ALTERATION OF THE VITAMIN D ENDOCRINE SYSTEM IN OBESITY:
THE ROLE IN PATIENTS UNDERGOING BARIATRIC SURGERY

by
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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
March 2015

ABSTRACT

We knew that: 1) vitamin D (VitD) deficiency is a burgeoning problem; 2) a major risk factor for VitD deficiency is obesity—also escalating; and 3) VitD deficiency is a side effect of some bariatric surgeries—the most durable treatment for obesity. We investigated the alteration of the VitD endocrine system in obesity as it relates to bariatric surgery patients.

We used two proxy measures, seasonality and latitude, to assess group VitD status in the Nationwide Inpatient Sample (NIS). We found that adverse outcomes following bariatric surgery are most common from January to March when VitD status is lowest (VitD Winter). The largest increased odds from VitD Summer (highest VitD status) to Winter were for wound infection. The strongest correlations were for dehiscence and extended length of stay (LOS). Adverse outcomes appeared to be more common in the North with LOS reaching significance ($p<0.001$). We replicated these findings in adolescents, a growing subset in bariatric surgery. We were only sufficiently powered to detect a difference in LOS by latitude, yielding a significant negative correlation between VitD status and LOS ($p=0.012$). VitD supplementation, an easy and inexpensive treatment, could potentially mitigate risks following bariatric surgery.

To assess pre-operative nutritional status in bariatric candidates at our center, we measured vitamins A, B-12, D, E-α, E-β/γ, thiamine, folate, and iron—the deficiencies seen most commonly post-operatively. We found that malnutrition in one or multiple micronutrients is pervasive, especially VitD and iron. VitD deficiency was present in 71.4% of candidates (81.3% in blacks). Minorities tended to have greater malnutrition overall. The effect of pre-operative supplementation should be explored.

We investigated VitD testing and therapy in our bariatric candidates. VitD testing was lower than clinically indicated. At least 44.5% have more than one risk factor for VitD deficiency, since these patients have high melanin concentration. However, we had serum 25(OH)D concentration for only 18.5% of candidates. A significant proportion had not been tested but are likely to be VitD deficient. Candidates who were tested were not different from those who were not. We would like to improve the rate of VitD testing in our candidates.

PREFACE

All the work henceforth presented was conducted in the Johns Hopkins Center for Bariatric Surgery (JHCfBS) at the Johns Hopkins Bayview Medical Campus (JHBMC) with support from the Johns Hopkins Center for Surgical Trials and Outcomes Research (CSTOR). All projects were approved by the Johns Hopkins Medicine Institutional Review Boards (IRBs) and were conducted in accordance with the federal Health Insurance Portability and Accountability Act of 1996 (HIPAA) and the Declaration of Helsinki. I was the lead investigator and was responsible for all key areas of these projects including original concept, data collection and analysis, and preparation of the manuscripts. I have no conflict of interests to disclose.

My mentor throughout this work was Kimberley Steele, MD, PhD, FACS, a bariatric surgeon and the Director of Research at the JHCfBS. My formal advisor during this work was Lawrence J. Cheskin, MD, a leader in the field of weight management and obesity research. I would also like to acknowledge Michael Schweitzer, MD, FACS, Director of Bariatric Surgery at JHCfBS, and Thomas H. Magnuson, MD, FACS, Chief of General Surgery and Founder of the JHCfBS, for their support and expertise throughout this work.

The expertise of Joseph Canner, MHS, the Senior Research Data Manager for CSTOR, was crucial in the creation of Chapters 2 and 3. Mr. Canner was able to add the latitude of each hospital into the dataset, yielding our second proxy measure of vitamin D status. We would like to acknowledge the Health Care Cost and Utilization Project Data Partners, listed at http://www.hcup-us.ahrq.gov/db/hcupdatapartners.jsp, without which the Nationwide Inpatient Sample (NIS) dataset used in Chapters 2 and 3 would not exist. Additionally, for Chapter 3, I would like to acknowledge my research assistants Rani Matuk, Bariatric Medical Tutorial Student, and Hatim AlSulaim, CSTOR Research Fellow.

The laboratory analysis of baseline nutritional status in Chapter 4 was possible due to the generous support of YASOO Health, Inc. as part of their clinical trial of a newly formulated chewable multivitamin/mineral supplement. This funding source played no role in the baseline study design, collection, analysis or interpretation of the data, manuscript preparation, or the decision to publish this manuscript in the future.

I would like to acknowledge Eva Kelly, Database Coordinator at JHCfBS, for her help in identifying patients for Chapter 5. I would also like to acknowledge the surgeons and staff of JHCfBS without whom this work would not have been possible.

I would also like to acknowledge my support system of family and friends including my parents, Connie and David Frame; my brother, David Frame, Jr.; my parents-in-law, Carolyn and Eric Peterson; my siblings-in-law, Laura and Daniel van der Bergh; and my best friend, Jennifer Schleiger. Finally, I would like to recognize my husband, Matthew Peterson, Esq., as the keystone in my support system. You drive me to be my best self.

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CHAPTER 1: INTRODUCTION

OBESITY

Obesity is a syndrome, a set of symptoms that occur together, due to excess body fat mass (adipose tissue). Adipose tissue is a highly active endocrine organ.[7-9] Excess adipose tissue leads to a pro-inflammatory state by altering production of cytokines such as interleukin-1β (IL-1β), IL-6, IL-33, and tumor necrosis factor-α (TNF- α) and adipokines such as leptin, adiponectin, and C1q/TNF-related protein 3 (CTRP3).[7-13] Such chronic inflammation predisposes an individual to chronic disease, e.g. metabolic syndrome, cardiovascular disease, non-alcoholic steatohepatisis (fatty liver disease), and cancer.[7-9;14]

PREVALENCE

In the United States (US), over 1/3 of adults have obesity (BMI ≥ 35 kg/m^2) according to the National Health and Nutrition Examination Survey (NHANES) 2011-2012.[15] There was no observed difference in obesity rates between men and women except in the subset of non-Hispanic blacks where 56.6% of women and 37.1% of men had obesity.[15] Non-Hispanic blacks and Hispanics are disproportionately represented in obesity rates of 47.8% and 42.5% respectively compared to 32.6% for non-Hispanic whites.[15] Non-Hispanic Asians had the lowest rate of obesity at just 10.8%.[15]There has been some indication that current cut-points for obesity are not suitable for patients of Asian-descent due to higher fat mass at lower BMIs, which may mask a higher rate of obesity in this population.[16;17] The overall rate of obesity shows no prospect of decline, remaining steady since NHANES 2003-2004.[15] Medical care for the more than 78 million American adults with obesity costs an estimated $147 billion annually.[18-20]

In NHANES 2011-2012, 20.5% of 12 to 19 year olds had obesity.[15] The incidence of adolescent obesity has more than quadrupled in the last 30 years.[15] Obesity was slightly more prevalent in non-Hispanic black (22.1%) and Hispanic (22.6%) adolescents than non-Hispanic white adolescents (19.6%).[21;22] Childhood obesity continues into adulthood—more than 3 in 4 obese children become obese adults.[23;24] The highest rate of obesity is in middle-aged adults (39.5%); however, obesity in childhood is particularly concerning due to long term exposure to the chronic inflammation associated with obesity. [15]

Globally, there is a trend towards increased BMI with the greatest increase in the top 5% of the distribution of BMI.[15] The overal rate of obesity has more than doubled in the last 30 years, affecting over 600 million adults worldwide in 2014.[25] The majority of human beigns on Earth now live in a country where overweight and obesity is a bigger killer than underweight.[25] Many of the countries newly suffering the effects of obesity are middle and low income countries where underweight and other malnutrition issues are concurrent in the same population and in many instances within the same family or even same individual.

COMORBIDITIES AND COMPLICATIONS

The high prevalence of obesity and the serious comorbidities, make obesity a major public health concern. Obesity is a leading contributor to global mortality and the burden of disease associated with diabetes, cardiovascular disease, musculoskeletal disorders such as osteoarthritis and back pain, and some cancers.[15;26;27] An increased susceptibility to infection such as surgical site infection and nosocomial (hospital acquired) infection is also a hallmark of obesity.[28] Individuals with obesity who contract an infection are also more likely to succumb to serious complications of that infection.[28] Class 3 obesity (BMI 40-49.9 kg/m^2) reduces life expectancy by 8 to 10 years, similar to the effect of regular cigarette smoking.[29]

Childhood obesity increases the risk of hyperlipidemia and hypertension and in turn the risk for cardiovascular disease.[25;30;31] As in adults, adolescents with obesity are more likely to be pre-diabetic or diabetic.[25;30] Approximately 215,000 Americans under the age of 20 have diabetes.[32-37] Other comorbidities that are more common in children with obesity include respiratory disease and poor bone mineralization.[25] Obesity is also associated with decreased quality of life and social exclusion, which can be particularly detrimental to a developing individual and their mental health.[23-25;38] Disability and premature death are more common in children with obesity.[25] Since most children with obesity become obese adults, childhood obesity is likely to lead to the chronic diseases associated with adult obesity. [23;24 39;40]

BARIATRIC SURGERY

Bariatric surgery is currently the most successful means of sustained long-term weight loss. During bariatric surgery, the digestive tract is restricted in size with focus on the stomach. There are various different procedures that have been developed over many decades, some of which also involve manipulating the intestines.

DEVELOPMENT

Patients who had significant portions of their stomachs or small intestines removed were observed to lose significant weight following such procedures, short-gut syndrome.[40;41] This observation, followed by much trial and error (see Figure 1), ultimately led to modern bariatric surgery. During development, bariatric surgery was thought to function based on two basic principles: restriction and malabsorption. Restriction occurs by decreasing the size of the stomach, and therefore mechanically limiting the amount of food required to create a sense of fullness. Malabsorption occurs when portions of the digestive tract are bypassed (removed from

the digestive process), such as the small intestines, decreasing the distance and thus time to absorb calories from food after it is ingested.

