changes (spondylosis) or compression fractures were excluded from calculation.

Clinical fractures were self-reported and registered in predefined case report forms. A low energy fracture was defined as a fracture resulting from minimal trauma quantified as forces equivalent to a fall from standing height or less [21]. All low energy fractures, except digital fractures, acquired during the 10 years after RYGB are reported.

### 2.3.3. Calcitropic hormones and supplements

Vitamin D deficiency was defined as serum 25(OH) vitamin D levels below 50 nmol/L and insufficiency as levels below 75 nmol/L [23,24]. Seasons were defined as; Summer: June to August, fall: September to November, winter: December to February and spring: March to May. SHPT was defined as a serum concentration of PTH above 7.0 pmol/L in the absence of serum ionized calcium above 1.33 mmol/L. Intake of vitamin supplements was self-reported. Patients were classified as taking calcium and vitamin D supplements if they reported intake of 1000 mg and 800 IU, respectively, or more, at least five days a week.

### 2.3.4. Menopausal status

Hormonal intrauterine devices made clinical evaluation of menstrual cycle challenging. For this reason a postmenopausal status was defined as a serum follicle stimulating hormone (FSH) of > 25 IU/L or higher [25].

### 2.3.5. Comorbidities

Preoperative type 2 diabetes (T2D) was defined as  $HbA_{1c} \geq 6.5\%$  and/or the use of one or more oral glucose lowering drugs with or without the use of insulin. Preoperative hypothyroidism was based on information given by the participant preoperatively, stated in referrals or based on use of levothyroxine substitution treatment.

## 2.4. Statistical analysis

Normally distributed continuous variables are presented as mean  $\pm$  SD, others are presented as median (range). Categorical data are reported as proportions (percentage). Subgroups were compared with an independent sample *t*-test, the Chi Square test or Fisher exact test as appropriate. Intra-class correlation coefficient (ICC) with 95% CI was used to assess concordance between the two DXA scanners (GE Lunar Prodigy and GE Lunar iDXA). ICC values of 0.75 or higher were considered excellent [26]. One way-ANOVA was used to explore possible seasonal differences in 25(OH) vitamin D values. To explore the association between PTH and aBMD z-scores, linear regression analyses were applied. The results from the linear regression analyses are presented as regression coefficients ( $\beta$ ) with 95% CI.

Logistic regression analyses were performed to identify factors associated with aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. All variables with a  $p < 0.25$  in the univariable analysis were entered into the multivariable logistic regression model using a manual backward stepwise elimination procedure. Multivariable analyses were preceded by estimation of correlation between risk factors. Predictors that correlated  $> 0.7$ , were not included in the model in order to avoid multicollinearity. The associations between factors and aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB at 10 year follow-up visit were quantified by calculating odds ratios (OR) with 95% CI. In linear regression analysis all continuous variables were checked for deviation from normality, non-linear effects, multicollinearity, and homoscedasticity. For logistic regression analysis they were checked for deviation of linearity of the logit and multicollinearity. Evaluation of the models predictive accuracy was assessed by calibration (Hosmer and Lemeshow goodness-of-fit test) and by the discriminatory capability (area under the ROC curve). Two tailed  $p$ -values  $< 0.05$  were considered statistically significant. All statistical analyses were performed using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY: IBM Corp).

## 2.5. Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics South-East Norway; 2015/142. Written informed consent was obtained from all participants. Preoperative data were registered in a database licensed by the Norwegian Data Inspectorate.

## 3. Results

Nine out of 203 patients died prior to the 10 year follow-up; thus 194 were eligible for the study. Of these, 124 attended the 10 year follow-up, and 122 (63%) were examined with DXA scans and were included in this study.

### 3.1. Participant characteristics

Participant characteristics are presented in Table 1. Mean age was 50.3 (SD 9.0) years, 118 (97%) were of Caucasian ethnicity. There were 94 females (77%), of whom 41 (44%) were postmenopausal. Of the 122 participants 63 (52%) were premenopausal females or males 49 years or younger, and 59 (48%) were postmenopausal females or males 50 years or older.

At 10 year follow-up, five participants received bone specific treatment for osteoporosis (oral bisphosphonates (2), intravenous bisphosphonates (2) or denosumab (1): one male older than 50 years of age and four postmenopausal females, they were all classified as osteoporotic). These participants had lower CTX-1 levels,  $0.23 \pm 0.11 \mu\text{g/L}$ , compared to  $0.49 \pm 0.23 \mu\text{g/L}$  for the remaining cohort,  $p = 0.034$ , but their PINP levels of  $51.0 \pm 14.7 \mu\text{g/L}$  were comparable to  $55.6 \pm 26.6 \mu\text{g/L}$  for the remaining cohort,  $p = \text{n.s.}$  Three participants reported treatment for rheumatoid arthritis, one had been treated for prostate cancer and one had undergone surgery for primary hyperparathyroidism. No participants had been diagnosed with breast cancer or Cushing's disease. Twenty-five participants (21%) had CKD stage II and three had CKD stage III (3%), no participant had CKD stage IV or higher. At the 10 year follow-up seven participants presented with low TSH levels ( $< 0.5 \text{ mIU/L}$ ), five had known preoperative hypothyroidism, while two had no known thyroid condition.

