The Effect of Sleeve Gastrectomy on Rates of Hyperparathyroidism in a Cohort of Patients Living with Severe Obesity in NL

by

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ABSTRACT

Purpose: Multiple studies show an association between hyperparathyroidism and obesity. The purpose of this study was to determine the effect of laparoscopic sleeve gastrectomy (LSG) on rates of hyperparathyroidism and its different subtypes in patients living with severe obesity.

Methods: The rates of primary, normocalcemic, and secondary hyperparathyroidism were derived from a cohort of 200 patients before and after LSG. Laboratory data and Vitamin supplementation were collected at an initial clinic visit (ICV) where lifestyle modification and supplementation were advocated, at a surgical pre-admission clinic visit (PACV), and at the last follow-up visit after surgery (LFV).

Results: The rates of primary, normocalcemic, and secondary hyperparathyroidism at ICV (n=134) were 2.23 % (n=3), 20.15% (n=27), and 17.91% (n=24), respectively. The same rates were 1.52% (n=3), 17.17% (n=34), and 10.10% (n=20) at PACV (n=198), and 0.72% (n=1), 25.90% (n=36), and 5.04% (n=7) at LFV (n=139). The rates of Vitamin D insufficiency at the ICV, PACV, and LFV were 41.84% (58/141), 19% (38/200), and 10.79% (15/139) respectively. 58% (n=116) of patients were taking supplements of Vitamin D, Multivitamin, or both at PACV.

Conclusions: The percentage of patients with primary and normocalcemic hyperparathyroidism peri-operatively remained stable. The percentage of patients with secondary hyperparathyroidism declined from ICV to LFV, as did the percentage of patients with Vitamin D insufficiency; the greatest change occurred between ICV and PACV. The results suggest that supplementation and lifestyle modification may impact secondary hyperparathyroidism and Vitamin D insufficiency in this population, and that LSG is not associated with a worsening of these conditions.

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BMI	Body Mass Index
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
GFR	Glomerular Filtration Rate
GERD	Gastroesophageal Reflux
HL	Hyperlipidemia
HREA	Health Review Ethics Authority
HTN	Hypertension
ICV	Initial Clinic Visit
LSG	Laparoscopic Sleeve Gastrectomy
LFV	Last Follow-up Visit
MV	Multivitamin
N/A	Not Available
NL	Newfoundland and Labrador
OR	Odds Ratio
OSA	Obstructive Sleep Apnea
p	probability value
PACV	Pre-Admission Clinic Visit
PTH	Parathyroid Hormone
RYGB	Roux-en-Y Gastric Bypass

Chapter 1: Introduction

1.1 Background and Rationale

1.1.1 Parathyroid Physiology. The parathyroid glands are small but important endocrine organs located in the neck in close vicinity of the thyroid gland (figure 1.1). The parathyroids play a key role in maintaining extracellular Calcium levels within a normal range. By being able to detect small changes in ambient Calcium concentration, the glands respond by either increasing or decreasing their secretion of Parathyroid Hormone (PTH) (Brown et al., 1991). This response is mediated by the Calcium-sensing receptor in the outer plasma membrane of the parathyroid cell; the receptor recognizes the Calcium ion as its ligand, and depending on the saturation of these receptors, the transcription and secretion of PTH by the cell is altered (Hebert et al., 1997; Randolph et al., 2004).

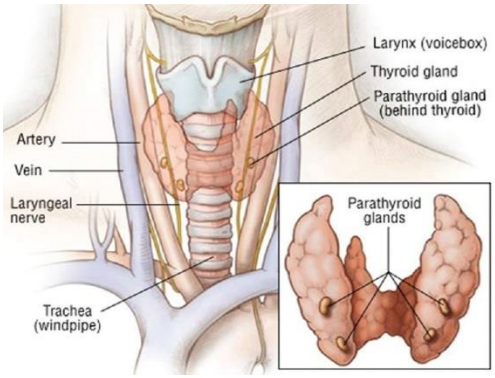

Figure 1. 1 ***The anatomical location of the parathyroid glands.***
(www.saintlukeshealthsystem.org: permission not required)

The relationship between Calcium and PTH follows an inverse sigmoidal curve under normal physiologic conditions: a small decrease in extracellular Calcium leads to an increase in

PTH secretion, while a small increase in extracellular Calcium leads to a decrease in PTH secretion, as illustrated in figure 1.2. When secreted, PTH acts on bone to stimulate osteoclast activity, which results in release of Calcium and Phosphate into the extracellular space (Silva & Bilezikian, 2015). At the level of the kidney, PTH acts on the distal tubule to conserve Calcium and decrease its secretion in urine (Quamme, 1982). Finally, PTH promotes the conversion of 25-OH Vitamin D to the active metabolite of Vitamin D, 1,25-OH Vitamin D (Nemere & Larsson, 2002). This metabolite then acts on the GI tract to increase Calcium absorption from the lumen. The net effect of these changes is an overall increase in serum Calcium levels, a phenomenon that is triggered in settings such as Calcium deficiency secondary to decreased oral intake, gastrointestinal loss of Calcium due to malabsorption syndromes, and loss of Calcium into urine due to renal failure. In the setting of irreversible phenomena like advanced chronic renal failure, the glands will not be able to compensate adequately, and serum Calcium will remain persistently low despite an elevated level of PTH.

The reverse physiological process occurs when Calcium levels rise too high: when PTH secretion is inhibited, mobilization of skeletal Calcium stores ceases, conversion of Vitamin D into the active form stops, and urinary secretion of Calcium resumes, leading to an overall decline in serum Calcium. This can occur in situations such as excessive Calcium or Vitamin D intake, hypercalcemia of malignancy, or sarcoidosis (Carroll & Schade, 2003). Again, in irreversible states such as malignancy, the Calcium levels will remain chronically high despite PTH levels becoming undetectable.

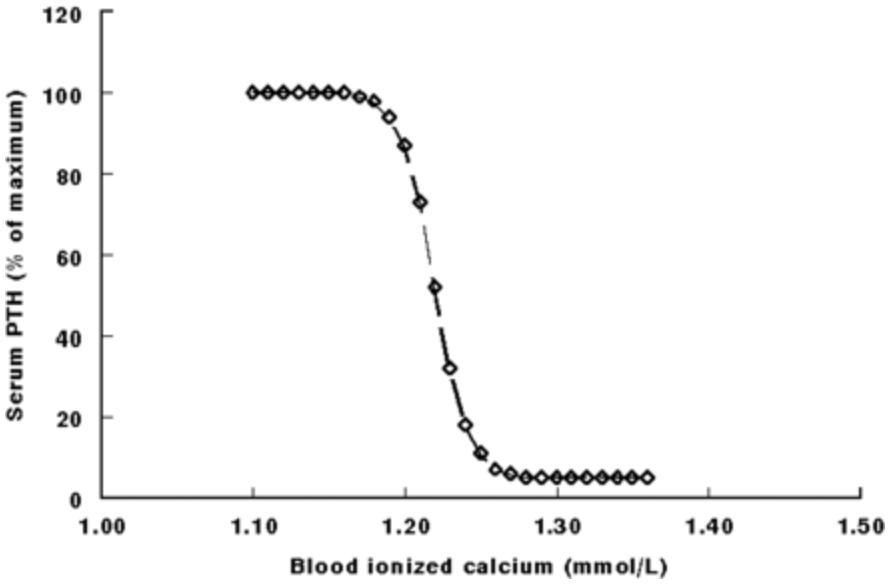

Figure 1. 2. ***The normal relationship between serum PTH and ionized Calcium. (****Chen & Goodman, 2004: permission not required). PTH=Parathyroid Hormone*

The above physiological relationship holds true provided that parathyroid gland function remains normal.

1.1.2 Hyperparathyroidism. In certain instances, PTH secretion can be stimulated not by extracellular Calcium levels, but by pathology of the parathyroid glands themselves - a phenomenon referred to as hyperparathyroidism.

Hyperparathyroidism is a state of excess PTH secretion by one or more parathyroid glands in the neck. This condition generally occurs in three main forms: primary hyperparathyroidism, its variant normocalcemic hyperparathyroidism, and secondary hyperparathyroidism. In primary and normocalcemic hyperparathyroidism the parathyroid glands undergo monoclonal growth and autonomously secrete excess PTH, whereas in secondary hyperparathyroidism the glands are triggered to secrete excess PTH by a physiologic stimulus, and can undergo diffuse hyperplasia as a result (Carling, 2001).

Primary hyperparathyroidism is the rarest form of the disorder. It is most common among postmenopausal women, where its prevalence has been reported to be as high as 21 in 1000 (Adami, 2002). The prevalence in the general population is much lower, and has been quoted as ranging from 1-7 per 1000 adults (Yeh et al., 2013; Wermers et al., 2006). However, for a number of reasons including earlier detection, the disease prevalence appears to be on the rise; in a recent study, the prevalence of primary hyperparathyroidism increased from 0.76 to 2.33 per 1000 women, and from 0.3 to 0.85 per 1000 men between 1995 and 2010 (Yeh et al., 2013).

In primary hyperparathyroidism, one or more of the parathyroid glands begin to grow and independently secrete abnormally high levels of PTH (Scwartz et al., 2004). PTH acts on end organs including the kidney, bone, and the gastrointestinal tract to raise Vitamin D and Calcium levels in the blood. Over time, this can have deleterious effects including formation of kidney stones, worsening renal function, osteoporosis, peptic ulcer disease, and pancreatitis (Duan et al., 2015). Several studies also provide evidence for an association between primary hyperparathyroidism and hypertension (HTN), increased risk of cardiovascular disease (CVD) and impaired glucose tolerance (Junghall et al., 1983; Stefenelli et al., 1997; Procopio et al., 2002; Andersson et al., 2004; Lind et al., 1991). Operative removal of the abnormal parathyroid tissue is the only cure for primary hyperparathyroidism, and there is general agreement among endocrinologists and surgeons that surgery is indicated for patients who are symptomatic, who show subclinical evidence of declining kidney function or bone mass, who are under the age of 50, or who have serum Calcium levels in excess of 0.25 mmol/l above normal (Eigelberger et al., 2004; Bilezikian et al., 2009; Silverberg et al., 2009). In patients who do not meet these criteria, observation with serial bloodwork and bone density measurement is recommended. However,

even in asymptomatic patients, multiple studies show that surgery is associated with measurable improvement in bone mineral density, psychological function, and quality of life (Zanocco et al., 2006; Sywak et al., 2002). In patients who have indications for surgery but who are poor surgical candidates, supportive treatment with bisphosophonates or PTH-lowering Calcimimetics can be considered (Marocci et al., 2014).

Normocalcemic hyperparathyroidism is a variant of primary hyperparathyroidism which was first officially recognized as its own entity by an international panel of experts at the Third International Workshop for Asymptomatic Primary Hyperparathyroidism in 1991 (NIH Conference, 1991). Patients with normocalcemic hyperparathyroidism manifest an elevated PTH in the face of normal Calcium - possibly representing an early form of primary hyperparathyroidism (Glendenning et al, 1998). The diagnosis can only be made after exclusion of all causes of secondary hyperparathyroidism where Calcium remains normal rather than being low – especially Vitamin D insufficiency, and the use of medications that alter Calcium homeostasis (e.g. lithium, thiazides) (Siprova et al., 2015). Although the biochemical profile of normocalcemic hyperparathyroidism differs from primary hyperparathyroidism, the treatment approach is generally the same as the same end organ processes are felt to occur (Tuna et al., 2015; Chen et al., 2015; Yener Ozturk et al., 2016; Pawlowska et al., 2015; Lowe et al., 2007; Yang et al., 2006).

Secondary hyperparathyroidism is the most common form of hyperparathyroidism. It is usually caused by Vitamin D (and sometimes Calcium) insufficiency due to inadequate intake, or more rarely, due to renal failure or gastrointestinal malabsorption (Duan et al., 2015). The lack of Vitamin D or Calcium is detected by the parathyroid glands, which then increase the secretion of PTH in an attempt to raise the levels of Vitamin D or Calcium in the blood. If the

insufficiency prevails, the glands undergo diffuse hyperplasia in an effort to maximize PTH production, and an exaggerated physiologic response results. In rare instances, the prolonged stimulus can lead to the emergence of a new, autonomous PTH-producing neoplasm, a phenomenon called tertiary hyperparathyroidism (Schwartz et al., 2004; Duan et al., 2015; Davies et al., 1968). Secondary hyperparathyroidism caused by overt Vitamin D deficiency, characterized clinically by osteomalacia or rickets, is now uncommon in most developed countries. However, subclinical Vitamin D insufficiency is frequent - especially in northern climates- and has been shown to be associated with development of osteoporosis and increased risk of fractures (Stechsulte et al., 2009; Zhu et al., 2008; Meier et al., 2004; Daly et al., 2006; Chapuy et al., 1992; Jackson et al., 2006). Several studies also suggest an association between Vitamin D insufficiency and impaired immune and cardiovascular function (Kendrick et al., 2009). Thus, the identification and treatment of Vitamin D insufficiency and secondary hyperparathyroidism by supplementation appears to be beneficial for musculoskeletal and possibly even extra-skeletal health, and is generally advocated (Tsai et al., 1987; Webb et al., 1988). The treatment of secondary hyperparathyroidism in refractory settings like chronic renal failure is more complicated, and while medical therapy remains the cornerstone, surgery may also be rarely indicated (Pitt et al., 2009).

