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The Effects of Bariatric Surgery upon the Maintenance of Calcium and Vitamin D Levels

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Abstract

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Obesity is a growing pandemic, and consequently the population that has undergone a bariatric procedure is steadily rising. Through various mechanisms, bariatric surgery alters the gastrointestinal anatomy, thereby obtaining weight loss while being associated with various nutrient deficiencies including vitamin D. Over time, vitamin D deficiency may lead to elevated levels of parathyroid hormone (PTH) and the depletion of bone, in order to maintain circulating calcium levels.

The aim of this thesis was to study the effects of bariatric surgery upon calcium homeostasis. In paper I we sought to investigate whether lifestyle factors could explain the difference in bone mineral density (BMD) seen among Roux-en-Y gastric bypass (RYGBP) patients. The second paper is a population-based study that examines the postoperative trends of vitamin D and PTH among patients that have undergone either a RYGBP or a sleeve gastrectomy (SG) (the two currently most popular bariatric procedures worldwide). In paper III the purpose was to explore whether medications to treat dyspepsia had an impact upon calcium homeostasis postoperatively among RYGBP patients. And finally, the purpose of the fourth paper was to study an intramuscular injection as an alternative method for the treatment of vitamin D deficiency among patients who had undergone a biliopancreatic diversion/duodenal switch (BPD/DS) procedure.

The results from paper I do not indicate that lifestyle factors are a cause of the reduced BMD observed after a RYGBP, therefore the procedure itself is a likely cause of reduced BMD. The analysis conducted in paper II points to the impact that bariatric procedures (specifically RYGBP and SG) have over time regarding vitamin D deficiency. The results showed both differences between procedures as well as a successive decline in vitamin D parallelled by a rise in PTH with time after surgery. In paper III our results indicate that regular PPI therapy is strongly associated with a much higher incidence of pathological PTH levels, suggesting that the combination of the procedure and the medication may have deleterious effects upon calcium homeostasis. Finally, when managing vitamin D deficiency after a BPD/DS procedure, we showed that intramuscular injections had a substantial effect upon both vitamin D levels and PTH levels.

Keywords: Bariatric surgery, Hypovitaminosis D, Obesity

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To my Ivar

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Stevens K, Hultin H, Hellman P, Sundbom M. “Low bone mineral density following gastric bypass is not explained by lifestyle and lack of exercise.” *BMC Surgery*, vol. 21, no. 1, 4 June 2021, https://doi.org/10.1186/s12893-021-01281-5.

II. Stevens K, Hultin H, Sundbom M. “Hypovitaminosis D and Hyperparathyroidism: A 5-year postoperative follow-up of 30,458 gastric bypass and sleeve gastrectomy patients.” *Surgery for Obesity and Related Diseases*, Feb. 2024, https://doi.org/10.1016/j.soard.2024.02.004.

III. Stevens K, Hultin H, Sundbom M. “Continuous PPI Treatment after Gastric Bypass Increases the Risk of Pathological PTH Levels at Ten Years Postoperatively.” (Manuscript)

IV. Hultin H, Stevens K, Sundbom M. “Cholecalciferol injections are effective in hypovitaminosis D after duodenal switch: A randomized controlled study.” *Obesity Surgery*, vol. 28, no. 10, 4 June 2018, pp. 3007–3011, https://doi.org/10.1007/s11695-018-3307-8.

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Abbreviations

25(OH)D	25-hydroxy vitamin D aka calcidiol
BMD	Bone Mineral Density
BMI	Body Mass Index
BPD/DS	Biliopancreatic Diversion/ Duodenal Switch
CaSR	Calcium Sensing Receptor
CI	Confidence Interval
CKD	Chronic Kidney Disease
COVID-19	Corona Virus Disease 2019
CSF	Colony Stimulating Factor
DEXA	Dual Energy X-ray Absorptiometry
eGFR	Estimated Glomerular Filtration Rate
EWL	Excess Weight Loss
FGF23	Fibroblast Growth Factor 23
hPTH	Hyperparathyroidism
IGF-1	Insulin-like Growth Factor 1
MDRD	Modification of Diet in Renal Disease $= 186.3*(\text{creatinine}/\ 88.4)^{-1.154} * \text{age}^{-0.203}$ (*0.742 for women)
OR	Odds Ratio
PPI	Proton Pump Inhibitor
PTH	Parathyroid Hormone
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand

RYGBP	Roux-en-Y Gastric Bypass
SG	Sleeve gastrectomy
SOReg	Scandinavian Obesity Surgery Registry
TRPV5	Transient Receptor Protein subfamily V member 5
TRPV6	Transient Receptor Protein subfamily V member 6
UVB	Ultraviolet-B Radiation
VDBP	Vitamin D Binding Protein

Introduction

This thesis studies the maintenance of calcium and vitamin D levels in patients who have undergone bariatric surgery due to obesity. The changes in gastrointestinal anatomy following bariatric surgery result in reduced peroral intake as well as gastrointestinal malabsorption. In addition to massive weight loss, various vitamin deficiencies are common postoperatively.

History

1. Vitamin D

At the beginning of the twentieth century the four essential components of the human diet were believed to be carbohydrates, protein, fat, and minerals. Conditions, including beri-beri, scurvy, and rickets, had been identified but their causes were still unknown. Experiments suggested that diet was a key player in these conditions, and in 1911 the work by Casimir Funk concluded that a "vital amine" may be needed. It was at this point that the term "vitamin" was born.[1]

Vitamin D was discovered shortly thereafter during a series of experiments that studied rickets (also commonly called "English disease" reflecting its high incidence in the United Kingdom).[2] Sir Edward Mellanby modelled the disease in dogs by feeding them a diet of exclusively oatmeal and inadvertently kept them indoors. He later supplemented the diet with cod liver oil, thereby curing the condition and suggested that vitamin A deficiency was the culprit. Elmer McCollum repeated the experiment with cod liver oil where vitamin A had been removed, and after arriving at similar results, he concluded that vitamin A was not the cure, and termed the new compound vitamin D.[2]

Rickets, characterized by bone deformations and skeletal fragility, was abundant among British (primarily Scottish) children prior to the twentieth century. During this time people moved away from the rural lifestyle and began living more indoors in cities where the air was polluted due to growing industrialization. This transition starved children of both ultraviolet radiation and a diverse diet, leading to a growing incidence of nutritional rickets, i.e., rickets caused by vitamin D deficiency. [2]

Vitamin D deficiency, discussed in further detail later, may cause low levels of serum phosphate and calcium, which in turn leads to faulty

mineralization of the growth plate and brittle osteoid structure in growing children.[3] Since the state of deficiency was discovered the fortification of food and supplementation of vitamin D has been implemented in several countries to meet the needs of growing children. Today rickets is rare among healthy children in developed countries, however vitamin D and states of deficiency remain topics of concern.[4]

2. Bariatric Surgery

In 1952 the Swedish surgeon Viktor Henrikson published his results after having performed an intestinal resection in an obese patient.[5] Shortly thereafter, Kremen et al contributed their results from a study where intestinal resections of various types were performed on canines. The purpose of the study was to investigate the procedure, performed then primarily to excise malignancies or ischemic tissue. Intestinal resections were at that time associated with catastrophic consequences, including malnourishment and bone disease. His results showed varying weight loss and fat absorption contingent upon which part of the intestine was resected.[6] A revolutionary change came about when the gastric bypass procedure was first documented by Mason in 1967. The procedure was a modification of the commonly performed Billroth II, which was then used to treat peptic ulcer disease. For the first time malabsorptive and restrictive qualities were combined and aimed at treating obesity. It was performed in twenty-four patients obtaining an average weight loss of 86 lbs (39 kgs).[7] Alongside this success, the report mentions mortality in two patient cases and cause for alarm due to complicated anesthesia among patients with obesity. The surgery was also reported to require roughly five hours to complete.[7]

Gastric bypass became increasingly popular, achieving not only reduction in body mass index but also improvements in obesity related conditions.[8] Already in 1992, a ten-year follow-up after gastric bypass reported remission in over 90% of patients with type-II diabetes mellitus. In addition, morbidity and mortality was cited at merely 1.2% of patient cases.[8] These drastic improvements fueled the procedure's growing approval and further developments. Additional bariatric procedures have since been introduced, including the sleeve gastrectomy (SG) and the biliopancreatic diversion/duodenal switch (BPD/DS).

Surgical procedures that entail modification of the small intestine are associated with reduced absorption of nutrients and thereby also states of deficiencies.[9] Bariatric procedures, resulting in both reduced gastric volume and reduced absorption due to a shortened small bowel, have successfully applied this to treat severe obesity. Vitamin D deficiency is of particular interest in this group due to the high prevalence of hypovitaminosis D among people with obesity, prior to bariatric surgery.[10, 11] This, followed by a state of malabsorption after bariatric surgery, leads to compounding risk factors

for severe deficiency and likely associated conditions. Despite lifelong multivitamin supplementation states of nutrient deficiencies are common, including vitamin D. [9]

Calcium Homeostasis

Calcium is an essential molecule in human physiology, of which 99% is found in bone. Stored here as hydroxyapatite, the molecule provides a robust structure in the midst of a meshwork of collagen fibers and cells. In circulation, either in its ionized form or protein bound, calcium is maintained within a strict concentration interval. Comparatively, a much higher concentration can be found extracellularly and due to this gradient calcium is used in multiple cellular processes including intra- and inter-cellular signaling.[12] The maintenance of circulating calcium is the result of an interplay between several components, including vitamin D, parathyroid hormone (PTH), phosphorous and FGF23 to name a few.

1. Vitamin D

Discovered by identifying the state of deficiency, vitamin D has been inaccurately labelled a vitamin due to the initial assumption that it was an essential additive to the human diet, i.e. a vitamin. The truth is however that humans have an endogenous production of vitamin D that is catalyzed by sunlight. Within the lipid bilayer of dermal and epidermal cells the compound 7-dehydrocholesterol can be found. It is here that the compound is exposed to ultraviolet-B radiation (UVB) from the sun, which converts it to pre-vitamin D3 followed by thermal isomerization to vitamin D3. This compound is sterically unfavorable to the lipid bilayer, therefore it is expelled from the membrane into the extracellular space, which leads to its transportation into the circulatory system. Once in the serum the vitamin is accompanied by vitamin D binding protein (VDBP), and to some degree albumin, during its migration through the circulatory system.[13]

Apart from the endogenous production, vitamin D can also be obtained, like true vitamins, through diet. The two forms of vitamin D, D2 (also known as ergocalciferol) and D3 (also known as cholecalciferol), are found in certain plants and fatty fish respectively. When consumed, the fat-soluble vitamin has a bioavailability estimated at the wide span of 55-99%, likely dependent upon both the type of food consumed simultaneously and dietary peptides released during consumption.[14] Absorption has long been presumed to occur by passive diffusion in the duodenum and jejunum, however recent studies suggest active channels may also contribute.[15]

Once in circulation, regardless of source, the vitamin travels bound to vitamin D binding protein (VDBP) and requires a two-step process to be activated. The first step is a hydroxylation reaction in the liver, which transforms the molecule into 25(OH)D. (Figure 1) This reaction is catalyzed by the enzyme CYP2R1, however knockout models suggest other enzymes also assist.[13] Studies of mice have also suggested reduced enzyme activity associated with obesity, yet no causal relationship has been established.[16] In addition,

conditions of inadequate hepatic function, including cirrhosis, have been associated with reduced 25(OH)D levels.[17, 18]

