Short-term changes in biochemical parameters following laparoscopic sleeve gastrectomy in patients from the Newfoundland & Labrador Bariatric Surgery Cohort Study

by

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A Thesis submitted to the

Schools of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science in Medicine

Clinical Epidemiology Unit, Faculty of Medicine

Memorial University of Newfoundland

May 2015

St. John's Newfoundland

ABSTRACT

Background Biochemical abnormalities associated with micronutrient status and non-alcoholic fatty liver disease (NAFLD) are often observed prior to bariatric surgeries, and the changes following surgery can be associated with either improvements or worsening. However, evidence on these changes following laparoscopic sleeve gastrectomy (LSG) is limited.

Purpose The purpose of this quasi-experimental study was to determine the baseline status of certain biochemical parameters associated with micronutrient status and NAFLD prior to LSG and compare them to the status post-LSG.

Methods A total of 188 patients, who underwent the LSG procedure between May 2011 and May 2014, consented to partake in this study. Laboratory measurements of select biochemical parameters associated with micronutrients (25-hydroxyvitamin D, PTH, vitamin B12, calcium, ferritin, hemoglobin and mean cell volume) and NAFLD status (bilirubin, ALP, ALT, GGT, HDL, LDL and triglycerides) were assessed at baseline and re-evaluated at 3 and 6 months post-LSG.

Results A subgroup consisting of 95 patients were included in the current thesis, as they had completed their baseline, 3 and 6 months evaluations. 82.1 % of patients were female with a mean BMI of 49.3 ± 6.8 kg/m^2. At baseline, 53 patients (55.8 %) presented with abnormal blood levels for the micronutrient-related parameters, and there was a non-significant decrease to 50.5 % and 46.3 % at 3 and 6 months post-LSG, respectively. Prior to surgery, the most common abnormalities were elevated PTH and low 25-hydroxyvitamin D (25-OH-D). The majority of the micronutrients and related parameters did not change significantly following LSG. A significant reduction in the proportion of patients with low 25-OH-D was observed, from 21.3 % at baseline to 1.4 % (1 patient) at the 6-month follow-up ($p < 0.001$), which may be attributed to increased supplement use. The majority of patients (80 %) presented with abnormal blood levels for one or more of the select parameters associated with NAFLD at baseline, the most common being abnormal GGT, triglycerides, HDL, LDL and ALT. There was a significant improvement post-operatively, with 60 % and 50.5 % of patients at 3 and 6 months post-LSG, respectively, presenting with abnormal levels for the select biochemical parameters ($p < 0.001$).

Conclusion Patients experienced an improvement in 25-OH-D post-LSG, which may have been attributed to increased use of supplements. An improvement in the majority of the biochemical parameters associated with NAFLD was also observed. The overall improvements observed may also be attributed to the significant weight loss following LSG.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Laurie Twells and my co-supervisor Dr. Deborah Gregory for their guidance and support throughout this process. I would also like to thank my committee member Dr. Christopher S. Kovacs for his advice and recommendations for this project.

I would like to thank the biostatistician connected to the Newfoundland and Labrador Bariatric Surgery Cohort study, Dr. William Midodzi, for his assistance with the statistical analysis plan for this study. I would also like to thank Dr. Timothy Koch, for his advice on parts of this study.

I would like to thank the nurse practitioner, the research nurse coordinator and the registered dietician, for inviting me to the bariatric surgery orientation sections, and providing information on the patient population and care pre- and post-surgery. I would also like to thank my fellow students, Hilary Price and Kendra Lester, for their support, advice and being sparring-partners.

Last but not least, I would like to thank my husband Peter Just Sørensen, for his continuous love and support throughout this process, even at times when the household chores fell upon him. This is for you.

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LIST OF SYMBOLS AND ABBREVIATIONS

BMI	Body Mass Index (kg/m^2)
WHO	World Health Organization
LSG	Laparoscopic Sleeve Gastrectomy
RYGB	Roux-en-Y Gastric Bypass
LRYGB	Laparoscopic Roux-en-Y Gastric Bypass
GB	Gastric Banding
LAGB	Laparoscopic Adjustable Gastric Banding
BD	Biliopancreatic Diversion
F	Female
M	Male
FLI	Fatty Liver Index
SI	Steatosis Index
HREA	Health Research Ethics Authority
HREB	Health Research Ethics Board
PTH	Parathyroid Hormone
MCV	Mean Cell Volume
LDL	Low-Density Lipoproteins
HDL	High-Density Lipoproteins
NAFLD	Non-Alcoholic Fatty-Liver Disease
NASH	Non-Alcoholic SteatoHepatitis
25-OH-D	25-hydroxyvitamin-D
GEE	Generalized Estimation Equation

ANOVA	Analysis Of Variance
WC	Waist Circumference
%	Percent
mmol/L	Millimol per litre
pmol/L	Picomol per litre
µmol/L	Micromole per litre
mg/L	Milligram per litre
U/L	Units per litre
g/L	Gram per litre
µg/L	Microgram per litre
fL	Femtolitre
mIU/L	Milli international units per litre
ng/L	Nanogram per litre
nmol/L	Nanomol per litre

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1. Introduction

1.1. Background and Rationale

There is an increasing worldwide obesity epidemic occurring, and there have never been as many overweight and obese individuals as there are today (Kazaks & Stern, 2013). While overweight and obesity used to be mainly an issue in Western countries, it is now a global problem, with increasing obesity rates occurring in most countries. Obesity is associated with multiple comorbidities, and today, the majority of the population live in countries where more people die due to overweight and obesity, than underweight [World Health Organization (WHO), 2013]. The increasing incidence rates places a burden on the global health care systems, due to increased visits to health professionals, hospital admissions, etc. (Kazaks & Stern, 2013; Twells et al., 2012).

There are several different treatment options for overweight and obese individuals, some more effective than others. Weight loss is best achieved through a combination of proper diet and an effective exercises routine. Exercise on its own is not enough, if there are no changes made to food intake (Kazaks & Stern, 2013; Lau et al., 2007). The majority of people trying to lose weight will attempt to make lifestyle changes, such as trying new diets and/or exercise routines, pharmacological agents or commercial weight loss programs (Kazaks & Stern, 2013; Lau et al., 2007). Every other year, there is a new diet promising to be the solution to overweight and

obesity. Some of these are the Atkins diet, soup diet, 5-2 diet (fasting two days of the week), the South Beach diet and the currently very popular Paleo diet (Everyday Diet, 2014). Even though these interventions might help some individuals, they often don't result in permanent weight loss for the majority of individuals living with overweight and obesity. An estimated 60 % of people who have lost weight due to either exercise, diet or both, will regain most of the weight they have lost within a year (Sharma & Padwal, 2009). Some of the more effective solutions are commercial weight loss programs, such as Weight Watchers and Jenny Craig. Through these types of programs, people often receive counseling or guidance in addition to being part of a group, resulting in weight loss of up to 10 % after the first year. The programs however are not a permanent solution, and people must adhere to the restrictions of the program to maintain the weight loss (Jenny Craig, n.d.; Weight Watchers, n.d.).

For the severely obese who have exhausted all lifestyle and medical weight loss options, bariatric surgery is the only solution resulting in significant reduction of weight and improvement or resolution of obesity-associated comorbidities (Kazaks & Stern, 2013). Bariatric surgeries available to obese patients are often classified as either restrictive or mal-absorptive (Salzman & Karl, 2013). The gold standard of bariatric surgeries is the Roux-en-Y gastric bypass (Shankar et al., 2010). In this restrictive and mal-absorptive procedure, a portion of the gastric pouch is

removed, and a section of the small intestine is bypassed. According to the 2011 Metabolic/Bariatric Surgery Worldwide report, the most commonly performed procedure is the Roux-en Y gastric bypass, 46.6 %, followed by the laparoscopic sleeve gastrectomy (LSG), 27.8 % and gastric banding, 17.8 % (Buchwald & Oien, 2013).

The relatively new procedure, the LSG, is becoming increasingly more popular, and now contributes to almost 30 % of all bariatric procedures (Buchwald & Oien, 2013). It is primarily a restrictive procedure, where the patient will have a large portion of the gastric pouch removed, leaving a much smaller tube, or sleeve (Roa et al., 2006; van Rutte et al., 2012). This surgery was initially performed as the first of a two-step procedure, where severely obese patients would undergo LSG in order to lose weight, before undergoing gastric bypass or biliopancreatic diversion [American Society for Metabolic and Bariatric Surgery (ASMBS), 2012; van Rutte et al., 2012; Snyder-Marlow et al., 2010]. However, the weight loss and improvement/resolution of comorbid conditions was significant with LSG on its own, and comparable to other bariatric procedures, which led to the procedure becoming its own independent surgery and was done as a stand-alone procedure for the first time in 2004 (ASMBS, 2012; Paluszkiewicz et al., 2012; Peterli et al., 2013). By 2011, LSG was the second most common bariatric procedure performed worldwide, with 94,689 surgeries done in that year alone (Buchwald & Oien, 2013).

1.2. Problem Statement

Studies have shown that many bariatric patients present with biochemical abnormalities prior to undergoing surgery. In addition, the changes observed for these parameters in the post-operative period can be associated with improvements, but also with worsening of certain pre-existing conditions (Koch & Finelli, 2010; Lovette et al., 2012; Salzman & Karl, 2013; Kopec & Burns, 2011, Mahaling et al., 2013; Paschos & Paletas, 2009; Hafeez & Ahmed, 2013).

Some of these biochemical parameters are associated with micronutrient status, and the proportion of micronutrient-related abnormalities might change following surgery, but there are some discrepancies as to if this change is a decrease or an increase (Aasheim et al., 2012; Coupaye et al., 2009). It is therefore important to assess individuals for micronutrient abnormalities prior to undergoing surgery, in order to treat any pre-existing abnormality (Aarts et al., 2012). According to the 2013 Clinical Practice Guidelines for bariatric surgery, the specific micronutrient parameters measured, varies depending on the type of surgery. For the mal-absorptive procedures, a more extended panel of parameters is advised post-operatively than is advised for the restrictive procedures (Mechanick et al., 2013).

Since LSG is considered a primarily restrictive procedure by not bypassing the intestine it is expected to only have a minimal influence on the micronutrient status. (Capoccia et al., 2012; Leivonen et al., 2011; Moize et al., 2012) However,

some studies have shown a change in micronutrient (vitamins and minerals) status following surgery suggesting the procedure may be mal-absorptive and restrictive, although there is not a general consensus of the direction of change (Aarts et al., 2011; Damms-Machado et al., 2012; Gehrer et al., 2010; Kehagias et al., 2011). The main purpose of this study is to determine baseline micronutrient status of select biochemical parameters in patients undergoing LSG, and compare it the short-term outcomes at 3 and 6 months following surgery in order to determine a possible change.

It is purported that one of the major causes of obesity is poor diet quality. A high intake of food that is high in calories and carbohydrates, but low in essential micronutrients, is often the most convenient and affordable (Lovette et al., 2012). This excess of food, but lack of nutrients, cannot only lead to overweight and obesity, but several comorbid conditions. Bariatric surgeries are often the only options for overweight and obese individuals to be healthier, as they are associated with significant weight loss and an improvement and/or resolution of certain co-morbidities (Kazaks & Stern, 2012; Snyder-Marlow et al., 2010). One of the major conditions associated with obesity is metabolic syndrome, which covers several comorbidities such as hypertension, dyslipidemia and diabetes.

The hepatic manifestation of the metabolic syndrome is a liver condition known as non-alcoholic fatty liver disease. Studies have shown that a large portion of the overweight and obese populations suffers from this condition (Franzini et al., 2011; Mishra & Younossi et al., 2012; Razavizade et al., 2012; Shifflet & Wu, 2009). It has been suggested that bariatric surgery might improve this condition as well and thus lead to improvements in the biochemical parameters associated with this condition. However, the majority of studies conducted on this topic have focused on the Roux-en-Y gastric bypass, gastric banding or biliopancreatic diversion (Pillai & Rinella, 2009; Hafeez & Ahmed, 2013). To date, there are only two identified published studies that examine the possible improvement of this liver condition following laparoscopic sleeve gastrectomy (Karcs et al., 2012; Mattar et al., 2005). Therefore, the secondary focus of this study will be to evaluate select biochemical liver parameters associated with non-alcoholic fatty liver disease pre- and post-LSG.

2. Literature Review

The purpose of the literature review is to review and identify the gaps in the current literature available on obesity and biochemical abnormalities following LSG. This chapter will cover the epidemiology of overweight and obesity worldwide as well as nationally and at the local provincial level, and the bariatric surgery treatment options available. The possible causes of biochemical abnormalities associated with bariatric surgery will be presented along with the associated medical conditions. An initial literature search was conducted using the PubMed, EMBASE, and CINAHL databases along with the Cochrane Library (Appendix 2. 1)

The MeSH terms and keywords used in the search were "sleeve gastrectomy, bariatric surgery, gastric bypass, gastric banding" in combination with one or more of the following: "micronutrient/s, vitamin/s, deficiency/deficiencies, NAFLD or NASH". The search resulted in 17 relevant articles on sleeve gastrectomy and 58 articles on bariatric surgery. Manual searches of references list from potentially relevant papers was performed in order to identify additional studies that may have been missed using the computer-assisted search strategy.

2.1. Obesity

The World Health Organization (WHO) defines obesity as: "*abnormal or excessive fat accumulation that may impair health*" (WHO, 2013). Overweight and obesity is most commonly classified according to an individual's body mass index (BMI), which is defined as weight in kilograms divided by height in meters squared,

or kg/m^2 (Table 2.1). The BMI is also used to define the population eligible for bariatric surgery. According to the Canadian Clinical Practice Guidelines, patients undergoing bariatric surgery must have a BMI > 40 kg/m^2 or a BMI ≥ 35 kg/m^2 plus risk factors (Lau et al.; 2007).

Table 2. 1

BMI classifications and risk factors.

BMI (kg/m^2)	Classification	Risk of comorbidities
< 18.5	Underweight	Increased
18.5 – 24.9	Normal weight	Minimal
25.0 – 29.9	Overweight	Increased
30.0 – 34.9	Obese I	High
35.0 – 39.9	Obese II	Very high
> 40.0	Obese III	Extremely high

Note: From Statistics Canada (a).

2.1.1. Causes of obesity

The causes of overweight and obesity are complicated, as there are numerous factors that contribute to the risk of development. The simplest explanation is that more calories are ingested compared to the amount of calories expended (Kazaks & Stern, 2013). The causes of overweight and obesity are often multifactorial; genetics, social-economics, cultural and environmental influences can play a significant role (Katzmarzyk & Mason, 2006; Lau et al., 2007; Sharma & Padwal, 2009).

2.1.2. Co-morbidities and risk of mortality

Overweight and obesity are associated with many comorbidities. Some of the main comorbidities related to overweight and obesity are diabetes, cardiovascular disease, sleep apnea, osteoarthritis, non-alcoholic fatty liver disease and certain cancers (Lau et al., 2007; Navaneelan & Janz, 2014). As BMI increases, so does the risk for developing these conditions (WHO, 2013). The risk of developing health problems is lowest when an individual is within the normal BMI range. The risks associated with BMI are often described by a J or U curve, with increasing risk of mortality seen at either end (Figure 2.1). There is a very low health risk for individuals with a normal weight, however, when the BMI values are within the three obesity classes, the health risk increases further (Twells, 2005; WHO, 2013).

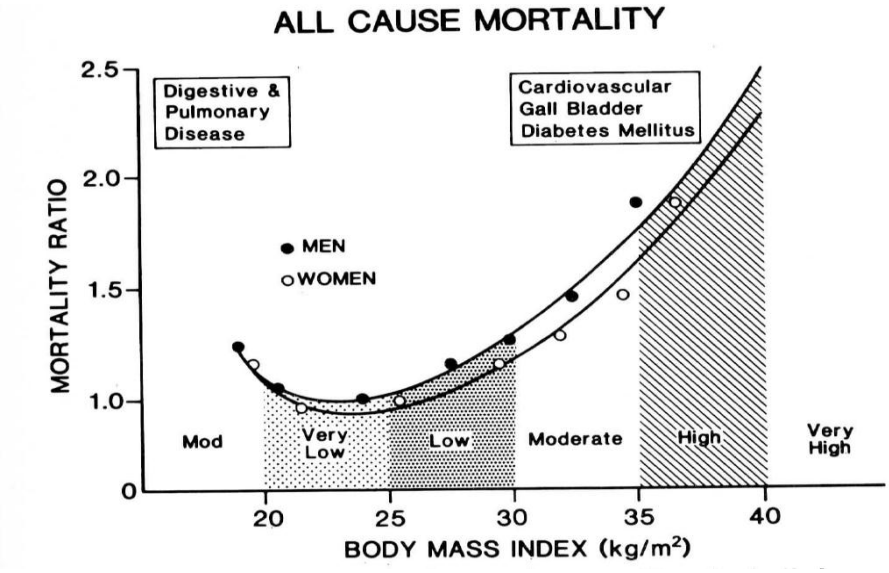

Figure 2. 1. **Mortality curve of BMI classes.**

Note: Used with permission from Gray, D. S. (1989) Diagnosis and Prevalence of Obesity. *Medical Clinics of North America.* Volume 73, issue. 1.

2.2. Epidemiology of obesity

The incidence rate of overweight and obesity is increasing worldwide, with more than 1.4 billion adult being overweight (WHO, 2014). People categorized as overweight or obese outnumber people with a normal weight, and the majority of the world's population now live in countries where more people die of overweight and obesity related causes than underweight (Kazaks & Stern, 2013; Lau et al., 2007). Even though the incidence is not increasing as much as in previous years, and may even have levelled off in some countries, the overweight and obesity rates remain high [Kazaks & Stern; 2013; Organisation for Economic Co-operation and Development (OECD), 2012]. The obesity epidemic is more prevalent in Western countries, with North and South America having the highest prevalence (62 % overweight and 26 % obese). The lowest prevalence is found among the Asian countries (Figure 2.2), with only 14 % overweight and 3 % obese (WHO, 2014). According to the 2012 Obesity Update from the Organisation for Economic Co-operation and Development (OECD), the overweight and obesity rates have been stable in Korea, Switzerland, Italy, England and Hungary over the past couple of years; however the rates are not decreasing (OECD, 2012). Although the epidemic used to be more prevalent in the developed countries, the rates are increasing in the developing countries as well (Lau et al., 2007).

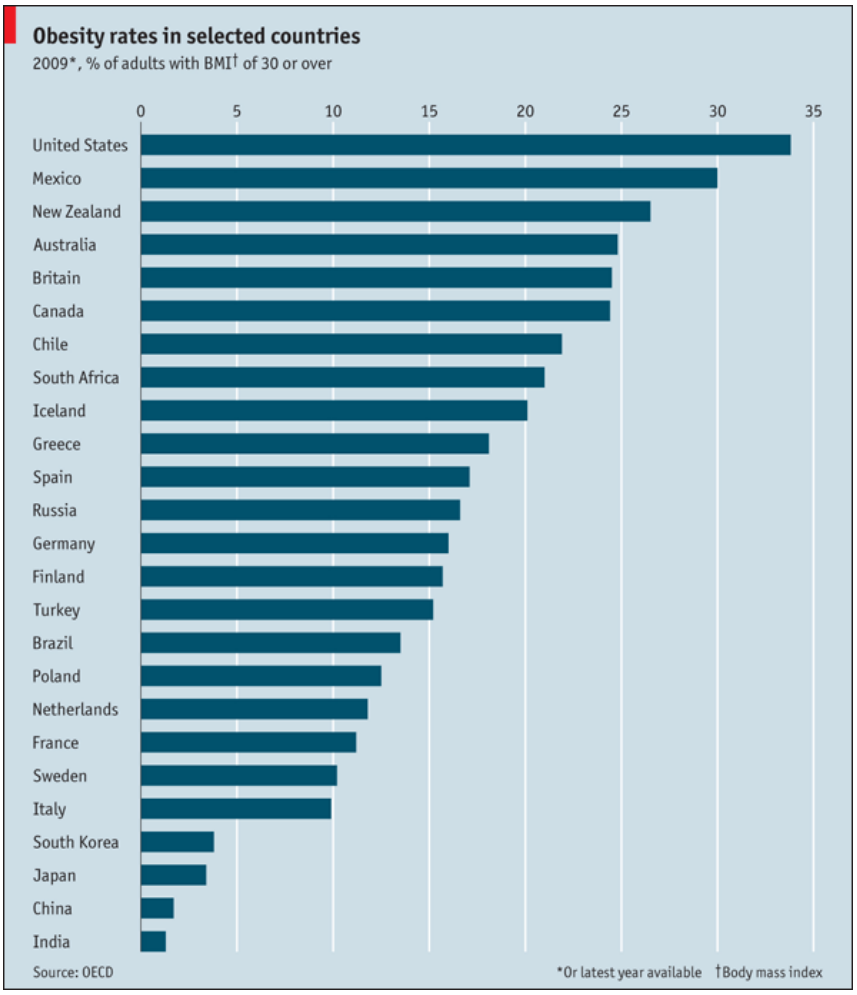

Figure 2. 2 **Global obesity rates.**

Note: Used with permission from The Economist, 2010. *"Obesity Rates. Fat of the lands"*

2.2.1. Canada

Canada is among the countries with the highest overweight and obesity rates and is following the global pattern with increased prevalence rates. According to a recently published study, the prevalence of obesity among the adult Canadian population in 2011-2012 was 24.8 %, which was a 17.5 % increase from 2003 (Navaneelan & Janz, 2014). In addition to this, the 2009-2011 Canadian Health Measures Survey (CHMS) Cycle 2 showed that 16.2 % of Canadians between 18 – 70 years of age were in the obese class I, 6.3 % were in the obese class II and 3.7 % were in the obese class III [Statistics Canada (b), 2013].

According to the 2011 Obesity in Canada report, the cost of overweight and obesity was about $4.6 billion in 2008, which was a 19 % increase from 2000. This estimate was done by including a number of chronic diseases associated with overweight and obesity, but the actual cost may be even higher if all related conditions are included [Public Health Agency of Canada (PHAC), 2011].

2.2.2. Newfoundland and Labrador

Newfoundland & Labrador has consistently been among the provinces with the highest rates of adult overweight and obesity, and the increase in prevalence rates occur at a much higher rate there than in other provinces. The rates are similar among other Atlantic Provinces; Prince Edward Island, Nova Scotia and New Brunswick along with the Northwest Territories [Human Resources and Skills Development Canada (HRSDC), 2014; Navaneelan & Janz, 2014; Twells et al., 2014].

In 2011-2012, the prevalence of obesity was second highest in this province, with 35.2 % of the population being considered as obese, only the Northwest Territories had a higher prevalence (35.3 %) than Newfoundland & Labrador (Navaneelan & Janz, 2014). If the current increasing trend continuous, it is estimated that more than 70 % of the Newfoundland & Labrador population will be overweight or obese in 2019 (Twells et al., 2014). Among the obese population in Newfoundland & Labrador, there is a significantly higher prevalence of visits to private physicians and general practitioners, when compared to the normal weight population. The obese population in Newfoundland & Labrador also have significantly more chronic conditions than the normal weight population (Twells et al., 2012).

2.3. Bariatric Surgery

There are several reports suggesting that obesity is associated with an increased risk of mortality due to the numerous obesity related conditions (Adams et al., 2007; Pontiroli & Morabito, 2011; Sjostrom et al., 2007; Walker et al., 1995). In addition, studies have shown that a significant weight loss improves many of these conditions, suggesting that weight loss might lead to a reduction of the mortality risk (Sjostrom et al., 2007; Sjostrom et al., 2012; Torgersen et al., 2014; Williamson et al., 1995). However, there continues to be conflicting reports as to whether weight loss is actually associated with the risk of overall mortality, and there is limited literature available (Adams et al., 2007; Pamuk et al., 1993; Sjostrom et al., 2012; Williamson et al., 2000).

While some studies are reporting that weight loss is associated with a reduction in overall mortality (Pamuk et al., 1993; Williamson et al., 1995; Williamson et al., 2000), the majority are reporting increased mortality being associated with weight loss (Pamuk et al., 1993; Walker et al., 1995; Wannamethee et al., 2000; Williamson et al., 1995). A limitation to these studies though, is the fact that they fail to distinguish between intentional weight loss and unintentional weight loss that might be a consequence of a condition leading to death, not the cause of it (Pamuk et al., 1993; Sjostrom et al., 2007; Walker et al., 1995; Williamson et al., 1995).

Many studies have reported that bariatric surgery is associated with a reduced risk of mortality, not the weight loss itself (Adams et al., 2007; Christau et al., 2004; Pontiroli & Morabito, 2001; Sjostrom et al., 2007; Sjostrom et al., 2012). In the Swedish Obesity Subjects study, undergoing bariatric surgery (gastric bypass) was associated with a 40 % reduction of all cause mortality, as well as a reduced risk of cardiovascular death, fatal myocardial infarction and stroke (Sjostrom et al., 2007; Sjostrom et al., 2012). The fact that bariatric surgery is associated with a reduced risk of mortality could suggest that it might be due to the weight loss. However, it might also be because bariatric patients are followed more closely by their health care professional, thus providing health care above the normal standard of care that their "control" would receive (Sjostrom et al., 2007). There continues to be a great need for studies assessing the association between intentional weight loss and the risk of mortality. In the end, a reduced risk of mortality following bariatric surgery is more important than the weight loss itself.

Bariatric surgery has been deemed the best treatment option available to the obese population in order to obtain and sustain significant weight loss. Patients tend to lose an estimated 20 -40 % of their initial body weight after bariatric surgery, which is the equivalent to an estimated BMI loss of 14 kg/m^2 (Salzman & Karl, 2013, Torgersen et al., 2014). Bariatric surgeries are usually classified into one of two categories; mal-absorptive or restrictive. Mal-absorptive procedures involve an anatomical alteration, usually a bypass of the intestines. By bypassing a section of the small intestine, the amount of absorption from the diet is limited, as much of the

micronutrient absorption takes place in the small intestine (Valentino et al., 2011). A restrictive procedure however, involves either a temporary or permanent reduction in the size of the gastric pouch. This reduction in stomach size reduces the amount of food that can be consumed, and may also have an effect on the absorption. Many of the enzymes and co-factors for micronutrient absorptions are produced in the gastric pouch (Salzman & Karl, 2013; Snyder-Marlow et al., 2010; Valentino et al., 2011).

2.3.1. Roux-en Y Gastric Bypass (RYGB)

The Roux-en Y gastric bypass is the most common bariatric procedure in North America, and is considered the gold standard of bariatric surgeries (Malone, 2009; Shankar et al., 2010). It can be performed as an open surgery but most are done as a laparoscopic Roux-en Y gastric bypass (LRYGB).