Over the past one hundred years, the clinical presentation of hyperparathyroidism has changed dramatically. Following the identification of the parathyroid glands as discrete organs in the late 1800s, von Recklinghausen is believed to have been the first to recognize the association between the parathyroid glands and osteitis fibrosa cystica. The first removal of a parathyroid adenoma occurred in a patient with this condition in 1925 (Mandl, 1926). However, it wasn't until the 1930s that hyperparathyroidism also became linked to nephrolithiasis, and

became classically described as a disease of ‘stones’ and ‘bones’ by Dr. Albright (Albright et al., 1934). The diagnosis was initially made only in progressive stages of the disease once end-organ damage became clinically apparent. This trend changed with the introduction of the multichannel auto-analyzer in the 1970s, which allowed for ready measurement of serum Calcium levels in the broader population (Randolph, 2004). As a result of this innovation, many more patients were diagnosed based on mild, often intermittently elevated Calcium levels and minimal clinical symptoms. Today, it is estimated that up to 80% of patients diagnosed with hyperparathyroidism are essentially asymptomatic (Bandeira & Griz, 2013; Bilezikian & Silverberg, 2004). It should be noted, however, that a significant proportion of patients with hyperparathyroidism that do not manifest the classical symptoms do report non-specific symptoms such as fatigue, weakness, depression, and mild cognitive dysfunction, and this has become an increasingly recognized phenomenon world-wide (Perrier, 2005; Lundgren et al., 1998; Chan et al., 1995; Mihoa & Sadler, 2008).

1.1.3 Hyperparathyroidism & Obesity.

Multiple studies have shown an association between morbid obesity and hyperparathyroidism, whether it be the primary, normocalcemic, or secondary subtype (Junghall et al., 1983; Yener Ozturk et al., 2016; McDermott et al., 1994; Clyde et al., 1992; Dalberg et al., 1996; Christiansen et al., 1999; Cortet et al., 2000; Gonelli et al, 2000; Ingle et al., 2002; Camozzi et al., 2003; Grey et al., 1994; Bolland et al., 2004; Ruiz-Tovar el., 2012; Vix et al., 2014; Carlin et al., 2006). The underlying mechanism at play has yet to be elucidated, and the theories to explain the association between the different types of hyperparathyroidism and morbid obesity are many. They include the development of chronic Vitamin D and Calcium insufficiency caused by fat-restricted diets, decreased UV light exposure, decreased production due to fatty liver disease, as well as increased sequestration of fat soluble Vitamin D in adipose tissue, with the persistent deficit ultimately inducing parathyroid gland growth and hyperparathyroidism (whether hyperplasia, as in secondary hyperparathyroidism, or possibly adenomatous growth as in primary and normocalcemic hyperparathyroidism) (Ruiz-Tovar et al., 2012; Vix et al., 2014; Carlin et al., 2006; Grethen et al, 2011). The possibility of a genetic association has also been investigated (eg. the link between the Bsm1 Vitamin D receptor polymorphism and the Calcium-Vitamin D-PTH axis) (Alexandrou et al., 2015). Ultimately, multiple variables likely play a role in this relationship, and at this stage we are unable to ascertain them all.

1.1.4 Hyperparathyroidism & Obesity Treatment.

Over the past decade, the introduction of bariatric surgery as a means of weight loss has had a dramatic impact on the treatment of morbid obesity. It has been shown to be both effective at inducing significant, long-term sustainable weight reduction, as well as reversing or improving obesity-related comorbidities such as CVD, HTN, diabetes (DM), obstructive sleep apnea (OSA), and hyperlipidemia (HL) (Shah et al., 2006; Colquitt et al., 2014; Laurino Neto et al., 2012; Schauer et al., 2012). Despite this, the effect of bariatric surgery on related endocrine disorders like hyperparathyroidism in this population is not well studied. Specifically, whether the higher rates of hyperparathyroidism in this population are affected by bariatric surgery like other comorbidities remains uncertain, and this topic forms the principal focus of this paper.

Laparoscopic sleeve gastrectomy (LSG) is a relatively new, restrictive bariatric procedure which has been shown to be equivalent to older, mal-absorptive bariatric procedures such as Roux-en-Y Gastric Bypass (RYGB) in terms of achieving weight reduction (Lakdawala et al., 2010; Benaiges et al., 2011; Franco et al., 2011). In LSG, up to 80 % of the stomach is removed, leaving behind a "sleeve" with a reduced gastric volume (Karmali et al., 2010; Poirier et al., 2011). The expected % excess weight loss within the first three years following LSG is 45-60%, and occurs most rapidly in the first year (Victorzon, 2012). Unlike RYGB, which bypasses the stomach and duodenum, LSG is mostly restrictive: gastric emptying is accelerated, but the food still comes in contact with all parts of the gastrointestinal tract, including the stomach and duodenum, where most Calcium is absorbed. As a result, insufficiency of micronutrients such as Calcium and Vitamin D is less likely to develop (Shah et al., 2006; Colquitt et al., 2014; Laurino Neto et al., 2012; Schauer et al., 2012; Lakdawala et al., 2010; Benaiges et al., 2011; Franco et al., 2011; Karmali et al., 2010; Poirier et al., 2011; Victorzon,

2012; Malinowski, 2006). With regard to minimizing any additional cofounding effects of the surgery itself, LSG thus presents itself as the more suitable procedure for analyzing the effects of weight loss on rates of hyperparathyroidism, and is the one selected for closer analysis in this study.

1.2 Purpose

Investigating whether and how LSG impacts the rates of different types of hyperparathyroidism in individuals living with severe obesity can provide useful information from a medical as well as an economic perspective. The treatment of hyperparathyroidism, whether surgical as in the case of primary and normocalcemic hyperparathyroidism, or medical as in the case of secondary hyperparathyroidism, is costly - as is the management of end organ damage that can result from untreated disease (Zanocco et al., 2006; Zanocco et al; 2011). If bariatric surgery favourably impacted rates of hyperparathyroidism among the morbidly obese, not subjecting an already vulnerable patient population to additional procedures and treatments would have substantial clinical benefits. Likewise, avoidance of these treatments could significantly reduce medical costs.

A closer analysis of the association between obesity and hyperparathyroidism in a clinical scenario where one of the variables is manipulated in a controlled fashion could also help shed more light on the physiologic mechanisms that underlie the relationship. The knowledge gained might then be used to help institute more appropriate preventative measures in the at-risk population. Finally, findings from this study could directly provide useful information on the most appropriate clinical management of these patients in both the pre and post-operative period.

To that effect, the present study was designed to investigate the effects of LSG on the rates of hyperparathyroidism in a cohort of morbidly obese patients undergoing the surgery in

Newfoundland and Labrador (NL). As Vitamin D supplementation, by potentially decreasing rates of secondary hyperparathyroidism, was found to be an important factor in the limited number of other studies analyzing the effects of bariatric surgery on hyperparathyroidism rates overall (see Literature review), the current paper also sought to investigate the role that peri-operative Vitamin D supplementation might have on the rates of hyperparathyroidism in this patient population.

The study aims to answer the following primary research questions:

1. Does the proportion of severely obese patients undergoing LSG that have hyperparathyroidism pre-operatively differ from the proportion of patients that have hyperparathyroidism post-operatively?
2. What proportion of these patients have primary hyperparathyroidism, normocalcemic hyperparathyroidism, and secondary hyperparathyroidism, and do the rates pre-operatively differ from those post-operatively?

The study also aims to answer the following secondary research questions:

1. Does the proportion of severely obese patients undergoing LSG that are Vitamin D insufficient pre-operatively differ from the proportion that are Vitamin D insufficient post-operatively?
2. Does Vitamin D supplementation affect rates of Vitamin D insufficiency and rates of primary, normocalcemic and secondary hyperparathyroidism in bariatric patients pre-operatively (independent of surgery)?

3. Does Vitamin D supplementation affect rates of Vitamin D insufficiency and rates of primary, normocalcemic and secondary hyperparathyroidism in bariatric patients who undergo surgery?

Chapter 2: Literature Review

The research that has been carried out on the topic of the effects of LSG on hyperparathyroidism in the severely obese population is quite sparse. The studies are often limited to either a comparison of the effects of LSG to another procedure like RYGB, or are constrained by the fact that the effects of LSG on hyperparathyroidism are a secondary outcome in an analysis designed to assess a different primary outcome.

The purpose of this literature review is to appraise and summarize the studies that have focused on the impact of LSG on hyperparathyroidism to date, specifically concentrating on the level of evidence available, and outlining any information gaps that persist. In order of increasing level of evidence in terms of the type of study performed, four relevant papers are reviewed.

2.1 Ruiz-Tovar et al. (2012)

In 2012, a retrospective study was published on a cohort of 30 patients analyzing the effects of sleeve gastrectomy on parameters of Calcium metabolism, Vitamin D, and PTH (Ruiz-Tovar et al., 2012). The study was conducted at a single center in Spain between 2008 and 2010. Patients had to be medically cleared for the procedure by a multi-disciplinary team using a set of standard bariatric surgery criteria. Any patients with gastroesophageal reflux (GERD) were excluded. GERD is thought to be exacerbated in patents with primary hyperparathyroidism secondary to high Calcium acting as a trophic factor for Gastrin, which then stimulates increased acid secretion by parietal cells (Gardner & Hersch, 1981). As GERD is common in obese patients as well, this selection bias could theoretically decrease the number of patients with primary hyperparathyroidism in the study group (Norman et al., 2015). All of the patients were females, and the mean BMI was 53.1 ± 8 kg/m^2 – in the 'super obese' range. This sampling bias

limits the generalizability of the results to the broader bariatric population undergoing LSG. No information was provided on the time of year – an important confounding factor that might influence Vitamin D and Calcium metabolism, as a significant part of Vitamin D synthesis occurs via UV radiation-mediated conversion of 7-dehydrocholesterol to previtamin D3 in the skin – a phenomenon that is more likely in the summer with increased sun exposure (Webb et al., 2010).

Along with other micronutrient levels and the patients' BMI, Calcium, Vitamin D, and PTH levels were obtained before operation and at 1, 3, 6, 9, 12, 18, and 24 months after surgery; the patients thus served as their own controls. The Calcium levels were simply reported as being normal for all patients at all stages. The patients' pre- and post-operative laboratory values were grouped as means, and there was no attempt at differentiating patients with hyperparathyroidism and normal Vitamin D values (possible normocalcemic hyperparathyroidism) from those with hyperparathyroidism with decreased Vitamin D levels (secondary hyperparathyroidism), who might respond differently to the surgical correction of obesity. The surgery was performed per standard protocol by different surgeons, which might introduce variability in the extent of stomach removed and the subsequent weight loss (performance bias). All patients were prescribed a multivitamin supplement post-operatively, with non-specified Vitamin D / Calcium content. This supplementation, while uniform, theoretically obscures the effect on subsequent Vitamin D insufficiency and hyperparathyroidism rates due to the surgery itself.

The data were complete for all patients at one year post-op, and for 73.3% of the patients at 2 years post-op. Before surgery, 96.7% of the patients had Vitamin D insufficiency, 20% had hyperparathyroidism, and Calcium was reported as normal in all (however, the definitions for Vitamin D insufficiency, hyperparathyroidism, and normal range for Calcium are not provided).

At 6 months post-op, only 13.2% of patients had Vitamin D insufficiency and 6.6% had elevated PTH, and at 12 months post-op, only one patient (3.3%) had Vitamin D insufficiency and elevated PTH. A statistical analysis of these rates is not provided. The initial Vitamin D insufficiency rate is one of the highest reported, and as 'normal' ranges and laboratory value cut-offs vary from one institution and one study to the next, an exact definition would be helpful (– especially in the context of a relatively low hyperparathyroidism rate).

The authors report a significant difference between pre-and post-operative PTH beginning 3 months from surgery (decrease of 16.6 pg/ml, 95% CI (2.6–30.6); $p<0.03$)), and a significant difference between pre-and post-operative Vitamin D beginning at 12 months from surgery (increase of 51.9 ng/dl, 95% CI (41.8-61.3); $p<0.001$)), respectively. As the subjects served as their own controls, a paired t-test could have been used to provide this analysis; however, no information on the statistical methods is provided.

This study suggests a positive effect of sleeve gastrectomy on rates of hyperparathyroidism, with a steady decline in PTH and steady rise in Vitamin D for up to 24 months post-operatively (with the changes becoming significant at 3 and 12 months, respectively). However, one has to take into consideration the usual biases inherent to a retrospective study and some of the previously mentioned issues: the study population analyzed in this article is unusual in its make-up given the high average BMI and the almost universal Vitamin D insufficiency (which may well be related to one another). These baseline patient characteristics limit the generalizability of the results to other study populations. As well, the patients are not stratified by type of hyperparathyroidism. The patients serve as their own historical control group, and while this design eliminates the problem of controlling for other baseline differences between experimental and control groups, environmental factors that change

with time (e.g. change in sun exposure with alternating season) cannot be accounted for. Most importantly, while uniform, the confounding effect of the Vitamin D and Calcium supplementation on the post-operative hyperparathyroidism rates is difficult to gauge: there is no pre-operative assessment other than the one immediately prior to surgery, and because supplements were uniformly started post-op, we do not know whether the observed increase in Vitamin D and decrease in PTH was due to supplementation, surgery, or both. Finally, while the follow-up is one of the longest in the literature, the sample size is very small.

2.2 Gehrer et al. (2010)

In 2010, Gehrer and colleagues published a prospective cohort study comparing pre- and post-operative micronutrient and Vitamin deficiencies and success of their treatment in patients undergoing LSG and RYGB (Gehrer et al., 2010). The study was carried out at a single institution in Switzerland between 2004 and 2006. RYGB was offered as the standard procedure to patients deemed candidates for bariatric surgery, while LSG was offered to patients felt to have contraindications to RYGB. These included a history of extensive abdominal surgery, large abdominal hernia, celiac disease, or super-obesity where bilio-pancreatic diversion was anticipated as a second-stage procedure if LSG did not produce sufficient weight loss. As in the previous article, patients with severe reflux or hiatal hernia were excluded from the LSG group. The grouping of super-obese patients and patients with celiac disease (more prone to Vitamin D insufficiency and secondary hyperparathyroidism) in the LSG group, with the simultaneous exclusion of patients with GERD (possibly more prone to primary hyperparathyroidism) introduces confounding variables that make the results more difficult to interpret (Norman et al., 2015; Merguiles et al., 2015). The inclusion of the first two factors could potentially raise the

baseline hyperparathyroidism rate in the LSG group, while exclusion of patients with GERD could lower it.