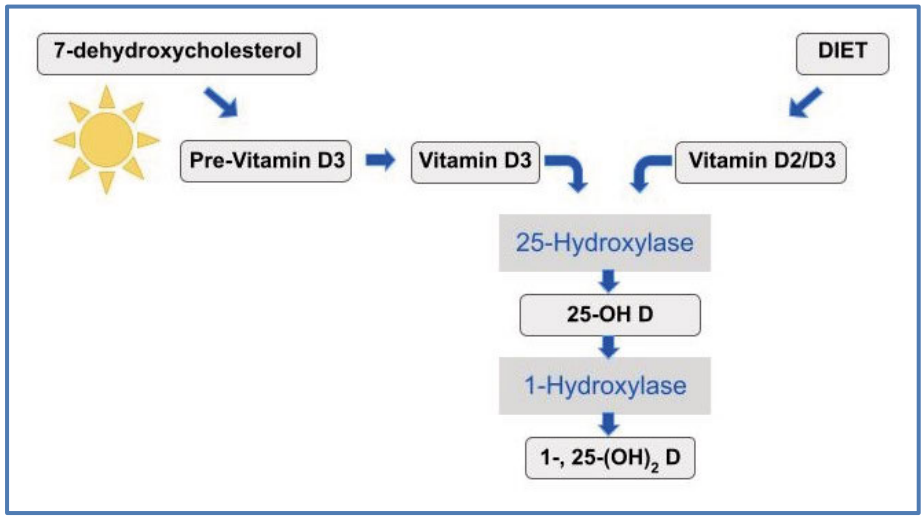

Figure 1. Activation pathway of Vitamin D

The second step is another hydroxylation reaction, this time by 1-alpha hydroxylase, also referred to as CYP27B1, located in the kidney. The enzyme is promoted by PTH and is inhibited by elevated levels of its product, active vitamin D.[19, 20] Clinically this enzyme is highly relevant in patients with chronic kidney disease (CKD). Reduced levels of active vitamin D are seen in patients with CKD, which is a contributing cause of secondary hyperparathyroidism and thereby osteomalacia (discussed further below).[21] As depicted in figure 1, the product of this second hydroxylation is 1-, 25-$(OH)_2$D (also known as calcitriol), the active form of vitamin D.

Historically the dietary contribution to overall vitamin D supply has been presumed to be minor, and possibly excessive and unnecessary, in comparison to the endogenous production. While this may be true for fair-skinned individuals at low latitudes with an outdoor lifestyle, the opposite likely applies to the greater majority. A study of seasonal variation in 25(OH)D levels among Swedish men showed a mean difference of 35.1 ± 16.3 nmol/L when comparing values of late spring to late summer.[22] Given reduced sun exposure due to urban living and increased awareness of skin cancer protection, it is possible that endogenous vitamin D is a minor component, and dietary sources should be considered the dominant supply. Accordingly, recommendations state that additional vitamin D should be primarily obtained through diet and dietary supplementation.[23]

In its active form, vitamin D increases serum levels of calcium by acting primarily in the gut, kidney and bone. Receptors (VDR) are found along the

small intestine, mainly the duodenum, where they signal additional calcium channels. The result is an increase in vitamin D dependent calcium channels, specifically TRPV6, that actively absorb calcium from the intestinal lumen.[21]

At the site of the kidney calcium is freely filtered in the glomerulus and then reabsorbed by the tubules. The majority (roughly 65%) of calcium reabsorption occurs passively in the proximal tubule. Additional reabsorption is vitamin D dependent and occurs in the distal tubule. Here vitamin D dependent channels, TRPV5, actively pump calcium from the lumen of the tubules. Experiments in mice have shown that the absence of TRPV5 channels leads to hypercalciuria, thereby illustrating their significance.[24]

Another key site for vitamin D activity is bone. Being the primary site for stored calcium, bone is constantly "remodeled" in adults. This is a continuous resorption and rebuilding process that allows the skeleton to mobilize calcium while strengthening areas in response to mechanical stress. For the process to occur without compromising the structural integrity there must be sufficient levels of substrate, specifically calcium, in circulation.[20] Calcitriol acts by activating both osteoclasts that resorb bone, and immature osteoblasts that eventually build bone. [25] In adults vitamin D deficiency causes impaired bone mineralization and thereby weak bones. Clinically, this condition is called osteomalacia, further discussed below. [25]

The active vitamin is also a key player in the calcification of cartilage at the growth plate, therefore fundamental for the development of bones in children.[24] When remodeling occurs at the growth plate in children, the lengthening of bones relies partly upon vitamin D dependent calcification of cartilage. In the absence of vitamin D, the lengthening of bones lacks the structural integrity needed to provide the strength and support needed. The result is the characteristic bowing of long bones seen in rickets.[28]

Outside of bone health, vitamin D has been implicated in several anti-inflammatory, immunomodulatory and anti-fibrotic pathways.[29] Vitamin D has also shown to promote the antimicrobial actions of monocytes and macrophages.[30] These findings have led to several studies that have identified an association between vitamin D deficiency and other disease states including cardiovascular disease, multiple cancer types, insulin insensitivity and respiratory tract illnesses.[24] Most recently, vitamin D deficiency has been associated to severity and mortality among COVID-19 patients.[31] It is important to note that causation has yet to be proven in most other associated diseases.

The definition of vitamin D deficiency has varied throughout the years and a true consensus has yet to be reached. Studies have shown that PTH levels rise significantly when vitamin D levels are below 60 nmol/L and a slight plateau has been shown at levels above 73 nmol/L.[32, 33] Bone mineral density has been shown to reduce significantly when 25(OH)D levels are below 62 nmol/L.[34] Lately increased attention has been paid to vitamin D deficiency and associated conditions. This has led to a drastic increase in supplementation, with and without a prescription from a health care provider. Despite this,

vitamin D toxicity is still very rare. A recent population-based study examined the prevalence of vitamin D toxicity in 20,308 individuals with 25(OH) D levels above 125 nmol/l. The study found no association between 25(OH)D levels and serum calcium levels despite elevated 25(OH)D levels. In addition, only one case of vitamin D toxicity was identified, in which the individual had a 25(OH)D level of 910 nmol/l (364 ng/ml).[35] These results illuminate the large span between vitamin D sufficiency and vitamin D toxicity.

2. Parathyroid

The calcium gradient is tightly monitored and controlled by the parathyroid, a small gland located in two pairs behind the thyroid gland. The parathyroid gland has calcium sensing receptors (CaSR) that monitor the extracellular concentration of the ion. In the case of low serum calcium the gland increases the release of parathyroid hormone and vice versa. Parathyroid hormone (PTH) works to replete the concentration of calcium in circulation by releasing calcium from bone, and by inducing 1-alpha hydroxylase at the kidney to activate more vitamin D. Sequentially, activated vitamin D (calcitriol) promotes the absorption of calcium from the small intestine and the reabsorption of calcium in the kidney. Receptors for PTH in bone are found in osteoclasts, cells specified to resorb bone and thereby release stored calcium.[36]

Excessive PTH secretion, known as hyperparathyroidism, has multiple causes. Primary hyperparathyroidism is caused by hyperplasia of parathyroid tissue, where the hyperactive secretion of PTH occurs despite raised serum calcium levels. Secondary hyperparathyroidism is a condition where PTH levels are raised to increase calcium in circulation in response to reduced serum levels. This may be caused by reduced calcium intake, and/or reduced vitamin D levels which in turn leads to reduced calcium absorption. In the case of prolonged raised PTH levels, bone is depleted due to excessive resorption.[36]

3. Phosphorus

Phosphorus is an essential building block in cell membranes, nucleotides, as well as a key component in many cellular reactions including energy production. Approximately 85% of the body's phosphorus content is stored with calcium in bone and teeth as hydroxyapatite. Through calcium dependent and independent processes, the element can be absorbed actively and passively primarily from the duodenum and proximal jejunum. Phosphorus levels are maintained at a narrow range by PTH, fibroblast growth factor 23 and vitamin D.[37]

4. Fibroblast Growth Factor 23

This hormone is synthesized primarily by osteocytes and works to promote phosphate excretion. Phosphate excretion is achieved primarily through the kidneys by reducing the expression of channels that reabsorb phosphate from the urine. Therefore FGF-23 is commonly characterized as phosphaturic. FGF-23 is also known to inhibit the activation of vitamin D, thereby reducing phosphate and calcium absorption from the intestine. This complicated pathway has further effects upon calcium levels as elevated FGF-23 levels have also been shown to decrease PTH production, thereby inhibiting mechanisms that increase calcium and phosphate levels.[36, 38]

5. Magnesium

This essential nutrient is a vital component in the production of energy for cells. Magnesium is also a cofactor for vitamin D activation, among many other pathways in the body. Roughly 60% of the body's magnesium is stored in bone, partially encapsulating the hydroxyapatite molecule. Magnesium deficiency has been associated with bone disease, likely because of its role in activating vitamin D, but also due to reduced osteoblast activity and increased osteoclast activity.[39]

Bone

Bone, by volume, is made up of approximately 10% cells and 90 % extracellular matrix. There are four types of bone cells: osteoprogenitor cells, osteoblasts, osteoclasts and osteocytes. Osteoprogenitor cells regulate the flow of minerals in and out of the extracellular matrix. They are also able to differentiate into osteoblasts, which are "bone building" cells. Osteoblasts reside on the surface of bone where they secrete proteins that bind phosphate and calcium, thereby regulating mineral deposition. Active vitamin D and PTH cause osteoblasts to secrete macrophage-CSF and RANKL, which are important cell signaling molecules in osteoclastogenesis.[38]

An osteoblast may also differentiate into an osteocyte, which is the mechanosensory cell of bone. When mechanical stress occurs deformation of the matrix leads to flow of fluid through the canalicular network. Osteocytes detect this flow and thereby control the remodeling of bone. They also have a role as endocrine cells, secreting FGF23 which leads to increased renal phosphate excretion, as mentioned previously.[36, 38]

Osteoclasts are bone resorbing cells which, through the secretion of enzymes and hydrogen ions, dissolve the deposited mineral and break down the type I collagen network. Osteoclasts are stimulated to resorb bone by PTH and inhibited by calcitonin.[38]

The bone matrix, by volume the largest component, is made up of organic and inorganic matrices. The inorganic bone matrix is responsible for the strength and durability of bone. This is the storage unit for calcium but also 85% of the body's phosphorus and most of the magnesium. Here, these molecules are mostly found in the compound hydroxyapatite. The organic matrix is the portion secreted by osteoblasts. It is composed of type I collagen, glycoproteins, proteoglycans and growth factors. This portion of the matrix protects it from tensile stress and gives bone its shape.[38]

During childhood bones continue to accrue mass, leading to a peak bone mineral density in approximately the third decade of life. Determinants of peak BMD include genetic factors, diet and physical activity to name a few.[40] After peak BMD bone continues to be remodeled. The maintenance of its robust architecture is dependent upon the balance between resorption and rebuilding by osteoclasts and osteoblasts respectively. As a result of complex cell signaling, the two different types of cells maintain the integrity of the skeleton while supplying the necessary calcium needed for cellular processes in the entire body.

1. Osteomalacia

Osteomalacia, also called "soft bone disease," is a condition where nutrient deficiency (vitamin D, calcium and/or phosphate) leads to faulty mineralization of bone during the remodeling process. The condition is often associated

with elevated levels of parathyroid hormone and leads to porous cortical bone and a net loss of cortical bone on the endosteal surface. The result, as evident by the name, is a more compliant structure that may begin to bend at weight-bearing sites. A patient with osteomalacia may experience vague pains from limbs where the bending of bone leads to stretching of the periosteum.[41] Finally, the condition results in pseudofractures, a radiological finding where the cortical surface exhibits a radiolucent line (due to reduced mineralization) perpendicular to the long axis of the bone.