RYGB is considered to be a combined restrictive and mal-absorptive procedure (Pillai & Rinella, 2009). The majority of the gastric pouch is resected and removed from the body, leaving only a small pouch in addition to the first portion of the small intestine being bypassed (Figure 2.3). Due to the small stomach size, patients will lose weight rapidly post-surgery due to a significant reduction in food intake (Koch & Finelli, 2010; Malone, 2009). It is the type of bariatric surgery associated with the greatest weight loss, making it very desirable (Lassailly et al., 2013; Mattar et al., 2005).

2.3.2. Adjustable Gastric Banding (AGB)

This type of procedure is more common in Europe than in North America, and is attractive due to the fact that it is reversible. However, there has been a worldwide decline in the use of this procedure over the years, decreasing from 145,563 procedures performed in 2008, to just 60,677 procedures in 2011 (Buchwald & Oin, 2013).

A band is placed around the stomach to create a smaller pouch at the site where food first enters the stomach (Malone, 2009). The band is adjustable through a subcutaneous port just above the abdominal muscles (Figure 2.4), meaning the size can be adjusted for certain occasions (Gasteyger et al., 2006).

2.3.3. Laparoscopic Sleeve Gastrectomy (LSG)

This relatively new surgical procedure was initially performed as a first step in a two-step operation for patients with high BMIs or increased risk of surgical complications (Roa et al., 2006). Patients would undergo LSG, followed by biliopancreatic diversion (BPD) or duodenal switch (DS). However, studies found that the weight loss after the first step was significant, and LSG became a stand-alone procedure (ASMBS, 2012; van Rutte et al., 2012; Snyder-Marlow et al., 2010). Studies have shown that the weight loss and improvement of co-morbidities is similar to that of the RYGB within the first year after surgery, with the exception of gastroesophageal reflux disease and the remission of type 2 diabetes, and in 2012,

the sleeve gastrectomy was recommended as an alternative to RYGB by the American Society for Metabolic and Bariatric Surgery. (Torgersen et al., 2014; Peterli et al., 2013)

Between 60 and 85 % of the stomach is removed, leaving a small "sleeve-like" pouch (Figure 2.5). The procedure is done laparoscopically using 5 or 6 trocars, and the size of the new stomach pouch is determined by a French bougie. The size of bougie varies among studies (e.g., 32 to 60), leaving the volume of the stomach between 75 – 120 mL (van Rutte et al., 2012). It is debated whether this procedure is purely restrictive, as there is an irreversible anatomical alteration of the stomach pouch (Andromalos, 2012; ASMBS, 2012; Snyder-Marlow et al., 2010).

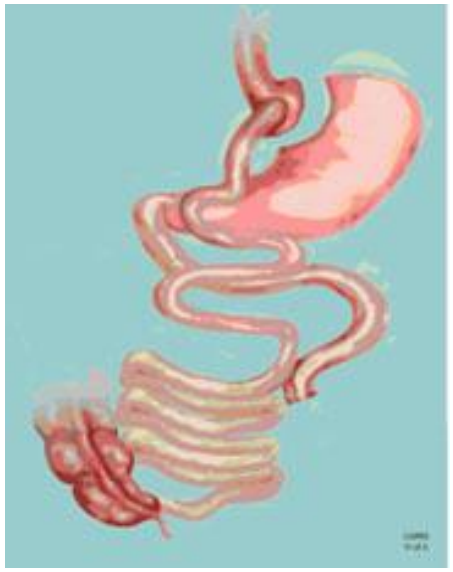

Figure 2. 3

Roux-en Y gastric bypass

Note: Used with permission from (Padwal et al., 2011)

Figure 2. 4

Adjustable gastric banding

Note: Used with permission from (Padwal et al., 2011)

Figure 2. 5

Laparoscopic sleeve gastrectomy

Note: Used with permission from (Padwal et al., 2011)

2.4. Origins of Micronutrient-related abnormalities

Micronutrients are essential in the functioning of the human body. They play a large role in metabolic processes and are factors of important cellular pathways and mechanisms. When the levels of these are either abnormally high or low, the consequences can be of serious concern. The origins of micronutrient abnormalities have several different causes. Micronutrient-related abnormalities can occur in the obese population prior to undergoing surgery, most often due to poor diet. Patients can also develop abnormalities following bariatric surgery, which may be related to a number of factors including reduction in the amount and quality of food consumed, altered digestion and/or absorption, non-adherence to diet regimens and recommended supplementation (Salzman & Karl, 2006), and any pre-existing abnormalities can improve or worsen following post-surgery as well (Lovette et al., 2012).

It is important to define the difference between clinical and biochemical abnormalities. A *clinical abnormality* is when an individual shows the symptoms associated with the specific deficit of a micronutrient and these can be psychological or physiological (Lovette et al. 2012; Salzman & Karl, 2013.) A *biochemical abnormality* is when the specific micronutrient is at a blood level below or above the normal reference range. As micronutrient-related abnormalities can be present both clinically and biochemically, a patient will usually consult with their primary care

physician when certain symptoms are present. If the doctor associates these symptoms with specific micronutrients, they will order tests to determine the blood levels of these parameters. It is the results from these tests that determine if the clinical symptoms are due to a micronutrient abnormality (Blake, 2012). However, many of the biochemical abnormalities are discovered incidentally through routine blood work, where patients might not be presenting with any symptoms.

2.4.1 Pre-operative

There are several possible causes of micronutrient-related abnormalities among individuals wanting bariatric surgery, the most common being poor diet (Salzman & Karl, 2013). The majority of overweight and obese individuals do not get the amounts of essential minerals and vitamins required to maintain proper levels in their body. There has been a significant increase in the amount of food people eat each year, but this increase is not in fruits and vegetables (Kazaks & Stern, 2013; Rickers & McSherry, 2012). The intake of food containing high-calories, carbohydrates and fat has increased among all gender and races. The poor diet is in many cases a reflection of the social-economic or cultural status, since nutritious and vitamin-rich foods are often more expensive than the processed, high-calorie options. Low-income households, which are at higher risk of being overweight or obese, may not focus as much on the quality of food, but more on the quantity (Lovette et al., 2012; PHAC, 2011; Sharma & Padwal, 2009).

According to the 2007/2008 National Health and Nutrition Examination Survey (NHANES), American adults over the age of 20 years did not meet the required intake of fibre, vitamins and minerals. Poor diet is not just common in the overweight and obese population, but a problem at a global level for normal weight people as well (Lovette et al., 2012).

One of the most common abnormalities found pre-operatively in the overweight and obese population, is low vitamin D (i.e. 25-hydroxyvitamin D). This can be due to insufficient vitamin D intake from food, such as oily fish (e.g. salmon, mackerel), fish-liver or dairy products, but may also occur from a lack of sun exposure. The main source of vitamin D is through sunlight exposure, as vitamin D is synthesized in the skin and converted into its active form in the liver. However, in some cases the release from the skin to the bloodstream may be mechanically altered (Dewey & Heuberger, 2011). Because of the stigmatization many obese individuals experience, many spend fewer hours outdoors, thus reducing the amount of ultraviolet exposure (Ruiz-Tovar et al., 2012; Swaminathan, 2011). In addition, people are generally advised by public health organizations to wear sunscreen and clothing to cover skin in order to reduce the risk of skin cancer, but this blocks the effect that ultraviolet light usually has in skin, to reduce the formation of vitamin D. However, if patients are exposed to adequate quantities of sunlight, the surgical procedure will be irrelevant and should not have an effect on the blood levels of 25-hydroxyvitamin D.

2.4.2. Post-operatively

Many studies have shown an increase in micronutrient-related abnormalities following bariatric surgeries, the causes of which may be complex. Some of the reasons identified by *Saltzman & Karl* (2013) were: reduced hydrochloric secretion from the gastric pouch, reduced intrinsic factor secretion from the gastric pouch, reduced absorption due to anatomical alterations and bacterial overgrowth in the small intestine.

Following most bariatric surgeries, there is a significant reduction in food intake, thus less nutrients are available to be absorbed. Even though most overweight and obese individuals do not have the most nutrient-rich diet pre-surgery, they might potentially ingest fewer nutrients afterwards (Aasheim et al., 2009; Salzman & Karl, 2013). Because diet following bariatric surgery is very restrictive, patients are not able to eat otherwise nutrient-rich foods (Rickers & McSherry, 2012). One of the hardest things for people to eat following bariatric surgery is red meat; however this food item contains important nutrients that the human body needs, such as iron, calcium, protein and several vitamins that one must now get from other sources (Salzman & Karl, 2013; Swaminathan, 2011).

Another explanation for the decrease of specific micronutrients is the reduced absorption that might take place post-operatively. In the mal-absorptive procedures, a part of the small intestine is bypassed. Since this is the main site of absorption, it can lead to possible low levels of certain micronutrient-related

parameters. In the LSG procedure, the majority of the gastric pouch is removed; however, important enzymes and co-factors for nutrient absorption are produced and secreted here. Either of these, or a combination, can potentially lead to micronutrient-related mal-absorption (Moize et al., 2012; Valentino et al., 2011).

Obese patients are at risk of developing these abnormalities and as such, the ASMBS recommend patients take a vitamin and mineral supplement routinely and immediately after surgery (Blume et al., 2012). However, the alteration of the gastric pouch and intestinal tract can potentially also contributing to impaired absorption of these supplements. This might be the explanation for the minimal effect of supplementation reported in some studies. These studies suggest that patients must take more than the required daily allowance of specific supplements in order to properly treat or prevent a deficiency (Aarts et al., 2012; Blume et al., 2012; Dalcanale et al., 2010).

It has been suggested that bacterial overgrowth of the gastric pouch and small intestine can have an effect on vitamin B_1, B_{12} and folate (Koch & Finelli, 2010; Lovette et al., 2012; Swaminathan et al., 2011). The mechanisms for this are not fully understood, but upper gut bacterial overgrowth has been observed after RYGB and AGB. Some studies indicate that the bacteria might ingest vitamin B_{12}, thus making a less amounts available for absorption and potentially causing a deficiency (Swaminathan, 2011). The effects of bacterial overgrowth can be present before and after bariatric surgery (Koch & Finelli, 2010; Lovette et al., 2012).

2.5. Current literature on micronutrient changes following bariatric surgery

Differences among the studies focused on micronutrient-related changes following bariatric surgery include varied patient populations, different follow-up time points, types of bariatric procedures, supplementation and micronutrient-related parameters measured (Toh et al., 2009). Because there are inconsistencies among the micronutrients studied, some of these variables have only been studied once for one surgical procedure, thus making it difficult to draw any overall conclusion to the changes following all bariatric procedures.

Despite the fact that micronutrient-related changes following bariatric surgeries have been studied by many, there are still many uncertainties regarding the direction of these changes from the different procedures (Ziegler et al., 2009). While the prevalence of some micronutrient-related abnormalities have been reported to increase in some studies (Coupaye et al., 2009; Toh et al., 2009), the same parameters have been observed to decrease or remain stable in others (Aasheim, 2012; Blume, 2012; Gasteyger, 2006).

2.5.1. Bariatric surgeries and micronutrient abnormalities

Micronutrient-related abnormalities are common and somewhat expected following mal-absorptive procedures, due to the alteration of the gastric pouch and the intestinal tract. LSG is typically associated with fewer micronutrient-related abnormalities than the mal-absorptive procedures, such as the RYGB (Gehrer et al., 2010; Salzman & Karl, 2013; Valentino et al., 2011). Some studies measure the micronutrient-related parameters at baseline and several times following the surgery, thus providing an estimated change over time. Other studies however, only present the baseline and the outcomes at one specific follow-up, e.g. at 6 or 12 months post-operative, or they might only show the results from the follow-up period.

While the changes following gastric bypass surgeries have been studied the most, there are few studies examining the micronutrient-related changes that follow LSG (Coupaye et al., 2009; Moize et al., 2012). The overall changes in select micronutrients following various bariatric procedures are presented in Table 2.2.

Table 2.2

Overall change in laboratory values following bariatric surgery.

	RYGB	**GB**	**LSG**	**DS/BPD**
Calcium	?	-	Increasing/?	Decreasing
Ferritin	Decreasing	Increasing/?	?	N/A
Hemoglobin	Decreasing	-	?	N/A
MCV	?	?	?	N/A
PTH	Decreasing	Decreasing	Decreasing	N/A
Vitamin B_{12}	Decreasing	-/Increasing	?	Decreasing
25-OH-D	Increasing	?	Increasing	Decreasing

Note: Estimates on changes based on current literature available. ? = Changes not clear from current literature, fluctuating levels post-surgery. N/A = Information not available from current literature. - = Stable levels, no change in levels post-surgery. (Aarts et al., 2012; Aasheim et al., 2012; Blume et al., 2012; Dalcanale et al., 2010; Gasteyger et al., 2006; Kehagias et al., 2011; Coupaye et al., 2009; Rickers & McSherry, 2012; Rojas et al., 2011; Toh et al., 2009; Schouten et al., 2010; Ziegler et al., 2009)

2.5.2. Laparoscopic Sleeve Gastrectomy and micronutrient-related abnormalities

In the literature search on this topic, a total of 11 studies examining changes in micronutrient and related parameters following LSG were identified. In all of the studies, the authors investigated micronutrient-related deficiencies; however, none of studies examined the clinical symptoms that would manifest for any of the parameters measured. It is important to note that whenever the authors used the term "*deficiency*", it meant the biochemical blood level of a specific micronutrient was lower than the normal range.

As with all the studies on this topic, a micronutrient-related deficiency is usually not diagnosed on the biochemical levels alone, but are be made in conjunction with corresponding clinical symptoms as well. The 11 studies identified on this topic measured a variety of micronutrients-related parameters. The following section describes the changes following LSG according to the 11 studies (Table 2.3).

Table 2. 3

Summary of the studies on micronutrient-related changes and abnormalities following LSG.

Study/Country	**Purpose**	**Design**	**Variables**	**Findings**	**Notes**
H. A. Hakeam et al. 2009 Saudi Arabia	Assess the micronutrient parameters associated with iron indices pre- and post-LSG.	Prospective study. 61 patients, mean BMI: 47.5 ± 9.6 kg/m^2.	Hemoglobin, MCV, ferritin and vitamin B_{12}.	Significant decrease in ferritin and vitamin B_{12} levels.	Majority were men, where other studies have a majority of women. No abnor-malities pre-LSG as inclusion criteria.
E. O. Aarts et al. 2010 The Netherlands	Evaluate micronutrients 12 months following LSG.	Prospective study. 60 patients, mean BMI: 56.8 ± 10.0 kg/m^2. Daily multivitamin instruction.	Hemoglobin, MCV, calcium, PTH and vitamin B_{12}.	Deficiencies at 12 months: Vitamin B_{12} (9%). Elevated levels: PTH (39%).	No pre-LSG measurements. Only descriptive data provided.
S. Gehrer et al. 2010 Switzerland	Determine nutrition deficiencies prior to surgery, and compare the outcome for LSG and LRYGB.	Non-randomized, prospective trial. 136 patients (50 LSG vs. 86 LRYGB). Mean BMI: 46.5 kg/m^2 (LSG) and 44.2 kg/m^2	Vitamin B_{12}, 25-hydroxyvitamin D (25-OH-D), calcium and PTH.	Pre-surgery: 57 % with at least one abnormality, 23 % 25-OH-D deficiency and 8 % elevated PTH. Post-LSG: 32 % 25-	Most micronutrient abnormalities were present prior to surgery.

Study/Country	Purpose	Design	Variables	Findings	Notes
		(LRYGB). Uniform multivitamin and mineral supplements prescribed.		OH-D and 18 % vitamin B_{12} deficiency, 14 % elevated PTH. Post-LRYGB: 58 % vitamin B_{12}, 52 25-OH-D and 33 % elevated PTH. Significantly higher abnormality prevalence after LRYGB.	
I. Kehagias et al. 2011 Greece	Comparison of mid-term outcomes for LSG and LRYGB.	Double-blind randomized trial. 60 patients (30 LSG and 30 LRYGB), mean BMI: 44.9 kg/m^2 (LSG) and 45.8 kg/m^2 (LRYGB). Uniform supplement prescribed for all LRYGB patients, only for the first 3 months for LSG	Hemoglobin, ferritin, vitamin B_{12}, calcium and PTH.	Results for baseline and 3 year presented. No significant difference between surgery groups, except for vitamin B_{12} (more deficiencies for LRYGB). LSG: 19 abnormalities pre-LSG (iron most common), 20 abnormalities post-LSG (hemoglobin,	Follow-up at 1, 3, 6, 12, 24 and 36 months post-surgery, but only 36 months data presented. More abnormalities following LRYGB, despite the use of supplements.

Study/Country	Purpose	Design	Variables	Findings	Notes
		patients.		iron and ferritin most common). LRYGB: 15 abnormalities pre-LRYGB (iron most common), 26 post-LRYGB (vitamin B_{12} most common).	
M. K. Leivonen et al. 2011 Finland	To assess whether older patients (≥ 59 years) would have similar outcomes as younger patients (< 59 years) following LSG.	Prospective study. 55 patients. 49.5 ± 8.1 kg/m2. Vitamin and calcium supplements throughout study duration.	Hemoglobin, MCV, 25-OH-D, vitamin B_{12} and calcium.	There was no sig. difference between age groups. 89% of older and 48% of younger patients had an abnormality at 12 months. 25-OH-D deficiency being most common. All mean levels within normal range.	No pre-LSG measurements. Only 12 patients > 59 years of age.
D. Capoccia et al. 2012 Italy	Analyze the role of weight loss on micronutrient abnormality	Prospective study. 138 patients, mean BMI: 44.4 ± 6.5 kg/m^2.	Hemoglobin, iron, vitamin B_{12}, calcium and vitamin D.	No significant change from baseline to 12 months follow-up. No signi-	No elaboration of the low vitamin D levels. No data analyses

Study/Country	Purpose	Design	Variables	Findings	Notes
	development.	Divided in 5 BMI groups. Uniform supplements to all patients for the first 6 months.		ficant difference between BMI groups. All micronutrient parameters within normal range pre- and post-LSG, except vitamin D.	techniques provided in the methods section.
A. Damms-Machado et al. 2012 Germany	Assess micronutrient deficiencies pre- and post-LSG.	Observational study. 54 patients, mean BMI: 51.0 ± 7.8 kg/m^2. Patients advised on supplementation.	25-OH-D, vitamin B_{12} and calcium.	51 % had at least one deficiency pre-LSG, most common being 25-OH-D (83 %). Decreasing levels: Vitamin B_{12} and 25-OH-D.	Different subgroups of patients had different laboratory measurements completed.
V. Moize et al. 2012 Spain	Comparison of dietary intake and micronutrient abnormalities between LSG and RYGB patients.	Prospective study. 355 patients, mean BMI: 51.6 ± 6.7 kg/m^2. Uniform supplement prescribed to all patients.	Hemoglobin, ferritin, calcium, vitamin B_{12}, 25-OH-D and PTH	No difference in dietary intake between groups. Low 25-OH-D and elevated PTH were the most common abnormalities. Baseline abnormalities more prevalent in LSG group. Abnormalities post-LSG	Significant difference in baseline demographics between groups. Long follow-up, 60 months.

Study/Country	Purpose	Design	Variables	Findings	Notes
				similar among procedure-groups.	
J. Ruiz-Tovar et al. 2012 Spain	Evaluate calcium parameters pre- and post-LSG.	Prospective study. 30 female patients, mean BMI: 53.1 ± 8.5 kg/m^2. Uniform supplements prescribed to all patients.	Albumin, ferritin, calcium, vitamin D, PTH and vitamin B_{12}.	Pre-LSG abnormalities: vitamin D (96.7 %), PTH (20 %). 12 months abnormalities: vitamin D (3.3 %) and PTH (3.3 %) – same patient. Significant inverse correlation between weight loss and vitamin D increase ($r = -0.948$, $p = 0.033$).	No mentioning of the statistical analysis used, except for Pearson correlation.
T. Saif et al. 2012 United States	To evaluate the micronutrient status in patients undergoing LSG	Prospective study. 82 patients, mean BMI: 55.7 ± 13.7 kg/m2. Advised to start multivitamin regimen.	Hemoglobin, ferritin, PTH, calcium, vitamin B_{12} and vitamin D.	Most common abnormalities pre-LSG: Vitamin D (75 %) and PTH (51.5 %). Increasing levels: Ferritin (F) and vitamin D.	Large drop-out rate, > 50 % at each follow-up.

Study/Country	Purpose	Design	Variables	Findings	Notes
				Decreasing levels: Ferritin (M) and PTH. Stable levels: Hemoglobin and calcium.	
P. W. J. van Rutte et al. 2014 The Netherlands	Establish the status and evolution of micronutrient-related deficiencies within 12 months post-LSG.	Retrospective study. 200 patients, mean BMI: 46.2 kg/m^2. Patients instructed to take multivitamins.	Hemoglobin, MCV, ferritin, calcium, vitamin B12, PTH and vitamin D.	Deficiencies at baseline: Hemo-globin (5%), MCV (5.5%), ferritin (7%, all female), calcium (0.5%), vitamin B_{12} (11.5), vitamin D (81%), PTH excess (28.5%). 12 months: Hemo-globin (6.5%), MCV (2%), ferritin (8%, all female), calcium (2%), vitamin B^{12} (11.5), vitamin D (36%), PTH excess (18%).	407 patients entered the study, only 200 had completed a follow-up visit.

Note: LSG = Laparoscopic sleeve gastrectomy. BMI = Body Mass Index. 25-OH-D = 25-hydroxyvitamin D. PTH = Parathyroid Hormone. MCV = Mean Cell Volume. M = Males, F = Females. 25-OH-D = 25 hydroxyvitamin

Hakeam et al., (2009). Obese patients who underwent LSG at a Saudi Arabian hospital were eligible to participate in the study, unless they presented with any inflammatory disorders, arthritis, were on steroids, had anemia, chronic infectious diseases, hypothyroidism or a pre-existing iron deficiency. Follow-up was at 6 months, and the laboratory measurements included hemoglobin, MCV, ferritin and vitamin B_{12}. All patients were given an iron-free supplement containing vitamin B_{12} post-LSG.

A total of 61 patients participated in the study, 31 of which were male, with a mean BMI of 47.5 ± 9.6 kg/m^2. The only abnormality found at baseline, was a vitamin B_{12} deficiency in five patients (8.1 %). At 6 months, there were 19 newly developed abnormalities, the most common being vitamin B_{12} deficiency (19.6 %). There was no significant change in MCV, hemoglobin and ferritin values, which stayed stable throughout the study.

A possible limitation of the study is the fact that all the patients had normal iron and hemoglobin values prior to surgery. Since many obese patients have micronutrient abnormalities pre-surgery, these patients might not be representative of the obese population in general. In addition, > 50 % of the patients were male, which is in contrast to most studies on this topic. This might have an effect on the study results, as women, especially those that are pre-menopausal, tend to be more at risk for developing low ferritin and hemoglobin levels.

E. O. Aarts et al. (2011) The aim of this Dutch prospective study was to assess the micronutrient abnormalities following LSG. The patients underwent LSG as a first step procedure, before possibly undergoing duodenal switch (DS). Within the first year post-LSG, six patients underwent DS and they were excluded from participating in the study. Patients were instructed to take a daily multivitamin, but it is not clear whether this supplementation was provided or not. The follow-up assessment after LSG was at 12 months, and the micronutrient parameters measured were hemoglobin, MCV, calcium, PTH and vitamin B_{12}.

A total of 60 patients enrolled in the study, 34 were males. The only micronutrient assessment done on patients prior to surgery was the values of hemoglobin and MCV, with 8.3 % having low MCV and 5.0 % having low hemoglobin values. At 12 months follow-up, the most common micronutrient abnormalities were low concentrations of hemoglobin along with elevated PTH (43 %, 26 % and 39 %, respectively). In addition, the study reported low MCV and high calcium values (15 % and 4 %, respectively) while one patient presented with low vitamin B_{12}.

S. Gehrer et al. (2010) The aim of this non-randomized, prospective trial was to assess the micronutrient deficiencies prior to surgery, and compare the outcome from LSG to those from LRYGB. 136 patients were enrolled in the study, 50 of which underwent LSG and 86 underwent LRYGB. Micronutrient assessment follow-up

were done at 3, 6, 12, 30 and 36 months, and the micronutrient parameters measured were vitamin B_{12}, 25-hydroxyvitamin D, calcium and PTH. Before undergoing surgery, 57 % of the patients presented with at least one micronutrient abnormality, the most prevalent being 25-OH-D deficiency and elevated PTH, 23 % and 8 % respectively. Any deficiency detected was treated accordingly.

The statistical analysis on the micronutrient changes pre- and post-surgery is done on a variable named *"post-operative"*, which appears to be an overall estimate of the results from 3 – 36 months post-surgery. There were significantly more deficiencies post-operatively among patients who underwent LRYGB; the most common deficiencies were vitamin B_{12} and 25-OH-D (58 % and 52 %, respectively), 33 % had elevated PTH levels. For patients in the LSG-group, the most common deficiencies after surgery were 25-OH-D, and vitamin B_{12} 32 % and 18 %, respectively. PTH was elevated in 14 % of the patients, while no abnormal values for calcium were found. There were a significant difference in the number of abnormalities between the two surgical groups for vitamin B_{12}, 25-OH-D and PTH. The majority of micronutrient abnormalities presented within the first year following both LSG and LRYGB. Targeting the specific micronutrient resolved the majority of micronutrient abnormalities, and the improvement/resolution rates were between 80 – 100 % for vitamin B_{12}, iron and 25-OH-D.

I. Kehagias et al. (2011) The aim of this study was to compare the outcomes for LSG and LRYGB patients. 60 patients were randomized to receive either LSG (30 patients) or LRYGB (30 patients) at a hospital in Greece. Only the surgeons performing the procedures were aware of the type of surgery the patients had, making it a double-blind, randomized trial, as neither the patients, caregivers or the individuals collecting data knew the surgery type in question.

All patients were prescribed a uniform vitamin and mineral supplement; however, patients who received the LSG procedure were only prescribed this for the first six months post-surgery. In addition, pre-menopausal women in the LRYGB group also received an oral iron supplement. Follow-up was done at 1, 3, 6, 12, 24 and 36 months post-surgery. The parameters measured in the study included hemoglobin, ferritin, vitamin B_{12}, calcium and PTH.

The number of micronutrient abnormalities at baseline was similar for the two groups, with iron and hemoglobin deficiencies being the most common. Only the results for baseline and 36 months follow-up were presented, and there was an increase in the number of abnormalities, 19 to 20 vs. 15 to 26 for LSG and LRYGB respectively. The most common abnormalities after surgery were ferritin and hemoglobin deficiencies for the LSG group, while vitamin B_{12} deficiencies were more prevalent among the LRYGB patients.

Except for vitamin B_{12}, there was no significant difference between the two groups in terms of micronutrient abnormalities. The number of abnormalities at baseline and 36 months follow-up are somewhat similar, especially for the LSG group. Patients in the LRYGB were more at risk for developing micronutrient abnormalities after surgery, despite the fact that they received vitamin and mineral supplementation for the duration of the study.