A total of 136 patients were analyzed in this study over the time period; of these, 86 underwent RYGB, and 50 underwent LSG. The groups were compared in terms of the baseline characteristics of age, gender and BMI, and were only similar in terms of age and gender, with a significantly higher BMI in the LSG group ($p=0.02$). Other factors such as skin colour and season were not considered. Finally, while the overall rates of pre-operative Vitamin D insufficiency and hyperparathyroidism in the cohort are provided (23% and 8%, respectively), there is no comparison of the baseline rates in each group - or even the mean values of Vitamin D, PTH, or Calcium in each group.

All patients were followed up at 3, 6, 12, 24, 30, and 36 months after surgery. The follow-up rate was 100% at 1 year, and the mean follow-up time was 24.4 months. Vitamin D, PTH, Calcium, and other micronutrient levels were measured at each follow-up visit for 'detection of deficiencies'. The ranges for micronutrient and Vitamin deficiencies were well-defined, and patients identified to have deficiencies pre-or post-operatively were treated immediately. However, as the baseline insufficiency rates are not provided, it is unclear how many patients in each group ended up getting supplements. Hyperparathyroidism without Vitamin D insufficiency was primarily attributed to Calcium deficiency (despite there being no patients with measured Calcium deficiency in this study), and was treated with Calcium supplements (500 mg Calcium/ 400 IE cholecalciferol twice daily *per os*). Again, this definition does not take into consideration the possibility of these patients having normocalcemic hyperparathyroidism - which would not be expected to respond to Calcium supplementation. When Vitamin D insufficiency was present, treatment consisted of the application of an oily

preparation of Vitamin D3 (300,000 IE) every 3 months. These supplements would continue until laboratory values on serial exam normalized – which was considered a treatment success.

Post-operatively, 32% of patients in the LSG group had Vitamin D insufficiency at some point and 14% had elevated PTH, while in the RYGB group, 52% of patients had Vitamin D insufficiency at some point and 33% had elevated PTH. The difference in the two values between the two groups was statistically significant ($p<0.02$). However, no information is provided on the post-operative values compared to the pre-operative values. Calcium was normal post-operatively in all patients in both groups. In the LSG group, 100% of the patients treated with Vitamin D for Vitamin D insufficiency, and 100% of the patients treated with Calcium supplement for hyperparathyroidism with normal Vitamin D values returned to normal PTH levels. In the RYGB group, Vitamin D supplementation for patients with Vitamin D insufficiency achieved an 84% response rate, while patients with secondary hyperparathyroidism without Vitamin D insufficiency treated with Calcium supplements achieved a 100% response rate.

In the LSG group, the number of patients with hyperparathyroidism was highest at six-month follow-up and then declined, while the number with Vitamin D insufficiency rose to its peak at 12 months and then declined. In the RYGB group, the number of patients with hyperparathyroidism was greatest at 3 months, while the number with Vitamin D insufficiency was highest at 24 months. However, the denominator (total number of patients in study at each time point) is not provided, and since we know that patient follow-up dropped off after 1 year, the rates of hyperparathyroidism or Vitamin D insufficiency at the different time points cannot be calculated.

Interval values for weight loss in each group are provided, however, no statistical comparison is made between the groups. The authors conclude that postoperatively, significantly lower rates of Vitamin D insufficiency and hyperparathyroidism were found in patients who had LSG compared to RYGB. However, as the groups were not matched for important confounding variables, and the baseline, pre-operative percentage of patients with these deficiencies in each group is unknown, the significance of these findings is questionable. The percentage of patients in each group that received supplements is also unknown, along with the timing of supplementation and its duration. This considerably obscures the effects, if any, that the different surgeries may have had on hyperparathyroidism rates. In addition, the number of patients lost to follow-up in each group is not documented. Overall, more rigorous documentation is needed in this study; the partial reporting of the pre-and post-op insufficiency rates and the lack of stratification of patients by supplementation vs. no supplementation makes the results of this study impossible to interpret.

2.3 Coupaye et al. (2012)

In 2012, Coupaye and colleagues published a prospective cohort study analyzing pre-and post-operative parameters of Calcium metabolism (including Vitamin D-25-OH, Calcium and PTH) following RYGB and LSG at a single French institution between 2005 and 2010 (Coupaye et al., 2012). This analysis was part of a much larger prospective study examining multiple peri-operative aspects of RYGB at this hospital.

A total of 202 patients undergoing RYGB were enrolled in the study, of whom 30 were matched to patients undergoing LSG during the same period. The groups were matched for weight pre-op and at 6 months post-op, age, gender, season, and skin color – all important factors influencing Calcium and Vitamin D metabolism.

The biochemical profile (serum Calcium, Albumin, Vitamin D, and PTH) was analyzed 3 months pre-op and 6 months post-op. Exact definitions of hyperparathyroidism, Vitamin D deficiency and Vitamin D insufficiency were provided. The baseline Calcium, Vitamin D, and PTH levels within 3 months before surgery were comparable in both groups, however the specific rates of hyperparathyroidism, Vitamin D deficiency and Vitamin D insufficiency were not provided for both groups. All patients were given standard supplements of Vitamin D and Calcium post-operatively, but if they demonstrated Vitamin D insufficiency pre-operatively, they underwent a specific, graded replacement regime for 6 weeks based on the extent of their deficit. It is not clear from the study whether this replacement was started before or after the surgery, only that all study patients were off of the replacements for at least 3 months by the time the levels were re-measured post-operatively. The surgeries were carried out by two different surgeons, possibly introducing some performance bias. Details of the breakdown of the procedures by surgeon are not provided.

As mentioned, all of the patients were started on a routine supplement post-op, but at 6 months, 13/30 LSG patients had discontinued this, while 3/30 RYGB patients had done the same. At 6 months, there was a statistically significant increase in the mean Vitamin D level in the LSG group compared to pre-operative levels (24.1 ng/ml ± 14.1 vs. 15.2 ng/ml ± 9.5, $p<0.05$, paired t-test), and a lesser, though also significant increase in the RYGB group (19.5 ng/ml ± 11.4 vs. 14.0 ng/ml ± 9.6, $p<0.05$, paired t-test). The PTH levels increased slightly in the RYGB group and decreased slightly in the LSG group, but the differences were not statistically significant. The serum Calcium levels also rose in both groups, but the increase was only significant in the RYGB group (2.27 mmol/l ±0.07 vs. 2.24 mmol/l ±0.09 pre-op, $p <0.05$, paired

t-test). Overall, at six-months post-op, there was no difference in Vitamin D, PTH, or Calcium levels between the 2 groups of surgical patients.

The authors conclude that in this study, patients in both the LSG and RYGB groups were shown to have significant improvements in mean Vitamin D levels at six-months post-op compared to their pre-operative baseline, without any significant accompanying change in PTH levels. While significant effort was made to ensure uniform distribution of the confounding factors at baseline, and thorough documentation and comparison of these factors was maintained both within and between the two groups, one of the most important extraneous factors introduced – the graded Vitamin D supplementation regime based on the extent of deficiency – was not recorded in detail or on a per-group basis. As such, it is difficult to ascribe the increase in Vitamin D purely to weight loss related to surgery given that all patients with Vitamin D insufficiency were supplemented with replacement regimes depending on the extent of their deficiency – and the levels were not re-measured until the six-month post-op time point. Finally, the number of patients in the LSG arm was very small, and the follow-up period was only 6 months, which seems short in comparison to the other studies.

2.4 Vix et al. (2009)

In 2009, Vix and colleagues conducted a randomized controlled trial to compare the post-operative outcomes of two bariatric procedures: LSG and RYGB at a single center in France (Vix et al., 2009). Patients were enrolled from a pool of bariatric surgery candidates at the University of Strasbourg: the patients had to be between 18 and 60 years old, have a BMI between 40 and 60 kg/m^2, have no preference for one procedure over the other, and have no history of prior gastrointestinal surgery. Reflux was not considered an exclusion criterion from the LSG group. The primary outcome in this study was % excess weight loss, and the sample

size was calculated using a non-inferiority trial design to detect a 60% decrease in excess weight loss with a power of 70% (not to measure change in secondary outcomes such as Vitamin D levels or PTH levels).

Based on the sample size calculation, one hundred patients were randomly assigned to RYGB (n = 45) or LSG (n = 55). The randomization to the two surgical arms occurred in a concealed fashion via envelope, and the baseline characteristics of the two groups (age, gender, BMI, Vitamin D levels, PTH levels, % with Vitamin D insufficiency, and % with secondary hyperparathyroidism) were not significantly different. The groups were not compared for skin colour or season.

The patients' Calcium, PTH and Vitamin D levels were measured pre-operatively and at 3, 6, and 12 months post-operatively. Secondary hyperparathyroidism was simply defined as an elevated PTH level regardless of the corresponding Calcium and Vitamin D level. Again, by doing so some patients with normocalcemic hyperparathyroidism may have been classified into this category as well, thus artificially increasing the number of patients thought to be suffering from secondary hyperparathyroidism. Primary hyperparathyroidism was not defined and not assessed in this study. The patients in this study who had a Vitamin D insufficiency (criteria clearly defined) were uniformly supplemented with 100,000 IU of Vitamin D month, and followed with serial bloodwork until levels normalized.

No information is provided with regards to whether one or more surgeons performed the procedure in either group: if multiple surgeons with varying skill sets were involved, this might theoretically introduce performance bias and influence the outcomes of the surgeries.

A per-protocol interim analysis was performed after completion of 12-months of follow-up. Complete data were not available for all patients in both groups at this point: 25% patients

had incomplete data in the LSG group, and 15% had incomplete data in the RYGB group - a loss to follow-up in excess of 10% per group, which further decreases the power of this already underpowered study. In addition, the uneven loss to follow up potentially introduces transfer bias.

Post-operatively, there was no statistically significant difference in % excess weight loss between the two groups. Mean Vitamin D values improved significantly in the LSG group at all assessment points compared to inclusion values, while values in the RYGB remained constant over time, with a transitory improvement at 6 months. There was no difference in Calcium levels before or after surgery in both groups (data not shown). There was a significant, progressive decrease in PTH levels in LSG patients at all point of post-operative assessment, while PTH levels remained unchanged in the RYGB group.

A significant difference was demonstrated between the two groups in absolute Vitamin D levels at 3 months (54.8 pmol/l RYGB vs. 61.6 pmol/l LSG, p=0.01) and again at 12 months (56.1 pmol/l RYGB vs. 59.8 pmol/l LSG, p=0.02). Likewise, a significant difference in absolute PTH levels was first seen between the groups beginning at 3 months (44.7 ng/l RYGB vs. 28.64 ng/l LSG, p=0.03), which was then maintained throughout the remainder of the study. The Vitamin D insufficiency rate in the LSG group dropped from 84% pre-operatively to 48% at 12 months, while the RYGB group rate remained unchanged from the pre-operative rate of 85%. Over time there was a trend towards greater need for Vitamin D supplementation in the RYGB group, (% of patients requiring supplements at each time point) but there was no statistically significant difference. Of note, these numbers are very small.

This is the only randomized controlled trial to date assessing hyperparathyroidism and Vitamin D insufficiency following sleeve gastrectomy, and as such should provide the strongest

level of evidence. The groups were well matched at baseline, though as with previous studies there was potential misclassification bias with no attempt at stratification of patients with secondary hyperparathyroidism and normocalcemic hyperparathyroidism. The measurement of serial laboratory values, deficiencies and extraneous micronutrient supplementation in each group at the different time points was well documented.

The main problem with the study is the small sample size (designed to measure the primary outcome of % excess weight lost, not rates of hyperparathyroidism), the high loss to follow-up, and the short study duration. A much larger, more sufficiently–powered study would need to be designed specifically to detect differences in hyperparathyroidism rates. As well, the uniform supplementation of patients with insufficiency peri-operatively makes the effects of supplementation and surgery difficult to distinguish.

2.5 Review Summary

Two of the four articles reviewed demonstrated decreased hyperparathyroidism rates following LSG compared to pre-operative values, as well as compared to RYGB (table 2.1). One article demonstrated no differences, and one article, while documenting decreased rates relative to RYGB, provided insufficient baseline information to draw any meaningful conclusions. In three of these articles, Vitamin D levels were demonstrated to rise post-operatively while Calcium levels remained unchanged, indirectly suggesting that the decrease in hyperparathyroidism rates was likely due to decreased rates of secondary hyperparathyroidism.

Table 2. 1 Summary of Studies Analyzing the Effects of Laparoscopic Sleeve Gastrectomy on Hyperparathyroidism and Vitamin D status:

Study	**Type**	**Sample**	**Results**	**Issues**
Ruiz-Tovar *et al.*	Retrospective Cohort	n=30 (LSG) Follow-up: 2 yr	-↑ Vit D, ↓ PTH post LSG -No change Ca	-Small sample -Sampling bias & generalizability -Confounding by supplements -Statistical analysis details?
Gehrer *et al.*	Prospective Cohort	n=50 (LSG) n=86 RYGB) Follow-up: 3 yr	-↓ Vit D Deficiency -↓ HP post LSG vs. RYGB	-Confounding baseline factors (pre-op Vit D, PTH levels) -Confounding by supplements -Internal Validity?
Coupaye *et al.*	Prospective Cohort	n=30 (LSG) n=202 (RYGB) Follow-up: ½ yr	-↑ Vit D post LSG -No change PTH or Ca	-Small LSG sample -Confounding by supplements -Short follow-up
Vix *et al.*	Randomized Controlled Trial	n=55 (LSG) n=45 (RYGB) Follow-up: 1 yr	-↑ Vit D, ↓ PTH post LSG -No change Ca	-Underpowered -(2°outcome Measures studied) -High loss to follow-up

It would thus appear, albeit based on the limited evidence, that LSG does not seem to be associated with the worsening of hyperparathyroidism rates post-operatively, and may, in fact, be associated with decreasing rates of hyperparathyroidism. Clearly, however, stronger evidence is necessary before more definitive conclusions can be drawn. Micronutrient and Vitamin D supplementation seems to be a common peri-operative strategy; however, in order to glean more meaningful results regarding the effects of the bariatric procedure itself, it would be helpful if

future studies more rigorously stratified patients based on whether they received peri-operative supplementation or not, or at least studied this phenomenon independent of surgery – as we attempted with the present study. It would also be helpful if patients with hyperparathyroidism were stratified into those with secondary, normocalcemic, and primary hyperparathyroidism, as with the current study, since one might expect these groups to respond to both surgery and supplementation differently. Ultimately, however, a randomized controlled trial with sufficient power and longer duration of follow-up would be the most ideal means of analyzing this topic further.