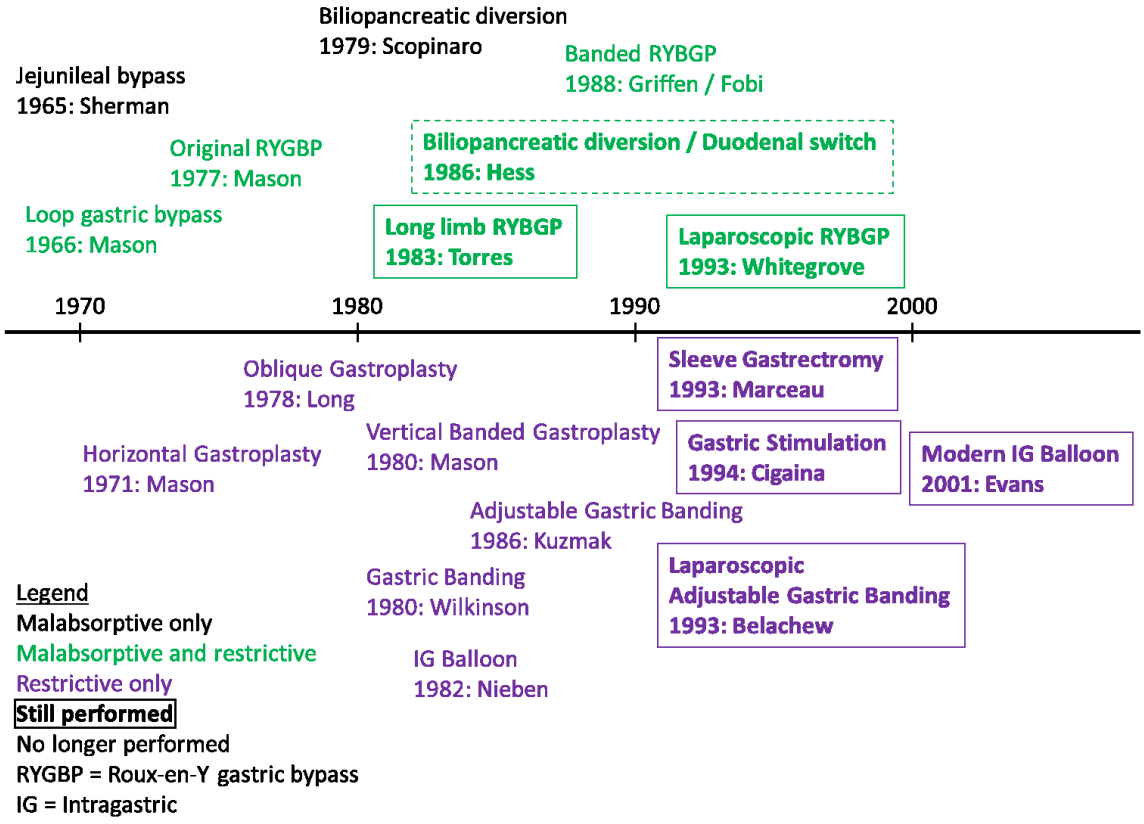

FIGURE 1 TIMELINE: DEVELOPMENT OF BARIATRIC SURGERY PROCEDURES

The earliest procedures functioned exclusively through malabsorption, but resulted in numerous complications such as severe macronutrient deficiency. With the development of restrictive procedures, many of the complications were eliminated or at least mitigated; however, the weight loss following these procedures was limited compared to those involving malabsorption. Eventually, combining the two principles would produce a safer and more effective bariatric surgery procedure.

With the advancement of bariatric surgery came improved understanding of the mechanism of action behind these procedures. While the classic understanding of malabsorption and restriction still play a key role in determining the best procedure for a patient, there is another category of action for these procedures. This category is the physiological alteration of the patient. For instance if the greater curvature of the stomach is removed the neuroendocrine activity along the gut-brain axis is substantially altered, allowing for drastic calorie reduction without activation of starvation mechanisms. The specific mechanisms behind the physiological effect of bariatric surgery on the gut-brain axis relates to alterations in adipokines and gut hormones, e.g. leptin, ghrelin, and GLP-1.[42] The rewards system in the brain is also affected by bariatric surgery with dopamine (D2) binding increasing after Roux-en-Y gastric bypass.[42;43]

Bariatric procedures utilizing malabsorption alone

All of the subsequent bariatric surgery procedures function primarily via alteration of the digestive tract to cause malabsorption. Malabsorption leads to reduced calories being gleaned from food but also to deficiencies in micronutrients, particularly fat soluble vitamins (A, D, E, and K).

1965: Jejunoileostomy

In the jejunoileostomy (JI) bypass procedure, the proximal jejunum is connected to the distal ileum—an anastomosis. The end-to-side configuration was discontinued in favor of an end-to-end configuration, which prevented reflux into the ileum. This reflux was the route of suboptimal weight loss in 10% of patients.[40] With improved weight loss came significant complications due to malabsorption of nutrients. Additionally, most patients developed atrophy of the intestines, leading to bacterial overgrowth (bypass enteritis) that could then lead to liver damage or death.[44] Most mortality in the JI bypass typically occurred within 2 years post-

operation at a rate of 4%, mostly due to liver failure.[40;45;46] The high rate of serious complications and mortality in the JI bypass not only led to the discontinuation of the procedure itself but for the field of bariatric surgery to be labeled as dangerous. The field would begin to resurge in the late 1970's with solely restrictive procedures to avoid the stigma of the JI bypass.

1979: Biliopancreatic diversion

Biliopancreatic diversion (BPD) entails a significant reduction in the size of the stomach into a pouch (partial gastrectomy) as well as the creation of a short common intestinal channel (Figure 2). This procedure, a modification of the JI bypass, was developed with the idea that all portions of the gut should be included in the digestion process. The partial gastrectomy results in a minor restrictive effect, but the key element for weight loss is the decreased transit time afforded by the short common intestinal channel.[46] BPD afforded approximately 70-75% excess weight loss in 90% of patients 1 to 8 years post-operation.[47;48] Key complications included anemia, stomal ulcer, protein malnutrition, and dumping syndrome.[40] Dumping syndrome is caused by too much food, particularly simple carbohydrates, reaching the small intestine, which leads to symptoms such as cramps, nausea, diarrhea, heart palpitations, dizziness, and

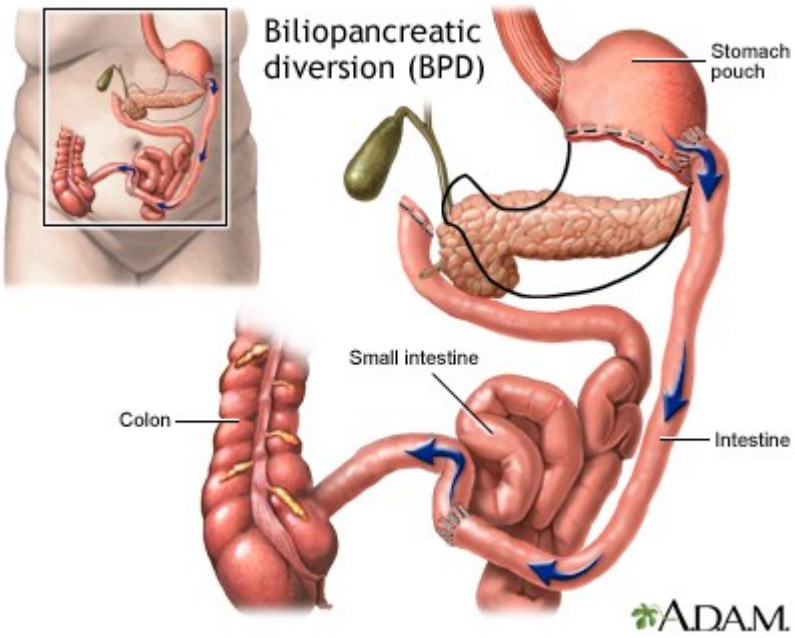

FIGURE 2 SCHEMATIC OF THE BILIOPANCREATIC DIVERSION PROCEDURE[2]

hypoglycemia. This procedure virtually eliminated bypass enteritis seen in the JI bypass.

Bariatric procedures utilizing restriction alone

The following bariatric surgery procedures function primarily via restriction of the size of the stomach, resulting in mechanical limitation of food intake. These procedures typically do not result in the severe nutritional deficiencies common with malabsorptive procedures, but they are less effective at producing weight loss. [40]

1971: Gastroplasty

In this procedure, the stomach is divided into a small upper pouch and a lower pouch. The channel between the two was later reinforced in various ways. In 1980, the vertical banded gastroplasty (Figure 3) was created, which quickly became the gold standard for this type of procedure. The benefits to gastroplasty include fewer complications: no malnutrition risk (not malabsorptive) and low leak risk (no anastomosis).[49] There is a risk of stomal stricture, which leads to nausea, vomiting, and poor apetite.[50] Excess weight loss was 50% in 50% of patients.[51;52] Gastroplasty did not prove to be durable, as the restrictive pouch would open and allow to weight regain.

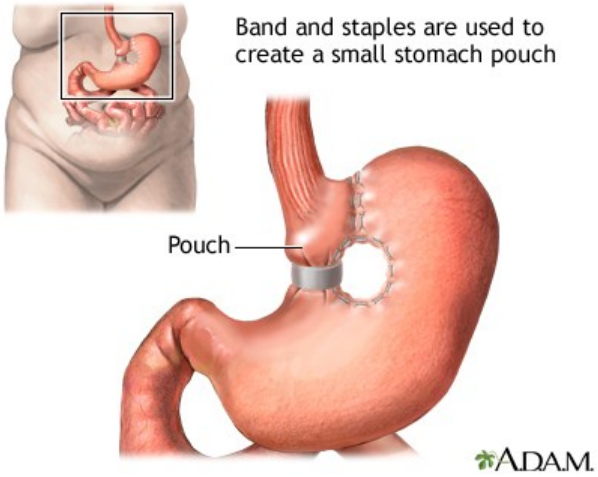

FIGURE 3 SCHEMATIC OF THE VERTICLE BANDED GASTROPLASTY PROCEDURE[4]

1980: GASTRIC BANDING

This is the only procedure in which the digestive tract is not surgically altered. Instead, a band is placed around the top of the stomach, which restricts the size of the initial portion of the stomach where food enters (Figure 4). The ability to adjust the restriction of this band came in 1986 with the adjustable gastric band (AGB). When an AGB is placed, a port is also attached under the skin of the patient. The port allows fluid to be delivered into the AGB via syringe to increase the pressure the AGB places on the stomach (restriction). The amount of restriction the AGB delivers can be altered without surgery or the band can be removed and the procedure completely reversed via surgery. The main complications associated with AGB are obstruction, band slippage, and band erosion. While the risk with AGB is minimal, so is the weight loss produced relative to other bariatric procedures.[53] A meta-analysis conducted by Garb et al. in 2009 showed almost 50% excess weight loss with AGB in 80% of patients.[42;54] While diabetes resolves in about 70% of patients after AGB placement, this procedure is far less effective with higher BMI patients, who have a remission rate of under 50%.[42] This decreased effectiveness at higher BMI is likely related to the lower weight loss and the fact that most patients have their bands removed at 9 years post placement (33%) or go on to have another bariatric procedure (24%).[42] Patients with lower BMIs may undergo the AGB while they may not be eligible for other bariatric procedures (BMI of 30 kg/m2 with . AGB had a period of popularity with the advent of laparoscopic placement in 1993 but has begun to fall out of favor.