Patients with osteomalacia may have elevated levels of bone turnover markers, such as ALP and PTH, as well as low levels of 25(OH)D, phosphate and/or calcium.[42] A reduction in bone mineral density may be evident as well.

2. Osteopenia and Osteoporosis

Osteopenia and osteoporosis are two related conditions of deteriorated bone density. The conditions are defined according to a statistical model, which compares an individual's bone mineral density scan to those of young healthy adults. Osteopenia is defined as a t-score between -1 and -2.5, and osteoporosis is a t-score below -2.5. There are multiple risk factors for osteopenia/osteoporosis including female sex, increased age, smoking, low physical activity and certain medications. Estrogen reduction, primarily associated with menopause, causes an increase in osteoclast activity, thereby increasing bone resorption. This occurs because of increased RANKL and M-CSF expression, which inhibits osteoclast apoptosis, leading to a net increase in the resorption of bone relative to the rebuilding process. Older age leads to a reduction in bone formation due to increasing levels of reactive oxygen species and PTH, as well as decreasing IGF-1 levels.[38]

3. DEXA

Dual-energy X-ray absorptiometry (DEXA) is a method used to measure bone mineral density. The method utilizes two low dose x-rays aimed at specific sites where the attenuation of the rays will reflect the types of tissues through which they have passed. When placed correctly, the difference in attenuation can differ between soft tissue and bone density, thereby allowing for the quantification of mineral content.[43] Bone mineral content, measured as g/cm^2, can then be compared to normal values. A statistical model uses this comparison to produce a T-score, which indicates how many standard deviations the individual measurement is from those of premenopausal women. As previously mentioned, T-scores that lie below one standard deviation of normal values indicate osteopenia, whereas T-scores lower than 2.5 standard deviations indicate osteoporosis.[44]

A DEXA scan usually focuses on sites that are also common for osteoporotic fractures, i.e. hip and lumbar vertebrae. The forearm is also measured in

case the aforementioned sites are unfavorable. Studies have repeatedly shown that DEXA scans can be misleading in individuals with obesity, particularly at the lumbar spine and femoral neck sites. This is partly due to overlaying tissue, which is calculated based on the assumption that the composition of soft tissue is homogenous. In individuals with obesity this tissue composition can vary, leading to greater precision errors.[45]

4. Proton Pump Inhibitors

As one of the most prescribed medications, this group has become a cornerstone for treatment of acid-related disorders. Proton pump inhibitors (PPIs) reduce the production of acid in the stomach. Specifically, these medications block a pump that secretes protons, hence the name. The pump can be found on parietal cells that line the stomach and is stimulated by the release of gastrin from G cells and the release of histamine from ECL cells.[46]

Proton pump inhibitors are intended for the treatment of acid-related disorders, but are also prescribed for prophylactic purposes, such as concomitant use of anticoagulants, NSAIDs, or corticosteroids. Certain formulations and dosages are also available without a prescription from a healthcare provider, making the usage both common and often underreported. A recent study reported that roughly 10% of bariatric patients regularly use proton pump inhibitors.[47]

Short-term use of PPIs is generally considered safe and therefore often recommended quite liberally by guidelines addressing acid-related disorders. Long-term use has however been linked to malignancies of the gastrointestinal tract and pneumonia, although causation has yet to be established.[48] The medication has also been associated with hypomagnesemia and vitamin B12 deficiency. Finally, several studies have reported elevated levels of PTH among patients using PPIs, where possible mechanisms include reduced calcium absorption and elevated levels of gastrin.[48]

Obesity

Obesity has been a growing problem over the past century, increasing at a nearly exponential rate during the past decades. Previously mainly a condition found among adults in high-income societies, overweight and obesity is now spanning the globe, socioeconomic statuses and occurring at younger ages. The World Health Organization reported that the obese population has close to tripled since 1975. Statistics from 2016 reported 39% of adults were overweight, and 13% were obese worldwide. Among children the numbers continue to grow. In 1975 barely 1 % of children aged 5-19 years old were obese whereas in 2016 6% of girls and 8% of boys were reported to meet the criteria for obesity.[49]

As these numbers continue to grow, so do the prevalences of diseases attributable to obesity. Related conditions include diabetes mellitus type 2, metabolic associated fatty liver disease/metabolic dysfunction-associated steatohepatitis, hypertension, coronary artery disease, and various malignancies to name a few.[50] Diabetes mellitus gains exceptional attention due to its increase in prevalence globally, but also mortality and suffering. In 1980 roughly 108 million people had been diagnosed with diabetes mellitus worldwide. Thirty-four years later an estimated 422 million people had received the diagnosis.[51] Following suite, data from 2017 report 6.28% of the world's population are diagnosed with diabetes mellitus. Over 1 million deaths per year can be attributed to diabetes alone, making it the ninth leading cause of mortality. Obesity is blamed for the drastic increase in these numbers.[10]

1. Vitamin D Deficiency and Obesity

There is a well-established association between vitamin D deficiency and obesity. Studies have shown a prevalence of vitamin D deficiency in the large span of 35-85% of people with a BMI > 40 kg/m^2.[11, 53] In addition, an inverse relationship between 25(OH)D and BMI has been reported by several studies. Thus, it is no surprise that patients eligible for bariatric surgery commonly present with vitamin D insufficiency/deficiency preoperatively.[54]

The cause of this association is however still unclear, and many mechanisms have been proposed. Because sunlight is a major source of vitamin D, outdoor activity and thereby exposure to UV radiation are positively associated with vitamin D levels.[55] Reduced exposure to sunlight due to clothing, and/or a more stationary lifestyle are plausible explanations for hypovitaminosis D, however this is difficult to objectively measure. Diet, the other source of vitamin D, is also a hypothetical cause of deficiency among people with obesity.

Because vitamin D is a fat-soluble compound, adipose tissue can sequester the nutrient. An increase in adipose tissue may therefore lead to more compartments for vitamin D to be stored. A study comparing the adipose tissue of

patients with obesity to the tissue of patients with normal BMI has provided evidence for this.[56] This theory is also supported by the fact that 25(OH)D levels tend to increase during the first years after bariatric surgery, which is also when weight reduction normally occurs. In addition, overall dilution due to a larger body volume has also been suggested. This theory further supports individualized dosing of vitamin D supplementation according to body weight to maintain adequate 25(OH)D levels.[57]

Finally, adipose tissue expresses enzymes that influence the activation of vitamin D. Both the VDR and 1-alpha-hydroxylase have been discovered in human adipocytes, suggesting that adipose tissue is a site for vitamin D activation.[58] When activated, vitamin D (1-alpha-25 hydroxy vitamin D) will prompt its own inactivation by inducing the enzyme 24-alpha hydroxylase (also referred to as CYP24A1). This enzyme is also expressed in visceral and subcutaneous adipose tissue in humans.[59] Combined, excess adipose tissue may lead to the depletion of circulating 25(OH)D and the inactivation of vitamin D in its active form.

Regardless, the fact remains that a large proportion of patients eligible for bariatric surgery are vitamin D deficient. Patients eligible for bariatric surgery commonly present with a history of being overweight or obese that spans years, or decades even, during which they have likely been vitamin D deficient as well.[60] Therefore, as vitamin D deficiency is linked to conditions that threaten bone health, patients eligible for bariatric surgery may, already prior to surgery, be at risk for reduced bone health.

2. Bariatric Surgery

Bariatric surgery is the most effective treatment option for people with obesity. Not only is weight loss obtained, but also the reduction of associated conditions. Staggering numbers have been reported in multiple studies, suggesting that bariatric surgery is far superior to medical therapy in terms of remission of type 2 diabetes. Studies have further suggested that the Roux-en-Y Gastric Bypass (RYGBP) and Biliopancreatic diversion with duodenal switch (BPD/DS) are the two bariatric procedures with the best results in long-term remission of type 2 diabetes. After a five-year post-operative period, several studies reported remission rates of 30-66% for RYGBP patients.[61-64] And although the numbers diminish slightly over time, ten-year remission rates have been reported at 25% for RYGBP and 50% for DS patients.[65] Similarly diabetic nephropathy reduces drastically after surgery, one study reported resolution of albuminuria in 51% of patients that had undergone bariatric surgery five years postoperatively.[66]

2.1. Roux-en-Y Gastric Bypass (RYGBP)

Of all bariatric procedures, the most common in the western hemisphere to date is the RYGBP. It involves a marked reduction of the gastric volume in addition to bypassing the duodenum and a portion of the jejunum. Due to this combination, it is described as both restrictive and malabsorptive. The malabsorptive characteristics arise both because there is a reduced area upon which nutrients can be absorbed, but also because digestive enzymes from bile acid and pancreatic secretions do not intersect with ingested nutrients until further along the gastrointestinal tract. Long term follow-up studies have shown that the RYGBP results in an excess weight loss (EWL) of roughly 50-60%.[67]

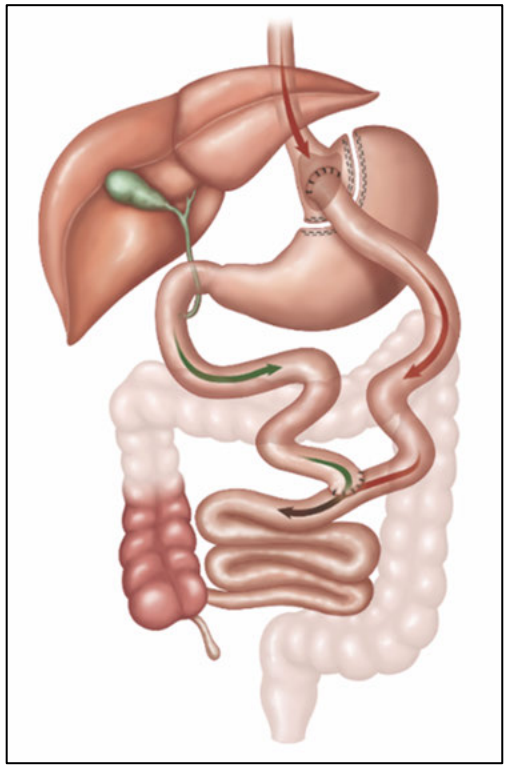

Figure 2. Illustration of Roux-en-Y Gastric Bypass (RYGBP) ©Ethicon, Inc. (2024) Reproduced with permission.

2.2. Sleeve Gastrectomy (SG)

In contrast to malabsorptive procedures, the SG procedure is considered solely restrictive. The stomach is reduced by excision of the major curvature, reducing the volume to roughly a third of what it was. This procedure has become increasingly popular during the past few years, producing EWL results similar to RYGBP.[67] During year 2023 31.1% of the bariatric procedures performed in Sweden were SG.[68]

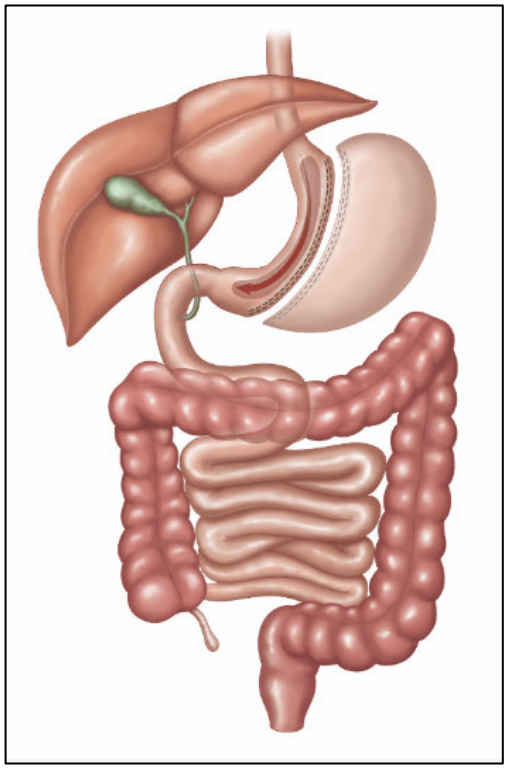

Figure 3. Illustration of Sleeve Gastrectomy (SG) ©Ethicon, Inc. (2024) Reproduced with permission.