Chapter 3: Methods

This chapter describes the study population and sample, methods of recruitment and data collection, definitions of the primary and secondary outcome variables, the statistical methods used in the analysis of the data, and finally, any ethical considerations. A standardized data abstraction form was used to collect patient demographics such as age, gender, BMI, medication use, and laboratory values such as Calcium, Vitamin D level, PTH, etc. The study, whose patient sample was drawn per protocol from the larger NL Bariatric Surgery Cohort Study, was conducted using a retrospective cohort design (Twells et al., 2016).

3.1 Population and Sample

The target population consisted of a cohort of 200 severely obese patients undergoing LSG between May 2011 and May 2014 who had consented to participate in the NL Bariatric Surgery Cohort Study. The patients in this study had given permission to have certain personal data collected for purposes of scientific research (including all of the data analyzed in the current study). The inclusion criteria for LSG in NL have been documented elsewhere, but essentially consist of patients with either BMI > 40, or BMI 35-40 with comorbidities (e.g., HTN, CVD, DM, OSA, GERD, or cancer) who had been deemed suitable surgical candidates following assessment by a bariatric nurse practitioner and a bariatric surgeon (Twells et al., 2016). The time period for the study was chosen such that, at the time of analysis, all patients included in the analysis would have been eligible for at least a 12-month follow-up appointment.

3.2 Recruitment

Patients were referred by their family physician to the NL bariatric surgery program at the Health Sciences Center at Eastern Health, the province's tertiary care center. Here, a bariatric program nurse practitioner performed a preliminary screening of patients and invited eligible patients to a pre-surgical education session. Information regarding the surgical procedure and general nutritional recommendations were provided at this session. Interested patients then met with the bariatric nurse practitioner individually at an initial clinic visit (ICV). They were encouraged to initiate a daily multivitamin (MV) regime, and asked to complete a trial of dietary modification while keeping a food diary, as well as completing a trial of liquid diet. Patients able to comply who were felt to be suitable candidates for surgery were informed of the NL Bariatric Surgery Cohort Study by a member of the clinical team. Those willing to participate then met with a research nurse who provided further information about the study, answered any questions, and obtained informed consent.

3.3 Data Collection

The electronic and paper medical records of patients who provided consent were retrospectively reviewed. Data was obtained as part of the NL Bariatric Surgery Cohort Study at the ICV, the pre-admission clinic visit (PACV) immediately prior to surgery, and at 3, 6, 12, 18 and 24 months after surgery. The last visit where the full set of data being extracted for the current study was available was labelled as the last follow-up visit (LFV). The specific dates of the individual visits were recorded.

Information collected for the present study included: patient age, gender, pre-operative BMI, and the use of any Vitamin and supplementation or antihypertensive medications peri-operatively. Serum levels of PTH, Vitamin D-25-OH, Calcium, and Albumin were collected at

the ICV, the PACV, and at the LFV. Creatinine levels were collected at the PACV. The majority of the biochemical assessments were performed at the Health Sciences Centre in St. John's, although some samples were collected elsewhere in the province and the results sent in. PTH was measured on Architect i2000sr immunoassay analyzer (Abbott Diagnostics), Vitamin D was measured by LC-MS/MS on an Acquity UPLC system coupled to a Xevo TQ Mass Spectrometer (Waters), and total serum Calcium and Creatinine were measured on Architect c16000 clinical chemistry analyzers (Abbott Diagnostics). Serum Albumin was used to correct serum Calcium levels if necessary.

3.4 Definitions

At each of the clinic visits, patients were categorized as either having hyperparathyroidism or being normal: those with an elevated PTH level were categorized as hyperparathyroid, while those with a normal PTH level were categorized as normal. Patients who had either PTH, Calcium, or Vitamin D 25-OH values missing were categorized as 'N/A' since a category could not be assigned. These patients were then excluded from subsequent analyses performed at the particular clinic visit.

The patients classified as having hyperparathyroidism were further categorized as having either primary, normocalcemic, or secondary hyperparathyroidism. Patients who had simultaneously elevated PTH and Calcium were categorized as having primary hyperparathyroidism. Patients with elevated PTH, normal Calcium, and normal Vitamin D 25-OH were categorized as having normocalcemic hyperparathyroidism. Patients with elevated PTH and either low Calcium and/or low Vitamin D 25-OH level / or high Creatinine were categorized as having secondary hyperparathyroidism). High Creatinine level was defined as

>91 μmol/l in females and > 113 μmol/l in males (Abbott Diagnostics: Architect c16000 clinical chemistry analyzers).

The assay method and the normal ranges of both Calcium and PTH changed several times during the study, and so a 'low' or 'high' or 'normal' value of the individual measurements relative to the given reference range at the time of testing was used to assign patients to their respective categories.

Vitamin insufficiency rather than 'deficiency' was used in this population as a more sensitive marker of Vitamin D shortage that has been shown to be associated with morbidity (Rosen, 2011). Insufficiency was defined as having a Vitamin D 25-OH level < 50 nmol /l (Abbott Diagnostics: Architect i2000sr immunoassay analyzer).

3.5 Data Analysis

Data was entered into STATA 14.0 (2015) for Windows, or SPSS for Windows Version 22.0 (Armonk, NY: IBM Corp) for analysis.

3.5.1. Analysis of Primary Outcomes. The number and percentage of patients categorized as 'normal' and 'hyperparathyroid' was calculated at each of the three time points - the ICV, the PACV, and the LFV. The distribution of patients classified as 'hyperparathyroid' vs. 'normal' at the three time points was then analysed by Cochran's Q Test for determining the association of dependent categorical variables over three time points. A post-hoc McNemar's analysis for determining the association of dependent categorical variables over two time points was also conducted separately between the ICV and PACV, the PACV and LFV, and ICV and LFV. As the LFVs occurred at different time intervals for different patients, the rates of hyperparathyroidism at each LFV time interval (3, 6, 12, 18, and 24 months) were also compared using Chi Square analysis for assessing the association of independent categorical variables. When the Chi Square Test assumption of an expected cell count of 5 or more in 80% of cells was not met, a Yates' correction was applied (Preacher, 2001).

Similarly, the number and percentage of patients categorized as being either 'normal', or having 'secondary hyperparathyroidism', normocalcemic hyperparathyroidism', or 'primary hyperparathyroidism' at the three time points was calculated. The distribution of patients across the diagnostic categories (primary hyperparathyroidism, normocalcemic hyperparathyroidism, secondary hyperparathyroidism, and normal) was then analyzed at the same three time points using the Kappa Test. A McNemar analysis was also conducted separately analyzing the proportion of patients with secondary hyperparathyroidism and normocalcemic hyperparathyroidism between the ICV and PACV, the PACV and LFV, and ICV and LFV.

A more detailed sub-analysis was performed on the patients initially categorized as having normocalcemic hyperparathyroidism to ascertain that they were truly patients with normocalcemic hyperparathyroidism, rather than patients with secondary hyperparathyroidism

and higher thresholds for vitamin D insufficiency - in whom increases in serum Vitamin D levels would subsequently lead to normalization of the biochemical profile. Those categorized as normocalcemic hyperparathyroid at the ICV were followed to the PACV and the LFV and their classification re-assessed (as primary hyperparathyroid, normocalcemic hyperparathyroid, secondary hyperparathyroid, and normal). Mean Vitamin D levels of those falling into the same category at PACV were then compared between the ICV and PACV. The analysis was performed using a Paired T-test for comparing continuous variables from correlated samples. The same analysis was also performed on those categorized as normocalcemic hyperparathyroid at the PACV who remained normocalcemic hyperparathyroid at the LFV.

3.5.2. Analysis of Secondary Outcomes. The number and percentage of patients classified as 'normal' versus 'Vitamin D insufficient' was calculated at the three different time points (ICV, PACV, LFV). The distribution of patients based on Vitamin D insufficiency status at the three time points was analyzed using Cochran's Q Test. A post-hoc McNemar's analysis (comparing the distribution of patients based on Vitamin D insufficiency) was also separately performed between the ICV and PACV, PACV and LFV, and ICV and LFV. The mean Vitamin D levels, with standard deviation, between the consecutive visits (ICV and PACV, PACV and LFV, and ICV and LFV) were compared using a Paired T-test. Patients with missing data were excluded from this analysis. As well, a one-way repeated measures analysis of variance (ANOVA) was conducted on the mean Vitamin D levels between the three visits.

The possible effect of Vitamin D / Multivitamin supplementation on patients' Vitamin D levels was studied separately. The number of patients supplemented versus not supplemented at each of the ICV, PACV and LFV was collected, and the mean Vitamin D levels of those supplemented versus those not supplemented were then analyzed at the three different time

points using a Student's T-test for testing the association between continuous data from unrelated samples. A mixed design repeated analysis of variance (ANOVA) was also performed to compare mean Vitamin D values among supplemented versus not supplemented patients at the three time points.

The possible effect of Vitamin D / Multivitamin supplementation on Vitamin D insufficiency status (Vitamin D insufficient versus normal), as well as on the classification of patients into the primary hyperparathyroid, normocalcemic hyperparathyroid, secondary hyperparathyroid, and normal categories *independent (i.e., pre-surgery)* -of surgery was analyzed by separately comparing those supplemented versus those not supplemented between the ICV and the PACV using the Pearson's Chi Square Test. As above, the Yates' test was used when the assumptions for the Chi Square Test could not be met.

In addition to this, the effects of age, BMI, gender, and initial Vitamin D level on Vitamin D insufficiency at PACV were analyzed, and multiple logistic regression analysis was used to derive Odds Ratios for the exposure variables on the outcome variables. A forward stepwise selection method was used: a simple logistic regression model was first fitted for each risk factor separately, and those risk factors whose inclusion reached a significance level of $p<0.2$ were retained. A multiple logistic regression model was then fitted using all of the retained risk factors. A *p value* <0.05 was considered significant (Jewell, 2003).

The possible effect of Vitamin D / Multivitamin supplementation on Vitamin D insufficiency status, as well as on the categorization of patients into the primary hyperparathyroid, normocalcemic hyperparathyroid, secondary hyperparathyroid, and normal categories *peri-operatively* was analyzed by separately comparing those supplemented versus

those not supplemented between the PACV and the LFV using Chi Square Analysis. The Yates' test was used when the assumptions for the Chi Square Test could not be met.

3.6 Privacy & Ethical Considerations

The study design and all of the outlined procedures were approved by the provincial Health Research Ethics Authority (HREA). A copy of this approval can be found in Appendix A. As previously noted, the patient sample in this study was part of a larger study assessing multiple short-term outcomes of LSG (NL BaSco study). For that study, patient data was de-identified by a bariatric research nurse and each patient was given a unique ID to ensure anonymity of the participants to the research staff. The patients' identity was protected using a computerized coding system. The master key corresponding to the patients' identities was kept on a hard drive in a password-protected computer in a locked office.

All paper data related to the study were stored in a filing cabinet in the Patient Research Centre to which only the bariatric research nurse had access. Access to the derived, coded databases was limited to the primary investigators and research staff.

Chapter 4: Results

This Chapter will review the results of the analysis in greater detail. The first two parts will focus on the primary outcomes, the crude rates of hyperparathyroidism before and after surgery, and the rates of the different subtypes of hyperparathyroidism (primary, normocalcemic, and secondary hyperparathyroidism) before and after surgery. The third part will review the rates of Vitamin D insufficiency and mean Vitamin D levels in the patient sample before and after surgery, and the fourth part will focus on the effects of the confounding factor of Vitamin D supplementation before and after surgery – examining both its relationship to Vitamin D insufficiency rates and its relationship to the distribution of patients into different HP subcategories.

4.1 Rates of Hyperparathyroidism

Two-hundred (n=200) patients were included in the study. The baseline characteristics of age, gender, BMI, initial Vitamin D level, supplementation, and supplementation type at the ICV were collected (table 4.1). The baseline variables of age, gender, BMI, as well as Vitamin D /mineral supplementation and type were available for all study subjects, while initial Vitamin D level was available for 141 (70.5%) of subjects at ICV.

Table 4. 1 Baseline Characteristics of Sample (n=200):

Variable	**Frequency (N)**	**Percentage (%)**
Gender:		
Male	37	18.5
Female	163	81.5
Supplementation:		
Yes	116	58.0
None noted	84	42.0
Supplementation Type:		
Vitamin D	25	12.5
Multivitamin	58	29.0
Both	33	16.5
None noted	84	42.0
	Mean	**Standard Deviation**
Age:		
	43.940 years	9.674
Initial Vitamin D level:		
	60.766 nmol/L	25.483
BMI:		
	48.786 kg/m^2	6.447

The average time to surgery from ICV was 6.3 months, while the average time to LFV from surgery was 14.6 months.