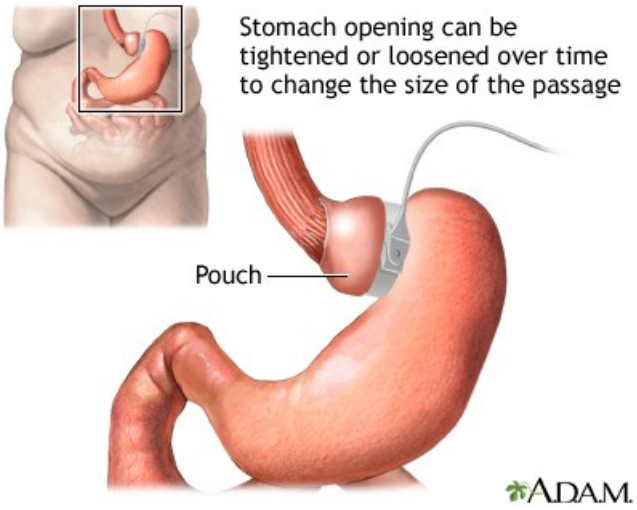

FIGURE 4 SCHEMATIC OF ADJUSTABLE GASTRIC BANDING[1]

1993: SLEEVE GASTRECTOMY

Also known as the vertical sleeve gastrectomy (VSG), this procedure was originally part of the duodenal switch as the restrictive component. VSG involves the removal of the greater curvature of the stomach only (Figure 5), leaving the intestines intact. The portion of the stomach remaining, the sleeve, is about 20% of the original. VSG was often used as the first step in a staged approach to duodenal switch or gastric bypass in order to have higher risk patients undergo initial weight loss before attempting a more complicated procedure. Beginning in 2009, VSG gained popularity as a bariatric procedure onto itself with 60% excess weight loss at 1 to 2 year post-operation.[42] At the Johns Hopkins Center for Bariatric Surgery (JHCfBS), VSG now comprises 70-75% of all cases. VSG may worsen acid reflux especially in patients with a history of gastroesophageal reflux disease (GERD); however, nutritional deficiencies are less common except for vitamin B-12.[42] Diabetes resolves in roughly 70% of patients after the VSG procedure.[42]

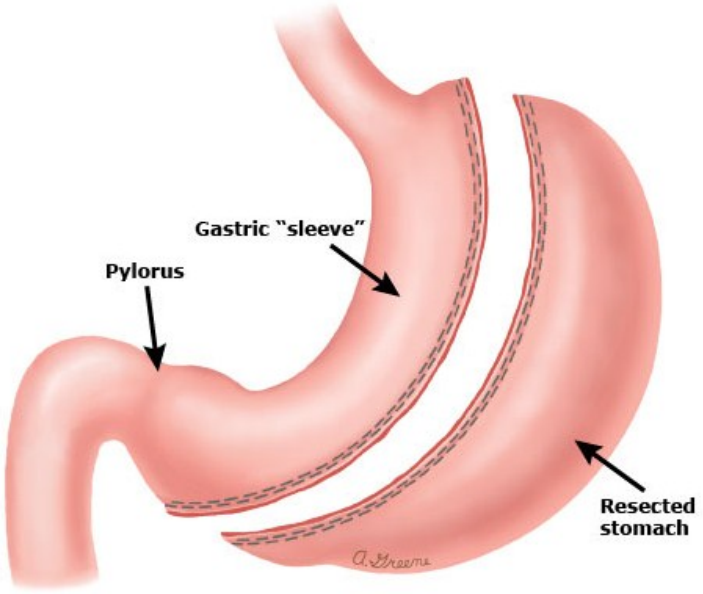

FIGURE 5 SCHEMATIC OF THE VERTICLE SLEEVE GASTRECTOMY PROCEDURE[6]

1994: Gastric Stimulation

In this procedure, electrodes are placed into the stomach wall (Figure 6) in order to stimulate portions of the stomach, for instance the vagal nerve, and therefore create a sense of satiety with less food. As with the AGB, gastric pacing has fewer risks and no permanent surgical alteration of the gastrointestinal tract. Gastric pacing provides even less weight loss than AGB, however. Results range from 20-25% excess weight loss.[55]

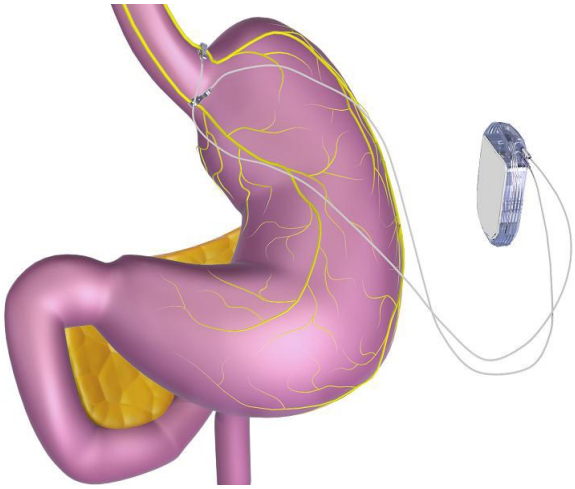

FIGURE 6 DEPICTION OF AN IMPLANTED GASTRIC STIMULATION DEVICE[56]

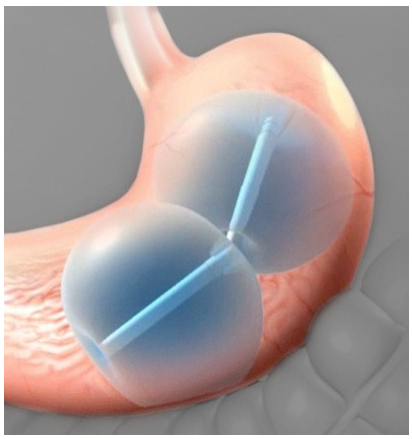

FIGURE 7 DEPICTION OF THE INVESTIGATIONAL RESHAPE DUO™ DUAL-BALOON SYSTEM[57]

2001: Modern Itragastric balloon

Endoluminal placement of balloon(s) inside the stomach was developed in an attempt to forgo surgery entirely. Intragastric balloons produce weight loss by limiting the amount of space inside the stomach and thus allowing for satiety with modest food consumption. These procedures are used alone or as initial weight loss prior to undergoing another bariatric procedure. Excess weight loss is about 30% at 1 year.[58] Figure 7 depicts an investigation dual-balloon system, which has shown potential for faster results than previous systems (1/3 excess weight loss at 6 months).[59] As with gastric banding, adjustable versions of the intragastric balloon have been

introduced with some improvement in weight loss results, around 45% excess weight loss at 1 year.[40] All balloon systems are only temporary means of weight loss that require a commitment to lifestyle change.

Bariatric procedures utilizing both malabsorption and restriction

The subsequent bariatric surgery procedures function using both malabsorption and restriction. They decrease the size of the stomach to limit the amount of food that can be eaten at one time and thus calories ingested. They will also limit the effectiveness of digestion in order to limit the absorption of calories (side effect being poor absorption of nutrients). These procedures tend to offer the greatest weight loss with a more moderate risk of side effects.

1977: Roux-en-Y Gastric bypass

In 1966, the loop gastric bypass (GBP) procedure was originally developed to restrict the stomach size (and thus meal size), reduce stomach acid (and thus digestion / absorption of calories), and shorten transit time to the small intestine (leading to dumping syndrome). After a few modifications, the Roux-en-Y GBP (RYGBP) was performed by creating a small stomach pouch and then connecting the distal part of the jejunum (the Roux limb) to this pouch,

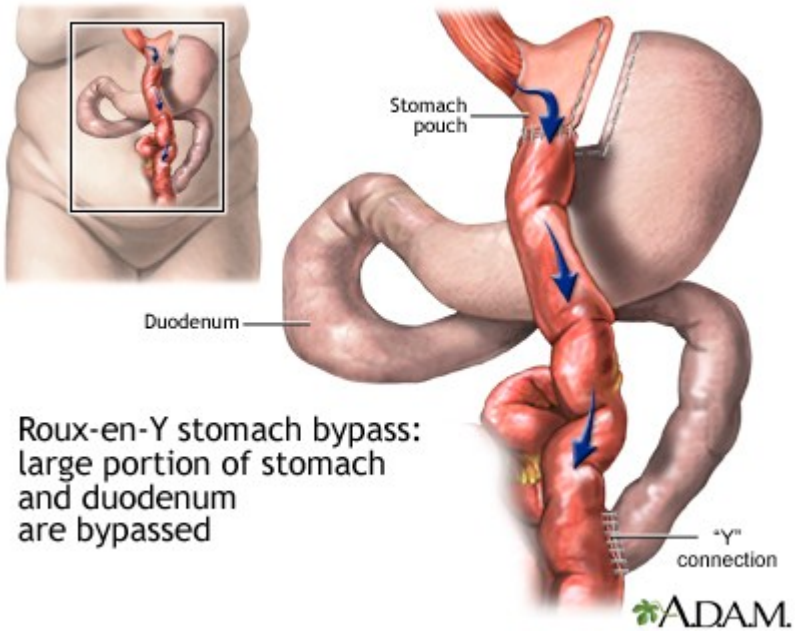

FIGURE 8 SCHEMATIC OF THE ROUX-EN-Y GASTRIC BYPASS PROCEDURE[5]

bypassing the duodenum (Figure 8). The duodenum is where much of the stimulation of hormones occurs following a meal.[42] The RYGBP, compared to the loop GBP, prevented bile from refluxing into the stomach and made the procedure malabsorptive in addition to restrictive, improving weight loss outcomes to around 80% excess weight loss.[42;60] Up to 90% of patients with diabetes go into remission following RYGBP.[42] The RYGBP has been one of the more prominent procedures and has stood the test of time. RYGBP is a highly effective weight loss procedure with lower complication rates compared to similar procedures.[42] Nutritional deficiencies are managed with supplementation and monitored with annual laboratory testing.

1986: Biliopancreatic diversion with Duodenal switch

Often called simply duodenal switch, this is a modification of the BPD procedure. In order to prevent stomal ulcers and limit dumping syndrome, the pyloric sphincter was preserved (Figure 9). Additionally, the stomach was further restricted from a pouch into a tube formed from the lesser curvature of the stomach. The additional restriction led to greater weight loss—80% excess weight loss was maintained after 8 years post-operation.[61;62] Complications such as micro- and macronutrient deficiencies and diarrhea remained. In many instances, the duodenal switch was limited for those with class 3 obesity due to the potential complications and the

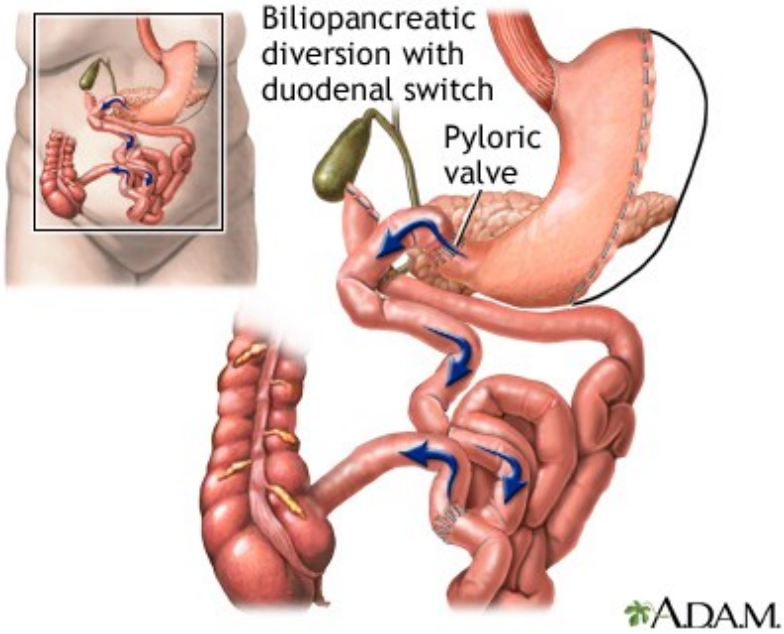

FIGURE 9 SCHEMATIC OF THE BILIOPANCREATIC DIVERSION WITH DUODENAL SWITCH PROCEDURE[3]

highest mortality rate of modern bariatric procedures.[42;63;64] Up to 99% of patients undergoing duodenal switch experience resolution of their diabetes post-operatively. This procedure gave rise to the VSG, which along with RY-GBP are the two most popular procedures today.