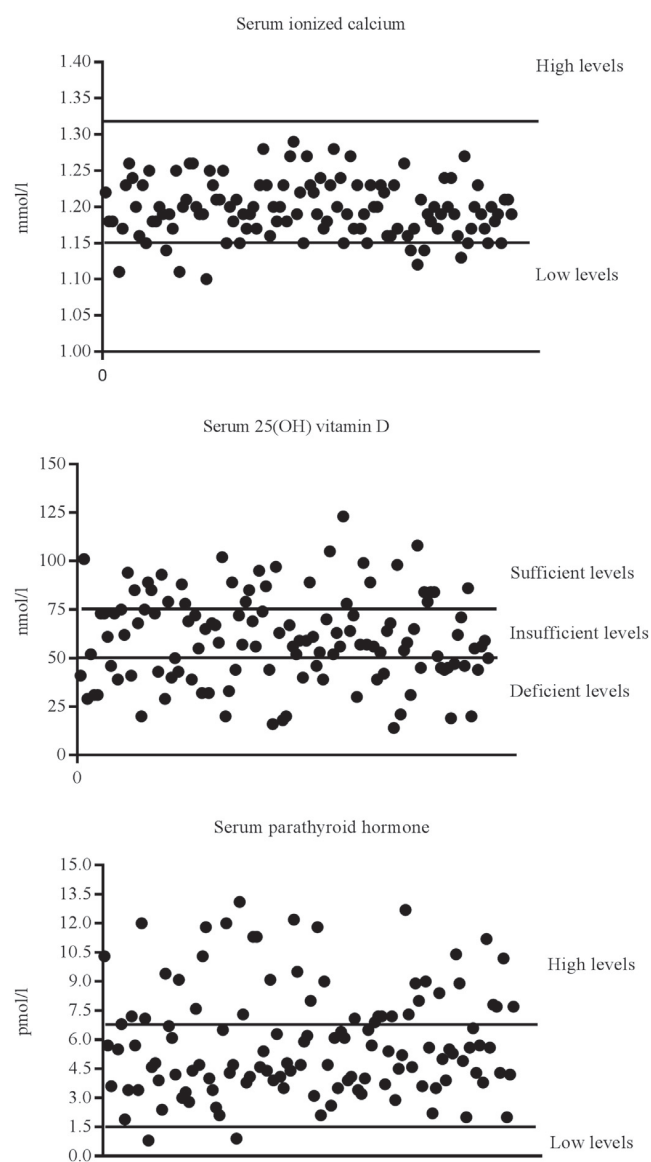
### 3.2. Calcium and calcitropic hormones

A total of 29 participants (24%) reported regular intake of calcium supplements and 38 (31%) reported regular intake of vitamin D supplements. Individual measurements of serum ionized calcium, PTH and 25(OH) vitamin D are shown in Fig. 1. Forty participants (33%) were vitamin D deficient and 91 (75%) insufficient, respectively. Among the participants reporting regular intake of vitamin D supplements, five (12.5%) had vitamin D deficiency, a significantly lower fraction when compared to participants not taking supplements where 33 participants (41%) were deficient,  $p = 0.002$ . 25(OH) vitamin D levels drawn during summer, fall, winter and spring season were  $61.9 \pm 20.5$ ,  $65.4 \pm 22.4$ ,  $52.8 \pm 24.4$  and  $56.8 \pm 22.9$ , respectively. No statistical difference was noted between the 25(OH) vitamin D levels of the different seasons.

No participant had serum ionized calcium or 25(OH) vitamin D levels above the reference range. SHPT was noted in 37 participants (31%). Participants with SHPT exhibited numerically lower levels of 25(OH) vitamin D compared to those without SHPT,  $54.0 \pm 3.9$  and  $62.7 \pm 2.4$ , respectively,  $p = 0.055$ .

### 3.3. Markers of bone turnover

Mean values for the bone turnover markers; CTX-1, PINP, BALP and osteocalcin, are given in Table 1 and individual measurements are



**Fig. 1.** Individual measurements of serum ionized calcium, parathyroid hormone and 25(OH) vitamin D in 122 participants 10 years after Roux-en-Y gastric bypass. Reference range is marked with lines.