Data for categorization by PTH status was available for 134 (67%) patients at ICV, 198 (99%) patients at PACV, and 139 (69.5%) patients at LFV. Fifty-four (40.3%) patients were hyperparathyroid at the ICV, 57 (28.8%) were hyperparathyroid at the PACV, and 44 (31.6%) were hyperparathyroid at the LFV. Figure 4.1 depicts the distribution of patients who had hyperparathyroidism versus those who had a normal biochemical profile at the three different time points (ICV, PACV, and LFV):

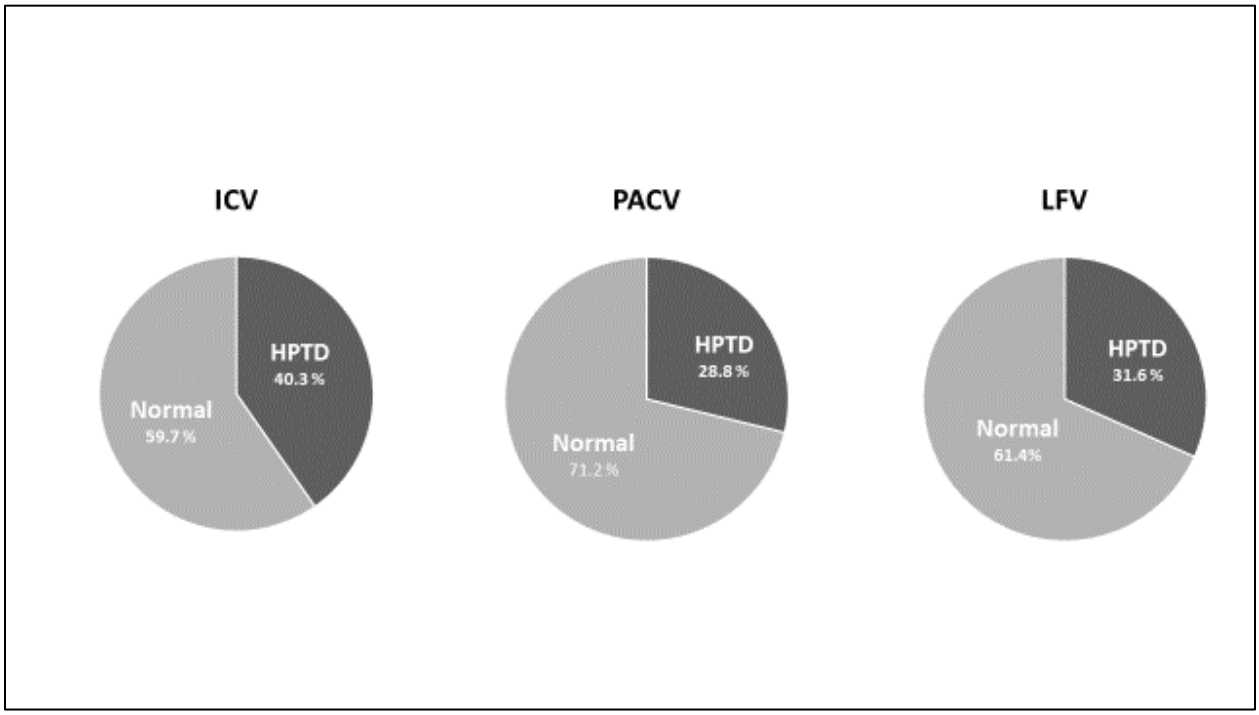

Figure 4. 1. ***Rates of Hyperparathyroidism at ICV, PACV, and LFV.*** *ICV = initial clinic visit, PACV = pre-admission clinic visit, LFV = last follow-up visit, HPTD = hyperparathyroidism*

There was no statistically significant difference in the distribution of patients into the two categories over the three time points (Cochran's Q = 3.77, p=0.152). On comparing ICV to PACV, PACV to LFV, and ICV to LFV, there was no statistically significant difference in the distribution of patients between categories using the McNemar's Test (data not shown). The rates of hyperparathyroidism at LFV were not significantly different when comparing rates at the possible LFV intervals (3 months: 0/7 (0%), 6 months: 10/28 (35.7%), 12 months: 9/38 (23.7%), 18 months: 13/33 (39.4%), 24 months: 12/32 (37.5%). Yates $\chi^2 = 3.600$, p=0.463).

4.2 Rates of Primary, Normocalcemic, and Secondary Hyperparathyroidism:

A diagnostic hyperparathyroid subcategory could be assigned for 134 (67.0%) patients at ICV, 198 (99.0%) at PACV, and 139 (69.5%) at LFV. Figure 4.2 depicts the distribution of patients with available data into the four different diagnostic subcategories (primary hyperparathyroidism, normocalcemic hyperparathyroidism, secondary hyperparathyroidism, and normal) over the three time points (ICV, PACV, and LFV):

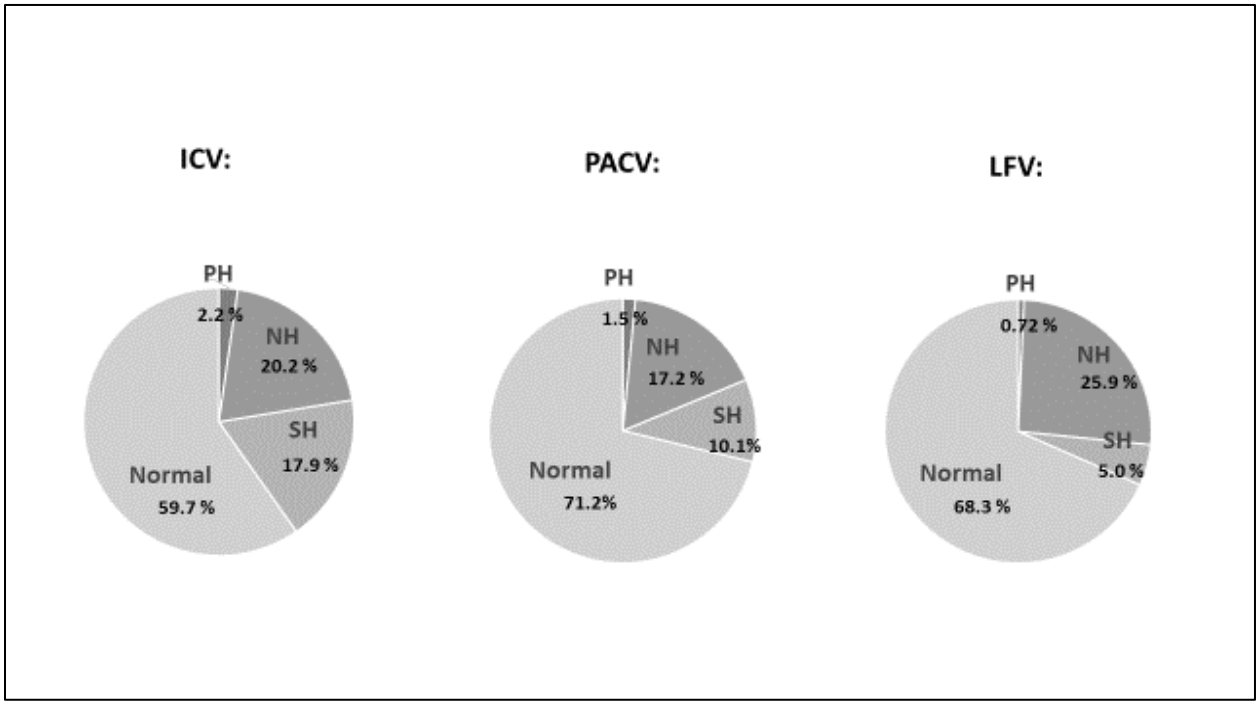

Figure 4. 2. ***Rates of Primary, Normocalcemic, and Secondary Hyperparathyroidism at ICV, PACV, and LFV.*** *ICV = initial clinic visit, PACV = pre-admission clinic visit, LFV = last follow-up visit, PH = Primary Hyperparathyroidism, NH = Normocalcemic Hyperparathyroidism, SH = Secondary Hyperparathyroidism*

There was a statistically significant difference in the distribution of the diagnostic categories over time (Kappa Z = 10.74, $p<0.001$). The rates of primary hyperparathyroidism declined over time; however, one patient underwent parathyroid surgery, and the numbers were

too small for statistical analysis. There was a statistically significant decrease in rates of secondary hyperparathyroidism over the three time points (Cochran's Q= 9.33, p=0.009). There was no statistically significant difference in rates of normocalcemic hyperparathyroidism over the three time points (Cochran's Q= 1.75, p=0.417).

The group of patients categorized as normocalcemic hyperparathyroid was analyzed more closely to ascertain whether increases in serum Vitamin D levels would be associated with changes in subtype of hyperparathyroidism over time.

Five of the 27 patients who were initially normocalcemic hyperparathyroid became secondary hyperparathyroid at the time of PACV. There was a decrease in their mean Vitamin D levels, though it was not statistically significant (67.200 vs. 41.800 nmol/l, p=0.120). Seven of the 27 patients who were initially normocalcemic hyperparathyroid acquired a normal profile at time of PACV. There was a minimal increase in their mean Vitamin D levels, which was not statistically significant (75.571 vs. 78.714 nmol/l, p=0.551). Fifteen of the 27 patients who were initially normocalcemic hyperparathyroid remained normocalcemic hyperparathyroid at PACV. There was a minimal increase in their mean Vitamin D levels, which was not statistically significant (76.867 vs. 78.867 nmol/l, p=0.718). Of these 15 patients, 6 also remained normocalcemic hyperparathyroid at LFV. The mean Vitamin D of these 6 patients at LFV were actually higher than at the ICV, however, the overall difference was not statistically significant (73.500 vs. 81.300 nmol/l, p=0.572).

4.3 Rates of Vitamin D Insufficiency and Mean Vitamin D Levels

Vitamin D status was available for 141 (70.5%) patients at ICV, 20 (100.0%) at PACV, and 139 (69.5%) at LFV. Figure 4.3 depicts the distribution of patients with available data into the two categories (Vitamin D insufficient versus normal) over the three time points (ICV, PACV, and LFV):

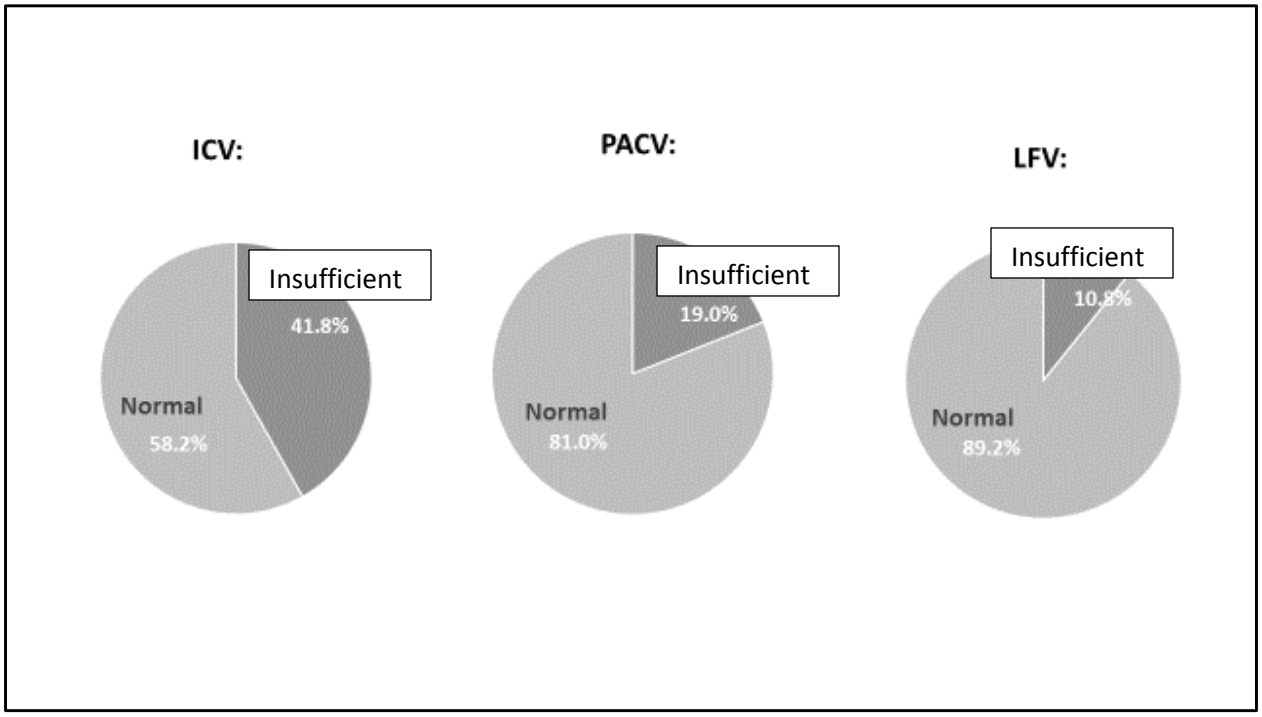

Figure 4. 3. ***Rates of Vitamin D insufficiency at ICV, PACV, and LFV.*** *ICV = initial clinic visit, PACV = pre-admission clinic visit, LFV = last follow-up visit*

There was a statistically significant difference in the distribution of patients who were normal versus those who had Vitamin D insufficiency over time (Cochran's Q = 29.522, $p<0.001$). The difference remained statistically significant when comparing ICV to PACV, PACV to LFV, and ICV to LFV with McNemar's Test (data not shown). The greatest difference

in consecutive time points occurred between the ICV and the PACV, *prior* to any surgical intervention.