Laparoscopic bariatric surgery

Minimally invasive surgery, laparoscopic surgery, was first introduced in the 1990's. In laparoscopic surgery a series of small incisions (1/4 to 1/2") are used instead of one long incision along the midline, traditional open surgery. These smaller incisions are less painful and heal faster and with fewer complications, e.g. infection, dehiscence, scarring, hernia. Laparoscopic procedures also require shorter hospital stays. Laparoscopic surgery is performed using instruments passed through ports in each incision while the surgery is viewed via a specialized camera, a laparoscope. Throughout the procedure, the abdomen of the patient is distended using carbon dioxide, providing sufficient room to perform the surgery. In some patients it may be required that a laparoscopic procedure be converted to an open procedure if scar tissue prevents the placement of the ports, visibility is poor, or excess bleeding occurs.

Complications

When following the candidate criteria set forth by the National Institute of Health (NIH), bariatric surgery is a safe, effective, and long-term treatment for obesity. The NIH guidelines state that patients with class 3+ obesity (BMI ≥ 40) or a BMI ≥ 35 and serious obesity-related comorbid conditions, who have failed traditional forms of weight loss, may be considered for bariatric surgery. In adolescents, it is recommended that patients have also achieved skeletal maturity due to concerns of nutritional deficiencies resulting from malabsorption, particularly poor absorption of calcium and vitamin D.

Any surgery comes with risks of complications related to anesthesia, bleeding, and infection. Mortality is very rare in bariatric surgery (< 2%). Acute complications are more common and include wound infection, dehiscence, abscess, leaks, intestinal obstruction, marginal ulcers, and deep vein thrombosis. There are numerous strategies for minimizing these risks. To prevent deep vein thrombosis, for instance, anticoagulation therapy is delivered prior to surgery, compression is used on the legs of the patient during and after surgery, and the patient is encouraged to ambulate as soon as possible after surgery.

Some chronic complications may require revision or reversal of the procedure. These included persistent vomiting, acid reflux, and sub-optimal weight loss. Complications following a revisionary procedure are more common. Patients may also develop symptomatic gallstones with weight loss. This can be treated with medications, but often results in the surgical removal of the gallbladder. It is not uncommon for a patient to have their gallbladder removed at the same time as the bariatric procedure.

Most chronic complications relate to nutrition since protein and micronutrients are poorly absorbed following many bariatric procedures. Malabsorptive procedures function by decreasing nutrient absorption, which works most effectively on the absorption of fat and fat soluble nutrients (vitamins A, D, E, and K). Greater fat intake increases the risk of diarrhea after such procedures. Another side effect of over-indulgence is dumping syndrome, which occurs if a patient eats too much starchy or sugary food. Many patients describe this as feeling like they are having a heart attack and endeavor to avoid it. Beyond fat soluble nutrients, deficiency in vitamin B-12, folate, and iron can also arise. Malnutrition can be managed with protein shakes, supplementation, and routine nutritional screening.

VITAMIN D METABOLISM

The top layer of the skin—the epidermis—is the sight of photoproduction of VitD (see Figure 10). Upon exposure to ultraviolet-B (UVB) radiation from the sun, the precursor to VitD that is present in the epidermis, proVitD, is photolyzed to preVitD, which spontaneously isomerizes to VitD. This VitD is then transported and further processed by the liver to produce the major circulating metabolite, 25(OH)D. Since VitD is a steroid hormone, it is fat soluble. Thus, 25(OH)D accumulates in adipose tissue (fat); however, access to this stockpile is limited. 25(OH)D is used clinically to determine VitD status. The active form of VitD, $1,25(OH)_2D$, can be produced from 25(OH)D by the kidneys or locally (extra renal) via CYP27B1, a mitochondrial enzyme. $1,25(OH)_2D$ is the ligand for the VitD receptor (VDR), the method of action for VitD. Extra renal processing of VitD into 25(OH)D and $1,25(OH)_2D$ takes place through various cells in the body, most notably keratinocytes in the skin. VitD activation, production of $1,25(OH)_2D$, is controlled by a negative feedback loop systemically via the enzyme CYP24A1, which produces inactive metabolites such as calcitroic acid. Additionally, $1,25(OH)_2D$ has a short half-life, about 7 hours versus a month for 25(OH)D.[65] In the kidney, additional factors contribute to the control of VitD activation including phosphorous and calcium concentration as well as parathyroid hormone (PTH).

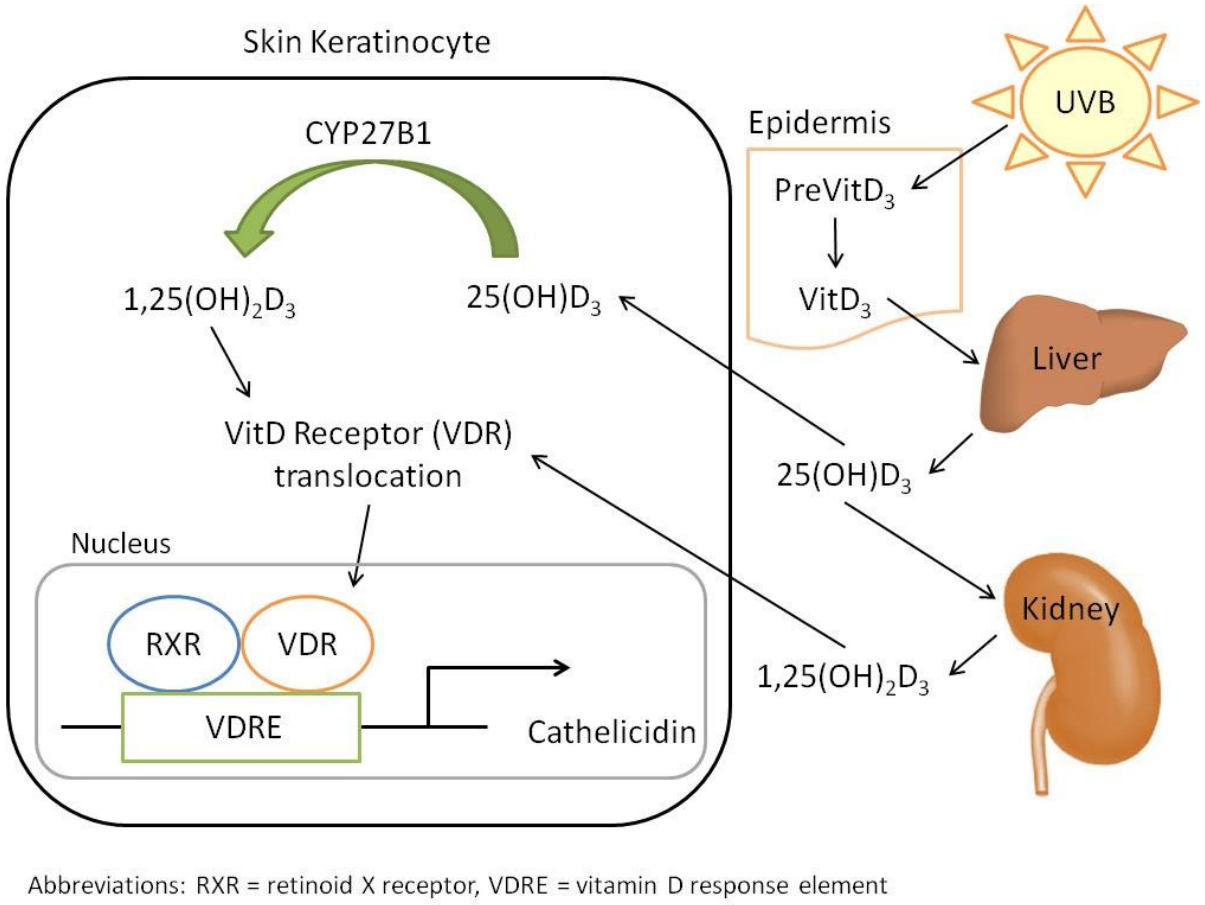

FIGURE 10 METABOLISM IN THE VITAMIN D ENDOCRINE SYSTEM

Once activated into $1,25(OH)_2D$, VitD demonstrates autocrine and paracrine effects primarily by binding with the VDR and translocating into the nucleus of a cell. In the nucleus, VDR with its bound ligand, $1,25(OH)_2D$, increases or decreases expression of genes by binding to their VitD response elements (VDREs). Other transcriptions factors, coactivators, and corepressors are required for VDR to effect gene expression, e.g. the retinoid X receptor (RXR). One corepressor, hairless, plays a key role in the regulation of epidermal proliferation and differentiation via $1,25(OH)_2D$ and the VDR.[66-68]

Figure 10 shows a skin keratinocyte, but most cells throughout the body have VDRs, yielding an effect via active VitD on tissues well beyond bone, e.g. skin, nerves, muscles, blood vessels, and cells primarily involved in innate as well as adaptive immunity.[69] VDREs have been discovered in most of the almost 2000 genes regulated by $1,25(OH)_2D$.[69;70] A classic example of the action of $1,25(OH)_2D$ includes stimulation of PTH release from the parathyroid glands. PTH acts to increase the amount of calcium present in the blood by increasing absorption in the intestine, re-absorption in the kidneys, and, if necessary, releasing calcium from bone. The effect of PTH is dependent upon the amount of calcium and phosphorous present in the body. VitD can also have more immediate action via the action of $1,25(OH)_2D$ on membrane-bound VDRs. This pathway has been more recently discovered, and thus is less well understood. The membrane bound VDR is thought to function in the action of $1,25(OH)_2D$ in rapid signal transduction via protein kinase C, phospholipase C, and calcium and chloride channels.[71-75]

Polymorphisms in the classic, nuclear VDR lead to systemic alteration of the VitD endocrine system. For example, the Fok1 polymorphism is a single base pair change at the start site of the VDR, leading to translation beginning at the next start codon, 3 codons later. This results in a shortening of the VDR protein by 3 amino acids. The phenotype displayed in an individual with this Fok1 polymorphism is controversial as some individuals show poor bone mineralization while others suffer from rheumatoid arthritis, hypertension, and cancers, e.g. breast.[69;76-81]

VDR expression in the skin is highly conserved among species including the model organism for developmental biology, *Xenopus*.[82] In the major cell type of the skin, keratinocytes, $1,25(OH)_2D$ bound to the VDR stimulates differentiation and limits proliferation.[82] Such alteration leads to the graduated differentiation of the epidermis and the development of the cornified envelope, the lipid envelop of the skin.[83-87] The cornified envelope is responsible for fluid retention and

protection, from radiation and other potential intruders—the barrier function of skin.[83-86] $1,25(OH)_2D$ bound to the VDR is also key in the cascade leading to the formation of the lipids producing the cornified envelope.[69;82;88] These varied and crucial roles is why skin is able to produce all elements of the VitD endocrine system without the aid of the kidneys or liver.