M. K. Leivonen et al. (2011) The aim of this prospective study was to compare the micronutrient changes in patients over and under 59 years of age, 12 months after LSG. A total of 152 patients had the LSG procedure at a hospital in Finland, but only 55 patients had been evaluated for the 12 months follow-up at the time the article was written. Of these 55 patients, 12 were older than 59 years, and all patients were provided a permanent supplement regimen post-surgery. Follow-up was done 12 months post-LSG, and the micronutrient parameters measured were hemoglobin, MCV, 25-OH-D, vitamin B_{12} and calcium.

At the 12-month follow-up, 89 % of the ≥ 59 years patients and 48 % of the <59 years patients had at least one micronutrient abnormality. However, all the mean blood values were within the normal ranges post-LSG. 25-OH-D deficiency was the most common abnormality found, and was more prevalent among the ≥ 59 patients; 25 % vs. 19 % when compared to patients < 59 years. However, this difference was not significant, and the micronutrient abnormalities occurred despite

multivitamin supplementation. The major limitation to this study is the lack of pre-LSG measurements. The biochemical values and number of abnormalities at 12 months are not informative in regards to the effect of LSG on these parameters, as there was no baseline assessment conducted.

D. Capoccia et al. (2012) The aim of this Italian study was to analyze the effect weight loss had on the development of micronutrient deficiencies, as well as the effect of supplementation. A total of 138 patients underwent LSG. They were divided into five groups determined by their pre-LSG BMI. Group A: BMI between 32 – 34.9 kg/m^2, 7 patients. Group B: BMI between 35 and 39.9 g/m^2, 29 patients. Group C: BMI between 40 and 44.9 g/m^2, 40 patients. Group D: BMI between 45 and 49.9 g/m^2, 33 patients. Group E: ≥ 50 g/m^2, 29 patients. All patients were provided uniform supplementation for the first 6 months. After the first 6 months patients received only vitamin B_{12} and vitamin D. The follow-up assessments were done at 3, 6 and 12 months; however, only the 12 months data are presented in the article. The parameters measured were hemoglobin, vitamin B_{12}, calcium and vitamin D.

There was no significant change observed in the biochemical parameters from baseline to the 12 months follow-up. In addition, there was no significant difference among the five BMI groups. All the mean micronutrient values were within the normal ranges pre- and post-LSG, except for vitamin D. The authors concluded that the supplements provided to the patients proved sufficient to avoid

any post-LSG abnormalities, except for vitamin D. The authors state that the vitamin D levels were low before and after surgery, but provide no estimate on the proportion of patients with low levels. Because the proportion of abnormalities is not provided, it is unclear if all patients had sufficient levels of hemoglobin, vitamin B_{12} and calcium, as suggested by the normal mean values.

A. Damms-Machado et al. (2012) The aim of this German observational study was to assess micronutrient deficiencies at baseline and at 12 months post-LSG. Patients had follow-up assessments done at 3, 6 and 12 months after surgery. Patients were advised to take a multivitamin immediately after surgery. The micronutrient parameters measured in the study were 25-OH-D, vitamin B_{12} and calcium. 54 patients were enrolled in the trial, and were measured for 25-OH-D and vitamin B_{12}. A subgroup consisting of 30 patients were additionally measured for calcium.

Of the 54 patients enrolled in the study, 39 were female (72 %). Prior to undergoing surgery, 51 % of the patients presented with at least one micronutrient deficiency, the most common being 25-OH-D and iron, (83 % and 29 %, respectively). The most commonly occurring abnormality following LSG was 25-OH-D deficiency. 3 months following LSG, 59.5 % of the patients presented with 25-OH-D deficiency, 2.4 % had low vitamin B_{12} levels, while no patients experienced low levels of calcium. At the 6-month follow-up, the proportion of patients with 25-OH-D

deficiency had increased to 76.2 %, and the prevalence of patients with vitamin B_{12} deficiency was 4.8 %.

The percentage of patients that reported taking some sort of micronutrient supplements following LSG were 41 %, 26 % and 22 % at 3, 6 and 12 months, respectively, the most common being an over-the-counter multivitamin (69 %). There were no significant differences in micronutrient parameters between patients taking supplements and those who did not, except for 25-OH-D, where supplement use seemed to have a deficiency-preventative effect.

V. Moize et al. (2012) The aim of this study was to compare the dietary intake (energy, macro- and micronutrients) and the micronutrient abnormalities for LSG and RYGB patients. The dietary intake was assessed on the basis of food records and diet journals, and a registered dietician guided the patients and evaluated their results. All surgeries were performed at a Spanish University hospital, and a total of 355 patients entered the study. The choice of surgical procedure was not done at random, but patients with a BMI > 55, increased operative risk or an enlarged liver underwent the LSG procedure. All patients were prescribed a uniform daily supplement, in addition to a monthly vitamin B_{12} injection. Patients were assessed at baseline, 6, 12, 24, 48 and 60 months post-surgery, and the micronutrient parameters measured were hemoglobin, ferritin, calcium, vitamin B_{12}, 25-OH-D and PTH.

Of the 355 patients enrolled in the study, only 61 patients underwent LSG, while 294 had the RYGB procedure. Due to inclusion criteria for the LSG procedure, there was a significant difference in the baseline demographics between the two groups. Patients in the LSG had significantly higher absolute weight, BMI and excess body weight. There were also significantly more micronutrient abnormalities among the LSG patients than the RYGB patients, which the authors contributed to the difference in baseline weight parameters.

The rates of micronutrient abnormalities were very similar between the two groups, with 25-OH-D deficiency and elevated PTH being the most common abnormalities. In this study, the values of 25-OH-D were divided into three categories; > 30ng/mL was sufficient, > 10 to < 30 ng/mL was insufficient and < 10 ng/mL was defined as being deficient. At baseline, 93 % of LSG patients and 91 % of RYGB patients had levels < 30 mg/mL. Despite the use of supplementation, the majority of study participants had insufficient or deficient 25-OH-D levels throughout the study. Similar to other studies, the values of the specific micronutrients tended to fluctuate throughout the follow-up period, thus making it difficult to assess whether patients actually improved or worsened. The drop-out rates were 16 %, 41 % and 66 % for the LSG patients at 2, 3 and 5 years, respectively making the long-term results less reliable.

J. Ruiz-Tovar et al. (2012) The aim of this prospective study was to evaluate the calcium metabolism parameter pre- and post-LSG. This Spanish study consisted of 30 female patients who meet with a dietician on a regular basis to ensure each patient was on a calorie-reduced and protein rich diet. In addition, a uniform vitamin and mineral supplementation was prescribed to all patients. Patients were assessed at 3 and 6 months post-surgery, and the micronutrient parameters measured were ferritin, calcium, vitamin D, PTH and vitamin B_{12}. Prior to undergoing surgery, 96.7 % of the patients had vitamin D deficiency and 20 % had elevated PTH. The proportion of abnormalities kept decreasing post-surgery. By 3 months, only 13.2 % had vitamin D deficiencies and 6.6 % had elevated PTH. However, 3.3 % (one patient) presented with newly developed ferritin deficiency. By six months, only one patient had vitamin D deficiency. The only significant changes following LSG was for vitamin D and PTH. A correlation analysis was done on weight loss and vitamin D following LSG. There was a significant ($p < 0.05$) inverse correlation after 3 months; as the weight decreased, the blood values of vitamin D increased. The study's authors also suggested that the vitamin and mineral supplements provided where capable of treating or avoiding most of the micronutrient abnormalities following the procedure.

Saif et al., (2012) The aim of this study was to evaluate certain micronutrient parameters following LSG. Eligible patients were divided into three groups, determined by their follow-up times; 1 year, 3 years and 5 years post-LSG. However, some patients were assessed on their micronutrient status more than once, and thus are entered in more than one follow-up group. No uniform supplementation was prescribed, but patients were advised to start a multivitamin regimen immediately after surgery. Adherence to this regimen was documented at each follow-up; 28.9 %, 42.9 % and 63.3 % at 1, 3 and 5 years, respectively. The micronutrient parameters measured were hemoglobin, ferritin, PTH, calcium, vitamin B_{12} and vitamin D.

A total of 82 patients entered the study, with the majority of patients being female (55 vs. 27). There was a significant drop-out rate at each of the three follow-up points, and only 35, 27 and 30 patients were available for assessment at 1, 3 and 5 years respectively. The most common abnormality at baseline was vitamin D deficiencies (75 %) and elevated PTH (51.5 %). There were no measurements for vitamin B_{12} at baseline, making it difficult to assess the follow-up data properly. Despite a change after the first and third year post-LSG for hemoglobin and PTH, the overall number of abnormalities at the 5-year follow-up was similar or higher than the baseline proportion. Overall, hemoglobin and calcium concentrations remained stable throughout the study. Ferritin (F) and vitamin D values increased, while ferritin (M) and PTH tended to decrease. There was no correlation between supplement use and blood levels of the vitamin D ($p = 0.37$) or PTH ($p = 0.74$).

P. W. J. van Rutte et al. (2014) The aim of this study was to determine the proportion of patients with micronutrient-related deficiency prior to LSG, and assess the evolution within the first 12 months following LSG. A total of 407 patients underwent the procedure at the same hospital in The Netherlands, but only 200 patients returned for a follow-up visit within he first 12 months post-operatively. Patients who did not return for a follow-up visit, and patients with a RYGB revision, were excluded from the study. Patients were restricted to a liquid diet in the first 3 post-operative weeks and instructed to initiate a supplement regimen consisting of multivitamins and minerals. Any micronutrient-related deficiency was defined as a blood value below the normal reference value, and the variables measured were hemoglobin, MCV, ferritin, calcium, vitamin B_{12}, vitamin D and PTH.

At baseline, the most common deficiency was vitamin D, with 81 % of patients presenting with blood values below 50 nmol/L. Of these patients, 55 % were severely deficient, meaning a vitamin D value below 30 nmol/L. Elevated PTH levels were found in 28.5 % of patients, the majority of whom also had deficient levels of vitamin D. Other deficiencies observed were vitamin B_{12}, ferritin (all female), MCV and hemoglobin with 11.5 %, 7 %, 5.5 % and 5 % of patients, respectively.

Within the first 12 months of surgery, significant improvements were observed only for vitamin D and PTH, 81 % vs. 36 % and 28.4 % vs. 17.9 %, respectively. The distribution of deficiencies 12 months post-LSG were: vitamin B_{12}

(11.5 %), ferritin (8 %, all female), hemoglobin (6.5 %), MCV (2 %) and calcium (2 %). The proportion of patients with other deficiencies did not change significantly post-LSG, although a significant number of patients (7 %) developed a vitamin B_{12} deficiency in this period. Overall, almost 20 % of patients developed a deficiency in the follow-up period, despite being supplemented. The most common newly developed deficiencies were vitamin D (15 %), vitamin B_{12} (7 %) along with excess PTH (6.5 %). Overall, patients presented with micronutrient-related deficiencies prior to LSG. These deficiencies were continuingly present within the first 12 months following LSG, along with a significant number of newly developed deficiencies.

The main limitation according to the authors, is the inability to determine of the improvements observed were due to weight loss or the use of supplementation. The dosages and brand of supplementation was not determined nor was the dietary intake history. However, the significant number of newly developed deficiencies post-LSG suggests that the supplementation used was not sufficient in treating and/or preventing these deficiencies.

2.5.2.1. Summary

Table 2. 4

Summary of changes in the biochemical parameters post-LSG from the 11 identified studies.

	25-OH-D/ Vitamin D	**PTH**	**Calcium**	**Ferritin**	**Hemoglobin**	**MCV**	**Vitamin B_{12}**
Aarts et al.	?	?	?	N/A	↑	↑	?
Damms-Machado et al.	↓	N/A	Normal	N/A	N/A	N/A	↓↑
Gehrer et al.	↑	↑	Normal	N/A	N/A	N/A	↑
Capoccia et al.	↓↓/-	N/A	↑↑/-	N/A	↓↓/- ↓	N/A	↓↓
Saif et al.	↑↑ ↓	↓↓ ↓	↑↑/- ↑	↓↓↑↑ ↑	-	N/A	?
Kehagias et al.	N/A	↓	↑	↑	↑	N/A	-
Ruiz-Tovar	↑↑	↓↓	↑↑	↑↑	N/A	N/A	↑↑
Hakeam et al.	N/A	N/A	N/A	↓↓/-	↓↓/- ↑	↓↓/- ↑	↓↓ ↑

	25-OH-D/ Vitamin D	PTH	Calcium	Ferritin	Hemoglobin	MCV	Vitamin B_{12}
Moize et al.	↓	↓	↓	↓	↑	N/A	↑
Leivonen et al.	?	N/A	?	N/A	?	?	?
van Rutte et al.	↓	↓	↑	↑	↑	↑	-
Overall	**2/3: ↑↑** **3=4/5: ↓**	**2/2: ↓↓** **4/5: ↓**	**3/5: ↑↑** **3/4: ↑**	**↓↑** **3/4: ↑**	**2/2: ↓↓/-** **5/6: ↑**	**↓↓/-** **3/3: ↑**	**2/4: ↓↓** **3/4: ↑**

Note: N/A = Parameter not measured in given study. ? = No baseline measurements presented. ↓ = Decreasing proportion of patients with abnormal blood levels. ↑ = Increasing proportion of patients with abnormal blood levels. ↑↑ = Increasing blood levels. ↓↓ = Decreasing blood levels. ↓↑ = Fluctuating results. - = Stable/No changes. Some studies report both changes in blood levels *and* change in status, thus results for both are presented in table.

There are some inconsistencies regarding the possible changes in micronutrients-related parameters following LSG (Table 2.4). This is mainly due to the variations of the study protocol, variables measured and the follow-up period. Not all studies measure the biochemical parameters prior to surgery, thus only presenting the data for the follow-up visits, while others report both baseline and post-LSG results. The duration of the studies varies, although the majority of the 11 studies report up to the 12-month follow-up. In addition, some of the studies report the number of deficiencies at each follow-up, while other report the laboratory changes in blood concentrations of the chosen parameters. This makes it difficult to compare results, as the number of abnormalities may not reflect the overall change of blood concentrations.

Overall, the studies reported increasing values of 25-OH-D/vitamin D and calcium following LSG, which corresponded to a decrease in the proportion of patients with abnormally low 25-OH-D/vitamin D values, but an increasing prevalence of abnormal calcium levels. Three out of four studies reported that the proportion of patients with elevated PTH levels decreased following LSG, due to decreasing blood levels. Although three studies reported an increasing prevalence of vitamin B_{12} abnormalities post-LSG, there were fluctuating results regarding the changes in mean levels. In addition, the majority of the studies also reported an increase in the proportion of patients with abnormal ferritin, hemoglobin and abnormal MCV.

2.6. Medical Conditions Induced by Micronutrient Abnormalities

The classification of micronutrient abnormalities is presented in Table 2.5.

Table 2. 5

Classification of micronutrient abnormalities.

Abnormality classification	
1. Cause	Primary
	Secondary
2. Type	Excess/Toxicity
	Insufficient/Deficient
3. Nutrition	Vitamin
	Trace element
	Mineral
4. Degree	Mild
	Moderate
	Severe
5. Duration	Acute
	Sub-acute
	Chronic
6. Outcome	Reversible
	Irreversible

Note: (McLaren, 1988)

2.6.1 Metabolic Bone Disease

Vitamin D deficiency can have a significant impact on bone health due to its role in calcium absorption. A decrease or deficiency of vitamin D leads to decreased calcium absorption. This initiates a chain-reaction leading to ostoemalacia (inadequate mineralization of the bone), hypocalcemia and eventually osteoporosis (Pressman & Adams, 1990; Salzman et al., 2013).

2.6.2. Anemia

Iron deficiency anemia is one of the most common complications following bariatric surgery with many patients requiring supplementation to treat this condition. It can occur even if the patient has normal iron, hemoglobin, ferritin and vitamin B_{12} blood values (Lovette et al., 2012). There are many possible explanations for this, and it might be a combination of more than one. One explanation for anemia and iron deficiency following bariatric surgery, is the fact that most mal-absorptive surgeries remove the part of the small intestine where iron is mainly absorbed. This could be why iron deficiency is more common following gastric bypass than the gastric banding, where the intestine is not altered. Other possible explanations are reduced hydrochloric acid from the gastric pouch, inflammation of the intestines and a lack of sufficient dietary sources (Koch & Finelli, 2010; Lovette et al., 2012; Salzman & Karl, 2013).

Intrinsic factor is needed in order for vitamin B_{12} to be absorbed. This is produced by the gastric parietal cells in the stomach pouch, which is removed in bariatric surgeries such as RYGB and LSG. This can potentially lead to pernicious anemia, which can be caused by the vitamin B_{12} deficiency related to the loss of parietal gastric cells (McLaren, 1988; Salzman & Karl, 2013).

2.7. Micronutrient-related parameters

Micronutrient-related parameters are often categorized as either direct or indirect nutrients. *Direct nutrients* are those that cannot be produced in sufficient quantities by the human body, thus they must be ingested through diet. *Indirect nutrients* can be produced in the human body, but may also be derived from diet (Biology Online, 2011). The indirect nutrients can reflect the micronutrient status in an individual, but could also reflect other aspects. For the parameters measured in this study, the direct parameters are calcium, vitamin B_{12} and ferritin, while the indirect parameters are vitamin D (25-OH-D), PTH, hemoglobin and MCV (Table 2.6). However, for the purpose of the current study, these parameters will be categorized according to their interaction with each other. Thus, bone health parameters are grouped as follows: 25-OH-D, calcium and PTH, while hematological parameters include ferritin, hemoglobin, MCV and vitamin B_{12}.

Micronutrient-related deficiencies can lead to numerous conditions, such as anemia, neurological conditions and impaired bone health, ranging from mild to severe and chronic in nature. Due to this, supplementation of multivitamins and minerals is very common following most bariatric procedures. However, excessive supplementation can potentially cause hypervitaminosis and toxic concentrations of micronutrients, which can lead to several other medical conditions, such as ataxia, alopecia and hepatomegaly (Aarts et al., 2011; Koch & Finelli, 2010; Pressman & Adams, 1990).

Table 2. 6

Summary of the select micronutrient and biochemical parameters measured in this study.

	Involvement	Low levels	High levels
Direct parameters			
Vitamin B_{12}	Released in the gastric pouch. Absorption assisted by intrinsic factor	Can lead to neurological, psychological, gastrointestinal and cardiovascular conditions	High levels without supplementation are unusual, but can occur with some liver disorders, leukemia and diabetes.
25-Hydroxyvitamin D (25-OH-D)	The active form (calcitriol) is made by converting 25-OH-D, which is determined by levels of calcium and PTH	Can lead to hypocalcemia, ostoemalacia and osteoporosis.	Abnormally high levels are unusual in non-supplemented individuals.
Calcium	Bone health, coagulation mechanism and neurological system. Involved in most bodily processes. Negative feed-back mechanism with PTH.	Can lead to hypocalcemia, ostoemalacia and osteoporosis.	Calcium levels increase as a response to high PTH levels.

	Involvement	Low levels	High levels
Indirect parameters			
Ferritin	Indicator of iron storage. Acute phase reactor.	Usually low when iron levels are low.	Can be elevated even when iron levels are normal. Can be high with infections.
Hemoglobin	Transportation of oxygen from lungs to tissue	Anemia	Can occur with bone marrow malfunctions, heart failure, liver and kidney cancer etc.
MCV	Average volume of the red blood cells	Can occur with iron deficiency	Can occur with Vitamin B_{12} deficiency
Parathyroid Hormone (PTH)	Important factor in calcium absorption	When calcium levels are too high, the amount of PTH secreted decreases. Low levels are seen with hypoparathyroidism and after thyroid surgeries.	When calcium levels are too low, the amount of PTH secreted increases

Note: [Christakos et al., 2013; Damms-Machado et al., 2012; Family Practice Notebook, 2013; Health Line, 2014; Health Link (a), 2013; Koch & Finelli, 2010; Lovette et al., 2012; Mayo Clinic (a), 2013; Mayo Clinic (b), 2014; McLaren, 1988; Medicine Net (a), 2013; Medicine Net (b), 2013; Pressman & Adams, 1990; Ruiz-Tovar et al., 2012; Saif et al., 2012; Salzman & Karl, 2013; Shankar et al., 2010; Shifflet & Wum, 2009; Swaminathan, 2011]

2.7.1. Bone health

Vitamin D is present in the human body in two forms; vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The vitamin D derived from either ultra violet light or diet is converted in the liver to 25-OH-D (calcidiol). When determining vitamin D concentrations in the blood stream, it is most often the concentrations of 25-OH-D that is reported, as it have a longer half-life. Many obese individuals have insufficient levels of Vitamin D, due to either poor diet or insufficient sun exposure (Coupaye et al., 2013; Swaminathan, 2011). Another explanation may be that because vitamin D is fat-soluble, some of it is sequestered in the fat cells and only a small portion reaches the blood stream. Absorbed vitamin D reaches the fat cells first and the remnant goes to the liver; thus the more fat cells, the more Vitamin D is sequestered and it has been reported that 25-OH-D is reduced by up to 57 % in obese individuals (Ruxton, 2011; Vix et al., 2014). Some studies suggest that as an individual loses weight, the levels of vitamin D increases. However, it is unclear whether this is due to a release of stored amounts, or because declining fat stores means that less vitamin D from the sun or diet is trapped in the fat (Coupaye et al., 2013; Damms-Machado et al., 2012; Ruiz-Tovar et al., 2012; Ruxton, 2011; Saif et al., 2012).

As vitamin D can be synthesized in the skin as a result of sun exposure, the blood levels can have seasonal variations. As people are more exposed to the sunlight during the summer months, the blood levels can be higher when measured in summer and fall as compared to winter and spring (Lefebvre et al., 2014). In addition, there might also be some geographic variation, as people living near the equator are more exposed to sunlight than those living in higher latitudes (Kimlin, 2008; Newhook et al., 2009). Studies have shown that the seasonal variations are more pronounced at higher latitudes, as the UV exposure needed to provide sufficient levels of 25-OH-D are reduced during the winter months (Kimlin, 2008; Huotari & Herzig, 2008). In fact, it has been reported that the UV exposure from in countries below 35° North latitudes, is sufficient to maintain sufficient 25-OH-D year round. On the other hand, the UV exposure is reduced north of the 35° latitude mark, and at latitude of 41.9° North, sufficient 25-OH-D synthesis from UV exposure is not possible from November to February (Ross et al., 2011; Tsiaras & Weinstock, 2011). With Newfoundland & Labrador being located at latitude of 46° - 61° North, there is a possibility of insufficient synthesis of 25-OH-D beyond November through February.

According to the 2009-2011 CHMS, Cycle 2, about one third of Canadians have blood levels of 25-OH-D below the normal reference value (< 50 nmol/L). This prevalence varied according to seasons, with 40 % classified as having insufficient levels (15 % of whom were deficient) from November to March, compared to 25 %

with insufficient levels (6 % of whom were deficient) from April to October [Jans & Pearson, 2013; Statistics Canada (b), 2013]. In addition, a study conducted in the Canadian province of Newfoundland & Labrador showed significant differences between the 25-OH-D levels in winter and summer months (Newhook et al., 2009).

The production of active vitamin D (calcitriol) is dependent on the levels of calcium and PTH, among others. When calcium is low and PTH is high, the production of calcitriol increases (McLaren, 1988; Ruxton, 2011; Swaminathan et al., 2011). About 30 % - 40 % of dietary calcium is absorbed in the intestinal tract, and 25-OH-D assists in the synthesis of a specific protein involved in this absorption, while the rest is usually excreted through feces (McLaren, 1988; Swaminathan et al., 2011). The amount of calcium stores in the body has an inverse relationship with parathyroid hormone (PTH). If calcium stores are too low, more PTH is secreted in order for the bones to release more calcium and vice versa [Health Link (a), 2013; Swaminathan, 2011]. This means, that if PTH is elevated, it is most likely due to insufficient intake or absorption of calcium or 25-OH-D, or both.

2.7.2. Hematological parameters

Ferritin is an indicator of iron storage and is often measured along with iron, total iron binding capacity and transferrin. Ferritin can be elevated even when iron concentrations are normal. Because it is an acute phase protein, the concentrations will usually increase when an infection or liver disease is present (Shifflet & Wum,

2009; Swaminathan, 2011; Web MD, 2013). In addition, ferritin can be elevated in certain chronic diseases, where inflammation prevents the irons stores in the bone marrow from being released.

Hemoglobin is an iron-containing protein in the red blood cells responsible for the transportation of oxygen from lungs to tissue. When the spleen removes the red blood cells, the iron is transported back to the liver by transferrin, where it will be used in the production of new red blood cells [Mayo Clinic (a), 2013; Medicine Net (a), 2013]. As the MCV can decrease with iron deficiency and increase with vitamin B_{12} deficiency, the MCV can be normal if both these conditions are present. However, if this were the case, hemoglobin concentrations would be lower than normal.

Vitamin B_{12} is also referred to as cobalamin and is the largest of the vitamins. It is bound to protein in the food we ingest and is released in the gastric pouch by the stomach acid. Intrinsic factor from the parietal gastric cells assists in the absorption that occurs in the small intestine (McLaren, 1988; Shankar, 2010). After the absorption, vitamin B_{12} reaches the liver where it is stored, until release is necessary due to an unexpected drop in circulating concentrations. Due to the relatively large storage capacity, vitamin B_{12} deficiency may not be detectable for some years after the elimination of the dietary source (Lovette et al., 2012; Koch & Finelli, 2010).

2.8. Non-alcoholic fatty liver disease

Many obese individuals also suffer from non-alcoholic fatty liver disease (NAFLD), which is an accumulation of fat in the liver cells not caused by alcohol consumption. Due to its increased prevalence, it is now the most common cause of liver disease in Western countries (Kopec & Burns, 2011). The risk factors associated with the development of NAFLD include age (the prevalence tends to increase with age), gender (men are at a higher risk than women), ethnicity (Hispanics have the highest prevalence, while African-Americas have the lowest) and the metabolic syndrome (Masuoka & Chalasani, 2012; Mishra & Younossi, 2012; Paschos & Paletas, 2009). In addition to this, NAFLD have been associated with an increased risk of not just all-cause mortality, but also specifically cardiovascular and hepatic related mortality (Calori et al., 2011; Koehler et al., 2013).

NAFLD is thought to be the hepatic manifestation of metabolic syndrome, which is the presence of three or more of the following: hypertension, obesity, dyslipidemia, increased waist circumference, Type 2 diabetes and insulin resistance (Franzini et al., 2011; Masuoka & Chalasani, 2013, Razavizade et al., 2012; Shifflet & Wu, 2009). Due to the fact that patients suffering from metabolic syndrome are at higher risk of developing NAFLD, treatment options are often targeted towards the resolution of this condition (Hafeez & Ahmed, 2012; Masuoka & Chalasani, 2012).