The mean Vitamin D levels at the three time points were also compared, as depicted in figure 4.4:

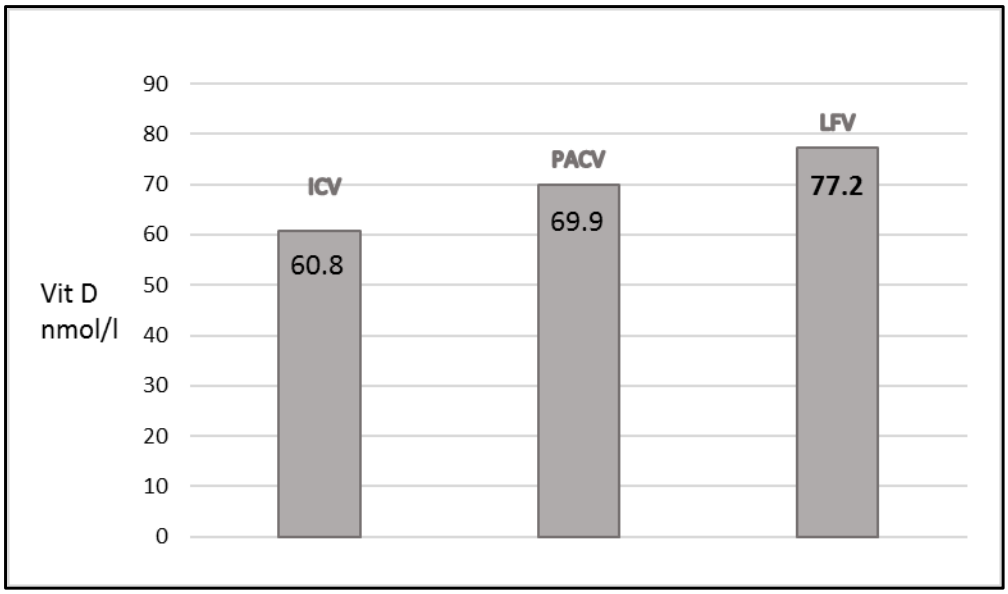

Figure 4. 4. ***Patients' Mean Vitamin D Levels at ICV, PACV, and LFV.*** *ICV = initial clinic visit, PACV = pre-admission clinic visit, LFV = last follow-up visit*

The mean Vitamin D levels were analyzed for consecutive visits in patients with available data. There was a statistically significant difference in mean paired Vitamin D levels between the ICV and PACV (n=141): 60.766 nmol/l vs. 69.184 nmol/l, $p<0.001$. There was also a statistically significant difference between the PACV and the LFV (n=131): 69.777 nmol/l vs. 77.201 nmol/l, $p<0.001$. A one-way repeated measures analysis of variance (ANOVA) was also conducted to evaluate whether there was any change in mean Vitamin D levels between visits (n=102). The results of the ANOVA indicate a significant time effect: Wilks' Lambda = 0.641,

$F(2, 100) = 28.017$, $p<0.001$, $\eta^2 = 0.359$. Follow-up comparisons indicated that each pairwise difference was significant ($p<0.001$).

Vitamin D Supplementation:

When the mean Vitamin D levels were compared at the three time points between those supplemented and those not supplemented, there was a statistically significant difference only at the level of the ICV (n=131): 57.630 nmol/l vs. 68.415 nmol/l, p=0.022. There was a trend of increasing mean Vitamin D levels in both groups over time, as demonstrated in figure 4.5.

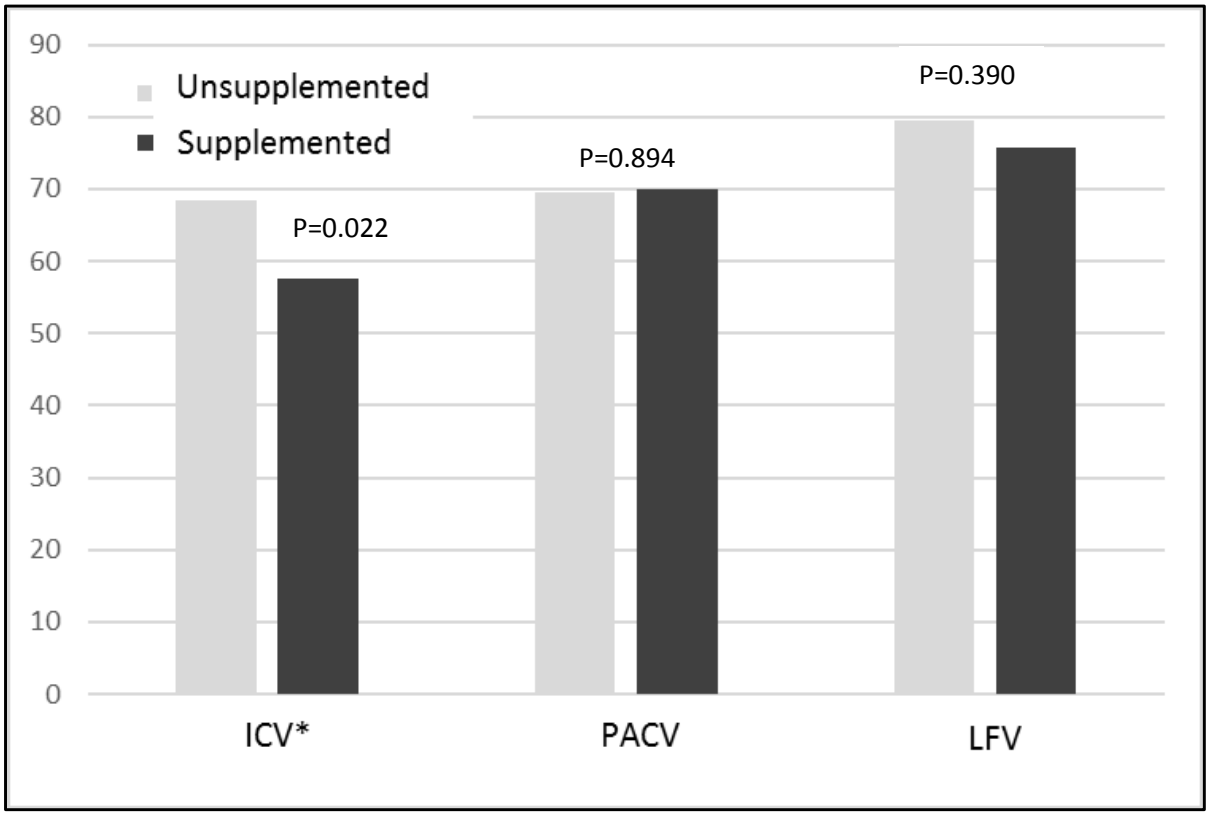

Figure 4. 5. ***Mean Vitamin D Levels at ICV, PACV, and LFV in Supplemented versus Unsupplemented Patients.*** *ICV = initial clinic visit, PACV = pre-admission clinic visit, LFV = last follow-up visit*

*Statistically significant ($p<0.05$)

A mixed repeat measures analysis of variance (ANOVA) was conducted to evaluate whether there was any difference in mean Vitamin D levels between visits among patients who were supplemented versus not (n=102). The results of the ANOVA indicated a significant time effect: Wilks' Lambda = 0.713, $F(2, 99) = 19.919$, $p<0.001$, $\eta^2 = 0.287$. Follow-up comparisons indicated that each pairwise difference was significant ($p<0.001$). When the analysis was applied between groups of patients who were supplemented versus not, there was no statistical significance ($p=0.208$).

There was no statistically significant difference among patients with Vitamin D insufficiency at ICV who were supplemented versus not, and the rates of Vitamin D insufficiency at PACV ($\chi^2 = 2.993$, $p=0.093$). Likewise, there was no statistically significant difference among patients with normal Vitamin D levels at ICV who were supplemented versus not, and the rates of Vitamin D insufficiency at PACV ($\chi^2 = 0.01$, $p=0.589$). -[data not shown].

To further analyze this phenomenon, a multivariate logistic regression analysis was carried out using the baseline characteristics of age, gender, BMI, initial Vitamin D level, supplementation, and supplementation type at the ICV, and the outcome variable of Vitamin D insufficiency versus normal status at PACV.

When simple univariate logistic regression was performed on the whole sample analyzing the impact of the factors of age, BMI, initial Vitamin D, gender, supplementation, and supplementation type (i.e. Vitamin D, Multivitamin, both, or none noted) on the odds of Vitamin

D insufficiency at PACV, only BMI, gender and initial Vitamin D level demonstrated statistical significance, with a p<0.2, as shown in table 4.2.

Table 4. 2 Univariate Logistic Regression Models of Baseline Initial Clinic Visit Variables:

Risk Factor	**Regression Estimate *(b)***	**S.E.**	**Wald Statistic**	**p-value**	**OR**	**95% C.I.**
Age	-0.008	0.019	0.196	0.658	0.992	0.956-1.029
BMI	0.098	0.030	10.902	0.001	1.103	1.041-1.169
Initial Vitamin D Level	-0.043	0.013	11.013	0.001	0.958	0.934-0.983
Gender	-0.580	0.424	1.866	0.172	0.560	0.244-1.287
Supplementation	0.138	0.363	0.144	0.704	1.148	0.563-2.339
Supplementation Type*			0.651	0.885		

BMI = body mass index

***** for Regression and Odds Ratios of the individual variables see Appendix.

The three factors of BMI, Initial Vitamin D level and Gender with $p<0.2$ were then included in the multivariate analysis, as demonstrated in table 4.3.

Table 4. 3 Multivariate Logistic Regression Model of Baseline Initial Clinic Visit Variables:

Risk Factor	**Regression Estimate *(b)***	**S.E.**	**Wald Statistic**	**p-value**	**OR**	**95% C.I.**
Initial Vitamin D Level	-0.037	0.013	7.844	0.005*	0.964	0.939-0.989
BMI	0.052	0.035	2.269	0.132	1.054	0.984-1.128
Gender	-0.163	0.555	0.087	0.768	0.849	0.286-2.519

BMI = body mass index

* Statistically significant (p<0.05)

Multivariate logistic regression analysis of these three variables demonstrated that only the initial Vitamin D level remained associated with a significantly decreased risk of Vitamin D insufficiency at PACV (OR 0.964, 95% C.I..939-0.989).

A separate analysis was performed to assess the relationship between ICV diagnostic category and supplementation, and PACV diagnostic category (table 4.4). There was no statistically significant difference among patients categorized as secondary hyperparathyroid at ICV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at PACV (Yates χ^2 = 1.282, p=0.527). There was no statistically significant difference among patients categorized as normal at ICV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at PACV (Yates χ^2 = 0.453, p=0.929). There was no statistically significant difference among patients categorized as normocalcemic hyperparathyroid at ICV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at PACV (χ^2 = 0.169, p=0.919). The numbers were too small to perform an analysis in the primary hyperparathyroid category.

Table 4. 4 Diagnostic Category at Initial Clinic Visit and Effect of Supplementation on Diagnostic Category at Pre-Admission Clinic Visit:

	Supplement	**PACV Diagnostic Category**		
ICV Diagnostic Category		SH	Normal	NH
SH				
	Yes	6	7	7
	No	3	0	1
Normal				
	Yes	2	49	3
	No	1	21	2
NH				
	Yes	3	5	12
	No	2	2	3

SH=Secondary Hypperparathyroidism, NH=Normocalcemic Hyperparathyrodisim, ICV=initial clinic visit, PACV = pre-admission clinic visit

To analyze the phenomenon of both Vitamin D supplementation and surgery on Vitamin D status, the patients between PACV and LFV were divided into those with Vitamin D insufficiency (serum Vitamin D $\leq$ 50 nmol/) and those with normal Vitamin D levels at PACV (serum Vitamin D > 50 nmol/l). The groups were then analyzed in terms of supplementation at PACV (table 4.5). There was no statistically significant difference among patients with Vitamin D insufficiency at PACV who were supplemented vs. not, and the odds of Vitamin D insufficiency at LFV ($\chi^2 = 0.270$, p=0.449). There was no statistically significant difference among patients with normal Vitamin D levels at PACV who were supplemented vs. not and the odds of Vitamin D insufficiency at LFV ($\chi^2 = 0.323$, p=0.445).

Table 4. 5 Vitamin D Status at Pre-Admission Clinic Visit and Effect of Supplementation on Vitamin D Status at Last Follow-up Visit:

	Supplement	**LFV Vitamin D Status**	
PACV Vitamin D Status		Normal	Insufficient
Normal			
	Yes	60	5
	No	39	2
Insufficient			
	Yes	13	5
	No	12	3

PACV=pre-admission clinic visit, LFV=last follow-up visit

A separate analysis was performed to assess the relationship between PACV diagnostic category and supplementation, and LFV diagnostic category (table 4.6). There was no statistically significant difference among patients categorized as secondary hyperparathyroid at PACV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at LFV (Yates $\chi^2 = 0.198$, p=0.906). There was no statistically significant difference among patients categorized as normal at PACV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at LFV (Yates $\chi^2 = 0.205$, p=0.903). There was no statistically significant difference among patients categorized as normocalcemic hyperparathyroid at PACV who were supplemented vs. not and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at LFV (Yates $\chi^2 = 0.671$, p=0.715). The numbers were too small to perform an analysis in the primary hyperparathyroid category.

Table 4. 6 Diagnostic Category at Pre-Admission Clinic Visit and Effect of Supplementation on Diagnostic Category at Last Follow-up Visit:

	Supplement	**LFV Diagnostic Category**		
PACV Diagnostic Category		SH	Normal	NH
SH				
	Yes	1	5	3
	No	2	4	1
Normal				
	Yes	3	46	8
	No	0	32	6
NH				
	Yes	1	7	10
	No	1	1	6

SH=Secondary Hypperparathyroidism, NH=Normocalcemic Hyperparathyrodisim, PACV=pre-admission clinic visit, LFV=last follow-up visit

Chapter 5: Discussion

This study examined the rates of hyperparathyroidism in patients undergoing LSG, specifically the rates of hyperparathyroidism in this population before surgery, and the rates of hyperparathyroidism after surgery. It also broke down patients with hyperparathyroidism into different subtypes – secondary hyperparathyroid, normocalcemic hyperparathyroid, or primary hyperparathyroid to better ascertain if any intrinsic factors (as in primary or normaocalcemic hyperparathyroidism) versus extrinsic factors (as in secondary hyperparathyroidism) might be at play if the distribution pre-op was found to be different from the distribution post-op. Finally, the study also examined rates of Vitamin D insufficiency pre- and post-operatively, and attempted to analyze the effects that Vitamin / mineral supplementation may have on the rates of hyperparathyroidism by studying separately its effects leading up to and after surgery.