A more recently discovered example of the action of $1,25(OH)_2D$ on the classic VDR, cathelicidin, is shown in Figure 10. Cathelicidin (LL-37 or hCAP-18) is an antimicrobial peptide (AMP), an endogenous substance that functions as a broad spectrum antibiotic with antibacterial and antifungal properties. Cathelicidin is found in many regions of the body, including saliva, sweat, and skin. While pharmacological antibiotic resistance is a growing public health concern, AMPs, like cathelicidin, have maintained their efficacy in numerous species and even plants in part due to their direct action of permeabilizing the membranes of pathogens. Studies in burn victims show that those individuals with lower expression of AMPs are at higher risk of infection.[70]

When VitD is photoproduced in the skin, high local concentrations of cathelicidin are present.[70] Injury or wounding also leads to increased production of AMPs like cathelicidin. If this response is swift and robust, the wound will heal without infection or long term consequences. If AMP production is insufficient, the wound will be vulnerable to infection, which may cause significant morbidity and even mortality due to sepsis or other complications. The disadvantage of an ineffective AMP response following injury is doubled by the secondary actions of AMPs. The initial action of AMPs is direct microbial killing. The secondary actions are two part 1) chemotaxic, meaning they signal immune cells to the site to continue to destroy invading pathogens, and 2) immunomodulatory. In the immunomodulatory function, AMPs stimulate the production of pathogen sensing receptors, Toll-like receptors, and alter the production of cytokines and chemokines by surrounding cells such as keratinocytes and granulocytes in order

to change the type of immune response.[69;89] Thus, an insufficient AMP response will not be able to kill all pathogenic invaders but will also not properly activate the second line of defense, adaptive immunity.

VITAMIN D REQUIREMENTS

CURRENT RECOMMENDED INTAKE

The dietary reference intakes (DRIs) for VitD were reconsidered in 2010. This was published in the Institute of Medicine *Dietary Reference Intakes for Calcium and Vitamin D Report* (2010 IOM Report).[90;91] DRIs include Recommended Dietary Allowances (RDAs), which are recommendations for dietary intake of a nutrient that is designed to cover the needs of at least 97.5% of the population, in this case the US. In the 2010 IOM Report, the RDA for VitD was set as 600 IU for all ages except infants aged 0 to 12 months and the elderly (71+ years of age). The elderly have an RDA of 800 IU.[90] Infants have an adequate intake (AI) of 400 IU.[90] An AI is a recommendation used in place of an RDA when and RDA cannot be determined because there is insufficient evidence to calculate and estimated average requirement. An AI is based on observed intakes in apparently healthy individuals. It is common to have an AI instead of an RDA in infants.

While the 2010 IOM report slightly increased the RDA, much of the report was spent discussing the dearth of high quality research available to accurately determine the RDA for extra-skeletal effects of VitD. Previously, the RDA was based on data regarding the prevention of Rickets, severe VitD deficiency leading to bone softening and potential skeletal deformities such as bowed legs. The 2010 IOM report also based its recommendation solely on bone health. The modest increase was sparked by newer research showing the effect of VitD on parathyroid hormone rather than severe deficiency markers such as Rickets. This, however, essentially ignored a vast portion of the VitD literature. A specific call was made in the 2010 IOM report for

more clinical trials, especially randomized controlled trials, to be conducted to investigate the precise effect of VitD on extra-skeletal effects. This data would be required to incorporate extra-skeletal outcomes into the next review of the RDA for VitD.

Dietary sources

Dietary sources of VitD are limited and vary greatly in composition over time and place.[90;91] $VitD_2$ (ergocalciferol) comes from plant sources and $VitD_3$ (cholecalciferol) from animal sources such as photoproduction in skin. The chief dietary source of VitD is seafood. Wild-caught salmon contains 500 to 1000 IU of $VitD_3$ per 3.5 oz serving while farm raised salmon, which is more common in the US diet, has just 100 to 250 IU of $VitD_3$ per 3.5 oz serving.[62;91] Cooking methods also affect VitD content. The most destructive method of cooking for VitD is frying (≈ 50% remaining) while baking (≈ 90% remaining) and microwaving (≈ 99% remaining) have modest effects on VitD content.[62] Fortified foods such as milk, cereal, orange juice, and bread supply minor amounts of VitD.[92]

Photoproduction in the skin

While dietary sources can be important, particularly in coastal regions where seafood is plentiful and affordable, photoproduction of VitD is the main source in most populations.[92-95] Photoproduction of VitD occurs when solar radiation in the wavelengths of 290 to 315 nm (UVB) hits the skin. This can occur for up to 20 minutes of exposure. UV exposure beyond 20 minutes, leads to degradation of both VitD and preVitD into the inactive forms lumisterol or tachysterol[95].

How much VitD is produced during sun exposure depends upon the angle of the sun with minimum production occurring at a solar zenith angle around 35° and maximum production occurring with a 15° or lower solar zenith angle.[90;96] The major contributors to the angle of the sun, and thus VitD photoproduction, are time of day, season, and latitude.[97] Altitude can also

affect photoproduction since there is less ozone to absorb UVB at higher elevations.[70] Beyond the environment, individual characteristics can affect VitD photoproduction. Melanin, skin pigment, acts as a natural sunscreen, absorbing UVB radiation before it can reach proVitD to produce VitD.[70] The same effect occurs with the application of sunscreen.[98]

VITAMIN D STATUS

DEFINING DEFICIENCY AND INSUFFICIENCY

In the literature, there is general consensus that VitD deficiency can be defined as a serum 25(OH)D concentration of less than 20 ng/ml (50 nmol/L). There is much more debate at what defines VitD insufficiency (sub-optimal VitD stores). Most studies currently use less than 30 ng/ml (75 nmol/L) or 32 ng/ml (80 nmol/L), but it has been suggested that serum 25(OH)D concentrations of 40 ng/ml (100 nmol/L) or even 48 ng/ml (120 nmol/L) are necessary for some of the extraskeletal health benefits, such as prevention of chronic disease and promotion of immune competence. The 2010 IOM Report concluded that a serum 25(OH)D concentration of 15 ng/ml (40 nmol/L) should meet the requirements of about 50% of the US population and 20 ng/ml (50 nmol/L) of about 97.5% of the population.[99] Since the DRIs are aimed at covering the requirements of 97.5% of the US population, the 20 ng/ml (50 nmol/L) cut point for deficiency has been widely adopted.[99] Since the 2010 IOM Report did not address extra-skeletal effects, they did not address a cut-point for VitD insufficiency.

RISK FACTORS

Since the majority of VitD status is determined by sun exposure resulting in VitD photoproduction[100], risk factors for poor VitD status include decreased sun exposure and lower ability to photoproduce VitD during sun exposure. Individuals who are at risk for insufficient sun exposure include: shift or night shift workers, office workers, those living in northern latitudes,

etc. If an individual does receive sun exposure, they can still be at risk of poor VitD status if they have high melanin concentration (darker skin pigmentation), daily sunscreen use, veiling or other body covering, age (especially being elderly).

Malabsorption may also lead to VitD deficiency. Disorders that cause poor fat absorption limit VitD absorption from dietary sources and supplements. Causes of malabsorption include Crohn's disease, Celiac disease, bowel resection, and some bariatric surgery procedures. Removal of a portion of the intestines may be due to cancer, enteritis, ulcers, etc. Such bowel resection limits the ability of the body to absorb fat and thus fat soluble nutrients (vitamins A, D, E, and K).

A third class of risk factors includes those that disrupt the function of the VitD endocrine system. These include polymorphism in the VDR and kidney disease. Inability of the VDR to function properly will lead to VitD-deficiency-like symptoms regardless of the serum concentration of 25(OH)D (VitD status) of the individual. Thankfully, VDR polymorphisms are rare. Kidney disease is more common, however. As the kidneys become more diseased, they become less able to activate 25(OH)D into 1,25$(OH)_2$D. This too limits the function of VDR beyond VitD status.

Prevalence of deficiency and insufficiency

Estimates of VitD deficiency in the US, Canada, and Europe range from 20 to 100%.[101-105] In 1988 the National Health and Nutrition Examination Survey (NHANES) began monitoring VitD status. In NHANES 2001-2006, 1 in 3 Americans over the age of 1 year had VitD deficiency, according to season adjusted serum 25(OH)D concentrations.[106] Lowest prevalence of VitD deficiency was seen in children from 1 to 8 years of age (Figure 11).[106] Prevalence of VitD deficiency significantly increased with age up to 14-18 years of age in females and 30 years of age in males, plateauing in females at age 51 and males at age 30.[106]

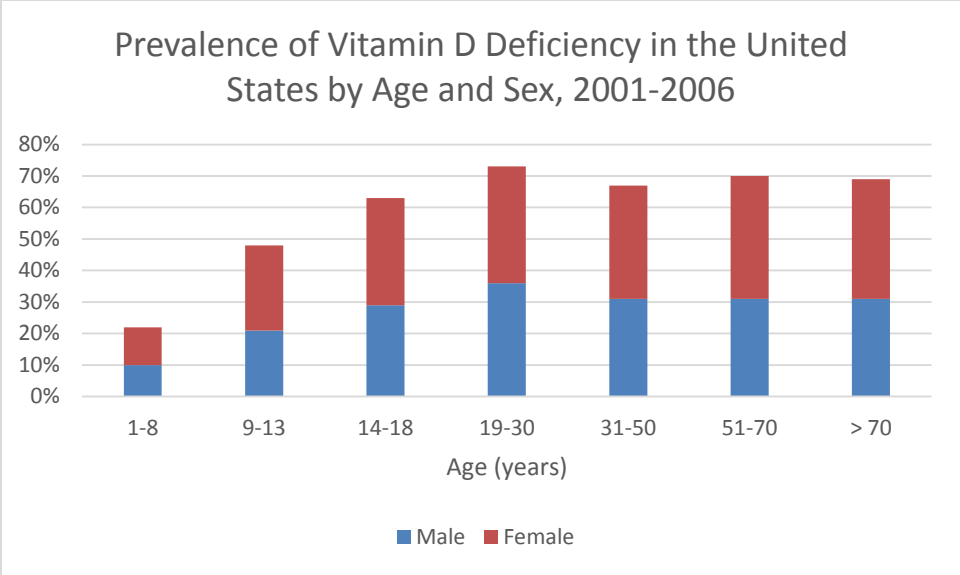

FIGURE 11 PREVALENCE OF VITAMIN D DEFICIENCY IN THE UNITED STATES BY AGE AND SEX, 2001-2006, SEASON ADJUSTED, ADAPTED FROM LOOKER ET AL. 2011[106]

The data from NHANES 2001-2005 also showed that prevalence was similar in males (41.1%) and females (42.0%).[107] The highest prevalence of VitD deficiency was in blacks (82.1%) followed by Hispanics (62.9%) and then whites (30.9%).[107] Being black was an independent risk factor for VitD deficiency (OR 9.6, $p<0.001$).[107] Those with a college education had significantly lower prevalence of VitD deficiency (36.7% vs. 48.2%).[107] Those who were not overweight or obese as well as those who were in better overall health had lower prevalence of VitD deficiency with hypertension and hyperlipidemia showing significant positive correlations with VitD deficiency.[107] Obesity was estimated to increase the odds of VitD deficiency by 1.9 times, $p<0.001$.[107]

National monitoring for VitD status occurs in select countries globally. In these countries, national VitD status falls below 75 nmol/L (30 ng/ml) for both adults (Figure 12) and children/adolescents (Figure 13). Countries farther from the Equator, such as Canada and

Argentina, show VitD inadequacy and deficiency at the country level.[99] Another epicenter of poor VitD status appears to be in Asian countries such as India, China, and Mongolia.[99] Given the large populations of India (1.252 billion)[108] and China (1.357 billion)[109] alone, the burden of VitD insufficiency and deficiency globally must be great and deserving public health action.