NAFLD is a general term used for a number of liver conditions, stretching from mild steatosis (fatty liver) to more severe conditions. When left untreated, NAFLD may progress to the more aggressive form, known as non-alcoholic steatohepatitis (NASH), which is estimated to occur in 5 – 37 % of the cases (Masuoka & Chalasani, 2013; Shifflet & Wu, 2009). The progression to NASH is thought to be a 2-step process, the first being simple steatosis – NAFLD. The second step is oxidative stress of the hepatic cells, which ends with inflammation, fibrosis, cirrhosis and eventually liver failure (Paschos & Paletas, 2009; Shifflet & Wum, 2009; Swaminathan, 2011). Due to the range in severity of the disease, NAFLD is often categorized as one of three states: simple steatosis (fatty liver), NASH with inflammation and ballooned hepatic cells *without* fibrosis or NASH with inflammation and ballooned hepatic cells *with* fibrosis (Chisholm et al., 2012; Mahaling et al., 2013).

Because NAFLD is mostly asymptomatic, the majority of obese individuals will never be diagnosed unless they are tested specifically for this condition. The clinical signs that might arise are vague, and include fatigue and abdominal pain (Kopec & Burns, 2011; Mahaling et al., 2013; Paschos & Paletas, 2009). Studies have shown that 17 – 40 % of the general population suffer from NAFLD, while the prevalence among the obese population might be as high as 75 – 95 % (Chavez-Tapia et al., 2010; Mishra & Younossi, 2012; Mummadi et al., 2008; Paschos &

Paletas, 2009; Schattenberg & Schuppan, 2011; Shifflet & Wu, 2009). The prevalence of NASH is more difficult to determine, as many studies combine NAFLD and NASH as simply "fatty liver disease', but is estimated to between 10 and 37 % of the obese population (Chisholm et al., 2012; Masuoka & Chalasani, 2013; Mummadi et al., 2008; Pillai & Rinella, 2009; Schattenberg & Schuppan, 2011). Among individuals with NASH as many as 15 – 50 % will progress to liver cirrhosis (Pillai & Rinella, 2009; Shifflet & Wu, 2009; Swaminathan, 2011). The prevalence of these conditions is expected to increase parallel to the increase in risk factors (Kopec & Burns, 2011; Pillai & Rinella 2009; Shifflet & Wu, 2009).

2.8.1. Diagnosis

To diagnose NAFLD or NASH requires a combination of tests, and the choice of diagnostic tools varies among studies. The gold standard is a liver biopsy, where the severity of the conditions can be determined, and it is also the only way to accurately establish the diagnosis of NASH (Chalasani et al., 2012; Hafeez & Ahmed, 2013; Kopec & Burns, 2011; Masuoka & Chalasani, 2013). Diagnostic imaging, such as ultrasound, CT or MRI scans are often used instead or in combination with biopsy, however, these can only determine if there is an accumulation of fat in the liver (Chalasani et al., 2012; Hafeez & Ahmed, 2013; Paschos & Paletes, 2009).

Certain biochemical parameters are also frequently used, including alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), bilirubin, low-density lipoprotein (LDL), triglycerides and high-density lipoprotein (HDL) (Kopec & Burns, 2011; Mahaling et al., 2013). Most often, a combination of two or more are used, in order to rule out any other suspicions. Most of these parameters are also found in other parts of the human body, and cannot alone indicate liver health (Paschos & Paletas, 2009; Swaminathan et al., 2011). In addition, it is important to note that some individuals with NAFLD or NASH might have normal levels of some of these parameters (Franzini et al., 2011; Masuoka & Chalasani, 2013; Paschos & Paletas, 2009).

There is currently no perfect diagnostic tool or a general guideline, as the gold standard is invasive and expensive. Liver biopsies are often performed on the basis of abnormal biochemical liver parameters or diagnostic imaging indications (Chalasani et al., 2012; Mahaling et al., 2013; Masuoka & Chalasani, 2013; Shifflet & Wu, 2009).

2.8.2. Treatment options

Several treatment options for NAFLD and NASH have been studied, including lifestyle (e.g. diet, exercise), pharmacologic (e.g. Thiazolidinedione or Metformin) and surgical interventions (e.g. gastric bypass). They all aim for either a reduction in weight, waist circumference or improvement of Type 2 diabetes and metabolic syndrome (Kopec & Burns, 2011; Masuoka & Chalasani, 2013; Schattenberg & Schuppan, 2011). The most promising option is significant weight loss, which shows an improvement in the biochemical parameters, fibrosis and inflammation. In one study, a weight loss of 10.1 % after 6 months of dieting resulted in a significant decrease in liver enzymes and lipids (Ghaemi et al., 2013). However, significant weight loss can be difficult to obtain and maintain through lifestyle changes alone (Chalasani et al., 2012; Pillai & Rinella, 2009; Schattenberg & Schuppan, 2011).

A significant and sustained weight loss can best be achieved by bariatric surgery, which also improves obesity related co-morbidities (Hafeez & Ahmed, 2013; Kazaks & Stern, 2013; Pillai & Rinella, 2009; Snyder-Marlow, et al., 2010). Although not used as a treatment tool in clinical practice, the weight reduction observed following bariatric surgery might improve the status of NALFD as well.

2.8.3. Biochemical parameters

As previously presented, there are several biochemical parameters used in the diagnosis of NAFLD and NASH. These included liver enzymes, lipids and other biochemical parameters. NAFLD is often associated with elevated levels of ALP, ALT, GGT and triglycerides, although none of these parameters are exclusive for this condition (Franzini et al., 2011; Hoofnagle et al., 2013; Kopec et al., 2011; Paschos et al., 2009; Razavizade et al., 2012). However, several studies have shown that these parameters tend to change following different types of bariatric surgical procedures. (Table 2.7)

Table 2. 7

Laboratory changes in select liver-related parameters following bariatric surgery.

	RYBG	**GB**	**LSG**
Bilirubin	?	?	?
ALP	?	?	?
ALT	Decreasing/?	Decreasing/?	Decreasing/?
GGT	?	?	?
LDL	Decreasing/?	Decreasing/?	Decreasing/?
HDL	Increasing/?	Increasing/?	Increasing/?
Triglycerides	Decreasing/?	Decreasing/?	Decreasing/?

Note: ? = Conflicting or unknown results. (Chisholm et al., 2012; Hafeez & Ahmed, 2013; Karcz et al., 2010)

ALP is typically produced in the bone, liver, placenta and intestine, but is more commonly known as an indicator for liver health. Elevated levels can be caused by a number of conditions such as bone disease, vitamin D deficiency, obstruction of the bile tract, hepatitis and cirrhosis (Merriam-Webster – ALP, 2006; Razavizade et al., 2012; Swaminathan, 2011). ALT is an enzyme found in most tissue throughout the body, but it is used in the evaluation of hepatocellular injuries when there is a destruction of the hepatic cells (Merriam-Webster – ALT, 2006). Hepatitis, diabetes, alcohol abuse and steatosis are some of the conditions that can cause elevated ALT levels (Cayman Chemistry – ALT, 2013; Swaminathan, 2011).

GGT is another enzyme used as a liver indicator. High concentrations can be found in the liver, kidneys and pancreas, but GGT is present throughout the body, except for the bones. The highest serum GGT concentrations are seen in patients with alcohol abuse, where as many as 70 % of alcoholics have elevated concentrations. Due to this, it is very important to determine the patient's alcohol use before diagnosing NAFLD or NASH. Bile obstruction, the use of certain drugs, cirrhosis and most forms of liver disease also increase serum GGT levels (Medline Plus, 2013; Swaminathan, 2011).

Total Bilirubin is mainly produced during the destruction of erythrocytes (red blood cells), and is transported to the liver bound to albumin. Here it is conjugated to form bilirubin mono- and deglucuronides before it is excreted to the bile. If there is an obstruction of the bile duct (cholestasis), the bilirubin cannot be excreted and is transported back to the blood. This deposition can cause jaundice seen as a yellowing of the tissue. Although bilirubin is often measured as part of the liver functioning test, it might be within the normal range despite the presence of liver disease [Kopec & Burns, 2011; Mayo Clinic (c), 2013; Swaminathan, 2011].

Most of the liver parameters are measured as a unit, as they might not be very indicative of anything on their own. For instance, GGT and ALP are often measured together due to the fact that they are both present in the liver. However, because ALP is also present in bones, while GGT is not, it assists in determining if the increased ALP is due to liver or bone conditions. Despite the increase in the parameters during liver disease, the increase itself does not necessarily indicate the severity or improvement/worsening of that specific condition [Mayo Clinic (c), 2013; Medline Plus, 2013; Paschos & Paletas, 2009; Swaminathan et al., 2011].

In addition to these conventional liver parameters, other biochemical parameters are measured as well in the assessment of NAFLD. Ferritin is an acute phase inflammation indicator, and elevated ferritin levels are common in patients with NAFLD, despite normal iron levels (Chalasani et al., 2012). One review suggests

that ferritin levels might be elevated in as many as 60 % of patients with NASH (Kopec & Burns, 2011). A lipids profile is also commonly used, as an improvement in LDL, HDL and triglycerides would be expected with a reduction of fatty liver due to the possible improvement/resolution of insulin resistance (Razavizade et al., 2012).

The improvement of NAFLD and NASH is often associated with an expected decrease in the blood concentrations of ALP, ALT, bilirubin, GGT, LDL and triglycerides, along with an expected increase in HDL blood concentrations (Chisholm, et al., 2012; Ghaemi et al., 2013; Mahaling et al., 2013; Razavizade et al., 2012). Two studies have compared liver parameters and lipids with three different grades of NAFLD, as diagnosed by ultrasound (Mahaling et al., 2013; Razavizade et al., 2012). Overall increases in ALP, ALT, LDL and triglycerides values, and significant decreases in HDL were observed in both studies, when the diagnosis went from grade 1 to 3 ($p = 0.001$). The increase in ALT concentrations was also significant in both studies ($p < 0.004$), while the ALP and LDL increases were non-significant. The study by Razavizade et al. (2012) found a significant increase in triglycerides concentrations as well ($p = 0.001$), but this increase was not significant in the study by Mahaling et al. (2013). In addition, Mahaling et al. (2013) found that the proportion of patients with abnormal lipid values increased with the severity of the NAFLD grade, for HDL, LDL and triglycerides.

Two studies have proposed novel approaches to determine the presence or absence of NAFLD, without having to use expensive, invasive and time-consuming methods like biopsy and ultrasound. (Bedogni et al., 2006; Borman et al., 2013) These novel methods use a combination of anthropometric measurements along with specific biochemical parameters. The first method was the Fatty Liver Index (FLI), which was proposed by *Bedogni et al.* (2006). Based on an Italian patient population consisting of 216 patients with and 280 patients without fatty liver disease, they created and evaluated a simple algorithm based on variables associated with fatty liver disease, such as ALT, GGT, BMI, waist circumference (WC), glucose, insulin, triglycerides and ethanol intake. BMI (kg/m^2) and WC (cm) were the strongest risk factors, and along with triglycerides (Trig, mg/dL) and GGT (U/L), these variables were included in the final model:

$$FLI = \left(\frac{e^{0.953\ x \ln(Trig) + 0.139\ x\ (BMI) + 0.718\ x \ln(GGT) + 0.053\ x\ (WC) - 15.745}}{1 + \ e^{0.953\ x \ln(Trig) + 0.139\ x\ (BMI) + 0.718\ x \ln(GGT) + 0.053\ x\ (WC) - 15.745}} \right) x100$$

The authors stated that this algorithm is easy to employ clinically, where it could be used as a "selection tool for ultrasonography" but not as a final diagnostic tool. They based their cut-offs on the accuracy of the FLI, specifically on the sensitivity, specificity and positive and negative likelihood ratios. The FLI is easy to use, as a FLI > 60 can be used to rule in fatty liver disease, while a FLI < 30 can be

used to rule it out. The limitation however, is the fact that it does not distinguish between fatty liver disease in general (hepatic steatosis), although ethanol intake was not found to be a significant factor in this patient population, and non-alcoholic fatty liver disease.

While the FLI has an excellent accuracy for hepatic steatosis detection based on ultrasound diagnosis, *Borman et al.* (2013) set out to externally validate the FLI, by comparing the FLI in their Canadian patient population with the results from liver biopsies. They determined that the FLI was only weakly correlated with the histological findings in regards to the percentage of lipid droplets in the liver cells ($r = 0.25$, $p < 0.001$), NAFLD Activity Score steatosis grade ($r = 0.28$, $p < 0.001$) and the fibrosis stage ($r = 0.18$, $p = 0.005$). In addition to this, the FLI had a poor ability to discriminate between steatosis grades. The authors proposed a new cut-off of 79, which would increase the sensitivity to 81 % compared to 61 % when using the cut-off of 60 as suggested by *Bedogni et al.* (2006). However, as this would decrease the specificity from 86 % to 49 %, they set out to establish a novel algorithm for the prediction of hepatic steatosis. Several variables were considered for this Steatosis Index (SI), including age, gender, BMI, ALT, GGT, ALP, triglycerides, cholesterol, glucose and the presence or absence of diabetes, but the final model included BMI (kg/m^2), triglycerides (Trig, mmol/L), glucose (mmol/L) and ALP (U/L):

$$SI = \left(\frac{e^{1.698\ x\ ln(Trig)+1.714\ x\ ln(Glucose)-1.044\ x\ ln(ALP)+3.879\ x\ ln(BMI)-10.940}}{1 + \ e^{e^{1.698\ x\ ln(Trig)+1.714\ x\ ln(Glucose)-1.044\ x\ ln(ALP)+3.879\ x\ ln(BMI)-10.940}}} \right)$$

When comparing the SI to the histological findings, there was a moderate correlation with the percentage of lipid droplets in the liver cells ($r = 0.45$, $p < 0.001$) and the grade of steatosis ($r = 0.46$, $p < 0.001$), and the SI outperformed the FLI when detecting significant steatosis. This patient population consisted of patients with confirmed hepatic disorders, whereas the patients in the study by *Bedogni et al.* (2006) consisted of patients with and without liver diseases.

Although the authors determined that the SI is superior to the FLI, they did not provide any cut-off for the SI scores. As the paper was published in 2013, it has not yet been externally validated. More studies utilizing both indices are needed, in order for them to be validated and for appropriate cut-off values to be determined and therefore clinically useful.

A third study by *Koehler et al.* (2013) set out to validate the FLI as a tool for identifying NAFLD. In this study 34.9 % of the study sample were diagnosed with NAFLD based on ultrasound. Based on the cut-off for of FLI > 60 the presence of NALFD as suggested by *Bedogni et al.* (2006), 32.6 % of the patients had NAFLD. In addition to this strong associated between FLI and the presence of NAFLD, the

sensitivity and specificity of a FLI > 60 predicting the presence of NAFLD in this study were 60.4 % and 82.3 %, respectively, which is similar to the 61 % and 86 % reported by Bedogni et al. (2006). As a secondary outcome in the study by *Koehler et al.* (2013), the authors also found a strong association between the FLI and the severity of NAFLD, which had not been reported previously.

2.8.4. Current literature on NAFLD and bariatric surgery

Studies looking at improvement in NAFLD and NASH following bariatric surgery have shown promising results, with an overall improvement in steatosis and fibrosis (Chavez-Tapia et al., 2010; Masuoka & Chalasani, 2013; Mishra & Younossi, 2012; Mummadi et al., 2008; Pillai & Rinella, 2009). The prevalence of NAFLD in patients seeking bariatric surgery has been estimated to be as high as 90 %, with 5 % of these patients presenting with cirrhosis (Chalasani et al., 2012). The impact of bariatric surgery on NAFLD and NASH is based on retrospective studies and case reports; however the surgical intervention, diagnostic tools, length of follow-up, outcome parameters and sample size varies greatly among the studies reviewed (Chavez-Tapia et al., 2010; Kopec & Burns, 2011; Mummadi et al., 2008; Pillai & Rinella, 2009). Most of the studies based their findings on histological results from liver biopsies, as this is the current gold standard. The outcomes most commonly reported in the studies were improvement of steatosis, fibrosis, inflammation, ballooning and NASH resolution/grade (Schattenberg & Schuppan, 2011; Pillai &

Rinella, 2013), while a few report worsening or progression of the same markers (Hafeez & Ahmed, 2012).

Two meta-analysis assessing the overall effect of weight loss post bariatric surgery on NAFLD have been published, that include retrospective and prospective cohort studies, as no randomized clinical trials have been conducted to date (Chavez-Tapia et al., 2010; Mummadi et al., 2008). Both studies highlight the limited amount of evidence on this topic in the literature. As with most of the literature on bariatric surgery, the majority of studies included in these meta-analyses are based on the RYGB procedure, and only one of the studies included the sleeve gastrectomy (Mattar et al., 2005).

The first published meta-analysis was conducted by *Mummadi et al.* (2008) and it included 15 studies. The main outcome was improvements and/or resolution in NAFLD, defined as steatosis, steatohepatitis and fibrosis. The authors pooled the data from the identified studies, to determine the overall effect of weight loss following bariatric surgery on NAFLD. Based on their findings, improvements were observed for steatosis (91.6 %), steatohepatitis (81.3 %), fibrosis (65.5 %), NASH (69.5 %) and resolution of steatohepatitis was observed in 69.5 % of patients, along with an overall BMI reduction of 19.11 – 41.76 %. However, due to varying biopsy protocols among the studies, there was no pooled analysis performed for fibrosis, and the 65.5 % improvement was based on only five studies that used needle biopsy.

Although 75 % of patients experienced an improvement of the histological features associated with NAFLD post bariatric surgery, the results may have been biased by several limitations identified by the authors. These include significant heterogeneity among the studies identified, significant publication bias, varying histological grading systems and different reasons for biopsies in the follow-up periods. In addition, because patients with diagnosed cirrhosis were excluded from the study, the effect of weight loss achieved by bariatric surgery is based on a patient population that was at a less advanced fibrosis stage.

The second meta-analysis by Chavez-Tapia et al. (2010) was based on 21 studies, 15 of which were also included in the meta-analysis by Mummadi et al. (2008), and focused on overweight and obese patients (BMI > 25 kg/m^2) with NASH. The follow-up period ranged from 1 – 5 years, and only two studies performed more than one liver biopsy during this time period. Of these included studies, improvements in the liver function tests (ALP, ALT, GGT and Bilirubin) was observed in 11 studies while seven studies reported improvements in one or more components of the metabolic syndrome. 18 of the 21 studies reported significant improvements of the histological parameters, including steatosis (18 studies), histological markers of inflammation (11 studies) and fibrosis (six studies). In addition to this, 14 studies did not report any adverse events in the follow-up period. However, four studies did report on a deterioration of the fibrosis stage, and

3 other studies observed a small proportion of patients with deterioration of histological and NASH global scores.

Although both meta-analyses reported that the majority of patients experience improvements and/or resolutions of their pre-existing NAFLD or NASH, neither study provides a definitive conclusion about the beneficial or adverse effects that weight loss following bariatric surgery might have on NAFLD or NASH. This is due to the lack of scientifically strong evidence that could be provided by randomized clinical trials.

Assessing the impact of other types of bariatric surgery on NAFLD is important. As the prevalence of LSG procedures increases, it is important to conduct studies examining the impact of LSG on NAFLD. Thus far, only two studies identified have included the LSG procedure. (Karcs et al., 2010; Mattar et al., 2005) *Mattar et al*. (2005) conducted a study to evaluate changes in liver disease following three bariatric surgery procedures; RYGB, LSG and LAGB (LapBand). Liver biopsies were performed during and post-surgery, with a mean follow-up of 15 ± 9 months. A total of 70 patients were included and had both the initial and second biopsy. Of the 70 patients, 23 underwent LSG, 41 LRYGB and six LAGB. In addition to the liver biopsies and clinical assessments, blood samples were also analyzed for ALT, triglycerides, LDL and HDL.

48 patients (68.6 %) were diagnosed with metabolic syndrome at baseline, which improved significantly, with only 10 patients (14.3 %) diagnosed post-operatively. A significant improvement of the histological findings was reported, as the severity of fibrosis and steatosis was reduced. 37 % of patients experienced a resolution of their previous steatosis and inflammation, while 20 % had a resolution of the pre-existing fibrosis. An important finding was that no patients experienced a progression or worsening of any pre-existing liver condition, which has been reported in other studies (Hafeez et al., 2012; Schattenberg & Schuppan, 2011).

Most of the biochemical measurements were within the normal range at baseline, and these improved significantly post-surgery in all except ALT. Because mal-absorptive procedures usually lead to more significant weight loss, patients in the study who underwent LRYGB had greater overall improvement in outcomes than patients in the LSG and LAGB groups.

Karcs et al. (2010) conducted a study consisting of 236 patients undergoing LSG, 223 of whom with liver biopsies performed. Among these 223 patients, 35 presented with normal liver histology, 77 with simple steatosis and 87 patients with NASH. There was a significant correlation between NASH and elevated ALT and low HDL levels ($p < 0.001$ and $p < 0.01$, respectively). There was a significant decrease in BMI, and the levels of ALT and triglycerides, along with a significant increase in HDL levels within the first year ($p < 0.001$) The study is limited by the fact, that no follow-

up liver biopsies were performed, thus making it impossible to draw any associations between the improvement in the biochemical parameters and NAFLD/NASH.

In summary, weight loss is currently the best method for improvements in NAFLD status, and the most efficient method for a significant and sustainable weight loss is bariatric surgery. The assessment of blood values for specific biochemical parameters would be a less invasive and less expensive method for diagnosis and management of NAFLD. However, with only two identified studies assessing the biochemical parameters associated with NAFLD following LSG, there is a great need for more research.

2.9. Research Questions

A. Primary research question

In patients ≥ 19 years of age with a BMI ≥ 40 kg/m^2, or a BMI ≥ 35kg/m^2 plus risk factors, who have undergone laparoscopic sleeve gastrectomy, is there a change in select micronutrients (vitamins and mineral) and related biochemical parameters pre- and post-surgery?

Specific primary research questions

From baseline to 3 and 6 months post-LSG, is there an improvement in the blood values of the biochemical parameters associated with bone health; 25-hydroxyvitamin D (25-OH-D), calcium and parathyroid hormone (PTH)?

From baseline to 3 and 6 months post-LSG, is there a decrease in the proportion of patients with abnormal values of the parameters associated with bone health; 25-hydroxyvitamin D (25-OH-D), calcium and parathyroid hormone (PTH)?

Is the change in 25-OH-D significantly associated with the seasonal variations in sunlight exposure or the use of vitamin-containing supplements?

Is there a correlation between weight lost and the change in 25-OH-D levels following surgery?

From baseline to 3 and 6 months post-LSG, is there a deterioration of the blood values of the hematological parameters; ferritin, hemoglobin, mean cell volume (MCV) and vitamin B_{12} or do they remain stable?

From baseline to 3 and 6 months post-LSG, is there an increase in the proportion of patients with abnormal values of the hematological parameters; ferritin, hemoglobin, mean cell volume (MCV) and vitamin B_{12}?

B. Secondary research questions

Do patients, who have undergone laparoscopic sleeve gastrectomy, experience an improvement in the biochemical liver parameters associated with non-alcoholic fatty liver disease within the first 6 months post-surgery?

Specific secondary research questions

From baseline to 3 and 6 months post-LSG, is there an improvement in the blood values of the biochemical parameters associated with non-alcoholic fatty liver disease; alanine phosphatase (ALP), alanine transaminase (ALT), gamma glutamyl transpeptidase (GGT), total bilirubin, high density lipoprotein (HDL), low density lipoprotein, (LDL) and triglycerides?

From baseline to 3 and 6 months post-LSG, is there a decrease in the proportion of patients with abnormal values of the parameters associated with non-alcoholic fatty liver disease; alanine transaminase (ALT), alanine phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, high density lipoprotein (HDL), low density lipoprotein, (LDL) and triglycerides?

Is there a correlation between weight lost and the change in the biochemical parameters associated with non-alcoholic fatty liver disease following surgery?

2.9.1. Hypotheses:

It is hypothesized that the biochemical values of 25-OH-D, PTH and calcium will improve post-LSG, the values of hemoglobin and MCV will decrease or remain stable and while the values of ferritin and vitamin B_{12} will fluctuate following LSG.

It is also hypothesized that the proportion of patients with abnormal values of 25-OH-D and PTH will improve post-LSG, while the proportion of patients with abnormalities for calcium, vitamin B_{12}, ferritin, hemoglobin and MCV will increase following LSG.

It is hypothesized that the biochemical values of the biochemical parameters associated with non-alcoholic fatty liver disease following LSG, mainly decreases in ALT, GGT, LDL and triglycerides along with increases in HDL.

It is also hypothesized that the proportion of patients with abnormal values of the biochemical parameters associated with non-alcoholic fatty liver disease, mainly ALT, GGT, HDL, LDL and triglycerides, will decrease following LSG.

3. Methodology

This chapter outlines the research design, the study population, the recruitment of the sample, study procedure and the surgical procedure. A standardized data abstraction form was used for data collection to gather the following information: patient demographics such as age, gender, employment status, level of education, history of co-morbid conditions; weight, height, waist circumference; fasting lipid levels, hemoglobin, MCV, ferritin; liver biomarkers including ALP, ALT, GGT, total bilirubin; other biochemical parameters such as calcium, PTH, vitamin B_{12}, 25-OH-D; and supplement use. The nutritional guidance patients received before and after surgery will be described along with the data analysis plan and ethical considerations.

3.1. Research Design

The Newfoundland and Labrador Bariatric Surgery Cohort Study (NL BaSCo) is a population-based prospective quasi-experimental study using a pretest-posttest design without a control group. It began in May 2011 when the first LSG surgery was performed in the province. All surgeries take place at the Health Sciences Centre in St. John's, Newfoundland. All analysis and results presented is from a sub-analysis conducted on data collected by others from the parent study.

3.2. Population and sample

General Practice physicians (GP), or specialists, referred all patients potentially eligible for LSG to the Eastern Health multi-disciplinary bariatric care clinic. The bariatric nurse practitioner at this clinic performed a preliminary eligibility screening of all patients, using a set of preliminary inclusion and exclusion criteria: patients must be between 19 and 70 years of age, they must have a BMI between 40 – 60 kg/m^2 (or BMI ≥ 35 kg/m^2 plus comorbidities) and they must have attempted a previous, non-surgical, weight loss.

Eligible patients were invited to attend a pre-surgical education orientation, where they were informed of the surgical procedure and its lifelong consequences. Participants, who were interested in proceeding towards bariatric surgery, were instructed to start keeping food records and start a food journal, which they were instructed to bring with them, along with a list of their medications, during their initial clinic visit. Each patient met with the nurse practitioner for further assessment, including blood work and a sleep study to identify and treat any sleep apnea disordered breathing if necessary. If any other medical concerns were identified, patients were consulted by the appropriate specialist (e.g. cardiologist, endocrinologist) based on their co-morbid condition.