2.3. Biliopancreatic Diversion with Duodenal Switch (BPD/DS)

The BPD/DS procedure is reserved for the treatment of extreme obesity (BMI > 50 kg/m^2). This procedure, although involving a reduction of the gastric pouch, is considered primarily malabsorptive. A combination of a vertical gastrectomy and a bypass of the duodenum leaves a gastric pouch at roughly 150 ml and total alimentary limb of approximately 40% of its previous length before entering the large intestine.[69] This greatly reduces the small intestinal surface area upon which nutrient absorption occurs, thereby achieving weight loss at the risk of essential nutrient deficiency. Despite multivitamin supplementation one study showed approximately 80% of patients suffered a micronutrient deficiency five years postoperatively and roughly 72% were vitamin D deficient.[70]

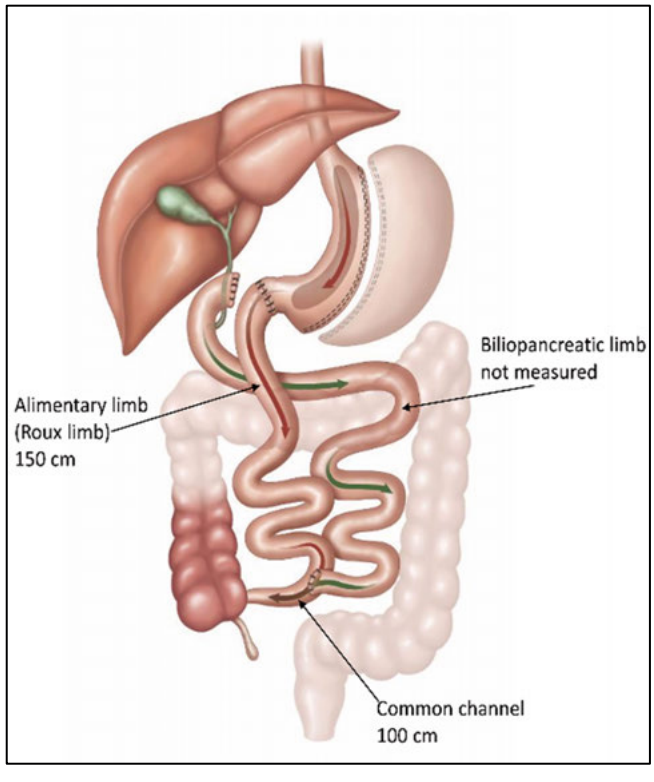

Figure 4. Illustration of Biliopancreatic Diversion with Duodenal Switch (BPD/DS) ©Ethicon, Inc. (2024) Reproduced with permission.

2.4. Vitamin D Deficiency after Bariatric Surgery

Of the bariatric procedures, the malabsorptive types are associated with the most significant weight loss, but also nutrient deficiencies.[9] Vitamin D deficiency is especially interesting in this group as the obesity pandemic reaches younger age groups. If vitamin D deficiency occurs prior to peak BMD is reached, bone mass will deteriorate from already reduced density levels. This will likely cause osteopenia and osteoporosis at younger ages. In addition, a common complication to type 2 diabetes includes chronic kidney disease, a condition that in itself may lead to vitamin D deficiency and thereby osteopenia. Studies show that 25(OH)D levels tend to increase the first year after surgery, followed by a gradual reduction. Few studies have followed patients for more than 3-4 years.[71] To date several studies have been published showing increased fracture risk among bariatric patients.[72-74] When comparing procedures, RYGBP and DS show a significantly higher risk of fracture versus strictly restrictive procedures.[75]

2.5. National Quality Registry for Bariatric Surgery

The Scandinavian Obesity Surgery Registry (SOReg) has been collecting data of bariatric procedures performed in Sweden since 2007. Data is reported into the registry at baseline, during the immediate postoperative period (up to 30 days after surgery) and at follow up appointments at one, two, five and ten years postoperatively. The registry contains more than 80,000 unique patients and has achieved a high acquisition rate and internal validity. Contents in the registry include surgery type, revisional surgery, age, sex, anthropometrics, specific medications including dietary supplements and results of blood samples analyzed for creatinine, hemoglobin, PTH and 25(OH)D.[76]

Main Hypothesis

Hypovitaminosis D is a common consequence after bariatric surgery, prevalence and severity varying between procedure types. Current supplementation regimes are not adequate in preventing vitamin D deficiency. Long term vitamin D deficiency may cause reduced bone mineral density, and thereby increase the risk for fracture.

Aims of the studies

- To compare lifestyle factors in two groups of premenopausal women, RYGBP-operated and controls, to eliminate these as culprits of the suspected difference in BMD. A secondary aim was to evaluate prognostic factors for low BMD in the RYGBP-group. (Paper I)

- The primary aim of this population-based study was to examine the trends of 25(OH)D and PTH levels among RYGBP and SG patients postoperatively. A secondary aim was to compare RYGBP and SG regarding 25(OH)D levels and the prevalence of hyperparathyroidism postoperatively. (Paper II)

- To compare the PTH values of postoperative RYGBP patients between those who report regular medication for acid-related diseases and those who do not. (Paper III)

- To evaluate intramuscular administration of cholecalciferol in patients with hypovitaminosis D after BPD/DS, despite standardized oral supplementation. (Paper IV)

Methods

Paper I

Women who had undergone RYGBP surgery at Uppsala University Hospital between 1996 and 2006 were invited to complete a DEXA scan and complete a short survey of lifestyle factors (see appendix I). The control group was obtained from a previous study conducted at Uppsala University where women had completed an identical survey and DEXA scan.[77]

Individuals were excluded if they met any of the following criteria: current smokers, menopause, or BMI < 20 kg/m^2. This left a final population of 71 RYGBP patients and 94 women in the control group.

The mean values for continuous variables were analyzed for normal distribution using Shapiro-Wilks test. Comparison of mean values was done by student's t-test for parametric data and Mann-Whitney U test for non-parametric data. Proportions were tested with a Chi^2 test. Significance was defined as $p< 0.05$ for these comparisons. A linear regression model was calculated using BMD as a dependent variable and independent variables were first entered individually where variables with a p-value < 0.1 were then included in a multiple variable model. To analyze the impact of time since surgery, a zero was entered for time since surgery for each individual in the control group.

The study was approved by the regional ethics committee in Uppsala Dnr 2005:295.

Paper II

The Swedish registry for obesity surgery, SOReg, was used to examine SG and RYGBP patients at one, two and five years after surgery concerning vitamin D and PTH levels. Data was extracted from 2008 to December 2021 (n= 81,036) but only RYGBP and SG patients with PTH and/or 25(OH)D values registered at some point were used for this study (total, n= 34,648; RYGBP n= 27,722 and SG n= 5,203). The MDRD equation was used to estimate glomerular filtration rate, and thereby exclude patients with reduced kidney function (defined as an eGFR < 60 ml/min/1.73 m^2). Only intact PTH was used, therefore patients were excluded if the postoperative center reported biointact PTH. Finally, patients that reported revisional surgery after their bariatric

procedure were excluded. The final study population was 25,385 RYGBP patients and 5,073 SG patients.

In the analysis of this data, mean values of 25(OH)D and PTH were compared from postoperative follow-up appointments. A PTH level > 7 pmol/L was defined as pathological, thereby creating a binary variable. This variable was entered as a dependent variable in logistic regression analyses for the two-year and five-year follow up data. Independent variables were entered in a stepwise manner, using the Hosmer-Lemeshow test as an indicator of goodness of fit. Finally, a linear regression model was calculated using only each patient's most recent follow-up appointment and calculating the amount of time since surgery to include as an independent continuous variable, thereby examining both the type of procedure as well as time since surgery as independent variables with an effect upon PTH levels.

The study was approved by the regional ethics committee in Uppsala Dnr 2021-06531-01.

Paper III

Again, using the SOReg population, patients that had undergone a RYGBP and had completed a ten-year postoperative follow-up were assessed. In this study the regular use of medications to treat acid-related disorders stratified the population to compare PTH levels. Only patients with 25(OH)D levels above 75 nmol/L were included, and other exclusion criteria were eGFR < 60 ml/min/1.73 m^2.

In a fashion similar to paper II, PTH levels were considered pathological if above 7 pmol/L and this classification was used as a binary variable. Logistic regression was performed similar to in paper II, but here the regular use of medications to treat acid-related disorders was entered into the model as an independent variable.

The study was approved by the regional ethics committee in Uppsala Dnr 2021-06531-01.

Paper IV

In this interventional study patients that had undergone BPD/DS surgery at Uppsala University Hospital between 2008 and 2010 were invited (n= 73). Patients were excluded if they had recently traveled or planned to travel to sunny climates, or if they received treatment for hypercalcemia, osteoporosis, primary hyperparathyroidism and/or renal failure. In addition, concomitant use of thiazides, corticosteroids, phenytoin, cholestyramine, phenobarbital and/or cardiac glycosides led to exclusion. Finally, pregnant and/or breast-feeding women did not partake in the study. Dietary supplementation with

vitamin D was restricted to 1400 IU in both groups and the study took place in December-May, thereby limiting the amount of UV-radiation obtained from sunlight.

The patients were randomly assigned (1:1) to either the treatment group or the control group. Thereafter, blood samples were taken from both groups, which were analyzed for 25(OH)D, intact PTH, calcium, creatinine and albumin. The treatment group received one intramuscular dose of 600,000 IU cholecalciferol and blood samples were repeatedly measured one, three and six months after injection. The values were compared using the Mann-Whitney U test and a p-value < 0.05 was considered significant.

The study was approved by the regional ethics committee in Uppsala Dnr 2012/201. EudraCT 2012-002217-19.

Results

Paper I

The RYGBP group was significantly older and had a higher average BMI than the control group. Roughly 7.5 years had passed since surgery (mean 7.55 ± 3.49). The RYGBP group also reported higher dietary calcium intake and no significance was seen when comparing physical activity. Despite this, reduced BMD was seen in the RYGBP group at the femoral neck and hip.

Table 1. Demographic and lifestyle information for groups and BMD measurements. Means ± Standard Deviations of continuous variables in each group compared using Student's t-test for parametric data, Mann-Whitney for non-parametric, and Chi2-test for categorical

Variable	**RYGBP** (n=71)	**Controls** (n=94)	**p-value**
Age (years)	42.4 ± 5.6	32.4 ± 5.0	<0.001
BMI (kg/m^2)	33.0 ± 5.8	23.9 ± 2.9	<0.001
Corticosteroid use (yes/no)	9/62	7/66	0.556
Menarche (years)	12.56	12.63	0.825
Calcium intake (mg/day)	681 ± 397	327 ± 126	<0.001
Activity, 5 y ago (min/week)	208 ± 248	287 ± 336	0.146
Present activity (min/week)	312 ± 356	315 ± 306	0.886
BMD Femoral Neck	0.98 ± 0.12	1.04 ± 0.13	0.004
BMD Hip	1.02 ± 0.12	1.07 ± 0.13	0.008
BMD Spine	1.22 ± 0.13	1.24 ± 0.14	0.487

Prognostic factors in RYGBP-patients

In a univariate regression analysis, younger age and not having undergone RYGBP were associated with higher BMD of both the femoral neck and total hip. No associations were seen for lifestyle factors. In multivariate analyses there was a trend towards an association between higher BMD of the femoral neck and younger age ($p= 0.09$), and a trend towards a negative correlation between BMD and time since surgery ($p= 0.06$). However, neither reached statistical significance in this study. For BMD of the total hip, each postoperative year was associated with a reduction of BMD by 0.003 g/cm^2, $p < 0.05$.