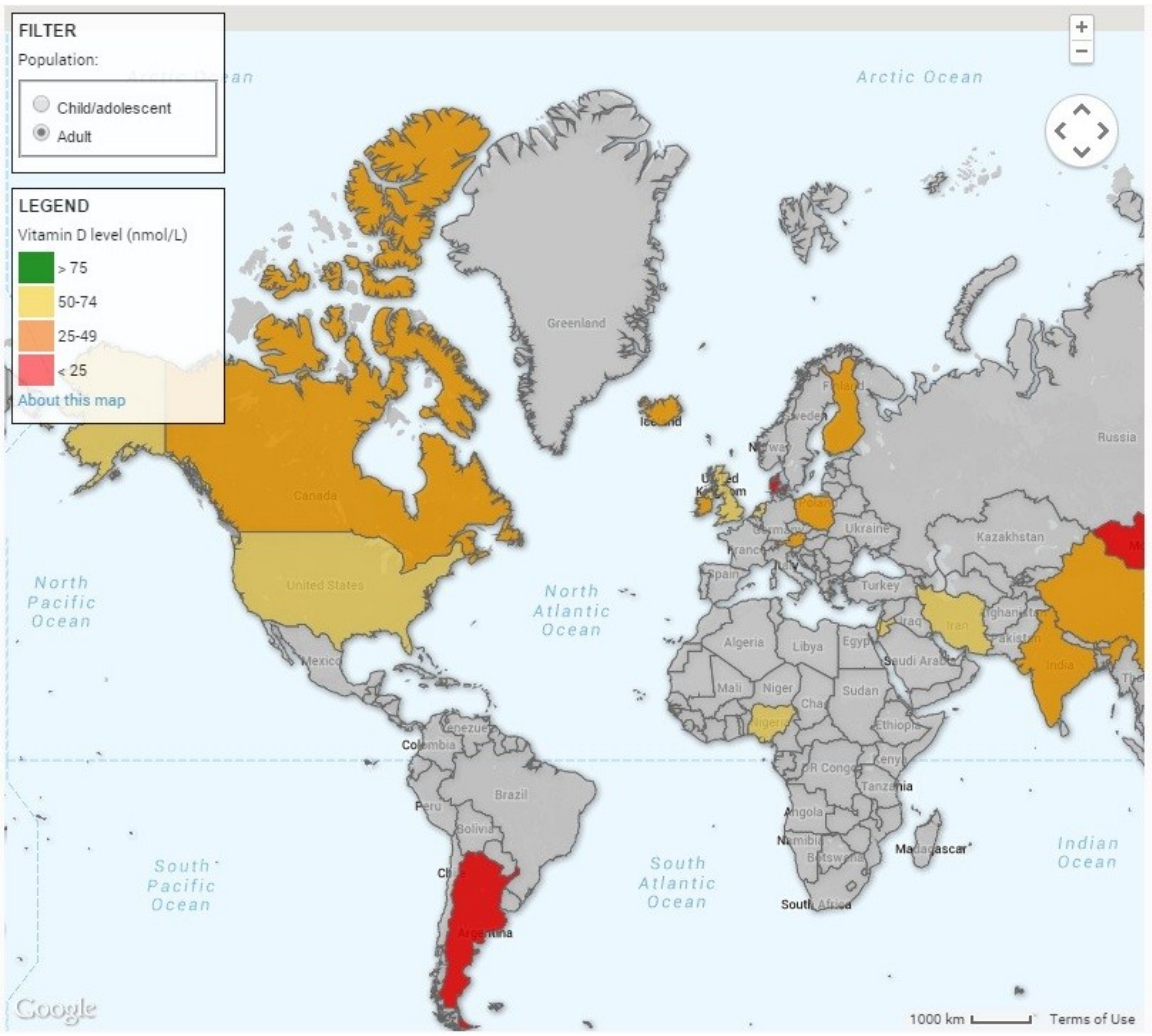

FIGURE 12 VITAMIN D STATUS AROUND THE WORLD - ADULT[99]

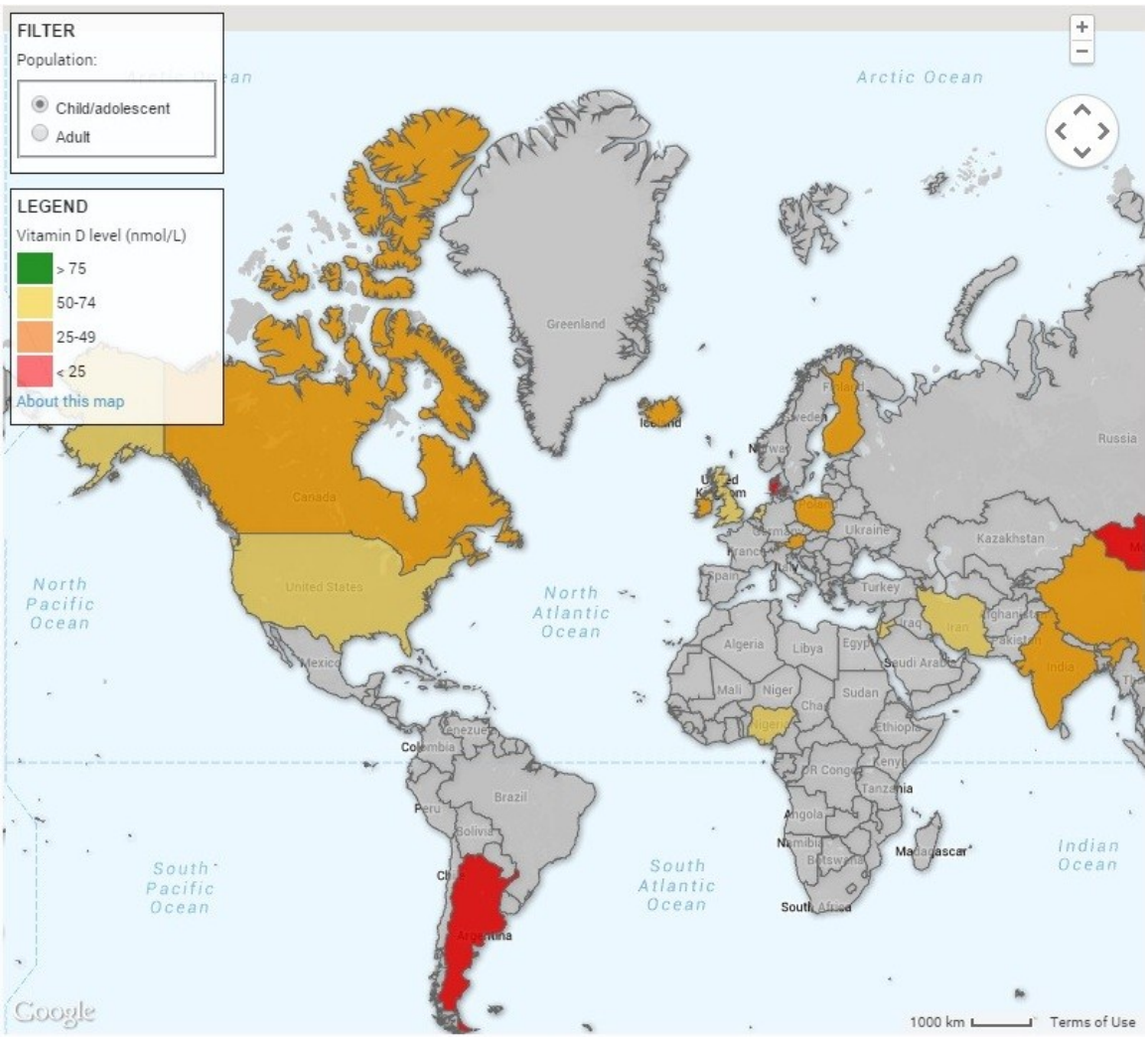

FIGURE 13 VITAMIN D STATUS AROUND THE WORLD - CHILD / ADOLESCENT[99]

TOXICITY

VitD toxicity may occur if an individual has VitD hypersensitivity syndrome.[110] Normal individuals rarely exhibit toxic levels of serum 25(OH)D due to multiple inactivation pathways including the negative feedback loop of $1,25(OH)_2D$ production. Excess VitD is excreted by the liver in the bile and removed from the body with the feces.

In the rare case of VitD toxicity, intakes were estimated to be upwards of 50,000 IU per day for an extended period of time and resulted in serum 25(OH)D concentration around 150 ng/ml (375 nmol/L).[110] This is an extraordinarily high concentration, considering the normative range of serum 25(OH)D is generally capped at 60 ng/ml (150 nmol/L), which is 2.5 times lower. Furthermore, 50,000 IU per day is greater than 80 times the RDA in the 2010 IOM Report. Toxicity requires massive daily supplementation doses over prolonged periods of time.

Prevention and treatment of deficiency in obesity

An additional complication relating to obesity and VitD status comes with supplementation. Given an identical dose of VitD, an obese patient will have a smaller increase in serum 25(OH)D concentration.[111] Ekwaru et al. showed that the trajectory of the relationship of 25(OH)D response to VitD supplementation differed according to body weight categories. Underweight individuals exhibited a sharp increase in status even with lower doses while obese individuals required large dose to significantly increase VitD status (see Figure 14).[111] In order to maintain VitD sufficiency in obesity, the estimated average daily supplementation is 1663 IU (95% CI: 1538-1790), according to Ekwaru et al., compared to 28 IU (95% CI: 0-115) for a normal weight individual or 534 IU (95%CI: 450-619) for an overweight individual.[111] Interestingly, underweight individuals required more VitD than normal weight individuals, estimated average daily supplementation of 151 (95% CI: 0-507).