Patients then met with a bariatric surgeon, and if deemed to be a surgical candidate, they signed a consent form to undergo bariatric surgery, specifically LSG. Patients had to meet a second set of inclusion and exclusion criteria before they could be considered a surgical candidate. They must be medically, psychologically and emotionally prepared, they must be committed and motivated for the follow-up requirements, they must be able to understand what the surgery is about and what it requires pre- and post-operatively, and female patients cannot be pregnant or plan on becoming pregnant within the first 24 months post-surgery (NL Bariatric Surgery Program, 2013).

3.3. Recruitment for the research study

Once the patients had been deemed eligible for surgery, a member of the clinical team approached eligible patients and informed them of the research study. Interested patients gave permission for the research nurse to approach them with additional information, such as a detailed explanation of the research study, an introductory letter from the researcher and a consent form, which contained more information. If permission was granted, patients met with the research nurse at their surgical appointment, where they would have had 24-48 hours to read through the material provided to them. Once the surgeon had given final approval for LSG and obtained written surgical consent, the research nurse met with the potential participant to answer any questions and obtain informed consent to participate in the cohort study. Baseline data was collected after informed consent was given.

3.3. Data Collection

Before undergoing the surgery, a standard of care routine blood work was completed for all patients for a large number of biochemical parameters. For the purpose of the current thesis, the focus will be on blood work completed in order to assess select biochemical parameters associated with micronutrient status and NAFLD (Table 3.1).

Table 3. 1

Select biochemical parameters measured in the NL BaSCo study and their normal reference values.

Parameter	Unit	Female Reference	Male Reference
ALP	U/L	< 150	< 150
ALT	U/L	< 55	< 55
Calcium*	mmol/L	2.12 – 2.62	2.12 – 2.62
Ferritin	µg/L	11 – 307	24 – 336
GGT	U/L	< 32	< 32
Hemoglobin	g/L	120 – 160	140 – 180
MCV	fL	81 – 99	80 – 97
PTH	ng/L	< 72	< 72
Total Bilirubin	µmol/L	< 20	< 20
Vitamin B_{12}	pmol/L	> 107	> 107
Vitamin D (25-OH-D)	nmol/L	> 50	> 50
Triglycerides	mmol/L	< 1.90	< 1.90
LDL	mmol/L	< 3.4	< 3.4
HDL	mmol/L	> 0.9	> 0.9

Note: *As albumin blood concentrations can influence calcium, the calcium blood values measured were later corrected for albumin.

The role of the candidate was to access data from the main database, in order to conduct the statistical analyses needed to answer the research questions. All statistical analyses were done with direct consultation with a biostatistician, but were carried out by the candidate. The candidate was not directly involved in the collection of any data used in the overall study, and did not directly interact with the patients. All baseline data were collected at the Health Science Centre in St. John's, Newfoundland by the research team. To accommodate patients from rural areas, follow-up assessments were also conducted via TeleHealth and blood work analysis ordered by GP's were collected at local hospital blood collection clinics. Results from any laboratory analysis outside the Health Sciences Centre were sent to the bariatric surgery clinic nurse practitioner.

3.4. Methods

Patients were assessed prior to undergoing LSG, as well as at several follow-ups post-LSG. Anthropometric assessments were performed at 1-month post-LSG, and were continued with biochemical assessments at 3, 6, 12, 18 and 24 months. However, for the purpose of this thesis, only the data from baseline, 3 and 6 months assessments were included due to small samples sizes at the remaining follow-ups.

3.4.1. Surgical Procedure

All surgical procedures took place at the Health Sciences Centre in St. John's, Newfoundland. On average, three surgeries were performed each week, with an estimated 100 surgeries conducted per year. A multi-disciplinary team consisting of three bariatric surgeons, a nurse practitioner, a dietician and other related health care professionals, provided care to patients pre- and post-surgery. The LSG procedure was performed under general anaesthesia. A total of six trocars were used, along with a 42 French bougie to determine the sleeve size. An endoscopic GIA stapler with tri-staple technology was utilized, and the product was produced and copyrighted by Covidien. The resection of the stomach pouch was initiated about 7 cm proximal to the pylorus, and an endoscopic air leak test was performed to confirm that the staple line is intact. If no leakage was detected, the resected part of the stomach pouch would be extracted from the abdomen, and the port sites are closed. On the first post-operative day, a gastrointestinal test was performed before introducing the patient to a liquid diet.

3.4.2. Micronutrient guidance

Pre-surgery

At the pre-surgical orientation, patients received information about the surgery procedure and information about the importance of nutrition and how to read labels on food products. At Eastern Health, patients were not provided a uniform multivitamin supplement, but they were strongly encouraged to keep food journals and initiate a multivitamin regime. They were provided with a list of suggestions for certain supplement brands such as Centrum or Swizz multivitamin, Ensure Nutritional Drinks, etc. Before the surgery, all patients accepted for surgery were required to adhere to a week-long full-fluid diet. The reason for this is three-fold (NL Bariatric Surgery Program: Orientation, 2013; Rickers & McSherry, 2012): to ensure patients were capable of consuming a very small amount of food and adhering to a very strict diet, to have patients lose some weight even before that surgery thus improving the outcome post-surgery and to shrink the liver before surgery in order to minimize the risk of complications during surgery.

Post-surgery

The day after the surgery, the dietician advised the patients on the strict diet that follows immediately after surgery, starting with an all-fluid diet for the first 4 weeks. The patients were instructed what foods they were allowed to eat in the advancement from full-fluid to solid-foods. Patients met with the dietician 4 weeks

after the surgery and with the surgeon at 6 – 8 weeks post-operative (NL Bariatric Surgery Program, 2013; NL Bariatric Surgery Program: Full Fluid Diet, 2013; NL Bariatric Surgery Program: Soft Diet, 2013).

3.4.3. Biochemical assessments

The majority of biochemical assessments were performed at the Health Sciences Centre in St. John's, but laboratory assessments were completed throughout the province. Due to this, there was varying analysis protocols used to analyze the same parameters. The analysis for 25-OH-D used the gold standard method of liquid chromatography coupled with tandem mass spectrometry, developed in-house (Personal correspondence with Dr. Edward Randell). Hemoglobin and MCV were both analyzed on the Beckman Coulter®HMX Hematology Analyzer, while ferritin, vitamin B_{12} and intact PTH were all analyzed on the Architect *i* System by Abbots Diagnostics. Calcium and the biochemical parameters associated with NAFLD were all analyzed on the Architect *c* System by Abbots Diagnostics, using different assays according to the parameters analyzed. In addition, the values of LDL were calculated on the basis of values of total cholesterol, HDL and triglycerides.

3.5. Data Analysis

All continuous data is presented as mean ± standard deviation, while descriptive and nominal data is presented as absolute numbers and percentages. The laboratory measurements are presented as mean ± standard deviation along with the minimum and maximum values. The proportions of patients with blood values higher or lower than the normal references will be listed, along with the number of patients with normal blood values. A repeated measures analysis of variance (ANOVA) was used to compare continuous data at baseline with the two follow-up times (i.e. 3 and 6 months). For non-normally distributed data, the non-parametric equivalent test (i.e. Friedman) was performed. This was done in combination with the Wilcoxon signed-rank test with Bonferroni correction with a significance value at 0.017. The McNemar test was used to determine any significant differences in categorical data, i.e. the proportions of biochemical abnormalities at baseline and post-LSG. One-way ANOVA was used to determine seasonal variations in 25-OH-D blood levels.

In order to analyse the changes in 25-OH-D over time, univariate and multivariate generalized estimating equation (GEE) regression models were performed. Levels of 25-OH-D can be affected by a number of variables, but the only variables available in the current study were seasonal variations and the use of vitamin D-containing supplements. Univariate analyses were conducted, and only significant predictors were adjusted for in the multivariate model.

The GEE is an expansion on the generalized linear model. A strength of the GEE model is that it does not assume that there is complete information on all patients, thus it includes all available data points regardless of individual missing data points. This means that if a patient has a missing data point at 3 months, the data from baseline and 6 months will stay in the model, whereas all data points on this patient would have been excluded by the repeated measures ANOVA. The model for these analyses used a normal linear link function, with a normal probability outcome distribution. The correlation structure was auto-regressive which assumes the correlation as of the time lag, and 25-OH-D was the dependent variable while time, seasons and supplement use were predictors (factors). The significance levels were determined by the Wald Chi^2 test which is carried out in the GEE analysis.

Univariate correlation analysis was used to determine any association between weight and 25-hydroxyvitamin-D status or any of the biochemical parameters associated with NAFLD. A repeated measures ANOVA was used to determine differences in weight change parameters and a one-way ANOVA was used to determine significant differences between seasonal variations in 25-OH-D blood levels. All statistical analysis was performed using IBM SPSS Statistics, version 19.0 (SPSS, Armonk, NY: IBM Corp). Apart from the Bonferroni correction, a p-value < 0.05 was considered significant for all analysis.

3.6. Ethical Considerations

The provincial Health Research Ethics Authority (HREA) approved the Newfoundland & Labrador Bariatric Surgery Cohort Study via the subcommittee The Health Research Ethics Board (Appendix 3. 1). Patients were assigned a unique ID prior to any data entry by the research nurse collecting the data, and electronic versions of the master list were stored on a password protected computer and external hard drive. All data was de-identified by the research nurse and the unique ID was to ensure anonymity of the participants to the research staff. Only the research nurse had access to the master list.

Data collected by the bariatric nurse practitioner and the research nurse, were forwarded in a de-identified manner to another member of the research team for entry into the database. Once the data was received, coded and entered into another database, copies of the coded abstraction forms were stored in a locked cabinet at the Patient Research Centre located at the Health Sciences Centre. The computer containing the databases was password protected and all files were backed up to an external hard drive as they were updated. Access to the databases was limited to the primary investigators and research staff. De-identified data used for analysis in the current thesis was obtained afterwards, from a separate database.

4. Results

The first section of this chapter will present the demographic and anthropometric characteristics of the study sample, such as weight, co-morbidities and socio-demographics. The second part will cover the changes in micronutrient-related parameters following LSG, by comparing the baseline values of the specific parameters to the values at 3 and 6 months post-LSG. The last section will address the changes in the biochemical parameters associated with NAFLD and NASH.

4.1. Characteristics of the Sample

By the end of May 2014, a total of 189 patients had undergone LSG and were approached to participate in the study. All but one patient consented, providing a 99.5 % participation rate. Of the total number of patients followed through the study, the number of patients who had completed the laboratory assessments were 127 (67.6 %), 109 (58 %) and 63 (33.5 %) at 3, 6 and 12 months post-LSG, respectively, but these numbers are expected to increase as the study progresses. A subgroup consisting of 95 patients had completed their baseline, 3 and 6 months assessments, and this thesis focuses on this subgroup of patients.

Table 4. 1

Baseline demographics of the study sample

	Subgroup (95 patients)	
	Mean ± SD	**± SD**
Age, years	44.9 **±** 9.6	22 - 66
BMI, kg/m²	49.3 ± 6.8	37.7 – 67.2
No. of comorbidities	5.8 ± 3.0	1 - 16
	n	**%**
Sleep Apnea	55	63.9
Dyslipidemia	54	58.7
Hypertension	48	51.1
Impaired Glucose Tolerance	37	39.8
Diabetes	33	35.1
Coronary Heart Disease	5	5.3
Fatty Liver	2	2.1
Female	78	82.1
Marital Status		
Married/Common-Law	71	75.5
Separated/Divorced	14	14.9
Single/Never Married	7	7.4
Widowed	2	2.1
Education Level		
College diploma or higher	45	48.4
Technical school/some university	25	26.9
High school diploma or less	22	23.7

	Subgroup (95 patients)	
	n	**%**
Employment status		
Full-time	48	51.1
Disability leave (Temp. sick, short/long term)	13	13.8
Retired	10	10.6
Other (part-time, school, home-maker, other)	23	24.5
Income		
>/= 80,000	24	25.5
50,000 – 79,999	23	24.5
30,000 – 49,999	20	21.3
15,000 – 29,999	11	11.7
< 15,000	3	3.2

Note: All data except BMI is self-reported. SD = Standard Deviation. N = Number of patients. % = Percent of the sample population.

The baseline demographics of the study sample are shown in Table 4.1. The majority of study participants were female (82.1 %), with a mean age and BMI of 44.9 ± 9.6 years and 49.3 ± 6.8 kg/m^2, respectively. The mean number of comorbidities was 5.8, with the most common being sleep apnea (63.9 %), dyslipidemia (58.7 %), hypertension (51.1 %), impaired glucose tolerance (39.8 %) and diabetes (35.1 %).

Almost half of the patients had achieved a college diploma or higher (48.4 %), while 23.7 % had completed high school or less. There was some range in the current household income for the patients, with half of the study population (50 %) having an income of $ 50,000 or more. The majority were married or in a common-law relationship (75.5 %) and working a full-time job (51.1 %).

4.2. Weight changes

At each follow-up the patients had their height and weight measured, which was then used to calculate their BMI. Absolute weight and reductions in BMI were calculated, along with the percentage weight loss (Table 4.2).

Table 4. 2*Change in body weight (kg), BMI (kg/m²) and waist circumference*

	Baseline	**3 months**	**6 months**
Absolute weight (kg)	135.7 ± 25.3	114.6 ± 21.8*	105.1 ± 20.6*†
Absolute weight loss (kg)	-	20.9 ± 5.7*	30.4 ± 8.5*†
% weight loss	-	15.4 ± 3.0	22.4 ± 4.5†
Absolute BMI (kg/m²)	49.3 ± 6.9	41.7 ± 6.1*	38.2 ± 5.7*†
Absolute BMI loss (kg/m²)	-	7.6 ± 1.8*	11.1 ± 2.8*†
Waist circumference (cm)	141.2 ± 16.6	122.1 ± 15.3 *	114.6 ± 13.9*
% waist circumference loss	-	13.5	18.8

Note: Repeated ANOVA analysis. * = p-value < 0.05 when comparing data to baseline. † = p-value < 0.05 when compared to 3 months. BMI = body mass index.

There was a significant decrease in absolute weight comparing baseline to both 3 and 6 months post-LSG ($p < 0.001$). The weight loss was significant, with patients losing an average of 20.9 kg and 30.4 kg of their initial weight after 3 and 6 months, respectively. The decrease in absolute BMI from baseline to 3 and 6 months post-LSG was also significant ($p < 0.001$), with patients loosing an average of 7.6 kg/m^2 by 3 months and 11.1 kg/m^2 by 6 months post surgery. In addition, a significant decrease in waist circumference was observed at both 3 and 6 months post-LSG, with patients having lost 18.8 % of their initial waist circumference 6 months following LSG.

4.3. Changes in micronutrient-related parameters

Micronutrient-related parameters were measured prior to surgery and at 3 and 6 months post-operative. The baseline values were collected after the bariatric orientation, initial full-fluid diet and nutritional guidance from the bariatric team at the Health Science Centre. Because not all biochemical parameters were part of standard blood work and needed to be ordered additionally, some patients did not have all biochemical parameters measured at baseline and at each follow-up. The proportion of missing data ranged from 2.1 % at baseline to 8.4 % at 3 months and 11.6 % at 6 months post-LSG. However, due to variation in routine laboratory protocols, 25-OH-D and PTH were not measured in all patients, with a proportion of missing data ranging from 1.1 % at baseline to 16.8 % at 3 months and 27.4 % at 6

months post-LSG. In order to reduce the impact of of missing data points, the GEE analysis was used to analyse the changes in mean blood values for 25-OH-D and PTH following LSG.

The mean values for the seven micronutrient-related parameters were all within the normal range at baseline. Despite a change in blood values post-LSG, the mean values remained normal at 3 and 6 months for all parameters, except for the mean value of PTH (Appendix 4. 1). Further examination determined that at baseline, 53 patients (55.8 %) presented with one or more abnormality, the most common being elevated PTH (26.9 %) and low 25-OH-D (21.3 %). Some patients experienced an improvement or resolution of any pre-existing biochemical abnormalities in the follow-up period, with the proportion of patients with one or more abnormality decreasing slightly to 50.5 % and 46.3 % at 3 and 6 months post-LSG, respectively ($p > 0.05$).

4.3.1. Bone health parameters

Table 4. 3

Changes in the proportion of patients with abnormal values of the parameters associated with bone health

	Normal Reference		Baseline n (%)	3 months n (%)	6 months n (%)
25-OH-D	> 50 mmol/L	N	94	79	69
		Normal	74 (78.7)	70 (88.6)	68 (98.6)
		Low	20 (21.3)	9 (11.4)	1 (1.4)*
PTH	< 72 ng/L	N	93	89	87
		Normal	68 (73.1)	55 (61.8)	55 (63.2)
		High	25 (26.9)	34 (38.2)	32 (36.8)
Calcium	2.12 – 2.62 mmol/L	N	93	93	88
		Normal	89 (95.7)	92 (98.6)	84 (95.5)
		High	4 (4.3)	1 (1.1)	4 (4.5)
		Low	0	0	0

Note: McNemar analysis. N = Number of patients. N = Total number of patients. High = Levels higher than upper reference value. Low = Levels lower than lower reference value. Normal = Levels within the normal reference values. * = p-value < 0.05 when compared to baseline.

From baseline to 3 and 6 months post-LSG, is there an improvement in the blood values of the biochemical parameters associated with bone health; 25-OH-D, calcium and PTH?

From baseline to 3 and 6 months post-LSG, is there a decrease in the proportion of patients with abnormal values of the biochemical parameters associated with bone health; 25-OH-D, calcium and PTH?

There were fluctuations in the mean blood levels of the bone health parameters throughout the study duration, but the individual changes will not be discussed here. The proportion of patients with 25-OH-D abnormality decreased post-operatively (Table 4.3), while the proportion of patients with elevated PTH increased initially ($p < 0.001$ and $p > 0.05$, respectively).

The mean values for 25-OH-D increased significantly from 69.6 nmol/L at baseline, to 78.4 nmol/L at 3 months and 81.1 nmol/L at 6 months post-LSG ($p < 0.001$). Mean PTH value increased initially by the 3-month follow-up from 62.9 ng/L at baseline to 74.1 ng/L ($p < 0.001$), before a modest decrease to 70.9 ng/L was observed at the 6-month follow-up ($p > 0.05$). There was a non-significant decrease in calcium concentrations by 3 months following LSG, however, an increase to 2.47 mmol/L was observed by the 6-month follow-up. This increase was significantly higher than both the baseline concentration of 2.44 mmol/L ($p > 0.05$) and the 3-month concentration of 2.43 mmol/L ($p = 0.007$). Despite these significant changes,

the mean concentrations were all within the normal range throughout the study (Appendix 4.1).

Prior to surgery, 20 patients (21.3 %) had a lower than normal 25-OH-D blood concentration (Table 4.3). By 3 months post-LSG, the proportion had decreased to 11.4 % ($p > 0.05$) and a further decrease to 1.4 % was observed at the 6-month follow-up ($p < 0.001$). At baseline, 26.9 % of the study sample presented with elevated PTH, but there was an initial increase to 38.2 % at the 3-month follow-up ($p > 0.05$) and small decrease to 36.8 % at the 6-month follow-up. ($p > 0.05$). Only 4 patients (4.3 %) presented with a calcium abnormality at baseline and there was a slight decrease to 1.1 % at 3 months and an increase to 4.5 % at 6 months following surgery, both non-significant.

4.3.1.1. 25-hydroxyvitamin D

Is the change in 25-OH-D significantly associated with seasonal variations in sunlight exposure or the use of vitamin D-containing supplements?

Due to the fact that vitamin D can be synthesized in the skin from sunlight exposure, and that there can be seasonal variations in 25-OH-D values, the blood levels were compared by season. The actual dates of laboratory measurements were divided into the four seasons according to the equinoxes and solstices: Winter (December 21st to March 19th) Spring (March 20th to June 20th) Summer (June 21st to September 21st) Fall (September 22nd to December 20th). The seasonal variations of

25-OH-D were not significant at the assessments at baseline ($p = 0.241$), 3 months ($p = 0.202$) or 6 months post-LSG ($p = 0.820$)

The use of supplementation may have an effect on the blood values of 25-OH-D, as many multivitamins and over-the-counter supplements contain vitamin D. As the research literature suggests, many overweight and obese individuals suffer from insufficient levels of certain micronutrients. As these can be treated with supplementation, it was important to identify the number of study participants who were taking one or more supplements.

There was no uniform supplementation provided to the patients participating in this study, but they were strongly encouraged to start a supplement regimen before they underwent LSG. More than half of the patients (55.3 %) were supplemented prior to undergoing surgery, and there was an increase in the overall use of overall supplements post-LSG, with almost all patients reporting taking one or more supplements at the 3 and 6 months follow-ups. (Table 4.4) The most commonly consumed supplement throughout the study was multivitamins. Apart from the multivitamin use, 18 patients (27.7 %) took a vitamin D supplement prior to surgery. The proportion of patients taking a vitamin D supplement increased post-operatively, with 35.5 % and 45.9 % taking vitamin D supplements at 3 and 6 months, respectively.

Only a small proportion of the study sample (7.4 %) was taking vitamin B_{12} supplements prior to surgery. Similar to many of the other supplements, vitamin B_{12} supplement use increased to 10.9 % at 3 months and 16.5 % at 6 months post-LSG. It is however important to note, that most multivitamins contain vitamin D, calcium and vitamin B_{12}. Some patients also reported using over the counter (OTC) supplements or herbals and 13 patients (13.8 %) took some form of OTC supplement or herbal at baseline. At the 3-month follow-up, 19.6 % of patients were on an OTC supplement regimen and this increased to 29.8 % at 6 months post-LSG.

Table 4. 4

Reported supplement use in study participants, n = 95

	Baseline	**3 months**	**6 months**
	n (%)	n (%)	n (%)
Calcium	5 (5.3)	4 (4.3)	7 (8.3)
Vitamin D	26 (27.7)	33 (35.5)	39 (45.9)
Vitamin B_{12}	7 (7.4)	10 (10.9)	14 (16.5)
Multivitamin	42 (44.7)	88 (94.6)	78 (91.8)
OTC or herbals	13 (13.8)	18 (19.6)	25 (29.8)
Overall	**52 (55.3)**	**92 (98.9) ***	**84 (97.7) ***

Note: n = number of patients. % = proportion of patients. All data is self-reported. OTC = Over the counter. % = Percentage of the study sample asked. * = p-value < 0.05 when compared to baseline.

The proportion of patients presenting with low 25-OH-D were assessed at each time-point, to determine the effect of supplement use. At baseline, the proportion of abnormalities was somewhat equally distributed among the supplemented and non-supplemented patients, with nine supplemented patients presenting with low levels compared to 11 patients with low levels who were not supplemented. However, the effect of supplement on the proportion of patients with low 25-OH-D was not able to be determined in the follow-up period, as all but one patient was taking a supplement that likely contained vitamin D. In addition, as most patients are only reporting if they are taking a supplement, and not the dosage of these, it is difficult to assess the effect properly. Due to this, supplement use was not included in the GEE analysis.

Three univariate GEE analyses were conducted to assess changes in 25-OH-D (Appendix 4. 2). According to the first univariate analysis, time since surgery was significantly associated with increasing 25-OH-D blood levels at 3 and 6 months post-LSG, $p < 0.001$. In the second univariate analysis, overall use of supplementation was associated with increasing 25-OH-D blood levels, $p < 0.001$. The third univariate analysis determined that seasonal changes were not significantly associated with the changes in 25-OH-D blood levels, $p = 0.575$.

A multivariate GEE analysis was then performed, to determine if the changes observed in 25-OH-D could be explained by the time since surgery or the use of supplementation. According to the multivariate analysis, time since surgery remained significant, p = 0.001, while the overall use of supplementation was no longer significantly associated with the increase in 25-OH-D blood values, p = 0.615. (Appendix 4.2.)

In addition, correlation analyses and regression models were performed to determine if the doses of vitamin D supplement specifically was associated with 25-OH-D blood levels. There were no significant associations determined at baseline (r = -0.024, p = 0.912), 3 months (r = 0.167, p = 0.416) or 6 months (r = 0.039, p = 0.840) post-LSG.

Is there a correlation between weight lost and the change in 25-OH-D levels following surgery?

In addition to the seasonal variation analysis for 25-OH-D, a correlational analysis was performed for 25-OH-D and weight parameters. In the first analysis, the relationship between body weight (kg) and 25-OH-D levels at baseline and at 3 and 6 months were analyzed. According to the analysis, there were moderate inverse correlations between weight (kg) and the levels of 25-OH-D at baseline (r = -0.343, p < 0.001), 3 months (r = -0.290, p = 0.01) and 6 months post-LSG (r = -0.295, p –

0.012). This indicates that both pre- and post-surgery, the higher the weight of a patient, the lower the 25-OH-D blood levels.

The changes in 25-OH-D levels from baseline to 3 and from baseline to 6 months were analysed for correlation with the changes in weight from baseline to 3 and from baseline to 6 months. There was a weak correlation between the weight lost and the increase in 25-OH-D from baseline to the 3-month follow-up (r = 0.242, p = 0.033). No significant correlation was demonstrated for the changes in weight and 25-OH-D from baseline to the 6-month follow-up.

4.3.2. Hematological parameters

Table 4. 5

Changes in the proportion of patients with abnormal values of the parameters associated with hematological status

	Normal reference		**Baseline n (%)**	**3 months n (%)**	**6 months n (%)**
Ferritin	M: 24 – 336 µg/L	N	95	88	90
	F: 11 – 307 µg/L	Normal	85 (89.5)	77 (87.5)	82 (91.1)
		High	9 (9.5)	7 (8)	3 (3.3)
		Low	1 (1.1)	4 (4.5)	5 (5.6)
Hemoglobin	M: 140 – 180 g/L	N	95	93	91
	F: 120 – 160 g/L	Normal	84 (88.4)	82 (88.1)	79 (86.8)
		High	1 (1.1)	1 (1.1)	2 (2.2)
		Low	10 (10.5)	10 (10.8)	10 (11)
MCV	M: 80 – 97 fL	N	95	93	91
	F: 81 – 99 fL	Normal	84 (88.4)	86 (92.5)	83 (91.2)
		High	2 (2.1)	1 (1.1)	3 (3.3)
		Low	9 (9.5)	6 (6.4)	5 (5.5)
Vitamin B12	< 107 pmol/L	N	95	87	90
		Normal	95 (100)	87 (100)	90 (100)
		Low	0	0	0

Note: McNemar analysis. n = Number of patients. N = Total number of patients. High = Levels higher than upper reference value. Low = Levels lower than lower reference value. Normal = Levels within the normal reference values. F = Female patients. Male and female abnormalities are combined for the baseline, 3 and 6 months columns.

From baseline to 3 and 6 months post-LSG, is there a deterioration of the blood values of the hematological parameters; ferritin, hemoglobin, mean cell volume (MCV) and vitamin B_{12} or do they remain stable?

From baseline to 3 and 6 months post-LSG, is there an increase in the proportion of patients with abnormal values of the hematological parameters; ferritin, hemoglobin, mean cell volume (MCV) and vitamin B_{12}?