Table 2. Regression analysis with Total Hip BMD as dependent variable.

	Univariate			Multivariate		
Variable	**Coefficient**	**Standard error**	**P-value**	**Coefficient**	**Standard error**	**P-value**
Age at present	-0.004	0.001	0.006	-0.001	0.002	0.412
Years since surgery	-7.58 E-3	0.000	0.001	-6.16 E-3	0.000	0.026
BMI	0.000	0.002	0.738			
BMI at 20 yrs	0.000	0.002	0.705			
Calcium intake	3.172 E-5	0.000	0.915			
Activity present (min/week)	-5.871 E-5	0.000	0.844			
Activity 5 yrs ago (min/week)	1.191 E-5	0.000	0.711			

Paper II

This study showed a progressive trend toward vitamin D deficiency paralleled by elevated PTH levels. A significant difference is evident when comparing SG and RYGBP patients regarding 25(OH)D and PTH levels. This is evident both in mean values and the proportion of patients with insufficient/deficient 25(OH)D levels. (figure 5 and figure 6)

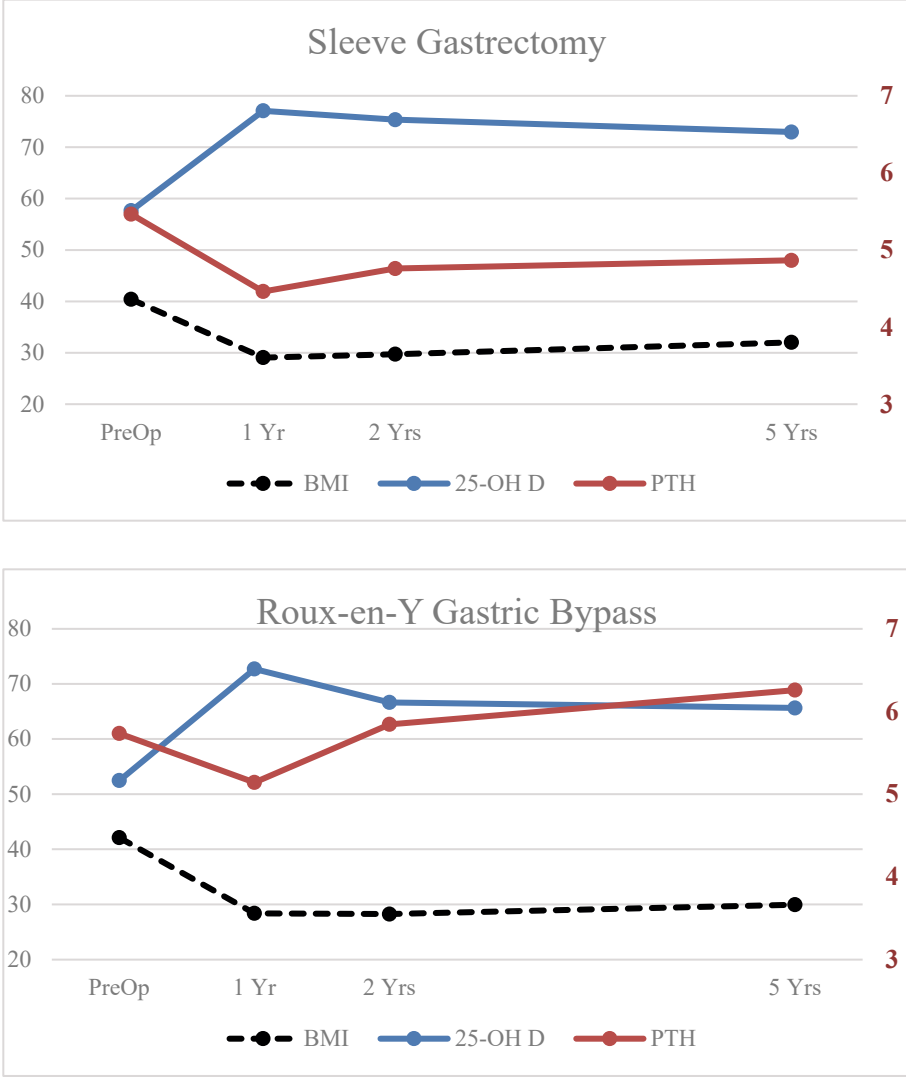

Figure 5. Mean values according to procedure.

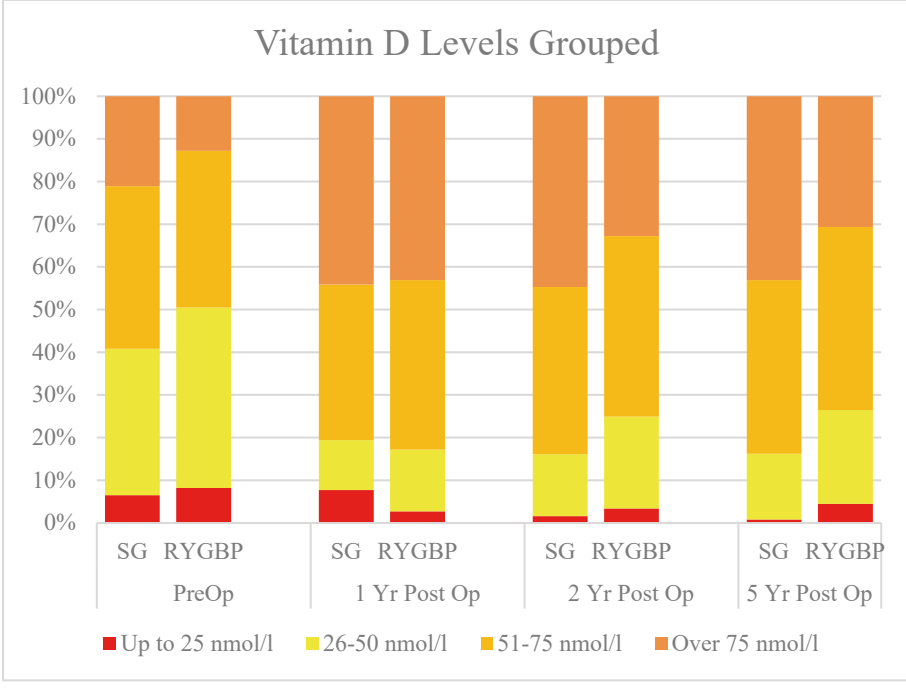

Figure 6. Proportion of patients according to 25(OH)D levels.

In an additional analysis, only the patients that had postoperative data for all follow-up appointments were compared. Similarly, lower 25(OH)D levels were evident at all data points mirrored by higher PTH levels among RYGBP patients.

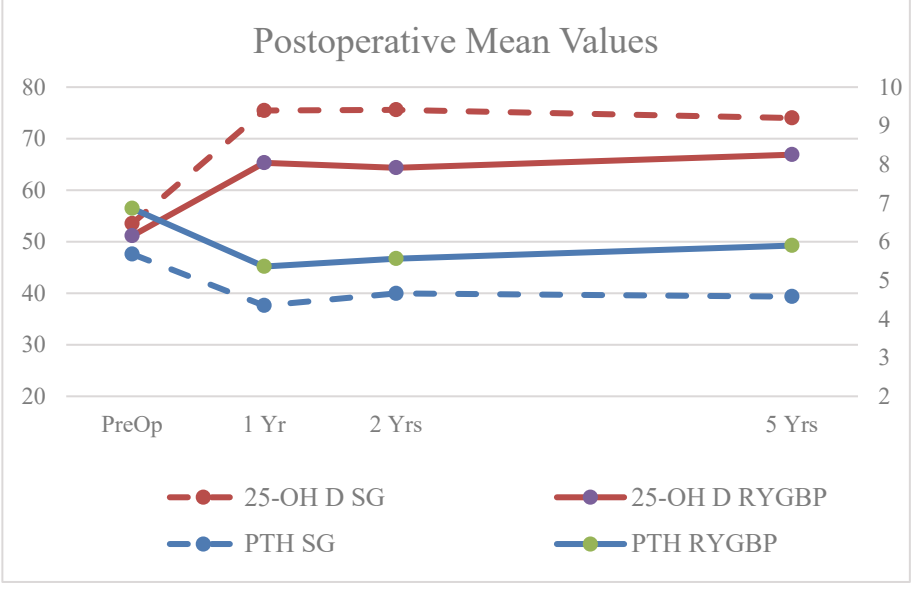

Figure 7. Mean values for patients with all data points. (SG n= 100; RYGBP n= 777).

In a logistic regression model, with pathological PTH levels defined as the outcome, independent variables known to be risk factors for elevated PTH levels (female, age, increase in BMI and low 25(OH)D levels) were all significant. In addition, the RYGBP procedure was a significant factor when compared to SG. (see table 3)

Table 3. Logistic regression model with pathological PTH levels as outcome.

	2 yrs				5 yrs			
	Total (%)	OR	95% C.I.	p-value	Total (%)	OR	95% C.I.	p-value
Sex								
Male	676 (23.9)	Ref.			891 (23.5)	Ref.		
Female	2156 (76.1)	1.44	1.6-1.8	<.01	2906 (76.5)	1.50	1.3-1.8	<.01
Age								
60.0-69.9	280 (9.9)	3.22	2.1-5.0	<.01	559 (14.7)	3.05	2.1-4.5	<.01
50.0-59.9	799 (28.2)	2.78	1.9-4.1	<.01	1155 (30.4)	2.36	1.6-3.4	<.01
40.0-49.9	898 (31.7)	3.02	2.1-4.4	<.01	1111 (29.3)	1.75	1.2-2.5	<.01
30.0-39.9	554 (19.6)	2.17	1.5-3.2	<.01	739 (19.5)	1.27	0.9-1.9	.22
20.0-29.9	301 (10.6)	Ref.			233 (6.1)	Ref.		
BMI								
20.0-29.9	1810 (63.9)	Ref.			1949 (51.3)	Ref.		
30.0-39.9	943 (33.3)	2.00	1.7-2.4	<.01	1647 (43.4)	1.73	1.5-2.0	<.01
40.0-49.9	79 (2.8)	4.19	2.6-6.8	<.01	201 (5.3)	4.06	2.9-5.6	<.01
Surgery								
SG	254 (9.0)	Ref.			291 (7.7)	Ref.		
RYGB	2578 (91.0)	3.41	2.2-5.3	<.01	3506 (92.3)	2.84	2.0-4.1	<.01
25(OH)D								
≤ 25	88 (3.1)	9.57	5.0-18.3	<.01	147 (3.9)	20.21	11.7-35.0	<.01
26-50	664 (23.4)	3.54	2.1-5.9	<.01	825 (21.7)	5.13	3.4-7.9	<.01
51-75	1271 (44.9)	1.94	1.2-3.2	.01	1705 (44.9)	2.22	1.5-3.4	<.01
76-100	645 (22.8)	1.38	0.8-2.3	.23	901 (23.7)	1.36	.9-2.1	.16
101-125	164 (5.8)	Ref.			219 (5.8)	Ref.		

Paper III

As presented in table 4, the groups did not differ significantly apart from PTH and 25(OH)D levels. There was also a significantly higher proportion of patients that presented with pathological PTH levels among those with regular PPI treatment (14.6% vs 38.9%; p = 0.018 as illustrated in figure 8).

Table 4. Comparison of patient demographics, PTH and Vitamin D levels in patients with and without daily use.