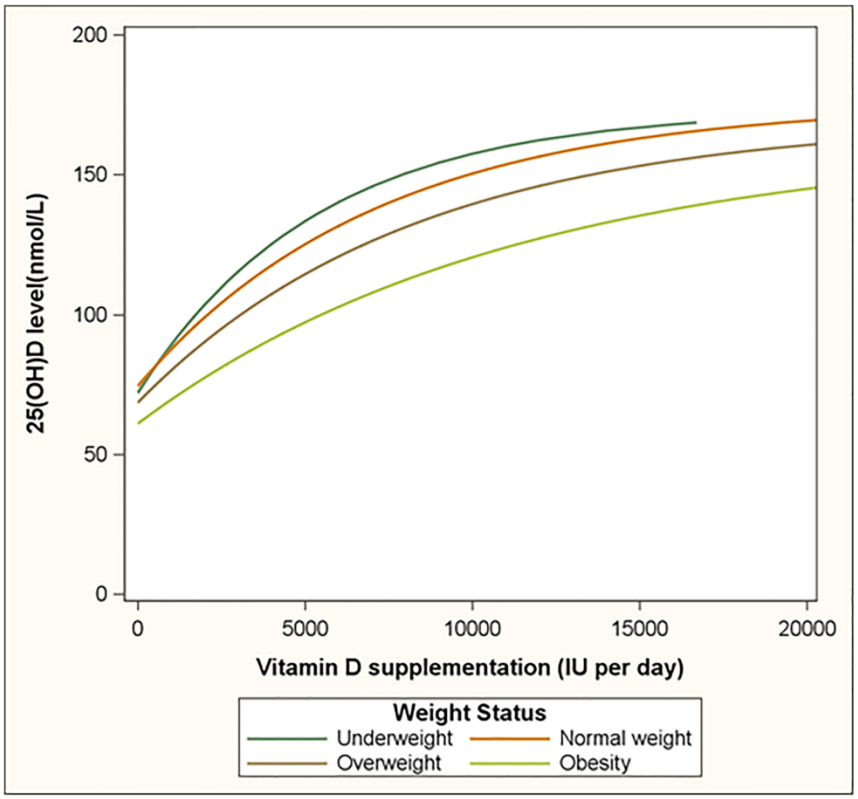

FIGURE 14 THE EFFECT OF BODY WEIGHT ON THE RESPONSE OF SERUM 25(OH)D TO SUPPLEMENTATION

Drincic et al. found that obese adults (BMI: 30 to 58 kg/m^2) require approximately 2.5 IU per kilogram of body weight to increase serum 25(OH)D status by 1 ng/ml (2.5 nmol/L).[111] This randomized, single-blind study by Drincic et al. also showed that 10,000 IU of $VitD_3$ daily for 21 weeks led to serum 25(OH)D concentrations ranging from 28.5 to 67.7 ng/ml (71.2 to 169.2 nmol/L) with a median value of 48.1 ng/ml (120.2 nmol/L), well above the insufficient cut point.[111] The baseline VitD status in these obese subjects was 23.2 ± 15.2 ng/ml (58.0 ± 38/0 nmol/L).[112] This is a 30% lower response compared to normal weight controls.[112-114] Despite this relatively high daily dose, no cases of hypercalcuria or hypercalcemia were observed.[112] These findings indicate that the following equation maybe helpful in determining the appropriate dose of $VitD_3$ to give an obese patient:

Additional daily vitamin D_3 (IU) = [weight (kg) x desired change in 25(OH)D (ng/ml) x 2.5] - 10

This means that an obese adult weighing 100 kg with a serum 25(OH)D concentration of 10 ng/ml would require supplementation with approximately 4990 IU of $VitD_3$ daily to reach 30 ng/ml (a 20 ng/ml increase).

Beyond body weight, serum 25(OH)D concentration and type of supplementation ($VitD_2$ or $VitD_3$) significantly modify of the effect of supplementation on VitD status.[115] The poorer the VitD status of an individual, the greater the effect supplementation will have on their serum 25(OH)D concentration. In other words, 1000 IU of $VitD_3$ given to an individual with serum concentration of 10 ng/ml (25 nmol/L) will have a greater impact than if that same individual had a baseline serum 25(OH)D concentration of 20 ng/ml (50 nmol/L). This has been shown in numerous studies.[116-123]

$VitD_3$ is more effective at increasing serum 25(OH)D concentration than $VitD_2$. A systematic review by Zittermann et al. showed that supplementation with $VitD_3$ resulted in an 8.08 ng/ml (20.19 nmol/L) greater increase compared to the same dose of $VitD_2$.[124]

THE EFFECT OF OBESITY & VITAMIN D ON THE IMMUNE SYSTEM

SKIN AND WOUND HEALING

Skin is the first line of defense. First, it is a physical barrier to the external world. The skin protects the body from a myriad of potential damages from invading pathogens to irritants. This barrier function is bidirectional, holding in nutrients and water. The skin is also an immune organ, facilitating the immune surveillance of lymphocytes and producing cytokines and other key substances to excite and sustain an immune response when necessary. Immune surveillance is a complex concept that encompasses all functions of the immune system, both innate and adaptive. Most often immune surveillance is a term used to denote the ability of the immune

system to detect, halt, and overcome a pathogen or diseased cell also known as immune competence. One element of immune surveillance that occurs in the skin is the production of compounds that are required in the event of an assault, such as antimicrobial peptides (AMPs).

Nutritional deficiencies can compromise the barrier function of skin and can lead to decreased immune competence.[124] VitD is one such nutrient that plays a crucial role in immunity in the skin. VitD aids in the barrier function of skin, in innate immunity including in the production of antimicrobial peptides, and in activation of adaptive immunity if necessary. VitD also aids in wound healing (re-epithelialization) through various mechanisms including keratinocyte proliferation and differentiation, matrix metalloproteinases (MMPs), and the antimicrobial peptide cathelicidin (LL-37 or hCAP-18).[125-127]

Alteration of inflammation in obesity

There are numerous changes to the structure and function of the body in obesity. The immune system is not resistant to these alterations. Obesity leads to excessive adipose tissue, especially visceral adiposity, leading to chronic low grade inflammation.[128] Such chronic inflammation increases risk for insulin resistance, type 2 diabetes, hypertension, cardiovascular disease, fatty liver disease, and cancer.[129] When the immune response is activated inappropriately, as in chronic inflammation, immunity to a real threat is dampened. This is why the immune system is less efficacious in obesity.[130;131]

Analysis in 23 patients from the Johns Hopkins Center for Bariatric Surgery (JHCfBS) has been performed to evaluate differences in inflammation before and after bariatric surgery (1 year post-operation). In these JHCfBS patients, inflammatory markers decreased with weight loss,[28;130;132-136] confirming the results of other studies.

Alteration of inflammation by vitamin D

VitD in both circulating and active forms--25(OH)D and 1,25$(OH)_2$D, respectively-- has widespread effects on the response and function of the immune system.[28;130;134-145] Generally, VitD activity stimulates steady-state conditions, meaning it stimulates anti-inflammatory cytokines and dampens pro-inflammatory cytokines. This is unless there is a threat present in which case it facilitates a robust immune response to eliminate the pathogen. The key in any immune response is the proper balance of cytokines in order to launch the correct form of attack for a specific pathogen. With insufficient VitD, the cytokine milieu will be faulty, leading to an inefficient or even detrimental immune response.

Immunity and surgical risk in obese individuals with poor vitamin D status

Obesity is a known risk factor for adverse surgical outcomes such as poor wound healing.[130] It is likely that obese patients will experience wound dehiscence, reopening of the wound, due to increased operative time, tension on the wound, and greater risk of wound infection, delaying wound healing.[146] Numerous studies have shown that obese patients are at higher risk of wound infections post-operatively than normal weight controls and that this relationship between BMI and risk of wound infection continues even among high BMI patients.[15] Chronic inflammation and obesity related comorbidities such as metabolic syndrome may contribute to this increased risk.[25] Furthermore, the subcutaneous tissue of obese patients is less oxygenated (poorly vascularized), which may increase infection risk especially following laparoscopic surgery.[20]

When the risk of adverse surgical outcomes in obesity is combined with that of poor VitD status, there is likely an additive or even synergistic effect. This means the risk and severity of such negative outcomes will increase, perhaps exponentially.

CHAPTER 2: PROXY MEASURES OF VITAMIN D STATUS CORRELATE WITH WOUND COMPLICATIONS AND LENGTH OF STAY AFTER BARIATRIC SURGERY IN THE NATIONWIDE INPATIENT SAMPLE, 2001-2010

INTRODUCTION

In the United States (US) National Health and Nutrition Examination Survey (NHANES) 2011-2012, over 1/3 of adults were obese (BMI ≥ 35 kg/m^2) [147-152]. Obesity is a leading contributor to global mortality and the burden of disease associated with diabetes, cardiovascular disease, musculoskeletal disorders such as osteoarthritis, and some cancers.[151] Class 3 obesity (BMI 40-49.9 kg/m^2) reduces life expectancy by 8 to 10 years, similar to the effect of regular cigarette smoking.[104]

Several studies have demonstrated that most obese adults are vitamin D (VitD) insufficient (<75 nmol/L, 30 ng/ml) or deficient (<50 nmol/L, 20 ng/ml).[153;154] A large study by Vimaleswaran et al. found a 10% increase in Body Mass Index (BMI) was correlated with a 4% decrease in VitD status.[125;155] The inverse relationship between BMI and VitD status is hypothesized to be due to sequestration of VitD by adipose tissue, reducing VitD bioavailability.

Classically, VitD maintains bone calcification, but a more varied role has been elucidated for this hormone. Insufficient VitD has been associated with susceptibility to infection, autoimmunity, cancer, and chronic disease.[156] VitD helps regulate and shows the potential to prevent inflammation that instigates chronic diseases.[130;157] Obesity is associated with chronic inflammation and increased risk of chronic diseases[158;159], which may contribute to adverse outcomes, e.g. improper wound healing[138] and wound infection[104]. Since VitD deficiency is also

associated with chronic inflammation, obese individuals with insufficient VitD have extraordinary risk of adverse outcomes, particularly delayed wound healing and infection due to the role of VitD in re-epithelialization and innate immunity.[160]

Bariatric surgery is currently the most successful means of long-term weight loss. During bariatric surgery, the digestive tract is restricted in size focusing on the stomach. There are risks of complications, which may lead to extended length of stay (LOS) post-operatively. In fact, wound infection is the most common hospital-acquired condition following bariatric surgery.[148] Since the indications for bariatric surgery are class 3+ obesity (BMI ≥ 40 kg/m^2) or class 2 obesity (BMI ≥ 35 kg/m^2) with one serious comorbidity related to obesity, bariatric surgery patients are at an increased risk of most adverse surgical outcomes. VitD deficiency is likely a risk factor that can be easily remedied pre-operatively to reduce the number and severity of such adverse outcomes.

Epidemiology has long used time of year as a proxy measurement for group VitD (VitD seasonality) since the majority of VitD nutriture comes from photoproduction in the skin exposed to solar ultraviolet-B (UVB) radiation.[161] In a recent study, Kasahara et al. confirmed that VitD status in the US peaked in August and troughed in February.[162] Thus VitD Summer, the time of highest VitD status, is July to September. The time of lowest VitD status is VitD Winter, which is January to March. This is a slight lag from traditional seasons with summer being June to August and winter December to February. For this study, we included the full spectrum of group VitD status (seasonality) by using VitD Spring (April to June) and VitD Fall (October to December) as an intermediary between the two extremities—VitD Summer and Winter.

A matched pairs study comparing pre-operative gastric bypass cases to non-obese healthy controls, found that pre-operative bariatric patients had significantly lower VitD status and

elevated parathyroid hormone.[162] The pairs were matched for age, sex, race/ethnicity, season (summer versus winter), and latitude (41°N). In summer (April to September), VitD status was higher than in winter (October to March). VitD seasonality has been explored as it relates to surgical outcomes in lung cancer patients, where patients with the highest VitD status (season plus diet) had better recurrence-free and overall survival.[160] Bariatric surgery patients have the combined risk of obesity with that of undergoing a surgical procedure, an additive or perhaps even synergistic effect.