A significant decrease in ferritin levels was observed at 3 and 6 months following surgery ($p < 0.001$) for both male and female patients (Appendix 4.1). The decrease in mean levels impacted the proportion of patients with abnormally low ferritin. Only one female patient presented with a low ferritin level at baseline, but by 3 months post-LSG, four female patients (4.5 %) had developed low levels and by 6 months post-LSG, five female patients (5.6 %) continued to present with this abnormality, $p > 0.05$ (Table 4.5). In addition to the female patients presenting with low ferritin, nine patients (9.5 %) presented with ferritin levels higher than normal at baseline, although this proportion decreased post-operatively ($p > 0.05$).

The mean hemoglobin values were within the normal reference range pre- and post-LSG, and they remained stable throughout the first 6 months following LSG. However, 10 patients (10.5 %) presented with low hemoglobin at baseline, indicating anemia. There continued to be 10 patients presenting with low hemoglobin at the 3 and 6-months follow-ups, although they were not all the same

patients (Table 4.5.). A very small proportion of female patients presented with hemoglobin concentrations higher than the normal reference value (> 160 g/L); 1.1 %, 1.1 % and 2.2 % at baseline, 3 months and 6 months, respectively ($p > 0.05$).

There was an overall significant increase in mean MCV from baseline to 6 months following LSG ($p < 0.001$) although mean MCV were within the normal ranges for males and females at each time-point. However, nine female patients (9.5 %) presented with lower than normal MCV at baseline, and a non-significant decrease to five female patients (5.5 %) at the 6-month follow-up was observed. In addition, two female patients (2.1 %) presented with higher than normal MCV at baseline, one patient had high MCV at the 3-month follow-up (1.1 %) and three patients (3.3 %) had high MCV at 6 months following LSG, $p > 0.05$ (Table 4.5).

The mean values of vitamin B_{12} varied throughout the study; increasing slightly from 359.9 pmol/L at baseline to 366.4 pmol/L at 3 months post-LSG ($p > 0.05$), before decreasing significantly to 324.9 pmol/L at 6 months post-LSG ($p = 0.006$). Despite these changes, the mean values were within the normal range and none of the patients in this study experienced insufficient levels of vitamin B_{12} (< 107 pmol/L) at any time-point throughout the study period (Table 4.5).

4.5. Parameters associated with NAFLD

At baseline, 76 patients (80 %) presented with abnormal blood values for one or more of the seven biochemical parameters associated with NAFLD. The proportion of patients with abnormal liver parameters decreased significantly in the follow-up period, with 60 % at 3 months ($p < 0.005$) and 50.5 % at 6 months ($p < 0.001$) post-LSG. The most common abnormalities prior to surgery were elevated GGT, 41.9 %, elevated triglycerides, 41.5 %, low HDL, 28.7 % and elevated LDL, 28.3 % (Table 4.8).

All mean values of the seven biochemical parameters were within the normal reference range both prior to – and following surgery, except for the mean value of triglycerides at baseline (1.98 mmol/L), which was slightly above the normal reference value of 1.9 mmol/L.

From baseline to 3 and 6 months post-LSG, is there a change in the values of the biochemical parameters associated with NAFLD; ALP, ALT, GGT, total bilirubin, HDL, LDL and triglycerides?

From baseline to 3 and 6 months post-LSG, is there a change in the proportion of patients with abnormal values of the biochemical parameters associated with NAFLD; ALP, ALT, GGT, total bilirubin, HDL, LDL and triglycerides?

Table 4. 6

Change in the proportion of patients with abnormal values of the parameters associated with NAFLD

	Normal Reference		**Baseline n (%)**	**3 months n (%)**	**6 months n (%)**
Bilirubin	< 20 µmol/L	N	93	94	92
		Normal	86 (92.5)	87 (92.6)	82 (89.1)
		High	7 (7.5)	7 (7.4)	10 (10.9)
ALP	< 150 U/L	N	93	91	91
		Normal	93 (100)	91 (100)	91 (100)
		High	0	0	0
ALT	< 55 U/L	N	93	93	90
		Normal	83 (89.2)	88 (95.7)	90 (100)
		High	10 (10.8)	4 (4.3)	0*
GGT	< 32 U/L	N	93	89	84
		Normal	54 (58.1)	70 (78.7)	75 (89.3)
		High	39 (41.9)	19 (21.3)*	9 (10.7)*†

HDL	> 0.9 mmol/L	N	94	93	92
		Normal	67 (71.3)	70 (75.3)	79 (85.9)
		Low	27 (28.7)	23 (24.7)	13 (14.1)* †
LDL	< 3.4 mmol/L	N	92	93	92
		Normal	66 (71.7)	74 (79.6)	70 (76.1)
		High	26 (28.3)	19 (20.4)	22 (23.9)
Triglycerides	< 1.9 mmol/L	N	94	93	92
		Normal	55 (58.5)	82 (88.2)	84 (91.3)
		High	39 (41.5)	11 (11.8)*	8 (8.7)*

Note: McNemar analysis. n = Number of patients. N = Total number of patients. High = Levels higher than upper reference value. Low = Levels lower than lower reference value. Normal = Levels within the normal reference values. * = p-value < 0.05 when compared to baseline. † = p-value < 0.05 when compared to 3 months.

Prior to undergoing the LSG procedure, two patients (2.1 %) presented with a self-reported diagnosis of NAFLD. The first patient presented with elevated GGT, LDL and triglycerides concentrations at baseline, but all parameters were within the normal range at the 6-month follow-up. The second patient with self-reported NAFLD had elevated GGT, ALT and triglycerides concentrations at baseline along with a low concentration of HDL. By the 6-month follow-up, all pre-existing abnormalities had resolved, except for a continuously low HDL.

The mean value of bilirubin increased significantly throughout the study from 12.2 μmol/L at baseline, to 13.2 μmol/L at 6 months post-LSG, $p = 0.008$ (Appendix 4.1). The proportion of patients with elevated bilirubin values (> 20 μmol/L) increased as well post-operatively from 7.5 % at baseline to 10.9 % at 6 months following surgery ($p > 0.05$). There was a modest, but non-significant, decrease in ALP levels in the follow-up period, however no ALP abnormalities were observed at any time-point through the duration of the study (Table 4.6).

ALT is one of the most common liver indicators measured and 10.8 % of the patients presented with elevated ALT levels (> 55 U/L) at baseline. There was a significant improvement post-operatively, as all patients experienced a resolution of their pre-existing ALT-abnormality by the 6-month follow-up, $p = 0.002$ (Table 4.6). This is supported by the significant decrease in mean ALT blood levels from 31.5 U/L at baseline to 18.1 U/L at 6 months post-LSG ($p < 0.001$).

A significant decrease was also observed for GGT, which decreased from 62 U/L at baseline to 23.9 U/L at 3 months, $p < 0.001$ and 21.4 U/L at 6 months post-LSG, $p < 0.001$ (Appendix 4.1). This was the most common abnormality prior to surgery, with 39 patients (41.9 %) presented with GGT levels above the normal reference (> 32 U/L), and there was a significant reduction in the proportion of patients with this GGT abnormality by 6 months, with only 10.7 % of patients, $p < 0.001$ (Table 4.6).

Despite a minor, non-significant decrease in the mean value of HDL at 3 months post-LSG, there was a significant increase from 1.04 mmol/L at baseline to 1.12 mmol/L at 6 months following LSG ($p < 0.001$). 28.7 % of patients experienced lower than normal HDL (< 0.9 mmol/L) prior to undergoing surgery. While the mean value of HDL had increased by 6 months post-LSG, the proportion of patients with low HDL had significantly decreased to 14.1 % ($p = 0.007$).

The mean values of LDL fluctuated post-operatively, with a significant decrease from 2.85 at baseline to 2.61 at 3 months post-LSG ($p = 0.006$) and then a significant increase to 2.79 mmol/L at 6 months post-LSG ($p = 0.008$). A large proportion of patients (28.3 %) presented with LDL values higher than normal (> 3.4 mmol/L) at baseline. There was a non-significant decrease by 3 months post-LSG, where 20.4 % of patients had high LDL, before a slight increase to 23.9 % was observed at the 6-month follow-up, $p > 0.05$ (Table 4.6).

There was a significant decrease in the mean levels of triglycerides throughout the study from 1.98 mmol/L at baseline to 1.42 and 1.33 mmol/L at 3 and 6 months post-LSG, respectively, $p < 0.001$ (Appendix 4.1). The mean level at baseline was the only mean blood value of the biochemical parameters associated with NAFLD outside the normal reference range, and 41.5 % of patients in this study experienced a higher than normal triglyceride level (> 1.9 mmol/L) prior to undergoing LSG. The majority experienced an improvement, as there was a significant decrease in the proportion of patients with elevated triglycerides levels at 3 (11.8 %) and 6 months (8.7 %) post-LSG, $p < 0.001$ (Table 4.6).

As previously mentioned, two studies proposed methods to more accurately predict the absence or presence of NALFD (Bedogni et al., 2006; Borman et al., 2013). Based on these methods, both the Fatty Liver Index (FLI) and the Steatosis Index (SI) were calculated for this patient sample (Table 4.7). Because waist circumference wasn't obtained as standard procedure at the beginning of the study, only 65 patients had the information required for the calculation of the FLI at baseline. In the algorithm for the FLI, the triglycerides is measured in mg/dL, however the triglycerides unit measured in the current study is mmol/L. Therefore, before calculating the FLI, the triglycerides values obtained were converted into mg/dL, by multiplying the values by 88. 57396.

Table 4. 7

Fatty Liver Index and Steatosis Index for the sample population

	Fatty Liver Index			**Steatosis Index**		
	Baseline (n = 65)	**3 months (n = 84)**	**6 months (n = 75)**	**Baseline (n = 91)**	**3 months (n = 88)**	**6 months (n = 86)**
Mean ± SD	99.16 ± 1.30	90.14 ± 13.30*	82.80 ± 19.13*†	0.96 ± 0.04	0.88 ± 0.11*	0.82 ± 0.13*†
Median	99.75	96.07	89.97	0.97	0.91	0.84
Min – Max	93.34 – 99.99	37.39 – 99.93	17.34 – 99.93	0.81 – 1.0	0.47 – 0.99	0.48 – 0.99
25th and 75th Quartiles	98.90 – 99.92	86.63 – 99.00	72.78 – 97.41	0.94 – 0.99	0.84 – 0.95	0.75 – 0.93

Note: Repeated measures ANOVA/Friedman's with Wilcoxon's. * = p-value < 0.05 when compared to baseline. † = p-value < 0.05 when compared to 3 months. (n) = total number of patients with available data for each time point.

A FLI > 60 would, according to the author (Bedogni et al. 2006), suggest the presence of fatty liver, while a FLI < 30 would indicate the absence. At baseline, all patients presented with a FLI > 60, indicating that all patients had fatty liver. A slight improvement was observed at 3 months, with 96.4 % of patients having a FLI > 60. At the 6 month follow-up, a significant improvement was observed, as 88 % of patients continued to present with a FLI > 60 while 2 patients (2.7 %) now presented with a FLI < 30, indicating the absence of fatty liver disease (p-value < 0.05).

The study by *Borman et al.* (2013) did not provide any cut-off to use with the SI, as it has yet to be validated against other indexes. Due to this, it was not possible to determine the proportion of patients with NAFLD according to the SI. However, a correlation analysis was performed for the FLI and the SI to determine if there was a relationship between the two indexes. Based on the scatterplots and analyses, there was a moderate positive correlation between the FLI and the SI at baseline ($r = 0.7611$, $p < 0.001$), as well as a strong correlation at 3 months ($r = 0.810$, $p < 0.001$) and 6 months ($r = 0.851$, $p < 0.001$) post-LSG (Appendix 4. 3).

4.5.1. Weight correlation

Is there a correlation between weight lost and the change in the biochemical parameters associated with NAFLD following surgery?

Univariate correlation analysis was conducted to determine any significant relationships between weight parameters and the select biochemical parameters associated with NAFLD. The first set of analysis assessed the correlation between body weight (kg) at baseline, 3 and 6 months post-LSG, along with the corresponding blood values of the seven biochemical parameters.

Significant inverse correlations between body weight and HDL levels were demonstrated for baseline (r = -0.230, p = 0.026), 3 months (r = -0.307, p = 0.003) and 6 months post-LSG (r = -0.342, p < 0.001). This indicates that as weight decreased, the HDL levels increased. In addition, a weak correlation between baseline weight and bilirubin levels (r – 0.296, p = 0.004), along with a significant correlation between the 6 months weight and GGT levels (r = 0.252, p = 0.023) were observed. The second set of correlation analysis was done for the change in body weight and the changes in the biochemical parameters. The only significant findings were modest correlations for the weight lost and the decrease in triglyceride levels from baseline to 3 months (r = 0.238, p = 0.023) and from baseline to 6 months post-LSG (r = 0.237, p = 0.026). This suggests that the greater the weight loss, the greater the decrease in triglycerides. No other significant correlations could be determined.

5. Discussion

One of the main findings in this study was a significant increase in 25-OH-D at both 3 and 6 months following LSG. Although time since surgery is likely to explain the increase observed, a possible benefit from supplement use cannot be ruled out. In addition, significant improvements in the majority of biochemical parameters associated with NAFLD were observed in the follow-up period. This was supported by the significant improvements observed in the Fatty Liver Index as well as the Steatosis Index.

5.1. Demographic profile

The current study sample had a mean BMI of 49.3 kg/m^2, which was similar to the mean BMI of 49.7 kg/m^2 found in the other studies identified on this area of research (Aarts et al., 2011; Damms-Machado et al., 2012; Gehrer et al., 2010; Hakeam et al., 2009; Kehagias et al., 201; Moize et al., 2012; van Rutte et al., 2014; Ruiz-Tovar et al., 2012). The baseline demographics of the current study sample were also similar to the baseline demographics found in those studies in regards to age (49.95 ± 10.8 years) and the distribution of comorbidities. The most common co-morbidities in these studies were dyslipidemia, hypertension and type 2 diabetes, similar to the common co-morbidities found in the current study. However, a larger proportion of this study sample was female (80.3 %) when compared to the

proportion of female patients found in the other studies (61.3 %), while the baseline weight (135.7 ± 25.3) was lower than the baseline weight among these other studies (142.3 ± 23.6).

In a study by *Padwal et al.* (2012), the demographic profiles of patients eligible for bariatric surgery in Canada were determined and compared with the patients actually receiving surgery. They found that patients receiving bariatric surgery were younger (43.6 vs. 45.8 years) and had a lower prevalence of comorbidities such as hypertension (13.1 % vs. 39.2 %), type 2 diabetes (2.4 % vs. 15.1 %) and dyslipidemia (21.1 % vs. 63.1 %) than patients eligible, but not receiving bariatric surgery.

The baseline demographic profile of patients in the current study sample is similar to patients receiving bariatric surgery in Canada for age and the percentage of female patients (44.9 vs. 43.6 years and 82.1 % vs. 82 %, respectively). However, the prevalence rates of comorbidities in the current study were much higher than patients in general receiving bariatric surgery in Canada for sleep apnea, dyslipidemia, hypertension, type 2 diabetes and coronary heart disease; 63.9 % vs. 10.9 %, 58.7 % vs. 2.4 %, 51.1 % vs. 13.1 %, 35.1 % vs. 21.1 % and 5.3 % vs. 0.9 %, respectively (Padwal et al., 2012). It is important to note, that these demographic characteristics are only for the 95 patients who, at the time of writing this thesis, had completed their baseline, 3 months and 6 months post-LSG assessments.

5.2. Changes in micronutrients and related parameters

All patients had their blood work done 1 to 2 days prior to undergoing surgery and this constitutes the baseline values. However, it is important to keep in mind, that these values are representative of the micronutrient status *after* the patients attended the orientation *and* completed the full-fluid diet. The full-fluid diet is likely to have improved patient's micronutrient status at baseline, as they were instructed to follow a strict diet prior to surgery. Due to this, the baseline values might be a slight underestimate of the actual micronutrient status in patients seeking bariatric surgery in Newfoundland & Labrador in general.

The overall mean blood values were within the normal reference for all variables both at baseline and at the 3 and 6-month follow-ups, except for the mean PTH value at 3-months post-LSG. Despite this, patients presented with abnormal blood values for one or more of the micronutrient-related parameters measured at each time-point. The majority of patients (57.7 %) presented with a micronutrient-related abnormality prior to surgery. This prevalence rate is almost identical to the findings by *Gehrer et al.* (2010) where 57 % presented with a baseline abnormality, and similar to the baseline prevalence of 51 % found by *Damms-Machado et al.* (2012). The 11 studies identified do not provide an overall estimate of the proportion of patients with one or more abnormality at each follow-up, so it is not possible to compare the prevalence rates of 52.1 % and 42.3 % (3 and 6 months, respectively) found in the current study to any other results reported.

5.2.1. Bone health parameters

Elevated levels of PTH (26.9 %) and low levels for 25-OH-D (21.3 %) were the most commonly found abnormalities in the current study, and consistent with other studies where elevated PTH and low 25-OH-D were the most commonly observed abnormalities prior to and after LSG. The prevalence of low vitamin D/25-OH-D in the studies assessing this parameter, ranges from 23 % to 96.7 % at baseline, (Gehrer et al., 2010; Ruiz-Tovar et al., 2012), but with the majority reporting a prevalence rate in the 75th quartile (Damms-Machado et al., 2012; Moize et al., 2012; Saif et al., 2012; van Rutte et al., 2014).

In regards to 25-OH-D, patients in the current study appear to be well managed prior to surgery, with the majority having sufficient levels despite the fact that only 27.7 % were taking a specific vitamin D supplement. The prevalence rate of low 25-OH-D among the current study sample (21.3 %) was considerably lower than the national rate (~ 32 %), but the mean baseline level of 69.6 nmol/L was similar to the estimated mean level of 63.5 nmol/L from the general population [Janz & Pearson, 2013, Statistics Canada (b), 2013]. In addition, the large proportion of patients with sufficient 25-OH-D levels (78.7 %) in the current study, is in contrast to the otherwise high prevalence of 25-OH-D deficiency in the general Canadian population, especially in Newfoundland & Labrador [Janz & Pearson, 2013; Newhook et al., 2009; Statistics Canada (b), 2013].

The bariatric patients might have been aware of this increased risk in the province, and could have sought out preventative options prior to seeking bariatric surgery, thus presenting with overall sufficient blood levels. According to the 2009-2011 Canadian Health Measures Survey (CHMS), Cycle 2, an estimated 32 % of all Canadians had 25-OH-D blood levels below 50 nmol/L. However, it might also be due to the 25-OH-D analysis used at the Health Sciences Centre. This in-house developed assay provides slightly higher values that the radioimmunoassays typically used at other sites and in most clinical studies (Hollis, 2012; Personal correspondence with Dr. Edward Randell).

The 21.3 % prevalence rate of low 25-OH-D in the current study, is similar to the findings by *Gehrer et al.* (2010), where 23 % of the patients presented with low 25-OH-D prior to undergoing LSG. In that study however, there was an increase in the proportion of patients with low 25-OH-D in the follow-up period, whereas a significant reduction was observed at 6 months post-LSG in the current study, with only one patient presenting with low 25-OH-D. A similar finding was observed by *Ruiz-Tovar et al.* (2012), where the 96.7 % prevalence rate at baseline was reduced to 13.2 % at 3 months post-LSG (11.4 % at 3 months post-LSG in the current study) and a further reduction to just one patient at the 6-month follow-up. The majority of the other studies assessing the changes in 25-OH-D following LSG, reported improvements in the follow-up period, although the prevalence rates of low 25-OH-D post-LSG ranged from 34.3 % to 77.2 % (Damms-Machado et al., 2012, Moize et al., 2013; Saif et al., 2012; van Rutte et al., 2014).

25-OH-D blood concentrations vary by seasons, as vitamin D is synthesized in the skin due to sun exposure. Mean concentrations of 25-OH-D are typically higher in summer when compared to winter, and differences in mean values ranges from 15.1 % to 35.2 % (Gozdzik et al., 2009; Newhook et al., 2009; Ross et al., 2011). The 2011 report on vitamin D from the Institute of Medicine, which analyzed multiple studies, reported a variation in mean 25-OH-D values of 25 nmol/L from winter to summer (Ross et al., 2011). According to the CHMS, Cycle 2, there is a difference of 37.5 % between individuals with insufficient 25-OH-D concentrations, and a difference of 60 % between individuals with deficient 25-OH-D concentrations when comparing summer to winter (Janz & Pearson, 2013).

However, the seasonal variations for 25-OH-D concentrations were not significantly different at any time in the current study, which was determined by two separate analyses. The reason for the non-significant variation of mean 25-OH-D concentrations may be due to a small sample size or the fact that nearly all patients were taking supplements following LSG. In the other studies assessing 25-OH-D following LSG, none mention seasonal variations or whether their results were corrected for these possible variations.

Elevated PTH were the most prevalent abnormality observed in patients prior to surgery with 26.9 % of patients presenting with higher than normal levels. Other studies have reported elevated PTH as being one of the most common abnormalities prior to surgery as well, with prevalence rates ranging from 6.7 to 62.5 % (Gehrer et al. 2010; Kehagias et al., 2011; Moize et al., 2013; Ruiz-Tovar et al., 2012; Saif et al., 2012; van Rutte et al., 2014). An initial significant increase in mean PTH was observed 3 months post-LSG, which resulted in an increase in the proportion of patients with elevated PTH in the same time-period (38.2 %), although this was not statistically significant. At the 6-month follow-up, a slight decrease in mean PTH and a corresponding decrease in the proportion of patients with elevated PTH (36.8 %) were observed. Only the study by *Gehrer et al.* (2010) reported an increase in the proportion of patients with elevated PTH levels, from 8 % at baseline to 14 % post-operatively. The other studies all reported improvements in PTH following LSG, with the prevalence rates for elevated PTH ranging from 0 % to 40.9 % (Kehagias et al., 2011; Moize et al., 2013; Ruiz-Tovar et al., 2012; Saif et al., 2012; van Rutte et al., 2014).

The initial unexpected increase in the proportion of patients with elevated PTH observed in this study, could be due to several factors. Usually when PTH levels are elevated, one would examine the 25-OH-D, to determine if the elevated PTH levels are due to insufficient 25-OH-D and thereby low calcium intake and decreased

post-operative absorption. But because the mean 25-OH-D levels were normal, the increase in PTH elevation cannot be attributed to reduced calcium absorption in some patients. Another possible explanation specific to this study, is related to technical issues with the assay used for the PTH analysis. The laboratory at the Health Sciences Centre in St. John's, Newfoundland, had been made aware by the manufacturer, that the assay might be malfunctioning, as shown by an upward drift in the reading of high control values. The manufacturer temporarily removed the assay from the market, but the issue has now been resolved. Although falsely elevated values were never detected at the main laboratory site connected to this study, it is possible that the assay contributed to the unexpected increasing prevalence of elevated PTH observed 3 months following LSG.

Due to difference in laboratory protocols, measurements for 25-OH-D and PTH were not part of standard blood work routine, and had to be ordered specially for the research study. This resulted in a decrease in the number of patients with 25-OH-D and PTH assessments completed in the follow-up periods. Measurements for 25-OH-D decreased from 94 completed assessments at baseline, to 79 and 69 assessments at 3 and 6 months post-LSG, respectively. The number of patients with complete PTH assessments decreased from 93 at baseline to 89 and 87 patients at 3 and 6 months, respectively. Because of this decrease, the results for 25-OH-D and PTH should be interpreted with caution, as the actual results might be slightly different if all patients had their 25-OH-D and PTH assessed properly pre- and post-LSG.

There was a modest, yet significant increase in albumin-corrected calcium concentrations from the 3-month follow-up to the 6-month follow-up, increasing from 2.43 mmol/L at baseline to 2.46 mmol/L. Only a few patients presented with higher than normal calcium concentrations throughout the course of the study, with fluctuating changes post-operatively. Calcium abnormalities are not very prevalent in patients prior to or following LSG, and while some of the other studies have reported a few patients presenting with low calcium concentrations, none have reported patients experiencing higher than normal calcium concentrations (Aarts et al., 2010; Capoccia et al., 2012; Damms-Machado et al., 2012; Gehrer et al., 2010; Kehagias et al., 2011; Moize et al., 2013; Ruiz-Tovar et al., 2012; Saif et al., 2012, van Rutte et al., 2014). Even though a significant increase in mean calcium was observed post-LSG in the current study, the slight increase in proportion of patients with high calcium concentrations at the 6-month follow-up (1.1 % to 4.5 %) was not statistically significant.

In summary, the proportion of patients with low 25-OH-D and elevated PTH were lower than the proportions found in other studies. 25-OH-D and PTH constitute the majority of the micronutrient-related abnormalities observed in the current study, which is similar to that reported by others (Damms-Machado et al., 2012; Moize et al., 2012; Ruiz-Tovar et al., 2012; Saif et al., 2012; van Rutte et al., 2014). There was a significant reduction in the proportion of patients with lower

than normal 25-OH-D post-LSG, which is consistent with the majority of other studies assessing 25-OH-D following LSG. An initial, non-significant increase in the proportion of patients with elevated PTH was observed in the follow-up period, which is in contrast to findings reported by other studies, where improvements in PTH is typically observed post-LSG. However, this increase in elevated PTH prevalence may be due to technical issues with the assay used for analysis.

As hypothesized, a significant improvements in 25-OH-D were observed following LSG in the current study, both in regards to the biochemical changes and the proportional changes. However, the findings regarding the change in PTH following LSG are inconsistent with the hypothesis of an improvement in PTH post-LSG, as both improvements and deteriorations were observed. In addition, the increase in calcium blood levels was consistent with the hypothesis.

5.2.1.1. Weight parameter correlations

The current study demonstrated a moderate, inverse correlation between the baseline weight of the study sample and their corresponding levels of 25-OH-D both pre- and post-LSG. This significant relationship indicates that patients with a higher baseline body weight had significantly lower levels of 25-OH-D. This relationship between weight parameters (e.g. BMI, total body weight, body fat) and 25-OH-D blood levels, has been observed by others as well (Ruxton, 2011). The present study also demonstrated a moderate inverse correlation between weight loss and 25-OH-D

increase in the first 3 months following LSG. This finding is similar to that found by *Ruiz-Tovar et al.* (2012) who also reported a significant inverse correlation between the weight lost and the increase in 25-OH-D levels observed after the first 3 months of follow-up.

The relationship between weight loss and 25-OH-D increase observed in the current study could further support the theory that the amount of 25-OH-D sequestered in adipose tissue is reduced following LSG. However, it remains unclear whether the increase in 25-OH-D levels observed is due to a release of stored 25-OH-D, a reduction in the amount stored before reaching the liver, or increased supplement use (Damms-Machado et al., 2012; Ruiz-Tovar et al., 2012; Ruxton, 2011).