Reference ranges: Serum ionized calcium mmol/L: 1.15–1.33; 25(OH) vitamin D nmol/L: 37–132; PTH pmol/L: 1.5–7.0.

presented in Fig. 2. Participants with an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB revealed higher levels of CTX-1 and PINP compared to participants with an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. However, only the association with PINP remained significant in the multivariable analysis (Table 2).

### 3.4. Bone mineral density

The percent estimates of aBMD z-scores, relative to expected aBMD of age, gender, and ethnicity, were below 90% in 35%, 43% and 27% of participants, in the total hip, femoral neck and lumbar spine, respectively (Fig. 3). An inverse association was noted between PTH and aBMD z-score of femoral neck;  $\beta -0.051$  95% CI ( $-0.099$  to  $-0.002$ ),  $p = 0.040$ , while the association with lumbar spine and total hip aBMD z-score were non-significant (Fig. 4).

**3.4.1. Osteopenia, osteoporosis and aBMD below expected range for age**  
**3.4.1.1. Premenopausal females and males 49 years or younger.** Of the 63 participants who were premenopausal females or males 49 years or younger, 5 (8%) revealed aBMD below the expected range for age (z-score  $\leq -2.0$ ) and 19 (30%) had osteopenia (z-score  $-1.1$  to  $-1.9$ ) of the spine, femoral neck or total hip 10 years after RYGB.

**3.4.1.2. Postmenopausal females and males 50 years or older.** Of the 59 participants who were postmenopausal females or males 50 years or older 16 (27%) had osteoporosis (t-score  $\leq -2.5$ ) and 30 (51%) had aBMD in the osteopenic range (t-score  $-1.1$  to  $-2.4$ ) of the spine, femoral neck or total hip 10 years after RYGB.

The prevalence of aBMD below the expected range for age and/or osteoporosis in the different skeletal locations (spine, femoral neck and total hip) are presented in Table 3. Of the total cohort 70 (57%) participants exhibited an aBMD z-score or t-score of  $-1.1$  or lower. Pre-operative hypothyroidism, or higher age, postmenopausal status, BMI  $< 35$  kg/m<sup>2</sup>, higher PINP value or SHPT at 10 year follow-up were all independent risk factors for aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB (Table 2). The Hosmer-Lemeshow's goodness of fit test for the multivariable model indicated a satisfactory fit of the model ( $p = 0.35$ ). The discriminatory capability (area under the ROC) between participants with and without a z-score or t-score of  $-1.1$  10 years after RYGB was 0.81 (95% CI: 0.73–0.89). When exploring risk factors for aBMD below the expected range for age, osteoporosis and/or fragility fractures (low energy fracture and morphometric vertebral fractures) only age was detected as a risk factor (Supplementary Table 1).