	PPI treatment (n=18)	No PPI treatment (n=157)	p-value
Men/Women %	16.7/83.3	19.8/80.2	1.00 [A]
BMI at 10 yrs	32.3 ± 7.7	30.7 ± 5.6	.385
Age (yrs)	49.6 ± 12.8	53.4 ± 10.8	.169
PTH (pmol/L)	6.56 ± 2.55	5.15 ± 1.95	.006
25(OH)D (nmol/L)	84.2 ± 10.8	90.3 ± 15.2	.049 [B]

[A] Comparison via Fischer Exact test, [B] Comparison via Mann-Whitney U test

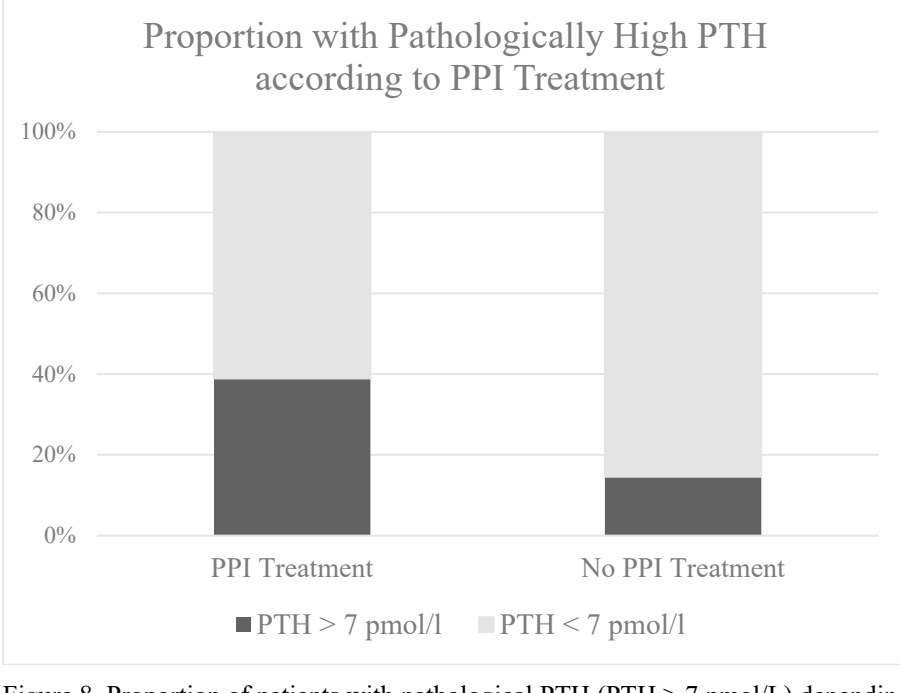

Figure 8. Proportion of patients with pathological PTH (PTH > 7 pmol/L) depending upon PPI treatment

Table 5. Logistic Regression Model, Pathological PTH as Dependent Variable.

		Simple Logistic Regression			Adjusted Logistic Regression		
	Total (%)	OR	95% C.I.	p-value	OR	95% C.I.	p-value
PPI treatment							
No	157	Ref			Ref.		
Yes	18	3.71	1.30-10.55	.014	3.97	1.35-11.70	.013
Age (yrs)							
≤ 52.93	103	Ref.			Ref.		
>52.93	72	2.72	1.15-6.33	.021	2.84	1.19-6.76	.018
BMI (kg/m^2)							
18.50-30.43	142	Ref		.			
> 30.43	33	1.96	0.87-4.40	.105			
25(OH)D (nmol/L)							
75-99	139	2.65	0.76-9.30	.128			
100 and up	36	Ref.					

Paper IV

At baseline, both groups had 25(OH)D levels below 50 nmol/l (injection 19.3; control 23.2 nmol/l) after more than 4 years postoperatively, despite oral supplementation. As demonstrated in table 6 and figure 10, the cholecalciferol injection resulted in elevated 25(OH)D levels at 1 month (65.4 vs. 29.2 nmol/l in controls, $p < 0.01$) and maintained for the duration of the study (67.4 vs. 29.2 nmol/l, $p = 0.04$).

Table 6. Effect of a single cholecalciferol injection from baseline to 6 months. (n= injection/controls). Data is presented as median value (interquartile range) and was compared by Mann-Whitney non-parametric U-test.

	Baseline (n=11/9)	**1 month** (n= 11/8)	**3 months** (n=7/6)	**6 months** (n=9/5)
25[OH]D (nmol/l)				
Injection group	19.3(9.3)	65.4 (6.3)	66.6 (11.0)	67.4 (16.1)
Control group	23.2 (22.5)	29.2 (13.4)	24.0 (23.0)	29.2 (31.4)
p-value	0.30	<0.01	0.03	0.04
Intact PTH (pmol/l)				
Injection group	12.3 (9.9)	6.4 (2.1)	7.3 (4.0)	9.6 (6.2)
Control group	9.5 (6.8)	8.5 (4.3)	8.4 (2.4)	9.1 (1.8)
p-value	0.20	0.15	0.23	0.90
Calcium (mmol/l)				
Injection group	2.2(0.14)	2.18 (0.13)	2.22 (0.13)	2.19 (0.06)
Control group	2.2 (0.10)	2.20 (0.20)	2.20 (0.20)	2.20 (0.10)

The largest increase in 25(OH)D levels varied between individuals, both in amount and point of occurrence. In table 7 the change in 25(OH)D levels (delta 25(OH)D) after injection is presented, which is further illustrated in figure 10. Table 7 also shows the large span across which delta 25(OH)D levels stretched after injection, which displayed an inverse linear relationship with BMI measurements (figure 9).

Table 7. Change in 25(OH)D levels after injection.

	Minimum	Maximum	Median (IQR)
BMI at study (kg/m^2)	22.0	43.1	32.2 (28.3-34.5)
Delta 1 month	11.8	64.1	45.7 (32.9-53.7)
Delta 3 months	27.4	69.1	47.3 (36.5-53.5)
Delta 6 months	21.5	95.3	48.9 (34.3-58.6)

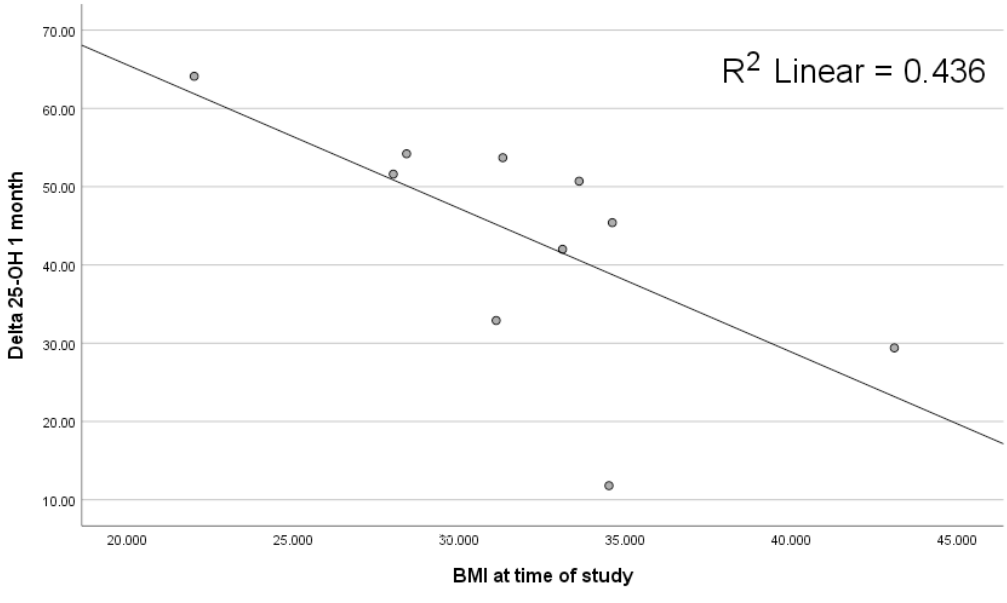

Figure 9. A scatterplot of change in 25(OH)D levels among patients having received cholecalciferol injections in relation to BMI.

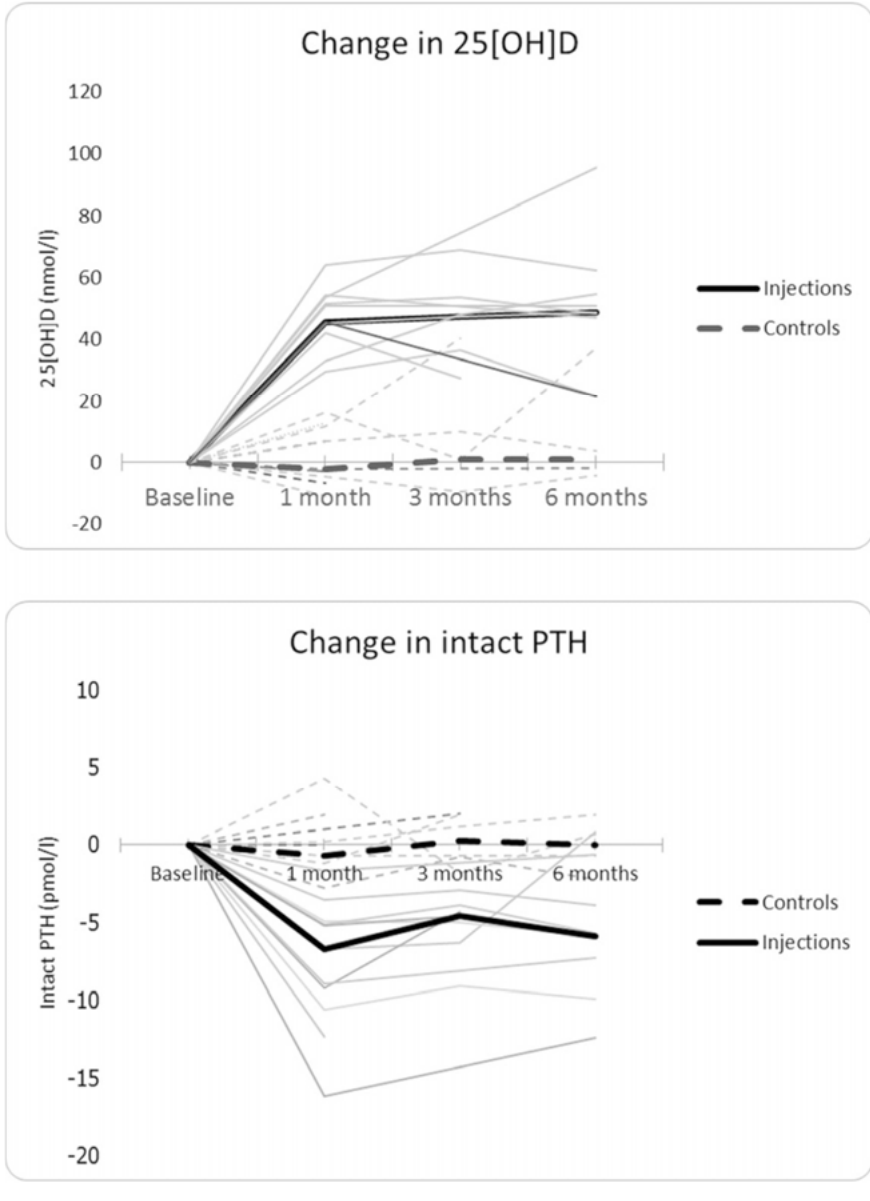

Figure 10. The changes in 25(OH)D and PTH levels for all patients in the two groups.