5.2.1.2. Supplement

Studies suggest that the use of supplementation is an important factor in post-operative care, and that the use should be supervised closely in order to prevent deficient or toxic concentrations of any micronutrient (Aarts et al., 2011; Capoccia et al., 2012; Damms-Machado et al., 2012; Gehrer et al., 2010; Saif et al., 2012). Despite patients not being provided a uniform supplement by the bariatric surgery clinic, more than half of the study sample (55.3 %) was taking one or more supplements prior to surgery, and nearly all patients reporting using supplements in the follow-up period, the most common being a multivitamin. In addition, there did

appear to be a relationship between the use of supplementation and the prevalence of low 25-OH-D levels, as the overall use of supplements increased while the prevalence of low 25-OH-D decreased in the follow-up period.

The effect of supplementation has been examined in several studies examining micronutrient-related changes following LSG, but there is currently no consensus on the magnitude of this potential effect. Two studies report that the use of supplementation did not have an effect on the blood values of the specific micronutrient-related parameters measured (i.e. 25-OH-D, vitamin B_{12}, calcium, PTH), and when supplemented patients were compared to those not supplemented, no significant differences were observed (Damms-Machado et al., 2012; Saif et al., 2012).

Two other studies by *Capoccia et al.* (2012) and *Gehrer et al.* (2010) however, reported a positive effect from supplement use, although the micronutrient-related parameters impacted varied within the studies (i.e. vitamin D/25-OH-D, vitamin B_{12}, calcium, PTH). *Gehrer et al.* (2010) also reported that any newly developed 25-OH-D deficiencies following LSG could be treated with supplementation in 100 % of the patients. There continues to be some inconsistencies regarding the effect of supplementation on the prevalence of low 25-OH-D levels. Despite the positive effect observed by *Gehrer et al.* (2010), the study by *Capoccia et al.* (2012) reported continuously low 25-OH-D levels throughout the study despite the use of

supplementation. *Damms-Machado et al.* (2012) reported a significant difference in the proportion of patients with low 25-OH-D levels, with the majority not being supplemented. However, the reported p-value was 0.049, indicating borderline significance.

All information on supplement usage was self-reported, and the majority of patients reported *if* they were using a supplement, but not the type of supplement or dose. It was possible to ascertain the dose of the vitamin D supplements based on each patient's medication profile. However, as the majority of patients were also taking a multivitamin or other supplement (e.g. calcium, some of which likely contain vitamin D), therefore, it was not possible to provide an accurate assessment of total consumption of vitamin D. The dose of vitamin D supplement alone could potentially underestimate the actual effect of supplement use on 25-OH-D levels.

The overall use of supplementation was significantly associated with the increase in 25-OH-D in the univariate GEE analysis, however it was not significant in the multivariate model. This suggests other factors such as dietary assessment and estimates of sun exposure, not assessed in the current study, may play an important role in impacting 2-OH-D blood levels. Despite an increase in the use of supplementation, patients continued to present with low 25-OH-D levels.

5.2.3. Hematological parameters

Abnormalities in the select hematological parameters were more prevalent among the female patients, which is expected, as they constituted the vast majority of the study sample and more significant blood loss occurs among pre-menopausal women (Hakeam et al., 2009). A significant decrease in mean ferritin concentrations, with a corresponding decrease in proportion of patients with high ferritin concentrations, was observed in the current study. However, as six patients experience a resolution of their pre-existing high ferritin, four other patients developed ferritin concentrations below the normal reference values.

A possible explanation for the proportion of patients with high ferritin prior to surgery, might be due to the fact that ferritin is also an acute inflammation indicator (Berkhan, 2002; Shifflet & Wum, 2009; Swaminathan, 2011). Several reports suggest that obesity causes a low-grade inflammatory response, which in turn can cause ferritin to increase (Lumeng & Saltiel, 2011; Emanuela et al., 2012). The improvements in patients' BMI following LSG in the current study, could improve the inflammation state and thus explain the decrease in the proportion of patients with high ferritin.

None of the studies identified in this area of research reported patients presenting with higher than normal ferritin concentrations, and just three reported increases in the proportion of patients with low ferritin in the follow-up periods (Kehagias et al., 2011; Saif et al., 2012; van Rutte et al., 2014). Of the three studies

presenting their findings at 6 months post-LSG, none observed abnormal ferritin concentrations (Hakeam et al., 2009; Moize et al., 2012; Ruiz-Tovar et al., 2012). The actual impact of LSG on ferritin is difficult to assess based on the current study, as both improvements and deteriorations were observed in the follow-up period.

10.5 % of patients in the current study presented with low hemoglobin levels at baseline, which is identical to the findings by *Moize et al.* (2012). They reported an increase to 12.1 % at 6 months post-LSG, which is similar to the 11 % found in the current study. The majority of studies examining changes in hemoglobin following LSG, reported an increase in the proportion of patients with low hemoglobin levels post-operatively, ranging from 0 to 13.3 % at baseline to 6.5 to 26 % post-LSG (Aarts et al., 2011; Hakeam et al., 2009; Kehagias et al., 2011; Moize et al., 2012, van Rutte et al., 2014). In the current study, one female patient presented with a hemoglobin level higher than normal at baseline and 3 months post-LSG, and at the 6-month follow-up two female patients had reportedly high hemoglobin levels. Elevated hemoglobin could be caused by dehydration, which can occur following bariatric surgery, as the fluid intake is reduced due to the small stomach size. None of the other studies have reported this finding.

MCV is not commonly measured in studies on micronutrient-related changes following LSG, and not many baseline findings from other studies are available for comparison to the findings from the current study. In our study, nine female patients (9.5 %) presented with low MCV, followed by a non-significant decrease to five female patients (5.5 %) at the 6-month follow-up. The studies by *Aarts et al.* (2011) and *van Rutte et al.* (2014) presented the proportion of patients with abnormal MCV pre- and post-LSG. Their findings showed a baseline prevalence of low MCV at 5 %, however one study reported an increasing prevalence to 15 % post-LSG (Aarts et al., 2011) while the other study reported a decrease in the proportion of patients with low MCV to 2 % (van Rutte et al., 2014).

In the study by *Hakeam et al.* (2009), low MCV was used as an exclusion criterion, thus ensuring a 0 % prevalence rate for MCV abnormalities at baseline. At the 6-month follow-up, two patients (3.2 %) in that study presented with low MCV. *Hakeam et al.* (2009) also reported decreasing MCV in the follow-up period, which is in contrast to the current study, where significantly increasing MCV was observed post-LSG. In addition, a couple of female patients continued to present with MCV above the normal reference value throughout the study, which has not been reported elsewhere.

Low levels of vitamin B_{12} is one of the most commonly found abnormalities prior to and after bariatric surgery. One of the possible causes post-operatively may be related to the fact that as the majority of the gastric pouch being removed during LSG, less intrinsic factor and hydrochloric acid will be produced and available post-operatively. These factors are necessary for the absorption of vitamin B_{12}, thus abnormal blood levels of this vitamin is likely to be observed following LSG due to a reduced absorption (Hakeam et al., 2009; Rickers & McSherry, 2012; Snyder-Marlow et al., 2010, van Rutte et al., 2014). In the current study though, no patients presented with insufficient levels of vitamin B_{12} at any time-point throughout the study. Only one other study reported all patients presenting with sufficient vitamin B_{12} levels throughout the study (Ruiz-Tovar et al., 2012), while the remaining studies reported a prevalence rate for vitamin B_{12} abnormalities between 2.7 % and 9.3 % at baseline (Damms-Machado et al., 2012; Gehrer et al., 2010; Hakeam et al., 2009; Kehagias et al., 2011; Moize et al., 2012, van Rutte et al., 2014). These studies all reported a consistent increase in the proportion of patients with low vitamin B_{12}, to as much as 19.6 % at 6 months post-LSG (Hakeam et al., 2009).

No patients presented with a vitamin B_{12} abnormality in the current study, despite the fact that the vast majority of patients (> 87 %) did not receive any vitamin B_{12} supplementation. However, as most multivitamins contain a wide range of vitamins and minerals, including vitamin D, calcium, iron and vitamin $B_{12,}$ and the majority of patients in the current study were taking a multivitamin pre and post-LSG, supplement use could be a reason for the sufficient vitamin B_{12} levels observed

(Zelman, 2011). Another possible explanation for the absence of any vitamin B_{12} abnormalities post-LSG, is the storage of this vitamin in the tissue. Usually the storage will contain enough vitamin B_{12} to ensure sufficient blood levels for years before an abnormality will present (Koch & Finelli, 2010; Lovette et al., 2012).

In summary, one of the more important findings regarding the hematological parameters was that no patients presented with low vitamin B_{12} levels at any time-point, as this is otherwise a commonly found abnormality observed pre- and post-LSG. As with the other studies assessing hematological parameters following LSG, only a small percentage of patients (< 10 %) experienced low ferritin and MCV. Some patients experienced values above the normal reference values for ferritin, hemoglobin and MCV, which have not been reported in other studies. Ferritin blood levels decreased, hemoglobin values remained stable, MCV increased and vitamin B_{12} levels fluctuated following LSG, but there were no significant changes in the prevalence of abnormalities.

In contrast to what was anticipated, no deterioration in vitamin B_{12} was observed following LSG. The blood levels of hemoglobin remained stable and no significant changes was observed, which is consistent with the hypothesis. Increases in MCV was observed post-LSG, which is in contrast to the hypothesis, along with the overall decrease in MCV abnormalities. The overall changes of ferritin were fluctuating, which is somewhat consisting with the hypothesis.

5.3. Non-alcoholic fatty liver disease

Only two patients (2.1 %) in the study sample presented with a self-reported NAFLD diagnosis prior to undergoing LSG. This is far less than the prevalence rates generally reported for the overweight and obese population. One study reported as many as 34.5 % of patients presented with steatosis (NAFLD) prior to undergoing LSG (Karcz et al., 2010), while the prevalence rate in the general obese population is as high as 75 – 80 % (Kopec & Burns, 2011; Mahaling et al., 2013; Paschos & Paletas, 2009). One of the possible explanations for this low prevalence rate observed in the current study, could be that the majority of NAFLD patients are asymptomatic and are therefore not yet diagnosed (Mishra & Younossi, 2012; Schattenberg & Schuppan, 2011; Shifflet & Wu, 2009).

This theory is supported by the large proportion of patients actually presenting with abnormal blood values for one or more of the biochemical parameters associated with NAFLD. The vast majority of patients (80 %) experienced abnormal blood values prior to surgery, suggesting the actual prevalence rate of NAFLD among the patients might be higher than the 2.1 %. Also, a study by *Chisholm et al.* (2012) investigated certain biochemical parameters in a group of bariatric patients undergoing laparoscopic gastric banding (LGB), and compared the biochemical values between patients with or without NASH. The values of the biochemical parameters were significantly higher among the NASH patients for GGT, ALT, bilirubin, triglycerides and lower for HDL.

Many patients in the current study experienced an improvement or resolution of their pre-existing abnormalities following LSG, with a significant reduction in the proportion of patients with abnormal blood values at 3 months (60 %) and 6 months (50.5 %) post-LSG. The most commonly abnormalities related to NAFLD, were elevated concentrations of GGT, triglycerides, LDL and ALT along with low concentrations of HDL, which is similar to findings reported by other studies (Chalasani et al., 2012; Chisholm et al., 2012; Franzini et al., 2011).

ALT is one of the most commonly assessed liver enzymes and studies have shown that it is often increased in obese patients seeking bariatric surgery (Chisholm et al., 2012; Franzini et al., 2011). In the current study, 10.8 % of patients presented with levels above the normal reference range for ALT at baseline, but there was a significant improvement as no patients experienced this abnormality at the 6-month follow-up. This is consistent with the results reported by *Mattar et al.* (2005), who assessed the ALT values after three bariatric procedures, one of them being LSG. They observed a significant decrease in ALT post-surgery, although they did not elaborate on the specific results for each procedure.

GTT levels above the normal reference was the most common abnormality of the biochemical parameters associated with NAFLD reported in the current study. 41.9 % of the patients presented with high GGT levels at baseline, but the majority experienced a significant improvement as only 10.7 % continued to have high GGT at

the 6-month follow-up. A significant decrease in mean GGT level, from 36.2 U/L at baseline to 21.4 U/L at the 6-month follow-up, was also observed in the current study, which is similar to finding reported by *Ghaemi et al.* (2013). However, this significant decrease in mean GGT level reported in that study, was observed after patients experienced significant weight loss due to micronutrient interventions, not bariatric surgery. Fatty liver disease due to alcohol consumption is often associated with elevated GGT levels accompanied with normal ALT levels, which was not observed in the current study.

Only a small proportion of study subjects experienced elevated bilirubin at baseline (7.5 %), but due to a significantly increased mean blood concentration, there was a non-significant increase to 10.9 % at 6 months post-LSG. Similar results for bilirubin following LSG have not been reported elsewhere. In addition no significant changes in ALP mean values were observed post-operatively, and none of the patients in the study sample presented with elevated ALP at any time-point.

Abnormal lipid concentrations were common among the current study sample, with high triglyceride levels being the most prevalent pre-LSG (41.5 %). Abnormal LDL and HDL levels were also highly prevalent at baseline, with 28.3 % and 28.7 % of the patients, respectively. All mean blood values were within the normal reference values throughout the study, except for the mean triglyceride level at baseline (1.98 mmol/L).

A significant increase in HDL levels and a significant decrease in triglycerides levels were observed in the follow-up period in the current study. This is consistent with the findings by *Mattar et al.* (2005), who also reported improvement in the lipid profile following bariatric surgery. The improvements in blood values observed in the current study, corresponded with significant decrease in the proportion of patients with abnormal HDL and triglycerides values from baseline to 6 months post-LSG (28.7 % to 14.1 % and 41.5 % to 8.7 %, respectively) *Mattar et al.* (2005) reported similar findings, where the improvements in blood values in that study, resulted in decreased proportion of patients with abnormal lipids, although the differences from baseline to the follow-ups were only significant for triglycerides.

The levels of LDL fluctuated in the follow-up period. There was a significant decrease from 2.85 mmol/L at baseline to 2.61 mmol/L at 3 months post-LSG, with a non-significant decrease in the proportion of patients with high LDL from 28.3 % to 20.4 %. This is consistent with the studies by *Ghaemi et al.* (2013) and *Mattar et al.* (2005), who both reported significant decreases in mean LDL levels following bariatric surgery. However, a significant increase in mean LDL level was observed at the 6-month follow-up in the current study, with the proportion of patients with high LDL increasing from 20.4 % to 23.9 %, although not statistically significant.

Studies have examined the relationship between the status of the parameters associated with NAFLD and the severity of NAFLD and/or NASH. They found that patients with NAFLD or NASH in general have significantly higher blood levels of ALT, GGT and triglycerides as well as significantly lower levels of HDL (Chisholm et al., 2012; Franzini et al., 2011). The studies conducted by *Razavizade et al.* (2012) and *Mahaling et al.* (2013) reported that the levels of LDL, triglycerides and ALT decreased while the levels of HDL increased, as the severity NAFLD or NASH intensified. In addition, one of the studies also showed that a significantly higher proportion of patients presented with liver enzyme or lipid abnormalities when the severity of NAFLD or NASH was greater (Mahaling et al., 2013).

As mentioned earlier, the improvement of these biochemical parameters alone is not a reliable tool in the diagnosis or estimation of NAFLD or NASH. The biochemical changes following LSG hold little merit without liver biopsy or diagnostic imaging to support the findings. The FLI and the SI were proposed as a first-step before assessment by ultrasound or liver biopsy, as they are more accurate than the biochemical parameters alone. Based on the calculations of the indexes, 100 % of patients in the current study presented with a FLI > 60, ruling in fatty liver disease (either fatty liver disease or NAFLD). This proportion of patients is close to the proposed prevalence rate of 95 % as suggested by *Feijo et al.* (2013) and the prevalence ranging from 85 – 95 % as suggested by *Machado et al.* (2006). This, along with the 80 % of patients in the current study presenting with abnormal

values for the biochemical parameters, provides further support that NAFLD is underdiagnosed.

Although significant improvements were observed for the mean SI values post-LSG, the lack of pre-determined cut-offs makes it impossible to determine the proportion of patients with or without NAFLD. The SI needs to be externally validated and cut-offs needs to be determined, before it can be used clinically. To fully establish the accuracy and validity of both the FLI and SI, more studies needs to be conducted. In order to fully determine the effect of weight loss following LSG on the NAFLD in this study, an assessment of the changes in liver health according to ultrasound or liver biopsy would have been necessary.

In summary, the actual prevalence of NAFLD among the study sample is most likely higher than the 2.1 % reported, due to the purported high prevalence of NAFLD among the obese population and the large proportion of patients with abnormal blood values (80 %) and a FLI > 60 (100 %). The overall improvement in liver enzymes and lipid profile is consistent with the findings observed in other studies. The significant improvements of the parameters associated with NAFLD observed in this study along with the significant improvements in FLI and SI, may be attributed to the significant weight loss and improved liver health. As hypothesized, significant improvements in the majority of the biochemical parameters associated with NAFLD, mainly for ALT, GGT, HDL and triglycerides, were observed following LSG.

6. Limitations, clinical relevance and recommendations

This section will present the limitations of the current study, the clinical relevance of the findings along with recommendations for future research on the micronutrient status and NAFLD in patients undergoing LSG.

6.1. Study limitations

With a recruitment rate of 99.5 % the possibility of selection bias is limited, indicating a study sample representative of the population of individuals seeking the LSG procedure in Newfoundland & Labrador. The longitudinal prospective cohort design was appropriate in order to detect any abnormalities for micronutrients or related parameters and assess the status of liver parameters at baseline, and to evaluate these abnormalities and parameters at 3 and 6 months following LSG.

A possible limitation is the fact, that out of the 188 patients enrolled in the study, only 95 patients had completed their 3 and 6 months follow-up thus far. However, there is no indication that this subgroup of 95 patients were significantly different than the entire study population, thus the study findings are sufficiently generalizable. Still, this project is part of an ongoing study, therefore the sample size at each time-point is expected to increase as time progresses. The study is based on a pretest-posttest design without a control group, which could pose a limitation to the findings. Patients serve as their own controls, but because there is no independent control group, it is not possible to determine if the changes observed following LSG are in fact due to the LSG procedure itself.

A major limitation of this study, was the inability to determine the doses of vitamin D in the diverse number of supplements used by patients. Only the doses for vitamin D supplements were obtainable, but the majority of patients were taking vitamin D, a multivitamin (which likely contain vitamin D), a calcium supplement that may or may not also contain vitamin D, or an over-the-counter supplement. The total vitamin D consumption is unknown and therefore it is difficult to assess the impact of vitamin D consumption on the changes observed in 25-OH-D. The lack of statistical significance from the multivariate GEE analysis may have been attributed to the inability to analyze dose-response relationship and/or that too few patients were not taking a supplement in the follow-up period.

Future studies assessing 25-OH-D levels post-bariatric surgery should aim to determine the doses of any supplement at each follow-up along with the dietary history and sunlight exposure. The findings on 25-OH-D and PTH need to be interpreted with caution, as the missing data could potentially underestimate the actual outcome. This concern will be addressed in the ongoing study.

The baseline measurements for the micronutrient and related parameters were done after the initial orientation session, when patients were advised on healthy and nutritious diets and supplements. This could be a confounder and as such, the current baseline values might not provide the most accurate picture of the state of the patients receiving LSG, as they might have made changes to their diet

and supplement regimen before presenting for surgery. In addition, not all of the select micronutrient and related parameters measured in this study were examined in other studies identified on this topic of research. This limits the number of studies to draw comparisons with, thus making it difficult to draw any firm conclusions. In addition, some of the published studies are reporting findings for vitamin D and unless otherwise stated, an assumption was made that is was 25-OH-D, so it could be used for comparison.

The blood concentrations of several of the biochemical parameters can be affected by diet. Patients in the study were advised of proper diet before and after undergoing LSG, which could have had an impact on the blood values. However, without a proper dietary history or assessments of intake (diet and supplements), the impact on the results presented remains unknown. The issues that arose with the PTH laboratory assay may have contributed to a false increase in elevated PTH prevalence. In addition, the assay used by the province for its 25-OH-D analysis differs from the radioimmunoassays (RIA) used in most other laboratories. This in-house developed procedure is thought to report slightly higher blood values than what would have been reported by RIAs, whereas the RIAs tend to underestimate the true values of 25-OH-D. This could explain the low prevalence rate for 25-OH-D abnormalities observed in this study.

As all co-morbidities are self-reported, the number of co-morbidities reported is most likely biased. An example of this is the large proportion of patients with abnormal liver markers at baseline, but only three patients reporting a NAFLD diagnosis. The prevalence of co-morbidities reported is most likely conservative and underestimates the actual picture.

6.2. Clinical relevance

Micronutrient deficiencies and toxicities can only be determined if both the biochemical and the corresponding clinical symptoms are present in patients. Since this study is based solely on the biochemical findings, we are only examining the biochemical abnormalities, not deficiencies or toxicities. The mean values found in this study all remain within the normal references at baseline and post-operatively for the vast majority of the measured parameters. If the larger cohort confirms these data when all patients have completed their 24 months follow-up, and by other independent studies, it could suggest that LSG does not have an adverse effect on these biochemical parameters for the majority of patients.

The diagnosis or staging of NAFLD and NASH are usually based on the findings from a liver biopsy or diagnostic imaging, not by the biochemical liver markers alone. However, liver enzymes and other liver parameters are often used as a helpful tool when combined with biopsy and imaging, such as ultrasound. The improvement of parameters associated with NAFLD in this study could possibly

indicate an improvement in overall liver health as well, but this would have to have been confirmed by the liver biopsy. The results from this study could be used in clinical practise, if future studies can confirm the relationship between liver improvement and liver marker improvement.

6.3. Recommendations for future research

Future research in this area is important in order to fully understand the consequences of bariatric surgery on patients' micronutrient and related parameters. This is important not only for the LSG procedure, but long-term studies for all bariatric surgery procedures, as there continues to be discrepancies on the changes in micronutrient and related parameters post-operatively. More longitudinal studies or randomized controlled trials should be conducted in order to determine the status in patients undergoing this procedure prior to and post-surgery. As these abnormalities can be present prior to surgery, assessments should be made at this time, and any abnormality should be treated accordingly. An assessment should also be performed at several time-points post-LSG, to diagnose and treat any developing abnormalities of the micronutrients and related parameters.

In addition, in order to fully assess the true impact of supplement use on changes in 25-OH-D blood levels post-LSG, the intake of vitamin D supplements needs to be quantified. Without this, it will not be possible to determine the relative effects of supplements, weight loss, and seasonal variations on the post-surgery 25-OH-D levels.

Prospective studies or a randomized controlled study aimed at the outcomes for NAFLD and NASH following LSG is greatly needed. It should be conducted with the use of diagnostic imaging, biochemical markers and possibly liver biopsies, all performed pre- or during surgery as well as at one or more follow-up time points. Overtime, this existing cohort of surgical patients will both grow in number and length of follow-up, adding clinically rich data that will help answer questions around the status of the biochemical parameter associated with micronutrients and NAFLD post-LSG.

7. Conclusions

A significant improvement in 25-OH-D was observed following LSG, but it remains uncertain whether this improvement was due to the LSG itself or the increased use of vitamin D-containing supplements. The status of calcium, ferritin, hemoglobin and MCV did not change significantly following LSG. Although there was an initial increase in the proportion of patients with elevated PTH levels, this may have been attributed to technical issues at the Health Sciences Centre. A significant inverse correlation between absolute weight and 25-OH-D was demonstrated both prior to- and following surgery, along with a significant inverse correlation between weight loss and the increase in 25-OH-D levels from baseline to the 3-month follow-up.

Significant improvements were observed for the majority of the biochemical parameters associated with NAFLD, with a decreasing proportion of patients with abnormal blood levels for ALT, GGT, HDL and triglycerides observed post-surgery. Significant inverse correlations were observed for body weight and mean HDL blood levels at all three assessments (baseline, 3 and 6 months), along with a weak correlation for weight and LDL changes from the 3- to the 6-month follow-up.

A significant improvement in the proportion of patients with a Fatty Liver Index > 60 was observed in post-operatively, along with significant improvements in the Steatosis Index. The improvements in the biochemical parameters associated with NALFD in addition to the improvements observed for both the Fatty Liver Index and the Steatosis Index, may be attributed to the significant weight loss observed post-operatively.

The weight loss achieved by LSG may have beneficial impacts on the biochemical parameters associated with micronutrient status and non-alcoholic fatty liver disease.

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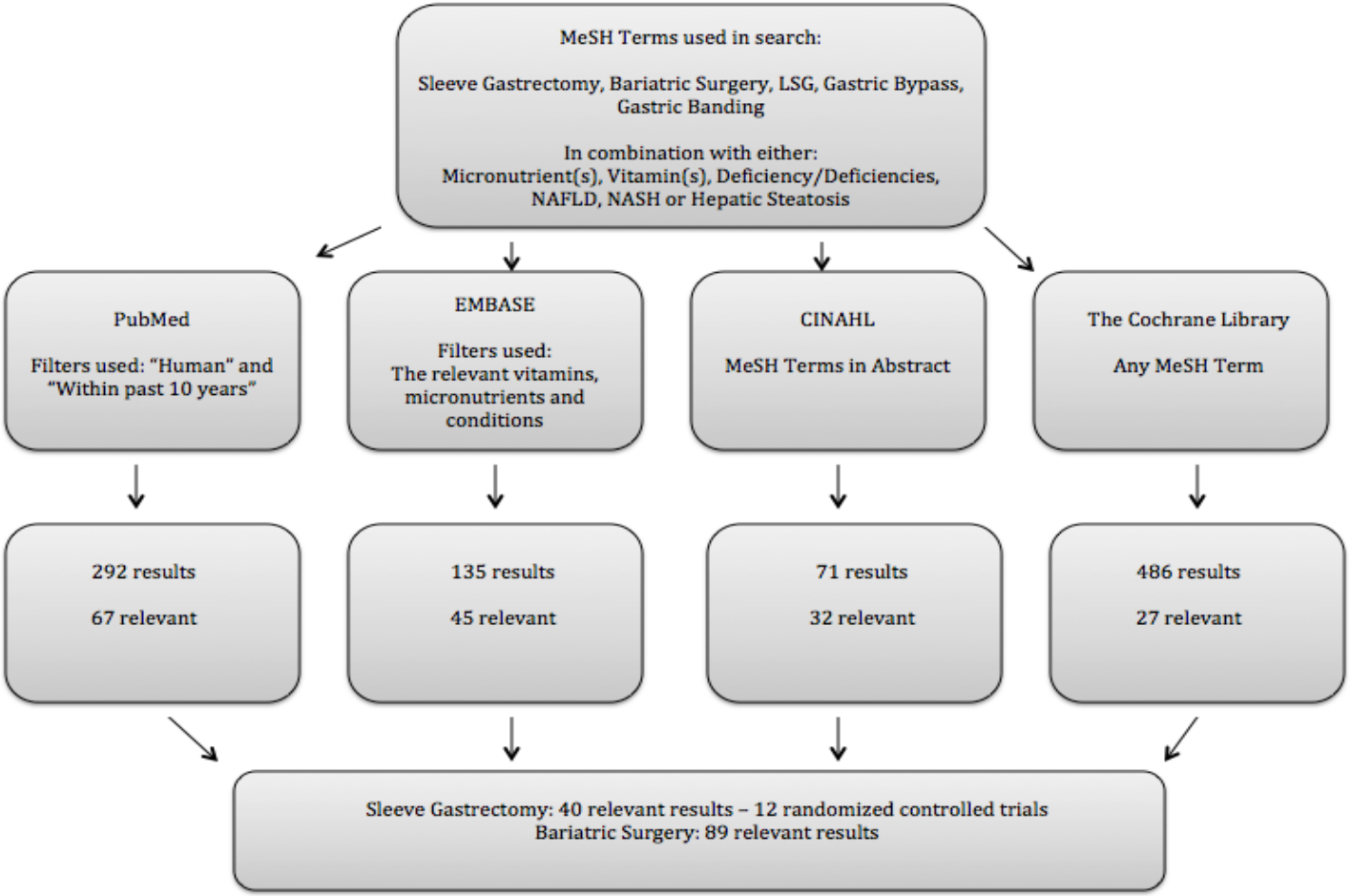
APPENDICIES

Appendix 2.1. Literature search



Appendix 3.1. Ethics Approval

Health Research Ethics Authority

received MAY 0 1 2013

Request For Ethics Renewal / Study Closure

- The Tri-Council Policy Statement- Ethical Conduct for Research Involving Humans (TCPS2; 2010) (article 6.14) requires ongoing review by the approving REB at least on an annual basis. The information provided in this form must be current to the time of submission and submitted to the HREA not less than 30 days nor more than 45 days before the anniversary of your approval date.
- Ethics approval is required if there is ongoing subject contact or data collection/transfer is active.
- Ethics approval is not required and the file may be closed if the project is in analysis or the writing stage.
- Please forward a summary of findings or published abstract to the HREA Office once the study is complete.
- Incomplete forms will not be accepted and may result in delay in the review and approval process
- (For clinical trials only) If the project is complete – please submit the applicable Study Closure form.