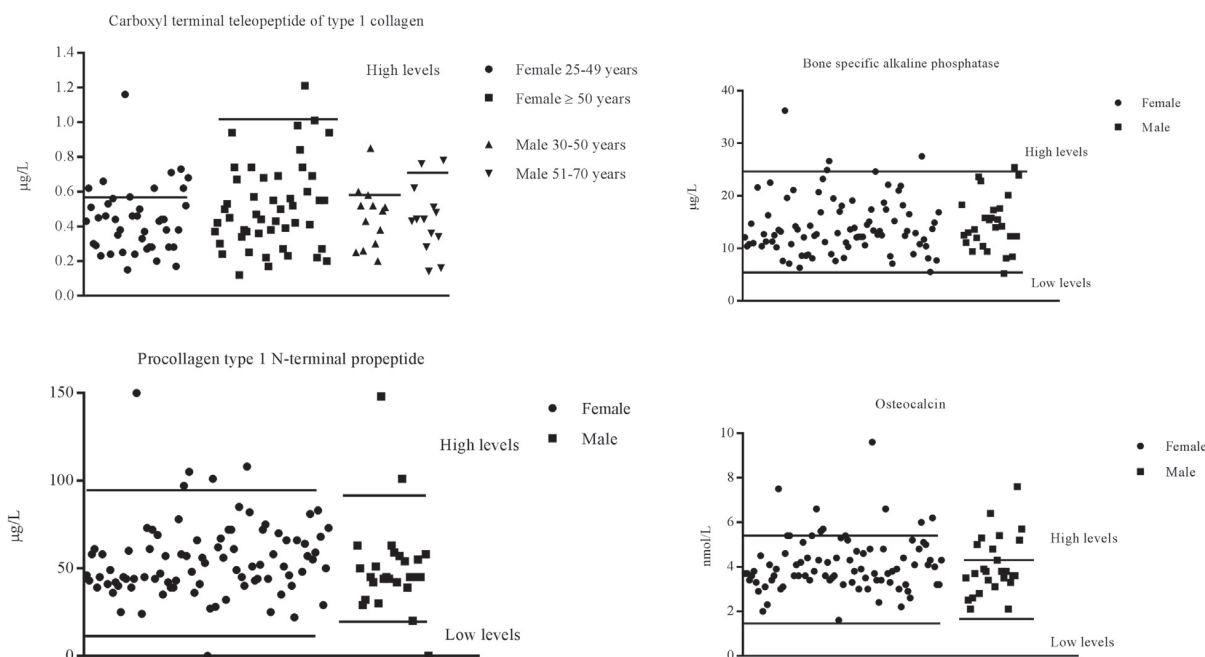
### 3.5. Fractures

Clinical low-energy fractures after RYGB were reported by 18 participants (15%) during the 10 years of follow-up, seven (11%) premenopausal females or males 49 years or younger and 11 (19%) postmenopausal females or males 50 years or older. Lower limb fractures were the most prevalent, followed by rib fractures (Table 4). The mean duration from RYGB to first low-energy fracture was 8.4 years  $\pm$  1.8. Participants with osteoporotic aBMD had a significantly higher prevalence of low energy fractures when compared to participants with normal or osteopenic aBMD ( $p = 0.023$ ). Vertebral fracture assessments of DXA revealed that three male (11%) and seven female (7%) participants had experienced one or more moderate to severe morphometric vertebral fractures. Of these 10 participants, one had presented with symptoms of a vertebral fracture.

## 4. Discussion

### 4.1. Areal bone mineral density below the expected range for age and osteoporosis

Among premenopausal females and males 49 years or younger the prevalence of aBMD below expected range for age was 8% and among the postmenopausal females and males 50 years or older the prevalence of osteoporosis was 27%. Duran et al. observed osteoporosis (t-score  $\leq -2.5$ ) in 13% in a study of 30 females, with a median age of 46 years, eight years after RYGB [13]. However, without knowing the prevalence of postmenopausal status it is difficult to directly compare this study to ours. The Tromsø study, a Norwegian population based study, reported a prevalence of osteoporosis of the femoral neck of 4.8% in males and 6.1% in females for subjects aged 50–69 years in contrast to our findings of 13% and 33%, respectively [27]. Furthermore, we noted that more than one in three had aBMD measures below 90% of expected for age, gender and ethnicity. Collectively, our findings suggest that RYGB may be a risk factor for aBMD below expected range for age and osteoporosis.



**Fig. 2.** Individual measurements of bone turnover markers in 122 participants 10 years after Roux-en-Y gastric bypass. Reference range is marked with lines. Reference ranges: Carboxyl terminal telopeptide of type 1 collagen (CTX-1),  $\mu\text{g/L}$ : females 25–49 years:  $\leq 0.57$ ,  $\geq 50$  years:  $\leq 1.01$ , males 30–50 years:  $\leq 0.58$ , 51–70 years:  $\leq 0.7$ ; procollagen type 1 N-terminal propeptide (PINP),  $\mu\text{g/L}$ : females  $> 25$  years: 11–94, males  $> 25$  years: 20–91; bone specific alkaline phosphatase (BALP),  $\mu\text{g/L}$ : 5.5–24.6; osteocalcin, nmol/L: females  $\geq 21$  years: 1.5–5.4, males  $\geq 21$  years 1.6–4.3. Missing (n): CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10).

#### 4.2. Bone turnover

We observed higher levels of bone turnover markers (CTX-1 and PINP) than those reported in preoperative cohorts of similar age [6,28,29] indicating elevated bone turnover 10 years after RYGB. Participants with an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB had higher levels of both CTX-1 and PINP, suggesting that longstanding accelerated bone turnover is a central mechanism underlying the development of decreasing aBMD after RYGB. Several studies comparing changes in aBMD and bone turnover markers, have shown that high turnover states increase bone loss and fracture rates [30].

#### 4.3. Mechanisms

The pathophysiology underlying the high rates of an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB is likely multifactorial. We noted that participants with SHPT had approximately three times higher odds for having an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB 10 years after RYGB, and we also observed an inverse association between PTH and aBMD z-scores of the femoral neck. Together these findings support our hypothesis that SHPT cause negative effects on skeletal health after RYGB. Studies have revealed significantly reduced intestinal calcium absorption after RYGB [7,31]. To what extent regular intake of calcium and vitamin D supplements could have prevented SHPT in our population is beyond the scope of the study. Notably, the standard recommendation of calcium supplementation was in the form of calcium carbonate, in contradiction to recent European and American recommendations of calcium citrate. Furthermore, the standard doses of 800 IU vitamin D3 daily is in line with Norwegian and European guidelines for post-bariatric management [32,33], but are considerably lower than the 3000 IU daily recommended by the American guidelines [24]. Additionally, we found that only one of three participants reported regular intake of the recommended dose of calcium and vitamin D supplements, that vitamin D

non-compliance was associated with vitamin D deficiency, and that participants with SHPT had numerically lower 25(OH) vitamin D levels compared to the remaining cohort. Other cohorts have also reported limited compliance in regard to use of recommended supplements after bariatric surgery [34,35].