General Discussion

Bariatric surgery is the only treatment that, to date, has been shown to lengthen life expectancy among patients with obesity.[78] Roughly 1,3 million people in Sweden fulfill the criteria for obesity and approximately 5000 bariatric procedures are performed annually.[68] Therefore, the number of patients that require postoperative follow up is on the rise and will likely continue. The results presented here reveal the prevalence of vitamin D deficiency and elevated PTH levels after bariatric surgery. Consequently, these patients are likely at increased risk for developing bone disease in the long term.

Paper I

The results from paper I showed no evidence that lack of exercise or diet is a major factor when accounting for the reduced BMD after a RYGBP. Although the RYGBP group had a significantly higher mean age, this is likely an insignificant factor since all women were premenopausal. In agreement, several studies have shown that little or no significant BMD loss is evident at the femoral neck or total hip sites prior to menopause.[79]

Importantly, the protective effect of increased BMI upon BMD was not evident after bariatric surgery. In studies of non-bariatric patients, it is well established that BMD increases with an increase in BMI,[80] although the question of bone quality often arises at higher BMI levels.[81] This relationship does seem to be saturable though as studies have also described a plateau above 30 kg/m^2 and even a point of inflection at 50 kg/m^2.[82, 83]

Finally, in the regression analysis with BMD at the hip as a dependent variable, time since surgery was a significant variable even when accounting for age. Therefore, the RYGBP procedure has a deteriorating effect upon BMD over time. These outcomes, alongside other results indicating increased prevalence of fracture, strongly support that the effect of bariatric surgery increases the risk of bone disease.[84]

Paper II

Following the two most common bariatric procedures, vitamin D deficiency increases in prevalence after the first postoperative year. In addition, this deficiency mirrors a gradual rise in PTH, which suggests a disruption in calcium homeostasis. Finally, this trend is much more evident among RYGBP patients compared to SG patients. This is evident in both the trends of the entire population (figure 5) as well as in the trends of the population followed from before surgery to the five-year follow-up (figure 7). This is reiterated and enforced by the logistic regression analysis, clearly identifying RYGBP as a risk factor for developing pathological PTH levels. Essentially, this indicates that the procedure leads to altered absorption of nutrients essential in maintaining calcium balance. As mentioned, reduced calcium absorption has been shown by other studies which is likely a key component in our findings.[85]

Finally, the results indicate that 25(OH)D levels need to be at least 75 nmol/L following bariatric procedures, which is higher than the commonly accepted 50 nmol/L.

Paper III

The regular use of PPI medications among RYGBP patients may be another opponent in the maintenance of calcium. Studies have shown a higher prevalence of hPTH among patients with PPI medications.[86, 87] In accordance with this, the results from paper III do show an increased prevalence of pathological PTH levels among PPI users, however the OR is much higher than previously referenced studies (OR 1.6 vs OR > 3.5). Given that this population is likely already at risk for elevated PTH levels, evident from paper II, PPI use may lead to a compounded detrimental effect upon calcium homeostasis. Further studies are needed however to both prove causality, but also to decipher effects in relation to dose and duration of therapy.

Combined, the elevated risk for bone disease encourages discretion and careful screening when prescribing regular PPI use for long term use.

Paper IV

Nutrient deficiencies can be difficult to treat in patients after malabsorptive procedures. In the case of vitamin D deficiency, its ability to exist quietly poses a risk for long-term effects. The results of paper IV show the accompanying rise in PTH levels in the presence of low 25(OH)D. This is further enforced by the decrease in PTH levels following the improvement in 25(OH)D levels in response to the intramuscular dose of cholecalciferol.

A similar study followed BPD/DS patients for 12 months after having received an equivalent dose cholecalciferol. This study produced results similar to ours, but a slightly lower average delta 25(OH)D value following injection. This study also showed that 25(OH)D levels peaked around 3 months but remained sufficient for nearly all patients during the entire 12 months.[88Eiarsdotti] Given the known association between vitamin D deficiency and BMI, this is likely to have an impact upon the pharmacodynamics of cholecalciferol injections. A clear inverse relationship between delta 25(OH)D and BMI was evident in our study, illustrated in figure 9. Considering the fluctuations in BMI during the first years after bariatric surgery, this is an important factor when considering its applicability among bariatric patients.

Due to the malabsorptive qualities of the procedure, alternatives to oral supplementation are essential. Considering vitamin D deficiency, a single dose showed improved 25(OH)D and PTH levels for at least a six-month period without risking toxicity. Therefore, this treatment method proved to be a valuable alternative when treating a deficiency that is both common and silent.

Strengths and Limitations

Overall, a strength of the works presented here is that all bariatric patients were encompassed by the same postoperative guidelines for follow-up and supplementation. Both paper I and IV are based on patient populations recruited from the same surgical center, which further indicates that the individuals have had identical postoperative follow-up regimes. In addition, the use of the SOReg registry (paper II and III) provided a study population with nationwide coverage. Finally, the acquisition rate has proven high (> 95%) as well as the validity of the data in the registry.[76]

The cross-sectional and observational design of paper I, II, and III limit our results to presenting association rather than causation. Vitamin D measurements were not available in the control group for paper I. Therefore, it is impossible to conclude that this is the cause for the observed differences in BMD. In addition, data regarding sun exposure and diet to better quantify sources of vitamin D, and calcium was lacking. As discussed, quantities of milk and cheese were surveyed only as rough indicators of calcium intake. This method is highly susceptible to recall bias, as was the survey of activity level. Although this would apply to both groups, individuals who have struggled with obesity may be more conscious of their diet and activity level compared to those who have not.

The only indicator for bone turnover in paper II, III and IV was PTH. As discussed, PTH is a hormone in the regulation of calcium levels, thereby indirectly an indicator of bone formation/resorption. Ideally, additional markers (such as ALP, CTx, NTx and DPD) would also have been used, which could have shown a more specific and direct impact upon bone health. In addition, calcium measurements were not available for paper II and paper III. This impedes the ability to characterize the etiology of the PTH elevation observed.

In both paper II and paper III the question regarding supplementation and compliance arises. The surgical centers included in the registry are encompassed by the same guidelines for postoperative supplementation. However, since the registry is nationwide there are likely regional differences that may influence the follow-up process for individual patients. Compliance with prescribed supplementation is however beyond the scope of this thesis.

Finally, in paper III dosages and medication type regarding PPI use, as well as duration of therapy was not available. Subsequently, it is not possible to further define a drug therapy that is associated with elevated PTH levels. In

addition, our findings have not considered what the indication for the PPI therapy is nor the potential risks of discontinuing PPI therapy. Therefore our conclusion cannot urge that PPI therapy should be avoided or discontinued.

Lastly, paper IV showed lasting effects of intramuscular injections without illustrating a clear inflection point towards a decline in 25(OH)D levels. Ideally, these patients would have been followed for a longer duration in order to thoroughly examine the effects of the therapy. Considering the impact BMI has upon 25(OH)D levels, a larger sample size grouped according to BMI would have been beneficial.

Conclusions

In each study there is evidence of disturbances in calcium balance following bariatric surgery, illuminating the risk for progression to bone disease among these patients. This aspect should be considered when evaluating patients for surgery as well as during a lifelong specialized follow-up program.

Future Perspectives

What impact does seasonal variation have upon 25(OH)D levels among individuals with obesity and among patients having undergone bariatric surgery?

Are there individual variations in PTH responses that can account for the elevated PTH levels observed among some, but not all, patients after bariatric surgery?

Does compliance with prescribed vitamin supplementation contribute to hypovitaminosis D and are there methods to improve this?

Can additional calcium supplementation prevent the rise in PTH levels observed among patients that have undergone malabsorptive bariatric procedures?

At what age do bariatric patients most often present with a fracture and does this differ to patients who have not had bariatric surgery? Is there a difference depending upon how old the patient was when the bariatric procedure was performed?

Is there an association between diabetes mellitus remission following bariatric surgery and hypovitaminosis D/elevated PTH levels?

Can bariatric procedures alter peak bone mineral density when performed in young adults?

Sammanfattning på Svenska

I takt med ökningen av antalet personer som lider av övervikt och obesitas ökar även antalet överviktskirurgier. Årligen genomförs uppskattningsvis 5000 överviktsoperationer i Sverige, och av dessa dominerar Roux-en-Y gastric bypass (RYGBP) och sleeve gastrectomy (SG). Oavsett typ av operation är vitamin- och näringsbristtillstånd vanligt förekommande postoperativt. Bland dessa återfinns D-vitamin brist vilket sällan upptäcks av individen själv. På grund av störd kalkomsättning leder långvarig D-vitaminbrist till en nedbrytning av skelettet. Syftet med denna avhandling var att undersöka sambandet mellan överviktsoperationer och D-vitaminbrist samt dess påverkan på skelettet.

Delarbete I

I det första delarbetet undersöktes premenopausala kvinnor rekryterade från Uppsala området. Syftet med studien var att kartlägga livsstilsfaktorer och bentäthet samt att jämföra dessa faktorer mellan RYGBP-opererade kvinnor och icke-opererade kvinnor.

Kontrollgruppen, bestod av kvinnor som hade deltagit i en studie av Ribom 2008 för att kartlägga bentätheten hos normalbefolkningen i Sverige.[77] Genom folkbokföringsregistret hade kvinnor mellan 20 och 39 år identifierats och 684 valdes slumpmässigt ut och bjöds in till studien. Kvinnor som var gravida, ej födda i Sverige eller hade flyttat exkluderades. I delarbete I inkluderades sedan endast de med ett BMI över 20 kg/m^2, var icke-rökare, och premenopausala. Detta ledde till en kontrolgrupp på totalt 94 kvinnor.

Den andra kohorten bestod av kvinnor som hade genomgått en RYGBP operation på Akademiska Sjukhuset mellan 1996-2006. Samtliga bjöds in och exklusionkriteria var identiska med den för kontrollgruppen. Kohort två bestod av 71 RYGBP patienter.

Alla studiedeltagare genomgick en bentäthetsmätning via DEXA och fyllde även i en enkät där de uppskattade hur mycket fysisk aktivitet de vanligtvis utförde nu och för fem år sedan. På samma sätt fick de uppskatta intag av ost och mjölk. Kohorten med RYGBP patienter lämnade även blodprover som analyserades för D-vitaminnivåer.

Resultaten visade lägre bentäthetsnivåer hos RYGBP gruppen där inga signifikanta skillnader i fysisk aktivitet eller kost kunde bidra till en förklaring. Högt BMI hade en positiv korrelation med bentäthet hos den icke-opererade gruppen men inte hos RYGBP gruppen. I en regressionsanalys visade det sig att tiden sedan operation var associerad med låg bentäthet.

Sammanfattningsvis kunde inga livstilsfaktorer förklara den lägre bentätheten hos RYGBP gruppen jämfört med icke-opererade. Slutsatsen är därför att operationen kan vara en orsak för lägre bentäthet och att denna påverkan ökar över tid postoperativt.

Delarbete II

Det svenska registret, SOReg, samlar data från patienter som genomgått överviktsoperationer i Sverige. Registret består av data innan operation och uppföljningsbesök vid ett, två, fem och tio år postoperativt. Syftet med delarbete II var att undersöka trender i D-vitamin (25(OH)D) och parathormon (PTH) efter RYGBP och SG operationer.

Från SOReg registret inkluderades patienter som genomgått en RYGBP eller en SG operation och som hade 25(OH)D eller PTH nivåer inrapporterade vid ett uppföljningsbesök. Patienter exkluderades vid tecken på nedsatt njurfunktion (eGFR < 60 ml/min/1.73 m^2). Detta resulterade i en studiepopulation på 25,385 RYGBP och 5,073 SG patienter.