HREB Ref Number: 11.101	Expiry Date of Current Approval 06/08/2013
Principal Investigator: Dr. Laurie Twells	
Title of study (with Protocol Number if applicable): The Newfoundland and Labrador Bariatric Surgery Cohort Study (The NL BaSco Study)	
Email of PI : ltwells@mun.ca	Email of Key Contact: Kimberley.manning@easternhealth.ca

Please chose one:		
OR	*I am requesting renewal of ethics approval for this file.*	X
	I am requesting to close this file.	

Dr. Laurie Twells — Name typed or printed

Signature of PI

04/29/2013 — Date (MM/DD/YYY)

For HREB Office Use Only:

This project was reviewed on May 6, 2013 (Date) *By Full Board Review* ☐ *By Expedited Review* ☑

Ethics approval for this project has been granted for a period of 12 months effective *From* June 9, 2013 *to* June 9, 2014

This research ethics board (the HREB) has reviewed and approved the study which is to be conducted by you as the qualified investigator/principal investigator named above. This approval and the views of this Research Ethics Board have been documented in writing. The Health Research Ethics Board operates according to *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, ICH Guidance E6: Good Clinical Practice: Consolidated guideline* 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.

This file has been closed as requested ☐

Signature Chair

APPROVED MAY 0 6 2013

Date

Page 1 of 3

Recruitment/Data Collection

Has the study started?	Yes	X	No	

If yes, please provide the following information as it applies to your project.

	Total planned for this site	Total to date *(if applicable)*	N/A
A. Number of Participants enrolled	200	152	
B. Number of Health Records reviewed			X
C. Number of tissue samples collected			X
D. Number of surveys returned			X

If more or fewer than expected, why?

Consent Form

Does this project have a consent form?	Yes	X	No	
If yes, Please give the date of the most recently approved consent form	06	14	2011	

For Clinical Trials Only *which are subject to ICH & Health Canada and required to report SAEs and SUSAR's to the REB*
Serious Adverse Event/s (SAE's) Or Suspected Unexpected Serious Adverse Reactions (SUSARS)

1. **Since Last Approval** a. Have DSMB/ QSR reports been submitted to HREB?	Yes ☐	No ☐
2. **Since Last Approval** **a.** Has there been amendments to this protocol as a result of safety reports ? *If yes, please provide a list amendment dates*	Yes ☐	No ☐
3. **Since Last Approval** a. Have you reported local SAE's? b. **If yes**, please provide number of **local** events:_____	Yes ☐	No ☐
4. **Since Last Approval** a. Have you reported deviations to the sponsor? b. **If yes**, please provide number of **Deviations:** _____	Yes ☐	No ☐
5. **Since Last Approval** a. Have you requested waivers? b. **If yes**, please provide number of **waivers:** _____	Yes ☐	No ☐

All Other Studies: Since Last Approval		
1. Have there been unexpected events or problems related to participant risk since original approval or last ethics renewal?	Yes [ ]	No [X]
2. Has there been amendments submitted for this project? Approved administrative amendments dated October 11, 2011, February 21, 2012,and May 29,2012.	Yes [X]	No [ ]
If yes, please describe the events/problems/amendments : (Add an addendum to this form if necessary)		

All Studies - Status At Local Site (check all that apply)	YES	NO	N/A
1. Intervention/data collection active	X		
2. Closed to recruitment/accrual		X	
3. Participants in follow up	X		
4. Site closed (clinical trials only)			X
5. **For secondary use of date only** is Data Transfer Complete			X

Knowledge Transfer	YES	NO	N/A
1. Have participants been informed of study findings?		X	
2. Have findings been presented/published? *Please indicate where: (Add an addendum to this form if necessary)*		X	

Additional Information :

Appendix 4.1. Laboratory changes of the biochemical parameters

Laboratory values for bone health parameters at each time-point.

	Normal reference	Baseline	3 months	6 months
25-OH-D	50-250 nmol/L	[a] 69.6 ± 24.7 (94) [b] 24 - 156	[a] 78.4 ± 23.9 (79) * [b] 35 – 160	[a] 81.1 ± 23.8 (69) * [b] 40 – 148
Calcium	2.12-2.62 mmol/L	[a] 2.44 ± 0.10 (93) [b] 2.2 – 2.8	[a] 2.43 ± 0.09 (93) [b] 2.2 – 2.7	[a] 2.46 ± 0.11 (88) † [b] 2.2 – 2.8
PTH	12-72 ng/L	[a] 62.9 ± 35.0 (93) [b] 11 – 256	[a] 74.1 ± 37.9 (89) * [b] 23 - 259	[a] 70.9 ± 30.2 (87) * [b] 33 - 201

*Note: Repeated ANOVA/Friedman's with Wilcoxon's or GEE for variables with significant missing data points. [a] Mean +/- SD (n). [b] Range (min – max). * p-value < 0.05 when compared to baseline, † p-value < 0.05 when compared to 3 months.*

Laboratory values for hematological parameters at each time-point.

	Normal reference	Baseline	3 months	6 months
Ferritin	Male: 24.0 – 336 µg/L	[a] 250.6 ± 206.6 (17) [b] 63 – 832	[a] 182.9 ± 128.2 (17) * [b] 55 – 493.9	[a] 136.2 ± 65.0 (17) *† [b] 37.8 – 366.7
	Female: 11.0 – 307 µg/L	[a] 130.2 ± 137.1 (78) [b] 6 – 947	[a] 110.9 ± 96.6 (71) * [b] 6 – 561	[a] 99.4 ± 97.4 (73) *† [b] 4 – 517
Hemoglobin	Male: 140 – 180 G/L	[a] 145.5 ± 12.8 (17) [b] 114 – 167	[a] 144.3 ± 11.8 (17) [b] 117 – 162	[a] 144.8 ± 12.2 (16) [b] 114 – 159
	Female: 120 – 160 G/L	[a] 136.9 ± 11.2 (78) [b] 105 – 163	[a] 136.4 ± 12.6 (76) [b] 98 – 177	[a] 134.8 ± 12.2 (75) [b] 86 – 171
MCV	Male: 80 – 97 fL	[a] 89.8 ± 2.7 (17) [b] 85.9 – 94.4	[a] 89.6 ± 2.9 (17) [b] 86.0 – 95.0	[a] 91.1 ± 2.9 (16) *† [b] 85.2 – 97.7
	Female: 81 – 99 fL	[a] 88.4 ± 4.9 (78) [b] 75.8 – 100.0	[a] 88.9 ± 5.2 (76) [b] 69.9 – 101.7	[a] 89.1 ± 5.6 (75) * [b] 67.0 – 101.7
Vitamin B12	107 – 675 pmol/L	[a] 359.9 ± 159.1 (95) [b] 157 – 1016	[a] 366.4 ± 178.3 (87) [b] 140 – 1332	[a] 324.9 ± 178.3 (90) † [b] 118 - 854

*Note: Repeated ANOVA/Friedman's with Wilcoxon's. [a] Mean +/- SD (n). [b] Range (min – max). M: Males, F: Females. * p-value < 0.05 when compared to baseline, † p-value < 0.05 when compared to 3 months.*

Laboratory values for parameters associated with NAFLD at each time-point.

	Normal reference	Baseline	3 months	6 months
Bilirubin	< 20 µmol/L	[a] 12.2 ± 5.1 (93) [b] 5 - 36	[a] 12.3 ± 5.2 (94) [b] 2.1 – 39.0	[a] 13.2 ± 6.2 (92) * [b] 5 - 48
ALP	40 – 150 U/L	[a] 85.7 ± 22.5 (93) [b] 36 - 145	[a] 82.2 ± 22.1 (92) [b] 38 - 144	[a] 82.5 ± 20.3 (91) [b] 32 - 144
ALT	< 55 U/L	[a] 31.5 ± 17.9 (93) [b] 9 - 111	[a] 26.0 ± 15.1 (92) * [b] 7 - 97	[a] 18.1 ± 7.5 (90) *† [b] 7 - 38
GGT	< 32 U/L	[a] 36.2 ± 22.8 (93) [b] 10 – 177	[a] 23.9 ± 13.9 (89) * [b] 6 – 100	[a] 21.4 ± 16.3 (84) *† [b] 5 – 136
HDL	> 0.9 mmol/L	[a] 1.04 ± 0.22 (94) [b] 0.52 – 1.69	[a] 1.02 ± 0.18 (93) [b] 0.64 – 1.49	[a] 1.12 ± 0.22 (92) *† [b] 0.76 – 1.81
LDL	< 3.4 mmol/L	[a] 2.85 ± 0.98 (92) [b] 1.03 – 5.12	[a] 2.61 ± 0.90 (93) * [b] 0.92 – 5.55	[a] 2.79 ± 0.88 (92) † [b] 0.66 – 5.33
Triglycerides	< 1.9 mmol/L	[a] 1.98 ± 0.92 (94) [b] 0.83 – 6.17	[a] 1.42 ± 0.44 (93) * [b] 0.63 – 3.02	[a] 1.33 ± 0.48 (92) *† [b] 0.54 – 3.52

*Note: Repeated ANOVA/Friedman's with Wilcoxon's. [a] Mean +/- SD (n). [b] Range (min – max). M: Males, F: Females. * p-value < 0.05 when compared to baseline, † p-value < 0.05 when compared to 3 months.*

Appendix 4.2. Univariate and multivariate generalized estimating equation models

25-OH-D and Time:

Test of Model Effects

Source	Type I			Type III		
	Wald Chi-Square	df	Sig.	Wald Chi-Square	df	Sig.
(Intercept)	1111.864	1	.000	1133.056	1	.000
Time	29.103	2	.000	29.103	2	.000

Dependent Variable: Vitamin D

Model: (Intercept), Time

Parameter Estimates

Parameter	B	Std. Error	95 % Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	69.896	2.5635	64.872	74.920	743.437	1	.000
[Time=3]	10.862	2.4666	6.028	15.697	19.393	1	.000
[Time=2]	8.799	1.7883	5.294	12.304	24.208	1	.000
[Time=1]	0[a]	.	.	.	.	.	.
(Scale)	584.173						

Dependent Variable: Vitamin D

Model: (Intercept), Time. a. Set to zero because this parameter is redundant

25-OH-D and Seasons:

Season 1. Winter: December 21st – March 19th

Season 2. Spring: March 20th – June 20th

Season 3. Summer: June 21st – September 21st

Season 4. Fall: September 22nd – December 20th

Test of Model Effects

Source	Type I			Type III		
	Wald Chi-Square	df	Sig.	Wald Chi-Square	df	Sig.
(Intercept)	1073.913	1	.000	1082.797	1	.000
Seasons	1.986	3	.575	1.986	3	.575

Dependent Variable: Vitamin D

Model: (Intercept), Seasons

Parameter Estimates

Parameter	B	Std. Error	95 % Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	73.608	2.9331	67.859	79.357	629.781	1	.000
[Seasons = 4]	2.294	2.6308	-2.862	7.450	.760	1	.383
[Seasons = 3]	3.814	2.9878	-2.042	9.670	1.630	1	.202
[Seasons = 2]	.560	2.5942	-4.524	5.645	.047	1	.829
[Seasons = 1]	0[a]	.	.	.	.	.	.
(Scale)	611.075						

Dependent Variable: Vitamin D

Model: (Intercept), Seasons

a. Set to zero because this parameters is redundant

25-OH-D and Overall Supplement use:

Test of Model Effects

Source	Type I			Type III		
	Wald Chi-Square	df	Sig.	Wald Chi-Square	df	Sig.
(Intercept)	1093.424	1	.000	909.489	1	.000
Overall Supplement Use	15.778	1	.000	15.778	1	.000

Dependent Variable: Vitamin D

Model: (Intercept), Overall Supplement Use

Parameter Estimates

Parameter	B	Std. Error	95 % Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	68.104	2.9611	62.300	73.908	528.977	1	.000
[OSU = 1]	8.884	2.2365	4.500	13.267	15.778	1	.000
[OSU =0]	0[a]	.	.	.	.	.	.
(Scale)	598.035						

Dependent Variable: Vitamin D

Model: (Intercept), OSU = Overall Supplement Use. 1 = Yes, 0 = No

a. Set to zero as this parameter is redundant.

25-OH-D, Time and Overall Supplement use:

Test of Model Effects

Source	Type I			Type III		
	Wald Chi-Square	df	Sig.	Wald Chi-Square	df	Sig.
(Intercept)	1081.2443	1	.000	855.033	1	.000
Time	26.377	2	.000	13.900	2	.001
Overall Supplement Use	.253	1	.615	.253	1	.615

Dependent Variable: Vitamin D

Model: (Intercept), Time and Overall Supplement use

Parameter Estimates

Parameter	B	Std. Error	95 % Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	68.990	3.0995	62.915	75.065	495.427	1	.000
[Time=3]	10.335	2.9856	4.483	16.186	11.982	1	.001
[Time=2]	7.943	2.4495	3.142	12.744	10.515	1	.001
[Time=1]	0[a]	.	.	.	.	.	.
[OSU = 1]	1.528	3.0394	-4.429	7.485	.253	1	.0615
[OSU = 0]	0[a]	.	.	.	.	.	.
(Scale)	599.954						

Dependent Variable: Vitamin D

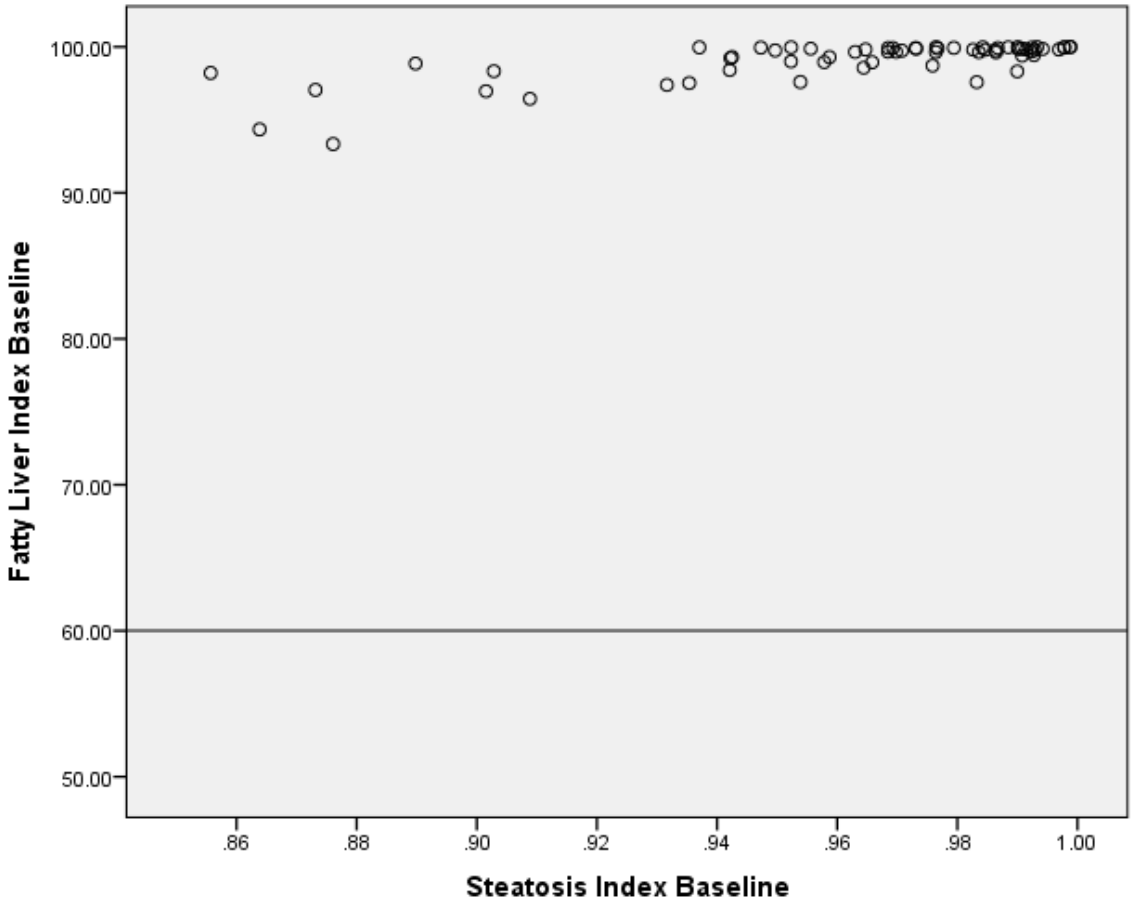
Model: (Intercept), Time and OSU = Overall Supplement Use. 1 = Yes, 0 = No.

a. Set to zero as this parameter is redundant.

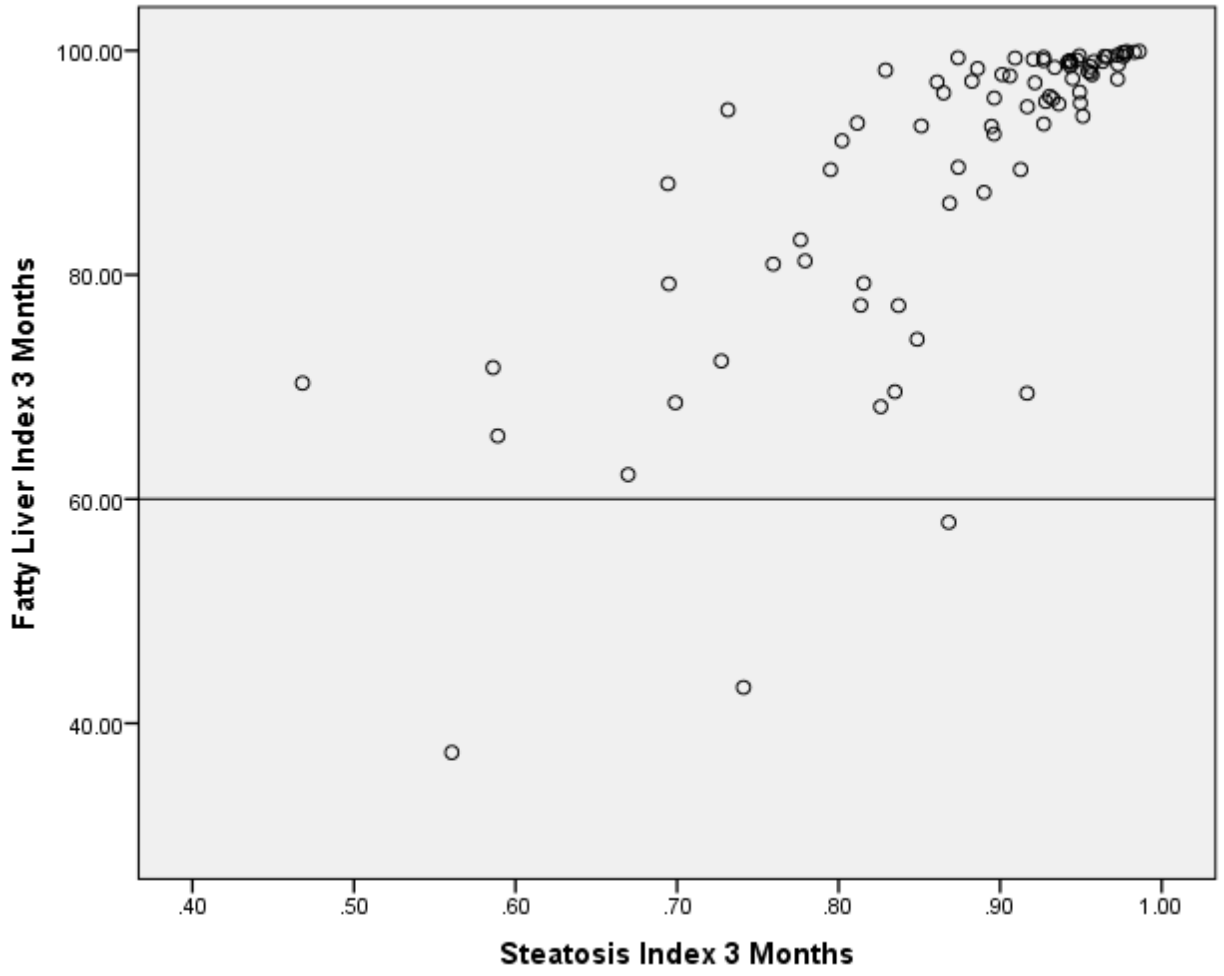
Appendix 4.3. Scatterplot and correlation analysis for the Fatty Liver Index and the Steatosis Index

	Baseline	**3 months**	**6 months**
r	0.611	0.750	0.851
p-value	0.000	0.000	0.000
Type of correlation	Moderate, Positive	Strong, Positive	Strong, Positive

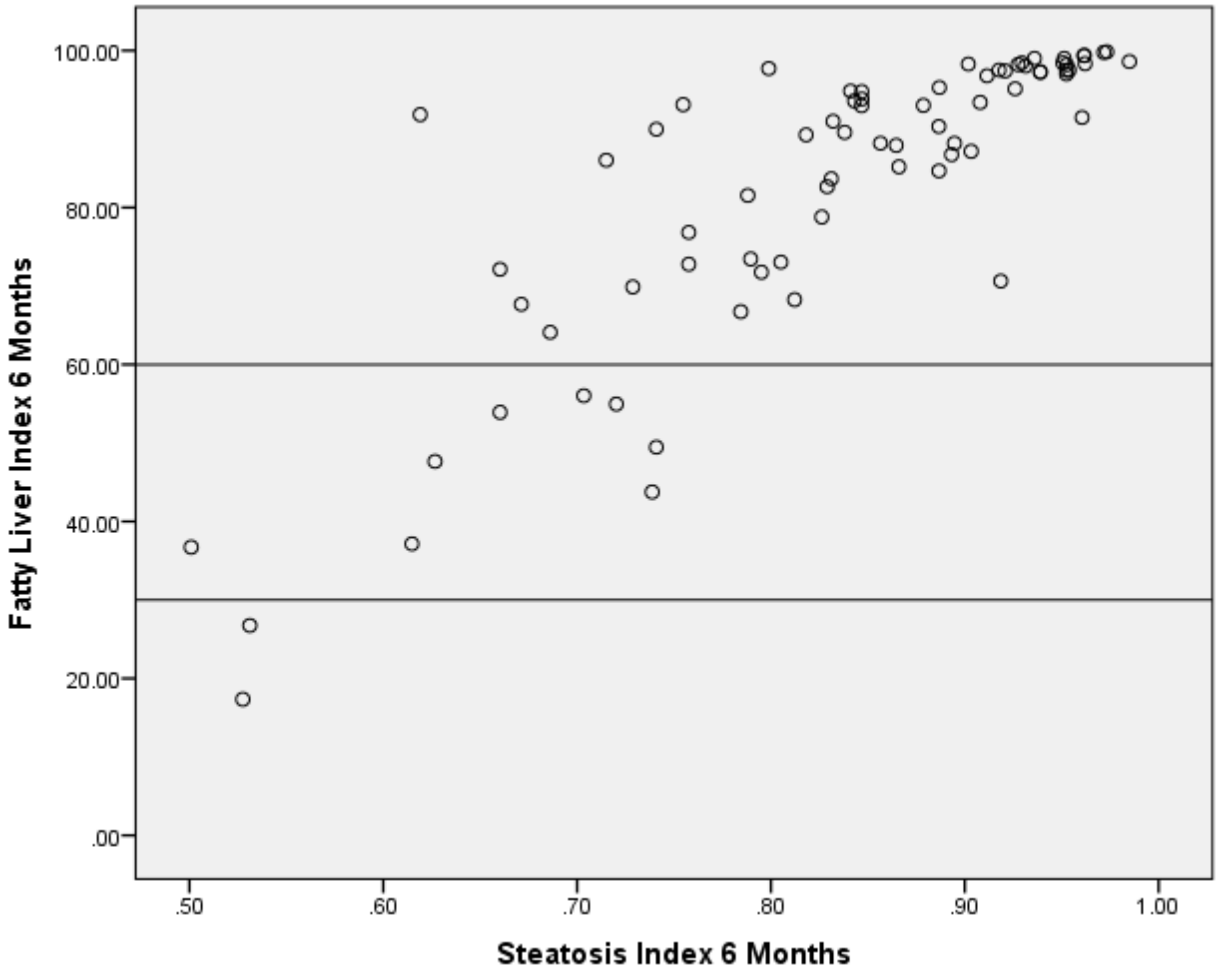
Baseline


Fatty Liver Index Baseline
100.00
90.00
80.00
70.00
60.00
50.00
.86
.88
.90
.92
.94
.96
.98
1.00
Steatosis Index Baseline

3 months


Fatty Liver Index 3 Months
100.00
80.00
60.00
40.00
.40
.50
.60
.70
.80
.90
1.00
Steatosis Index 3 Months

6 months


Fatty Liver Index 6 Months
100.00
80.00
60.00
40.00
20.00
.00
.50
.60
.70
.80
.90
1.00
Steatosis Index 6 Months