Participants with preoperative hypothyroidism revealed four times higher odds for having an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB. In adulthood, the thyroid hormone is important for bone mass maintenance and strength [36]. Untreated hypothyroidism is associated with reduced bone turnover and a positive bone balance [37]; which generally protects against bone loss and fracture. Due to simple, reliable biochemical diagnostic tools, patients are quickly diagnosed and put on thyroid substitution therapy. Studies of subjects receiving substitution treatment for hypothyroidism have revealed discordant results regarding whether or not it is associated with a lower aBMD [38,39], but supraphysiological doses of substitution therapy have been shown to exert negative effects on bone in hypothyroid patients [40]. In addition a hyperthyroid state preceding hypothyroidism could have negatively affected bone health in this group. We therefore favor the notion that excessive doses of thyroid hormone or possible hyperthyroidism preceding hypothyroidism, after thyroiditis or surgical/radioiodine treatment of hyperthyroidism, constitute plausible reasons for our findings.

Mechanical loading of bone plays a key role in determining bone mass, strength and size [41,42]. In line with this we noted that participants with BMI  $< 35 \text{ kg/m}^2$  10 years after RYGB had a doubled risk of having an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB, however this association did not remain significant in the multivariable analysis, indicative of limited importance in the long-term after RYGB. As for the general population, postmenopausal status and older age were associated with an increased risk of having an aBMD z-score or t-score of  $-1.1$  or lower 10 years after RYGB [21]. Our findings are supported by Schafer et al. who described that postmenopausal females had a larger decrease in bone mineral density after RYGB when compared to premenopausal females and males [29]. Older age was the

**Table 2**  
Factors associated with aBMD z-score or t-score  $-1.1$  or lower 10 years after Roux-en-Y gastric bypass (RYGB).

	Univariable		Multivariable			
	aBMD z-score or t-score of $-1.0$ or higher 10 years after RYGB n = 52	aBMD z-score or t-score of $-1.1$ or lower 10 years after RYGB n = 70	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-Value
Gender, female	39 (75%)	55 (79%)	1.2 (0.52–2.9)	0.64		
Heredity osteoporosis	5 (10%)	17 (24%)	3.0 (1.0–8.8)	<b>0.044</b>		
Preoperative						
Hypothyroidism	4 (8%)	18 (27%)	4.4 (1.4–14.0)	<b>0.012</b>	4.1 (1.1–16.3)	<b>0.041</b>
Type 2 diabetes	13 (26%)	21 (34%)	1.5 (0.66–3.4)	0.34		
BMI > 50	19 (37%)	21 (31%)	0.79 (0.37–1.7)	0.55		
10 years after RYGB						
Age, years	47.4 ± 8.0	52.5 ± 9.1	1.1 (1.0–1.1)	<b>0.002</b>	1.1 (1.0–1.1)	<b>0.016</b>
Postmenopausal	7 (14%)	35 (52%)	6.7 (2.6–16.9)	< <b>0.001</b>	4.7 (1.6–14.1)	<b>0.005</b>
Δ BMI	-10.0 ± 6.6	-11.6 ± 6.7	0.96 (0.91–1.0)	0.18*		
BMI < 35 kg/m <sup>2</sup>	20 (39%)	40 (57%)	2.1 (1.0–4.4)	<b>0.043</b>		
Loss of height, cm	-1.4 ± 1.9	-1.6 ± 1.9	0.95 (0.78–1.1)	0.57		
Smoking	15 (29%)	26 (37%)	1.5 (0.67–3.2)	0.34		
Muscular or skeletal pain	32 (62%)	47 (67%)	1.3 (0.63–2.8)	0.52		
Secondary hyperparathyroidism	13 (25%)	24 (35%)	1.6 (0.72–3.6)	<b>0.25</b>	2.8 (1.0–7.9)	<b>0.045</b>
25(OH) vitamin D insufficiency	41 (79%)	50 (71%)	0.67 (0.29–1.6)	0.35		
Low-energy fracture after RYGB	7 (13%)	11 (16%)	1.2 (0.43–3.3)	0.73		
CTX-1, µg/L	0.39 ± 0.17	0.54 ± 0.26	34.1 (3.9–295)	< <b>0.001</b>		
PINP, µg/L	48.1 ± 17.2	60.7 ± 30.3	1.0 (1.0–1.0)	<b>0.016</b>	1.0 (1.0–1.1)	<b>0.006</b>

aBMD z-score or t-score of  $-1.1$  or lower 10 years after includes:

i. Premenopausal females and males 49 years or younger

aBMD in the lower range of normal: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score <  $-1.0$  to >  $-2.0$ ).

aBMD below expected range for age: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score  $\leq -2.0$ ) [20].

ii. Postmenopausal females and males 50 years or older [21]

Osteopenia: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score <  $-1.0$  to >  $-2.5$ ).