The first section of this chapter compares the baseline characteristics of the current study population to other study populations analyzed in the literature. The second section examines the observed primary and secondary outcomes in comparison to outcomes published in other studies to date.

5.1 Review of Baseline Demographics and Laboratory Values

The current study was larger than any of the other studies previously published on the subject (Ruiz-Tovar et al., 2012; Gehrer et al., 2010; Coupaye et al., 2013; Vix et al., 2014). The male to female distribution was 18.5% to 81.5%, which is slightly lower than three of the other studies, but higher than the Ruiz-Tovar study, which enrolled all females. The average age was 43.9 years, which is comparable to the other studies, which ranged from 35.1 to 47.7 years. The mean pre-operative BMI in the current study was 48.8 kg/m^2, which again is comparable to three

of the other studies, where mean BMI ranged from 45.6kg/m^2 to 49.6 kg/m^2, but somewhat lower compared to the Ruiz Tovar study, where mean BMI was 53.1 kg/m^2. With regards to crude rates of hyperparathyroidism, the pre-operative rate in the current study was 28.8% - higher than any of the other studies, where the pre-operative hyperparathyroidism rate ranged from 8.0% to 28.1%. The rate of pre-operative Vitamin D insufficiency was 19.0% - similar to the Ruiz-Tovar study (20.0%) and the Gehrer study (23.0%), but different from the Coupaye and Vix studies – 80.0% and 84.6%, respectively. It should be noted, however, that the Vix study used a higher threshold for Vitamin D insufficiency – 60 nmol/l rather than 50 nmol/l.

5.2 Review of Primary and Secondary Outcomes

The current study demonstrated no statistically significant difference overall in rates of crude hyperparathyroidism over the three peri-operative time points (43.0% vs. 28.8% vs. 31.6% at the ICV, PACV, and LFV, respectively. On separately comparing the rates between ICV and PACV, PACV and LFV, and ICV and LFV, there was no statistically significant difference, however the greatest decline occurred between ICV and PACV - suggesting that the effect of supplementation, lifestyle and dietary change in the absence of any surgical intervention may have the greatest effect on hyperparathyroidism rates in these patients. On comparing the PACV and the LFV, the crude rate of hyperparathyroidism actually rose slightly from 28.8% to 31.6%. That the hyperparathyroidism rate remained relatively unchanged post-operatively may be due to the fact that the weakly mal-absorptive effects of LSG became counterbalanced by the cumulative effects of weight loss at the LFV (on average 14.6 months post LSG) – a time by which most of the excess weight loss is expected to have occurred (Victorzon, 2012). In other words, any Calcium or Vitamin D deficiencies and subsequent state of secondary hyperparathyroidism, may, by one-year post-op, be counterbalanced by the overall loss of

adiposity, along with all of the putative benefits on rates of hyperparathyroidism this is expected to have.

While there was no temporal difference with regards to overall hyperparathyroid status, there was a statistically significant difference in the distribution of the diagnostic categories of 'secondary hyperparathyroidism', 'normocalcemic hyperparathyroidism', 'normal' or 'primary hyperparathyroidism' overall. Between ICV and LFV the rates of secondary hyperparathyroidism progressively decreased over time, likely reflecting the rise in the % of patients with normalizing Vitamin D status. Interestingly, there was no statistically significant difference among patients categorized as secondary hyperparathyroid at ICV who were supplemented vs. not, and their categorization as secondary hyperparathyroid, normocalcemic hyperparathyroid, normal or primary hyperparathyroid at PACV. However, the numbers were quite small, which inevitably affects the power of these statistics. As well, there may be an element of recall bias as Vitamin D insufficiency rates did decrease progressively over time, while mean Vitamin D levels increased overall (figures 4.3 and 4.4). The rates of primary hyperparathyroidism also declined over time, however, the numbers were too small for statistical analysis. Also, one patient underwent parathyroid surgery. The rates of normocalcemic hyperparathyroidism remained unchanged.

The fact that the meanVitamin D levels at ICV were higher in those unsupplemented vs. supplemented likely reflects the retrospective nature of the study – with those reporting supplementation at the PACV perhaps being more likely to have been supplemented based on their Vitamin D level at the ICV.

These results are quite different in comparison to some of the data published in the past. In the study by Ruiz-Tovar et al., the 20% rate of hyperparathyroidism pre-operatively declined

to 3.3% at 6 months post-operatively, becoming significantly lower at 3 months post-operatively (Ruiz-Tovar et al., 2012). In that study, all of the patients were given Vitamin D and Calcium supplements. In the current study, 58% of patients were *recorded* to be taking either Vitamin D supplements, Multivitamin, or both at PACV. However, this percentage could be a gross underestimation given the possibility of supplementation simply not being reported by either the patient or study nurse at PACV. The Ruiz-Tovar study reported an increase in Vitamin D levels from pre-operative values which became statistically significant at 12 months post-op. There was felt to be a significant inverse correlation between the weight loss and Vitamin D increase, which could be demonstrated from the third month after surgery onward. In the current study, there was a statistically significant difference in the distribution of patients who were normal versus those who had Vitamin D insufficiency over time, which began in the *pre-operative* period. The difference remained statistically significant upon comparing ICV to PACV, PACV to LFV, and ICV to LFV. The decrease in Vitamin D insufficiency was mirrored by a decrease in rates of secondary hyperparathyroidism, however, the overall rates of hyperparathyroidism did not change over time – indicating that a larger proportion of patients either remained or became normocalcemic hyperparathyroid.

In the study by Gehrer et al., 8% of all patients were reported to be hyperparathyroid immediately pre-op (compared to the 28.8% recorded in the current study) (Gehrer et al., 2010). Patients identified to be hyperparathyroid or to have Vitamin D insufficiency pre-or post-operatively were treated immediately with additional supplementation on top of a uniformly issued Multivitamin. However, as the baseline hyperparathyroidism and Vitamin D insufficiency rates in the LSG group were not provided, it is unclear how many patients ended up getting extra supplements. Post-operatively, 14% of patients in the LSG group had elevated

PTH, and 32% had Vitamin D insufficiency. The number of hyperparathyroid patients was highest at 6 months of follow-up and then declined, while the number with Vitamin D insufficiency rose to its peak at 12 months and then declined, with a mean follow up of 24.4 months. In our study, the crude hyperparathyroid rate was lowest immediately prior to surgery (28.8% in the PACV), and rose post-operatively to 31.6% at LFV (which, on average, occurred 14.6 months post-op. The rates of hyperparathyroidism at LFV were not significantly different at 3, 6, 12, 18, and 24 months. Again, the Vitamin D and Multivitamin supplementation in this study was regimented and targeted to those with deficiencies, while the supplementation in our study was merely recommended. However, the average follow-up time in our study was shorter, and it is possible that with a longer duration of follow-up, an overall decline in the rates would have eventually been seen in our study as well.

It is difficult to compare this study to the study by Coupaye et al., as that study only followed mean laboratory values of PTH, Calcium and Vitamin D taken 3 months pre-operatively to 6 months post-operatively without actually providing rates of hyperparathyroidism (Coupaye et al., 2013). The Coupaye study did show that at 6 months, there was a decrease (though statistically insignificant) in mean PTH level among the LSG group from 50.1 ng/l to 47.4 ng/l, and a statistically significant increase in the mean Vitamin D level in the LSG group compared to pre-operative levels (24.1 ng/ml ± 14.1 vs. 15.2 ng/ml ± 9.5, $p<0.05$). The units used in this study differ from ours, using ng/ml rather than nmol/l. When converted to nmol/l, the corresponding values are 60.2 nmol/l post-op and 38 nmol/l pre-op – compared to our 77.2 nmol/l post-op and 60.8 nmol/l pre-op. All patients were given standard supplements of Vitamin D and Calcium post-operatively, but if they demonstrated Vitamin D insufficiency pre-operatively, they underwent a specific, graded replacement regime for 6 weeks based on the

extent of their deficit. Unfortunately, no additional biochemical analysis was performed pre-operatively (as in the present study) to evaluate the effect of this supplementation. The pre-op Vitamin D levels in our study were likely higher because of the supplementation and lifestyle changes that would have been introduced at the ICV, on average 6.3 months pre-operatively.

In the study by Vix et al., the number of patients actually undergoing LSG was also quite small (n=55) (Vix et al., 2014). The pre-operative hyperparathyroidism rate was 28.1% - comparable to the 28.8% hyperparathyroid rate in the current study. The initial rate of Vitamin D insufficiency was 84.6% (vs. 41.8% in our study) and patients presenting with Vitamin D deficiency were supplied with cholecalciferol (Uvedose 100,000 IU, once per month) until normalization was achieved in the post-operative period. The mean Vitamin D values improved significantly at all assessment points compared to inclusion values, and there was a progressive decrease in PTH levels which became significant at 6 months. Hyperparathyroidism rates declined at all points of post-operative assessment - 24 % at 1 month, 8 % at 2 months, 0 % at 6 months, and 12 months (compared with 43% to 28.8% to 31.6% at ICV, PACV, and LFV in our study). Mean Vitamin D values improved significantly at all assessment points compared to inclusion values, and the percentage of patients with Vitamin D deficiency dropped significantly from 84.6% at inclusion to 35 % at 6 months and then rose slightly to 48 % at one year. This is in comparison the Vitamin D insufficiency rates of 41.8% at ICV, 19.0% at PACV and 10.8% at LFV in the current study. Again, the supplementation in the Vix trial was regimented, whereas the peri-operative supplementation in our study was only recommended. However, there was no opportunity to assess the effects of supplementation only in the Vix study as there was in our study - by comparing values at the ICV and at the PACV. The change in hyperparathyroidism

rates and Vitamin D status reflect possible effects of supplementation as well as surgery, once again making the contribution of each factor difficult to analyze.

The overall comparison of the 4 studies with the current study highlights an interesting point – which is that while all 4 studies (as well as ours) showed a relative improvement in Vitamin D levels pre-op compared to post-op (whether supplementation was recommended, routine, or administered only to those with insufficiency), only our study showed persistent rates of crude hyperparathyroidism at LFV which were not statistically different from those measured at ICV.

This raises the possibility of a different subtype of hyperparathyroidism being prevalent in our study – specifically, a higher proportion of patients with primary and normocalcemic hyperparathyroidism in our study, who would not be expected to normalize their PTH levels with Vitamin D replacement.

The current study did show a higher than expected prevalence of primary hyperparathyroidism among the study population, although the patient numbers are very low (figure 4.2). The total number of patients in the study initially identified as having classical primary hyperparathyroidism was 3/200 (1.5%). Primary hyperparathyroidism rates have been reported to be as high as 21/1000 (2.1%) in the population at highest risk for the disorder (females aged 55-75), however, the prevalence in the general population ranges from 1-7 per 1000 adults (0.1 to 0.7 %) (Adami et al., 2002; Yeh et al., 2013; Wermer et al., 2001). The average age in the present study was 47.0 +/- 9.72 years, with 17 % males and 83% females (tables 4.1). The average age of patients with primary hyperparathyroidism in the study was 56, and 1 out of the 3 was male.

The three patients with primary hyperparathyroidism were initially identified based on bloodwork at the ICV (elevated Calcium and PTH, and normal Vitamin D). At the subsequent PACV, the PTH normalized in one of these patents, but this patient was unfortunately then lost to follow-up. The second of the three patients initially identified went on to have parathyroidectomy. The third patient continued to demonstrate primary hyperparathyroidism at LFV. A fourth patient was then identified to have classic primary hyperparathyroidism at the PACV clinic which had not been apparent at the ICV – however this patient was lost to follow up as well.

The numbers, admittedly, are very small, and based on a limited number of laboratory results with margins for error. However, the higher proportion of men and the younger average age of the patients with primary hyperparathyroidism in this study (a group not normally associated with the highest frequency the disorder) raise the possibility of an increased susceptibility to primary hyperparathyroidism in the morbidly obese population. As already mentioned, the association between primary hyperparathyroidism and increased body weight has been demonstrated in the past. In a study comparing patients with primary hyperparathyroidism to age-matched, eucalcemic controls, Grey et al. demonstrated that patients with primary hyperparathyroidism were on average 9.2 kg heavier than healthy aged-matched controls (Grey et al., 1994). The majority of this weight difference was due to increased fat mass (7.2 kg of 9.2 kg). A similar finding was observed in a meta-analysis of 17 studies by Bolland et al., where patients with primary hyperparathyroidism were on average 3.3 kg heavier than age- and gender-comparable eucalcemic controls (Bolland et al., 2004). One would expect that this phenomenon would become even more pronounced in the morbidly obese population.

The underlying explanation for the positive relationship between PTH and fat mass is not entirely clear, however the theory that elevated PTH levels arise as a consequence of increased body weight is generally favoured (Bolland et al., 2006). The Grey et al. study showed that women with PH were heavier throughout their entire adult lives compared to age-matched controls, supporting the theory that increased body weight predispose to PTH excess. The alternative explanation would be that elevated PTH levels predispose to increased fat mass; while there is some experimental evidence from *in vitro* studies suggesting that higher levels of extracellular Calcium inhibit adipolysis in fat cells, if hyperparathyroidism predisposed to the development of obesity, one would expect that its correction (for example by parathyroidectomy) would lead to reduction of body weight (McCarty & Thomas, 2003). However, there does not appear to be any data in the published literature supporting this.