Resultat visade att genomsnittsvärdet på D-vitamin (eller 25(OH)D) var högre ett år postoperativt hos både SG och RYGBP opererade patienter. Därefter var genomsnittsvärdet lägre för varje uppföljnsingsbesök, och tydligt lägre hos RYGBP jämfört med SG gruppen. Dessa värden speglades även i en successiv ökning av PTH värden efter första uppföljningsbesöket. När operationsmetoderna jämfördes visade RYGBP gruppen signikant högre PTH nivåer. Även här sågs PTH värdet öka med tiden efter operation. 25(OH)D nivåer under 75 nmol/L, vilket skiljer sig från den generella gränsen för D-vitaminbrist på 50 nmol/L, visade sig även korrelera med höga PTH nivåer.

Sammantaget så skiljer sig D-vitamin nivåerna mellan SG och RYGBP grupperna efter operation, och detta påverkartillsynes även kalkbalansen. I och med att RYGBP operationen innefattar en justering av tunntarmen så kan detta vara en anledning till de lägre D-vitaminnivåerna och den påverkade kalkbalansen.

Delarbete III

I delarbete III var syftet att undersöka om användningen av protonpumpshämmare (en läkemedelsgrupp som hämmar produktionen av magsyra) påverkar kalkbalansen hos överviktsopererade. Även i detta delarbete användes SOReg

för studiepopulationen. Här undersöktes patienter som genomgått en RYGBP-operation och där data rapporterats från tioårsuppföljningen. Patienter med 25(OH)D nivåer under 75 nmol/l exkluderades för att undika direkt D-vitaminbristpåverkan på PTH. Detta ledde till en studiepopulation på 175 patienter.

Den grupp som regelbunde konsumerade PPI had ett högre genomsnittligt PTH värde, samt en större andel patienter med förhöjd PTH nivå. PPI användingen ökade även sannolikheten för förhöjda PTH nivåer nästan fyrfaldigt. Slutsatsen blir därför att PPI användning är associerat med högre PTH nivåer hos RYGBP opererade vilket eventuellt kan bero på att läkemedlet försämrar upptaget av kalk från tarmen.

Delarbete IV

I det fjärde delarbetet studerades ett alternativt sätt att tillföra D-vitamintillskott. Till skillnad från övriga studier undersöktes här patienter som genomgått en BPD/DS operation. BPD/DS är en operationsmetod som används för att behandla patienter med den högsta graden av obesitas, (BMI $> 50\ kg/m^2$). Operationsmetoden förminskar magsäcken, men framförallt omkopplas en större del av tunntarmen så att födan endast transporteras genom en bråkdel av denna. På grund av detta är operationen förknippad med grava bristtillstånd, inklusive D-vitaminbrist.

I detta delarbete undersöktes patienter som genomgått BPD/DS operation på Akademiska Sjukhuset Uppsala mellan 2008-2010. De patienter som rapporterat nedsatt njurfunktion, hade rest eller planerade resa till soliga länder, var gravida/ammade, och de som ordinerats läkemedel som kunde påverka kalkbalansen exkluderades. Slutligen återstod 20 patienter som lottades slumpmässigt till en kontrollgrupp och en injektionsgrupp som erhöll en dos 600 000 IE kolecalciferol intramuskulärt. Båda grupper fick begränsa D-vitamintillskotten peroralt till 1400 IE dagligen. Därefter lämnades blodprover 1, 3 och 6 månader efter injektion för att undersöka 25(OH)D, kalk och PTH nivåer.

Vid första blodprovstagning efter injektionen hade 25(OH)D nivåerna stigit signifikant hos injektionsgruppen jämfört med kontrollgruppen. Dessa värden speglades även i PTH nivåerna som sjönk efter injektion. De förbättrade värdena bibehölls hos injektionsgruppen vid tre- och sex-månadersprovtagningen. Ingen studiedeltagare visade toxiska nivåer av 25(OH)D under studietiden.

Slutsatsen från delarbete IV är att en injektionsbehandling kan erbjuda ett bra alternativ för patienter med D-vitaminbrist och försämrat upptag av kosttillskott.

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I might be the one the spotlight's on
But I didn't get here alone
-Kenny Chesney

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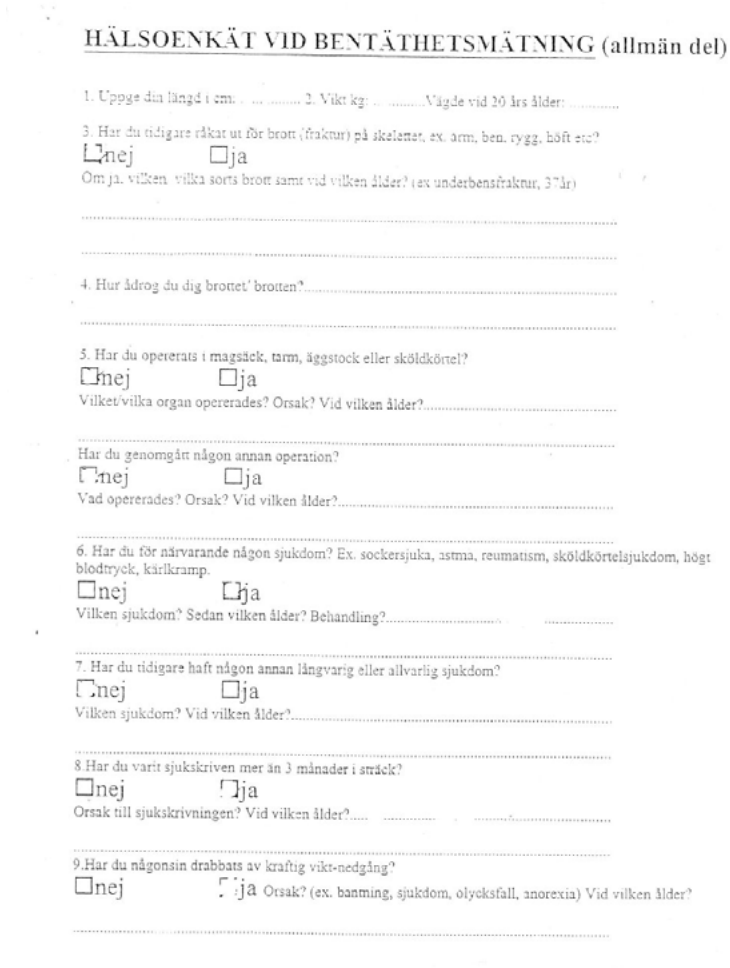
My honey, Ivar Stevens- Because of you all of this was possible, because of you anything is possible. I love you.

Appendix A

Hälsoenkät vid bentäthetsmätning



HÄLSOENKÄT VID BENTÄTHETSMÄTNING (allmän del)

1. Uppge din längd i cm. ..... 2. Vikt kg: ..... Vägde vid 20 års ålder: .....

3. Har du tidigare råkat ut för brott (fraktur) på skelettet, ex. arm, ben, rygg, höft etc?
☐nej ☐ja
Om ja, vilken vilka sorts brott samt vid vilken ålder? (ex underbensfraktur, 37 år)

.....

.....

4. Hur ådrog du dig brottet/ brotten?.....

.....

5. Har du opererats i magsäck, tarm, äggstock eller sköldkörtel?
☐nej ☐ja
Vilket/vilka organ opererades? Orsak? Vid vilken ålder?.....

.....

Har du genomgått någon annan operation?
☐nej ☐ja
Vad opererades? Orsak? Vid vilken ålder?.....

.....

6. Har du för närvarande någon sjukdom? Ex. sockersjuka, astma, reumatism, sköldkörtelsjukdom, högt blodtryck, kärlkramp.
☐nej ☐ja
Vilken sjukdom? Sedan vilken ålder? Behandling?.....

.....

7. Har du tidigare haft någon annan långvarig eller allvarlig sjukdom?
☐nej ☐ja
Vilken sjukdom? Vid vilken ålder?.....

.....

8 Har du varit sjukskriven mer än 3 månader i sträck?
☐nej ☐ja
Orsak till sjukskrivningen? Vid vilken ålder?.....

.....

9.Har du någonsin drabbats av kraftig vikt-nedgång?
☐nej ☐ja Orsak? (ex. banning, sjukdom, olycksfall, anorexia) Vid vilken ålder?

.....

Frågorna på denna sida riktar sig enbart till kvinnor

10. Hur gammal var du när du hade din första menstruation?

.....år.....mån

11. Har du fortfarande menstruationer?

☐nej.

menstruationerna upphörde helt när jag var: .....år

☐ja.

under sista året har jag haft antal menstruationer:

☐10 (eller flera) ☐5-9 ☐3-4 ☐1-2

Min senaste menstruation började (datum): .....

12. Har du haft någon period med oregelbundna menstruationer?

☐nej ☐ja

Vid vilken ålder? Hur länge?

.....

13. Har du haft något menstruations-uppehåll längre än 3 månader (graviditet räknas inte)?

☐nej ☐ja

Vid vilken ålder? Hur länge? Någon orsak känd?

.....

.....

14. Hur många gånger har du varit gravid? .....

15. Antal fullgångna graviditeter? .....

16. Använder du eller har du använt P-piller/ P-spruta

☐nej ☐ja

Hur länge? Vid vilka åldrar? Preparat-namn?

.....

.....

17. Använder du eller har du använt någon typ av östrogen-preparat (kvinnligt könshormon) annat än P-piller?

☐nej ☐ja

Hur länge? Vid vilka åldrar? Preparat-namn? Tablett/ plåster?

.....

.....

18. Har du regelbundet tagit någon medicin? Var vänlig och fyll i detta formulär!
För östrogenpreparat (kvinnligt könshormon) och P-piller finns frågor på annan sida i enkäten.

Medicintyp	*Nej*	*Ja, tar nu*	*Tagit förut*	*Hur länge*	*Orsak till medicineringen?*	*Började vid ålder*	*Preparat-namn*
Epilepsi-medicin							
Sköldkörtelmedicin, ex Levaxin							
Magkatarr-medicin, ex Novalucol							
Cortison, tabl,spruta, spray							
Urin-drivande medicin							
Annan typ medicin?							
Annan typ medicin?							
Calcium-tablett (kalk-tabl)							

(glöm inte att vissa vitamintabletter innehåller calciumtillskott)

Egna kommentarer: .....

.....

.....

.....

.....

19. Har du någonsin rökt regelbundet, dvs dagligen eller nästan dagligen?

☐nej ☐ja

Hur gammal var du när du började röka? .....år

Röker du fortfarande regelbundet?

☐nej

Vid vilken ålder slutade du? .....år.
Hur många cigaretter per dag (i genomsnitt) rökte du innan du slutade? .....

☐ja

Hur många cigaretter per dag röker du? .....

32. Hur pass fysiskt aktiv var du för fem år sedan?
Var vänlig och ange detta i nedanstående formulär:

Aktivitet	*Aldrig*	*Utfördes antal mån/år*	*Antal ggr per månad*	*Minuter per gång*	*Kommentar*
Promenad (även till jobbet)					
Cykling (även till jobbet)					
Längskidåkning					
Friluftsliv, ange typ:					
Inline rollerbiade					
Jogging					
Orientering					
Bollsport, ange typ:					
Kampsport, typ:					
Aerobics/step-up					
Styrketräning					
Utförsåkning (inkl. snowboard)					
Annan träning/fysisk aktivitet?					
Exempel: Tennis		*12mån*	*15ggr*	*180min*	*Var elitspelare, distriktsmästare 2ggr*

33. Fråga till dig som är 23år eller äldre: Var du under tonåren idrottsaktiv?
☐nej ☐ja
Beskriv typ idrott/sport, timmar per månad, ev. tävlingsmeriter:

.....

.....

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