Osteoporosis: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score  $\leq -2.5$ ).

Heredity of osteoporosis was defined as having a first degree relative with osteoporosis diagnosis.

The results are given as number (proportion in per cent) for categorical variables, mean (SD) for continuous variables with normal distribution. Patient characteristics associated with aBMD <  $-1.0$  10 years after RYGB were studied with univariate regression. Any variable associated with  $p \leq 0.25$  from the univariable analysis were entered into a multivariable logistic regression model using a manual backward stepwise elimination procedure. In total, 8 potential predictors were examined (p-values written in bold). \* Δ BMI not included in multivariable analysis due to high degree of association with BMI < 35 kg/m<sup>2</sup> at 10 year.

Abbreviations; Body mass index (BMI), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), bone specific alkaline phosphatase (BALP).

Missing: CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10).

Definitions: Postmenopausal; follicular stimulating hormone > 25 IU/L, 25(OH) vitamin D insufficiency; serum 25(OH) vitamin D < 75.0 nmol/L.

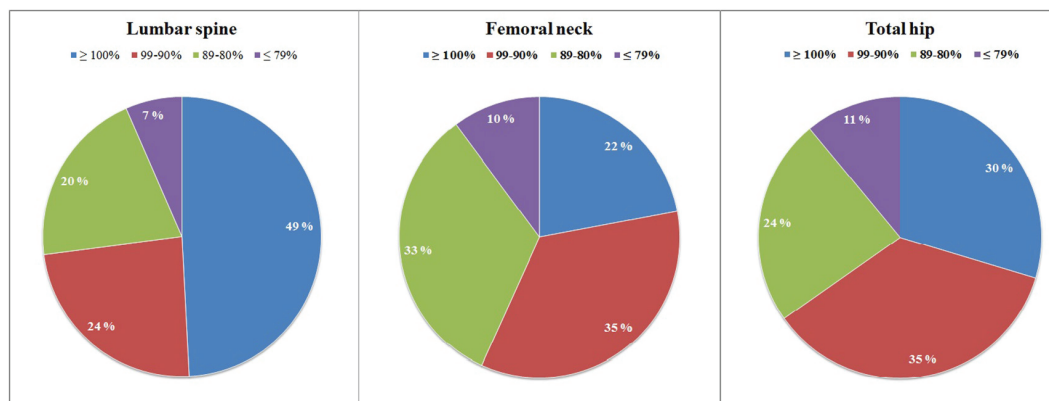
Postmenopausal females are compared to premenopausal females and men.

only independent risk factor for having aBMD below the expected range for age, osteoporosis or a fragility fracture, stressing the importance of screening older subjects in a bariatric population.