It is possible the hyperparathyroidism -obesity phenomenon may be mediated by altered Vitamin D metabolism; multiple studies have shown an inverse relationship between body weight and/or fat mass and serum Vitamin D 25-OH. (Yanoff et al., 2006; Snijder et al., 2005). Vitamin 25-OH D is fat soluble, leading to increased tissue sequestration in people with increased body fat (Mawer et al., 1972). It has also been suggested that overweight people have decreased exposure to sunlight because of decreased exercise levels and mobility (Coupaye et al., 2013). The secondary hyperparathyroidism that would result from the decreased availability of Vitamin D could, if chronic, increase the risk of developing parathyroid adenomas, a phenomenon that is well documented in renal failure with the development of tertiary hyperparathyroidism (Schwartz et al., 2004; Duan et al., 2015; Yang et al., 2006). However, the interdependence with Vitamin D status has not been consistently demonstrated in all studies. In a second analysis by Bolland and colleagues, adjustment for the presence of Vitamin D

insufficiency or Vitamin D 25-OH levels did not substantially change the relationship between PTH and fat mass (Boland et al., 2006). Ultimately, more research is required to help elucidate the exact underlying mechanisms at work.

Unfortunately, other studies looking at hyperparathyroidism rates pre- and post LSG did not report the rates of the different subtypes of hyperparathyroidism, and thus a comparison between our data and the published literature cannot really be drawn. However, given the rarity of primary hyperparathyroidism (even in this patient population) and the smaller patient samples, one would not expect a substantial number of patients to have been identified in other studies. Of note, the bariatric surgery did not have any immediate impact on the rates of primary hyperparathyroidism in our study, as might be anticipated with an intrinsic disease of the parathyroid glands.

In our study, a substantial number of patients were also identified as having normocalcemic hyperparathyroidism. Twenty-seven patients were identified to have normocalcemic hyperparathyroidism at the ICV. Fifteen (55.5%) of these retained this biochemical profile; the remainder either normalized (7/27 or 25.9%), or developed hyperparathyroidism secondary to Vitamin D insufficiency (secondary hyperparathyroidism) (5/27 or 18.1%). Amongst those that normalized, there was an overall increase in mean Vitamin D levels between ICV and PACV, however this difference was not statistically significant (75.6 nmol/l vs. 78.7 nmol/l, p=0.551). Amongst those that developed secondary hyperparathyroidism, there was an overall decrease in their mean Vitamin D levels between ICV and PACV, but this difference was not statistically significant (67.2 nmol/l vs. 41.8 nmol/l, p=0.120). There was no significant difference in the distribution of patients into different diagnostic categories at PACV when supplementation versus no supplementation was considered

($\chi^2 = 0.169$, p=0.919). This, however, seems again likely attributable to the imprecise recall by both patients and nurses at PACV.

The 15 patients who remained in the normocalcemic hyperparathyroidism category were analyzed more closely, and 6 retained the biochemical profile at LFV despite non-significantly increasing Vitamin D levels. This suggests that they were likely not simply patients with secondary hyperparathyroidism and higher thresholds of Vitamin D insufficiency who would be expected to respond to increasing Vitamin D levels, but more likely patients with true normocalcemic hyperparathyroidism whose course over time would not be expected to alter with either consistent, regimented supplementation or bariatric surgery.

The identification of patients with normocalcemic hyperparathyroidism is relevant, as several studies suggest they seem to suffer from the same myriad of complications as patients with classical primary hyperparathyroidism, including comparable rates of renal stones and decreased bone density (Tuna et al., 2016). Several small observational studies also suggest that patients with normocalcemic hyperparathyroidism may be prone to similar metabolic disturbances linked to higher cardiovascular risk like patients with primary hyperparathyroidism (Yener-Ozturk et al., 2016). As a result, it is generally recommended that any patient with a high PTH and normal Calcium at least needs further evaluation of their ionized Calcium and Vitamin D 25-OH levels, as well as evaluation of kidney function in the form of Creatinine or Glomerular Filtration Rate (GFR) levels. If either of these are abnormal, secondary hyperparathyroidism must be suspected and ruled out – for example by supplementation with Vitamin D, or amelioration of renal function, if possible. In our study we did more closely analyze the Creatinine levels among the patients at PACV – however, only 3% had decreased renal function (GFR < 50 ml/min), and none of these were classified at any point as normocalcemic

hyperparathyroidism. Thus, this only leaves to rule out the possibility of secondary hyperparathyroidism in the setting of a normal Vitamin D: in some instances, there may be differences in individual set points for 'normal' and 'abnormal' levels of Vitamin D that vary from the laboratory cut-off values. Therefore, a certain percentage of patients may not fall into the standard range, and thus cannot be analyzed in the same manner.

Several attempts have been made to further define those patients who truly fall into the 'pathologic' normocalcemic hyperparathyroid category. Infusion of IV Calcium over 3 hours and the corresponding response in PTH secretion were tested by Titon and colleagues to determine a PTH suppression cut-off that distinguished patients with hyperparathyroidism from normal control subjects (Titon et al., 2007). Unfortunately, performance of these tests involves a long, invasive intervention, and as such may not be very practical. Given the high prevalence of Vitamin D insufficiency in the normal population, some authors have tried to re-assign a lower reference cut-off range for PTH However, this cut-off range was assigned in patients who had bone disease, and the same results could not be replicated in normal patients (Jackson et al., 2006).

Noting that patients with 'borderline' primary hyperparathyroidism did not really fit the sigmoidal curve relationship of Calcium and PTH that had been historically described, Harvey and colleagues set out to find a nomogram to establish a 'normal' vs. abnormal biochemical profile in this particular patient population (Harvey et al., 2012). The group based their initial analysis on a cohort of 222 parathyroidectomy patients and healthy normal subjects. Multivariate analysis was used to determine those factors that correlated with PTH in the cohort. Significant independent predictors of PTH were total serum Calcium, age, and Vitamin D-25-OH. The group then developed a nomogram which, incorporating serum Vitamin D levels,

Calcium levels, and age provides a "maximum" PTH level that a patient would be expected to have if they have normal PTH axis physiology: upper limit PTH (ng/ml) = 120 - [6 × Calcium (mg/dL)] - [0.5 × Vitamin D -25(OH)D (ng/mL)] + [0.26 ×age (years)]. If the patient's serum PTH level was above this 'upper limit' of PTH, the patient was felt to have primary rather secondary hyperparathyroidism. Application of the nomogram to a series of patients with surgically confirmed primary hyperparathyroidism resulted in the correct identification of 94%, while identifying 95% of the healthy patient cohort as 'normal'. 32% of those identified by the nomogram to have primary hyperparathyroidism (and confirmed to have the disease at operation and histologically) had a biochemical profile of normocalcemic hyperparathyroidism. Overall, the sensitivity, specificity, positive predictive value, and negative predictive value of the PTH nomogram to diagnose primary hyperparathyroidism were 94%, 95%, 97%, and 92%, respectively.

When this nomogram was applied in our study to the 6 patients who remained normocalcemic hyperparathyroid at LFV, they all fell within the primary hyperparathyroidism scale (although a degree of interassay variability in applying this single cut off to different hospital populations must be taken into consideration). As with the patients in the classical primary hyperparathyroidism category, these patients' hyperparathyroid status was not affected either by surgery or supplementation, providing support for a deeper intrinsic underlying cause which does not appear to be susceptible to extrinsic manipulation. Our study brings this group of patients to light as a subpopulation that is unlikely to respond to supplementation and LSG, and that might more appropriately be targeted with parathyroidectomy, as deemed appropriate.

With regards to the patients with secondary hyperparathyroidism in our study, this proportion decreased steadily over time, as seen in figure 4.2. As discussed previously,

secondary hyperparathyroidism occurs when the parathyroid gland(s) appropriately responds to a reduced level of extracellular Calcium or Vitamin D. There were no recorded instances of hypocalcemia in our study population at any of the three measured time points, and closer analysis of Creatinine levels at PACV revealed only 1 patient with secondary hyperparathyroidism and decreased renal function, which persisted from the ICV to the LFV. As such, 95% of the secondary hyperparathyroidism observed was attributed to Vitamin D insufficiency. The gradual decrease in secondary hyperparathyroidism rates in our study mirrored the decrease seen in the other studies analyzed above; however, our study was the only study that separately analyzed patients' responses to dietary modification and supplementation even before surgery, and the rate of decline pre- and post-operatively was similar. The pre-operative decline in secondary hyperparathyroidism could be a result of lifestyle modification after the ICV – with adherence to recommended dietary modifications, repeated trials of clear fluid diet, and increased efforts at exercise all leading to early changes in adiposity and increased availability of Vitamin D – as well as adherence to increased Vitamin supplementation. It is quite possible that the slope of the decline in both Vitamin D insufficiency and rates of secondary hyperparathyroidism post LSG is partly dampened by the partially mal-absorptive nature of the surgery, which, as mentioned previously, would then counterbalance the gains in Vitamin D availability caused by decreases in adipose tissue.

Chapter 6: Strengths, Limitations & Future Research

The current study analyzed hyperparathyroidism rates in 200 patients undergoing LSG - which, to the best of our knowledge, is the largest patient cohort analyzed to date. It was also the only study that looked at sub-categories of hyperparathyroidism in this patient cohort, shedding light on some of the underlying mechanisms affecting hyperparathyroidism rates over time. Finally, it was the only study that analyzed the possible effects of lifestyle modification (decreased caloric intake, increased exercise, and increased Vitamin and mineral supplementation) on hyperparathyroidism rates independent of surgery – revealing that lifestyle modification in and of itself may play a substantial role.

One of the main limitations of this study is its retrospective nature, which did not allow a more detailed, quantitative analysis of the effects of supplementation on hyperparathyroidism rates. In our study, Vitamin D and mineral supplementation was merely encouraged at the ICV, compared to other studies where it was mandatory and regimented. However, due to the fact that our study followed patients for an average of 6.3 months before surgery (when only the effects of lifestyle modification and supplementation could be observed) as well as following them for 14.6 months after surgery, any changes seen in the cohort pre-LSG served as a baseline control from which conjectures could be drawn in the post-operative period. However, it is difficult to draw conclusions about the individual contributions of each of supplementation versus dietary change or other lifestyle changes.

Information was not gathered on the patients' skin colour, or time of year – important confounding factors that might influence Vitamin D and Calcium metabolism. Finally, the study had a relatively high loss to follow-up. As with any retrospective study, it is difficult to

speculate whether there was any difference between the group of patients who were lost to follow-up, and patients with complete data sets. Specifically, the possibility that those lost to follow-up may have had better or worse outcomes in terms of their surgery remains unanswered – which could then possibly have affected the results in terms of hyperparathyroidism rates.

The findings of the study do emphasize the importance of testing for hyperparathyroidism (in its multiple forms) both pre- and post-operatively in this patient population. Identification of those with primary hyperparathyroidism (which may be more frequent in this patient population) as well as those with true normocalcemic hyperparathyroidism can help to single out a group of patients whose biochemical profile would not be expected to normalize with lifestyle modification or bariatric surgery, and who might ultimately benefit from parathyroidectomy. As per the results of this study, lifestyle modification alone in those patients who have secondary hyperparathyroidism appears to be most effective, even in the pre-operative state. The optimal regime of mineral and Vitamin D replacement has yet to be determined, and would be an important topic of additional research.

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APPENDIX A: HREA APPROVAL

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

March 2, 2015

Linda Bohacek
20 Dartmouth Place
St. John's, NL
A1B 2W2

Dear Dr. Bohacek

Reference #15.039

Re: The Effects of Sleeve Gastrectomy on Hyperparathyroidism in Morbidly Obese Pateitns

Your application received an expedited review by a Sub-Committee of the Health Research Ethics Board and **full approval** was granted effective **February 27, 2015.**

This approval will lapse on February 27, 2016. **It is your responsibility to ensure that the Ethics Renewal form is forwarded to the HREB office prior to the renewal date; you may not receive a reminder, therefore the ultimate responsibility is with you as the Principle Investigator.** *The information provided in this form must be **current to the time of submission** and submitted to the HREB **not less than 30 nor more than 45 days** of the anniversary of your approval date.* The Ethics Renewal form can be downloaded from the HREB website http://www.hrea.ca.

This is to confirm that the following documents have been reviewed and approved or acknowledged (as indicated):

- Application, approved
- Letter of Request, acknowledged

The Health Research Ethics Board advises THAT IF YOU DO NOT return the completed Ethics Renewal form prior to date of renewal:

- *Your ethics approval will lapse*
- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*

email: info@hrea.ca Phone: 777-6974 FAX: 777-8776

Lapse in ethics approval may result in interruption or termination of funding

"This is your ethics approval. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approval from the Regional Health Authority or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals."

You are also solely responsible for providing a copy of this letter, along with your application form, to the Office of Research Services should your research depend on funding administered through that office.

Modifications of the protocol/consent are not permitted without prior approval from the Health Research Ethics Board. Implementing changes in the protocol/consent without HREB approval may result in the approval of your research study being revoked, necessitating cessation of all related research activity. Request for modification to the protocol/consent must be outlined on an amendment form (available on the HREB website) and submitted to the HREB for review. This research ethics board (the HREB) has reviewed and approved the research protocol and documentation as noted above for the study which is to be conducted by you as the qualified investigator named above at the specified site. This approval and the views of this Research Ethics Board have been documented in writing. In addition, please be advised that the Health Research Ethics Board currently operates according to *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans*; *ICH Guidance E6: Good Clinical Practice* and applicable laws and regulations. The membership of this research ethics board is constituted in compliance with the membership requirements for research ethics boards as defined by *Health Canada Food and Drug Regulations Division 5; Part C*

Notwithstanding the approval of the HREB, the primary responsibility for the ethical conduct of the investigation remains with you.

We wish you every success with your study.

Sincerely,

Dr Fern Brunger, PhD (Chair Non-Clinical Trials)
Ms. Patricia Grainger, (Vice-Chair Non-Clinical Trials)
Health Research Ethics Board

email: info@hrea.ca Phone: 777-6974 FAX: 777-8776