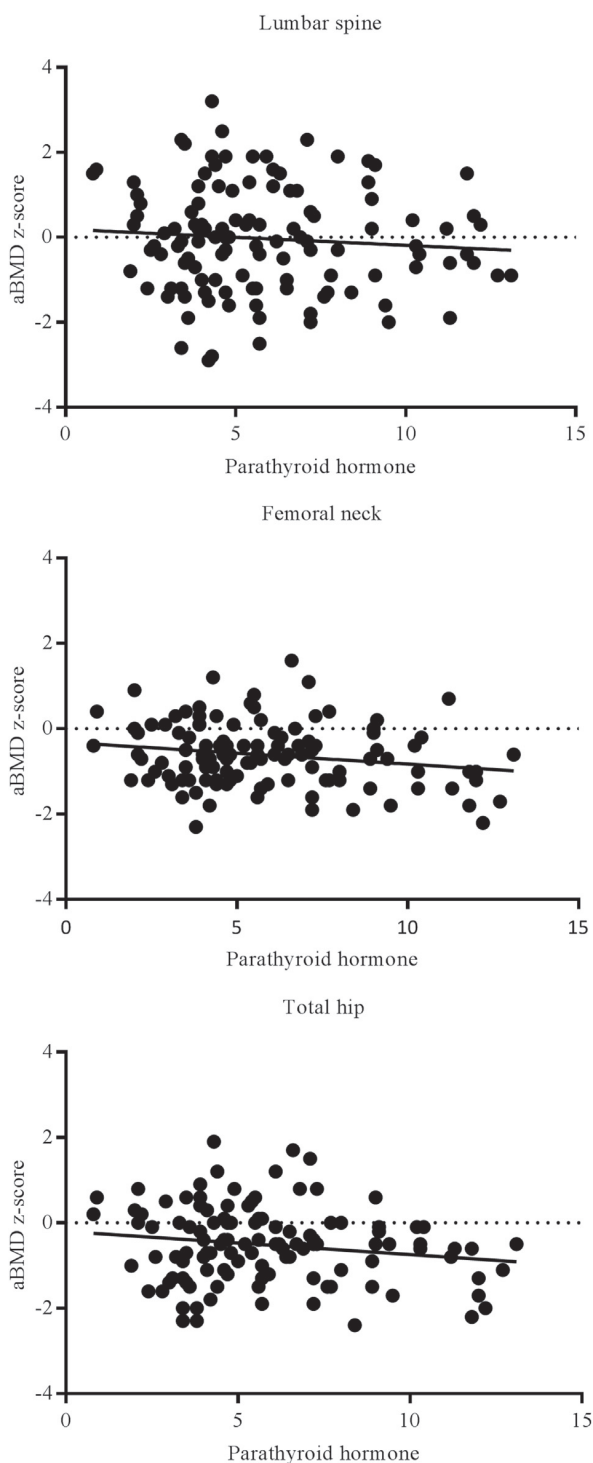
#### 4.4. Fractures

Emerging data indicate that obesity is associated with a site specific

increased fracture rate [43,44] despite a normal aBMD [45], implicating that obesity is associated with reduced bone quality. A study comparing subjects pursuing bariatric surgery to obese controls revealed an increased fracture rate in the bariatric population, prior to the surgical intervention [46]. To what extent the RYGB surgery may have contributed to the high fracture rate noted cannot be fully answered by our study. However, two years or more after RYGB patients have a 43%



**Fig. 3.** Percent of expected areal bone mineral density in 122 patients 10 years after Roux-en-Y gastric bypass when matched with a reference population (NHANES and Lunar given by manufacturer GE Lunar) in regard to age, gender and ethnicity.



**Fig. 4.** Inverse association between parathyroid hormone and areal bone mineral density (aBMD) z-scores of lumbar spine ( $\beta -0.38$  95% CI  $(-0.12$  to  $0.044)$ ,  $p = 0.36$ ), femoral neck ( $\beta -0.051$  95% CI  $(-0.099$  to  $-0.002)$ ,  $p = 0.040$ ) and total hip ( $\beta -0.054$  95%CI  $(-0.11$  to  $0.005)$ ,  $p = 0.072$ ) in 122 participants 10 years after Roux-en-Y gastric bypass.

higher risk of skeletal fractures compared to subjects after adjustable gastric banding surgery, supporting the notion that RYGB itself may pose a negative effect on bone health [10].

During follow-up 15% of our participants experienced clinical low-energy fractures, similar rates have been reported for subjects exposed

**Table 3**

Prevalence of areal bone mineral density below the expected range for age or osteoporosis assessed by dual energy X-ray absorptiometry in 122 patients 10 years after Roux-en-Y gastric bypass for subgroups based on menopausal status or age, subdivided for gender.

	Areal bone mineral density below the expected range for age in premenopausal females and males younger than 50 years of age n = 63			Osteoporosis in postmenopausal females and males 50 years or older n = 59								
	Total		Male	Female		Total	Male	Female				
	n	%	n	n	%	n	%	n	%			
Any skeletal location	5/ 63	8%	1/ 12	8%	4/ 51	8%	16/ 59	27%	2/ 16	13%	14/ 43	33%
Lumbar spine <sup>a</sup>	3/ 63	5%	1/ 12	8%	2/ 51	4%	11/ 59	19%	1/ 16	6%	10/ 43	23%
Femoral neck	0/ 61	0%	0/ 11	0%	0/ 50	0%	6/ 57	9%	1/ 15	7%	5/ 42	12%
Total hip	2/ 61	3%	0/ 11	0%	2/ 50	4%	5/ 57	9%	0/ 15	0%	5/ 42	12%

Premenopausal females and males younger than 50 years of age: Areal bone mineral density below expected range for age; z-score  $-2.0$  or lower.

Postmenopausal females and males 50 years or older: Osteoporosis; t-score  $-2.5$  or lower.

Missing: Femoral neck and total hip; total n = 4, male younger than 50 years of age (1), male 50 years or older (1), premenopausal female (1), postmenopausal female (1).

<sup>a</sup> Vertebrae with osteoarthritic changes (spondylosis) or compression fractures were excluded from calculation.

to long-term glucocorticoid treatment [47]. In addition 11% of male participants and 7.4% of females had a moderate or severe morphometric vertebral fracture detected by vertebral fracture assessments of DXA. These rates are higher compared to prevalence of 7.5% in males and 3% in females reported in the general Norwegian population (38–59 years) [48]. The presence of morphometric vertebral fractures is a known risk factor for future fracture [49], yielding clinical relevance to our finding. Median duration to low energy fracture post RYGB was more than eight years. Together, our findings indicate an increased risk of fracture 10 years after RYGB and support the need for long term follow-up focusing on bone health.

**4.5. Strengths and limitations**

Strengths of our study include the large and well defined cohort, the comprehensive clinical examination during individual consultations, clinical interviews according to predefined case report forms, DXA scans and retrieval of bone turnover markers of all participants. The single center design may be a strength of the study as all patients received the same type of surgery, supplementation, counseling, and follow up, but this design may also limit the external validity of our findings.

The study is limited by the lack of preoperative bone specific medical history, bone turnover markers and DXA measurements. Possible effects of changes in neuro-hormonal axis on bone health were not evaluated. A total of 37% of potential participants were lost to follow-up. In the letter inviting patients to participate in the study we informed that the 10 year follow-up would include evaluation of bone health. This might have influenced subjects suspecting bone disease to participate in the study. The introduction of a new DXA machine during the study could have affected the aBMD results. Of the 122 participants, 51 had their examination with the GE Lunar Prodigy and 71 with the GE Lunar iDXA, but proper cross calibration was performed during transition from one machine to another. Additionally, the definition of aBMD z-score of  $-1.1$  to  $-1.9$  as aBMD in the lower range of normal



**Table 4**

Prevalence of low-energy fractures acquired during 10 years of follow up after Roux-en-Y gastric bypass (RYGB) in 122 participants.

Fracture site	Premenopausal females and males younger than 50 years of age n = 63			Postmenopausal females and males 50 years or older n = 59		
	aBMD higher range of normal n = 39	aBMD in the lower range of normal n = 20	aBMD below expected range for age n = 4	Normal aBMD n = 13	Osteopenia n = 30	Osteoporosis n = 16
Spine	1	1	0	–0	–0	2
Femoral neck/hip	–0	–0	–0	–0	1	1
Rib	–0	–0	–0	–0	1	1
Upper limb	1	–0	–0	–0	–0	2
Lower limb	2	–0	1	1	1	–0
More than one location	1	–0	–0	1	–0	–0
Proportion of participants with clinical low energy fracture after RYGB	5 (13%)	1 (5%)	1 (25%)	2 (15%)	3 (10%)	6 (38%)
Morphometric fractures of spine	2 (%)	3 (15%)			3 (10%)	2 (13%)

Definitions: aBMD: areal bone mineral density.

Premenopausal females and males younger than 50 years of age.

*aBMD in the higher range of normal*: 1.0 standard deviations lower than the expected aBMD of age, gender and ethnicity or higher (z-score > –1.0).*aBMD in the lower range of normal*: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score < –1.0 to > –2.0).*aBMD below expected range for age*: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score ≤ –2.0).

Postmenopausal females and males 50 years or older:

*Normal aBMD*: 1.0 standard deviations lower than the peak bone mass of young women or higher (t-score > –1.0).*Osteopenia*: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score < –1.0 to > –2.5).*Osteoporosis*: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score ≤ –2.5).

Upper limb fractures: Distal radius (n = 1), humerus (n = 2).

Lower limb fractures: Proximal tibia (n = 1), ankle (n = 1), tarsal (n = 1), metatarsal (n = 2).

More than one location: Ankle and distal radius (n = 1), carpal bone and costa (n = 1).

Morphometric fractures of spine revealed by vertebral fracture assessments by DXA.

was defined by the authors and is not a part of ICDS guidelines. This new definition may have inflated the number of patients considered to have abnormal aBMD. Lastly, information concerning etiology of hypothyroidism and repeated TSH measurements could have further elaborated the association between an aBMD z-score or t-score of –1.1 or lower 10 years after RYGB and preoperative hypothyroidism.

## 5. Conclusion

Ten years after RYGB 27% of postmenopausal females and males 50 years or older were osteoporotic, and 8% of premenopausal females and males 49 years or younger exhibited aBMD below the expected range for age. The prevalence of low-energy fractures was high. Hypothyroidism prior to, or SHPT, higher PINP levels, older age or postmenopausal status at 10 year follow-up were all risk factors for aBMD z-score or t-score of –1.1 or lower 10 years after RYGB.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bone.2019.07.014>.

## Authors' roles

Conceptualization; IKBH, TM, SH, MCK, and JAK. Data curation; SH, IKBH and MCK. Formal analysis; IKBH and CB. Funding acquisition; EFE. Investigation; IKBH. Methodology; IKBH. Project administration; MCK, TM, SH, JAK, IKBH and HLG. Supervision; TM, JAK, EFE, HLG, Visualization; IKBH, Writing - original draft; Writing; IKBH, review & editing; IKBH, SH, MCK, HLG, EFE, TM, JAK, CB.

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