Another classical proxy measure of group VitD status is latitude, as individuals closer to the Equator have more opportunity to photoproduce VitD. Generally, latitudes closer to the poles receive insufficient UVB radiation to photoproduce VitD. Webb, Kline, and Holick determined that skin exposed to sunlight on a cloudless day in Boston (42.2°N) from November to February did not photoproduce VitD.[163;164] Furthermore, this lack of photoproduction occurred over a longer period of time farther north; in Edmonton (52°N), no photoproduction occurred from October to March. Conversely, VitD photoproduction occurred all year long at 34°N (Columbia, South Carolina) and 18°N (US Virgin Islands) [165]. To study the effect of latitude, we choose the cut-point of 37°N (Virginia Beach, Virginia). At and above 37°N, VitD photoproduction cannot occur year round, meaning that deficiency is more likely during VitD Winter. Therefore hospitals located at or above 37°N would be included in the North cohort, the low VitD status group, and those located below 37°N in the South cohort, the high VitD status group.

The aim of our study was to investigate the association between proxy measures of group VitD status (seasonality and latitude) and the risk of adverse outcomes following bariatric surgery.

METHODS

We conducted a retrospective cohort study using the Nationwide Inpatient Sample (NIS), a database established as part of the Healthcare Cost and Utilization Project and sponsored by the Agency for Healthcare Research and Quality. NIS contains all-payer data on inpatient stays from over 1,000 hospitals in the US each year. NIS approximates a 20% stratified sample annually, meaning every US hospital will be sampled at least once in any 10 year period. Our analysis was performed using inpatient stays from 2001 to 2010, one 10 year period.

We restricted our analysis to bariatric surgery patients aged 18 to 65 years using International Classification of Diseases, 9th Revision, (ICD-9) codes. The codes 446.8 and 449.5 are sufficient to indicate bariatric surgery alone. The code for class 3 obesity (278.01) must be used in conjunction with the following codes to indicate bariatric surgery: 438.9, 443.1, 443.8, and 443.9. Furthermore, we excluded stays including cancer (150, 151, 152, 157, and 199), ulcer (531 and 533), or revision codes (442.1-2, 437, 445, 446.9, 449.6-7).

PRIMARY EXPOSURE AND OUTCOMES

We used two classical epidemiological methods to indirectly asses group VitD status in bariatric surgery cases. The first technique, the primary exposure, is season, utilizing the principle that the vast majority of VitD status is determined by sun exposure (photoproduction). VitD season was defined by the following groups: VitD Summer (July to September), Winter (January to March), and Fall/Spring (October to December and April to June). These groups are based upon refined estimates of seasonality from Kasahara et al., who used serum 25(OH)D (directly measured individual VitD status).[166;167] VitD status is highest from July to September (VitD

Summer) and lowest from January to March (VitD Winter). VitD status is moderate from October to December (VitD Fall) and April to June (VitD Spring).

Latitude, the second classic technique, is based on the principle that individuals closer to the equator have more opportunity to photoproduce VitD and thus have higher VitD status as a group. For all analysis, we used the latitude of the sampled hospital. Latitude was computed based on the hospital ZIP-code from the NIS. SAS version 9.3 PROC GEOCODE (SAS Institute, Cary, NC) was used to convert ZIP-codes to latitudes. We created a dichotomous variable to distinguish between locations at or above 37°N (North) and those below 37°N (South).

The primary outcomes were adverse surgical outcomes: non-healing wounds, wound infection, fascial dehiscence, suprafascial dehiscence, delayed wound healing, any complication, and prolonged length of hospital stay. Outcomes were determined using ICD-9 codes, grouped as in

Table 1. Suprafascial dehiscence is defined as the early stage of dehiscence where wound separation is limited to skin and subcutaneous tissue while fascial dehiscence occurs once wound separation has deepened to include fascia and possibly muscle. Delayed wound healing is defined as the occurrence of non-healing wound, wound infection, or suprafacial dehiscence. We also used the LOS variable in NIS to create the dichotomous variable extended LOS (> 3 days versus ≤ 3 days).

TABLE 1 DEFINING ADVERSE OUTCOMES FOR BARIATRIC SURGERY IN THE NATIONWIDE INPATIENT SAMPLE

Outcome Variable	ICD-9 Codes	Description
Non-healing wound	998.83	Non-healing surgical wound
Wound infection	998.51	Infected postoperative seroma
Fascial dehiscence	998.31	Dehiscence of surgical wound involving the fascia and/or muscle
Suprafascial dehiscence	998.3, 998.32	Dehiscence of surgical wound involving external tissues, i.e. mucosa, skin, subcutaneous tissues
Delayed wound healing	998.3, 998.32, 998.51, 998.83	All above except fascial dehiscence
Any complication	998.3, 998.31, 998.32, 998.51, 998.83	All above

ICD-9 = International Classification of Diseases, 9th Revision

STATISTICAL ANALYSIS

Statistical analysis was performed using Stata 12.1 (StataCorp, College Station, TX). We compared the occurrence of adverse outcomes over the spectrum of VitD seasonality. Since our outcome is dichotomous (outcome / no outcome) and our independent variables are a mix of continuous and categorical, X^2 tests and logistic regression models were used. In our logistic regression models, we utilized Charlson Comorbity Index (CCI) to adjust for comorbidities. CCI interprets multiple pieces of data to predict the 10 year mortality for an individual, allowing comparison between myriad of diseases in a cohort with multiple comorbidities. We utilized ZIP-code income quartile (ZIQ) to adjust for socioeconomic status (SES). ZIQ is an approximation of the SES of the area in which an individual lives, which is predictive of the SES of an individual. To determine cost per case differences, we performed linear regression using the NIS variable TOTCHD, total charges (cleaned).

Due to the differences among groups, we utilized multiple methods of analysis. Missing data was treated as missing at random and imputed where possible. We set α=0.05 and β = 0.20 for all analysis and used two-sided tests. Power calculations were performed for the primary outcomes (Table 2). There is sufficient power to detect a difference between VitD Winter and Summer for all adverse outcomes except non-healing wounds. Power decreased for latitude analysis with non-healing wound and wound infection being under powered.

TABLE 2 POWER FOR PRIMARY OUTCOMES IN OBSERVED PROPORTIONS

	Season		**Latitude**		**Sex**		**Race**	
Non-healing wound	0.0%		0.0%		0.0%		100.0%	*
Wound infection	100.0%	*	0.0%		0.0%		100.0%	*
Wound dehiscence	100.0%	*	93.0%	*	100.0%	*	100.0%	*
Wound separation	100.0%	*	99.7%	*	100.0%	*	27.7%	
Delayed wound healing	100.0%	*	99.4%	*	100.0%	*	100.0%	*
Any complications	100.0%	*	99.4%	*	100.0%	*	100.0%	*
Length of stay > 3 days	100.0%	*	100.0%	*	11.7%		100.0%	*

* $\beta < 0.20$

RESULTS

We identified 932,091 bariatric surgeries in the NIS dataset from 2001-2010 (Table 3). Most surgeries (51%) occurred during the moderate VitD season (Spring/Fall) while 26% occurred during high VitD season (Summer) and 23% during low VitD season (Winter). The sampled hospitals were mostly urban (83.3%), which was unchanged throughout the seasons. The median ZIQ was 2.68 with no variation between the seasons. More surgical procedures were performed in the North (64.8%) and particularly during VitD Winter ($p < 0.001$). The majority of procedures were performed in women (81.2%) and in whites (74.4%). Sex was unequally distributed among the seasons, p=0.005. Median age was 43.0 years in all season.

TABLE 3 NATIONWIDE INPATIENT SAMPLE BARIATRIC SURGERY DEMOGRAPHICS BY SEASON

	Total	Winter	Spring/Fall	Summer	p-value
Surgeries	932,091	215,060 (23%)	475,186 (51%)	241,845 (26%)	0.000*
Urban ZIP-code	83.3%	83.7%	83.3%	82.8%	0.152
Income Quartile	2.68 ± 0.06	2.68 ± 0.07	2.68 ± 0.06	2.68 ± 0.06	0.628
North (≥37°N)	64.8%	66.3%	64.3%	64.4%	0.007*
Female	81.2%	80.8%	81.0%	81.6%	0.005*
Age (years)	43.0 ± 0.18	43.0 ± 0.21	43.0 ± 0.19	43.0 ± 0.21	0.000*
Race/ethnicity					0.634
White	74.4%	74.8%	74.2%	74.6%	
Black	12.6%	12.6%	12.7%	12.4%	
Hispanic	8.4%	8.0%	8.5%	8.7%	
Asian/PI	0.5%	0.6%	0.5%	0.5%	
Native Amer.	0.6%	0.6%	0.7%	0.5%	
Other	3.4%	3.4%	3.4%	3.3%	

* = significant at the $p < 0.05$ level

PI = Pacific Islander

Table 4 shows the demographics displayed by latitude (North versus South). We found the North cohort to be slightly wealthier ($p<0.001$) and perhaps more rural ($p=0.062$). The large proportion of females (81%) and median age (43.0 years) is consistent between geographic regions. The North cohort is more prominently white (77.1% versus 70.4%, $p < 0.001$), largely due to fewer blacks and Hispanics.

TABLE 4 NATIONWIDE INPATIENT SAMPLE BARIATRIC SURGERY DEMOGRAPHICS BY SEASON & LATITUDE

	Total	North (≥37°N)	South (<37°N)	p-value
Surgeries	932,091	603,940 (65%)	328,151 (35%)	0.000 *
Urban	83.3%	81.7%	85.7%	0.062
ZIP-code Income Quartile	2.68 ± 0.06	2.75 ± 0.08	2.55 ± 0.08	0.000 *
Female	81.2%	81.3%	80.9%	0.444
Age (years)	43.0 ± 0.18	43.0 ± 0.23	42.9 ± 0.29	0.677
Race/ethnicity				0.000 *
White	74.4%	77.1%	70.4%	
Black	12.6%	12.0%	13.6%	
Hispanic	8.4%	6.6%	11.3%	
Asian/PI	0.5%	0.5%	0.6%	
Native Amer.	0.6%	0.4%	0.9%	
Other	3.4%	3.5%	3.3%	

* = significant at the $p < 0.05$ level

PI = Pacific Islander

Table 5 shows the frequency of adverse outcomes by season, ranging from n=72 for wound infection and non-healing wounds to n=367,245 for prolonged LOS. Fascial dehiscence, suprafascial dehiscence, delayed healing, and any complication are significantly different by season (Figure 15). All outcomes except non-healing wound decrease in frequency with increasing VitD season.

TABLE 5 FREQUENCY OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY BY SEASON

	Total		Winter		Spring / Fall		Summer		
	n	%	n	%	n	%	n	%	p-value
Non-healing wound	72	0.01	24	0.01	34	0.01	14	0.01	0.627
Wound infection	76	0.01	36	0.02	35	0.01	< 11	0.00	0.060
Fascial dehiscence	666	0.07	174	0.08	385	0.08	107	0.04	0.030*
Suprafascial dehiscence	804	0.09	289	0.13	358	0.08	157	0.07	0.001*
Delayed wound healing	948	0.10	349	0.16	427	0.09	172	0.07	<0.001*
Any complication	1608	0.17	523	0.24	811	0.17	274	0.11	<0.001*
Length of stay > 3 days	367,245	39.40	88,757	41.27	184,187	38.76	94,301	38.99	<0.001*

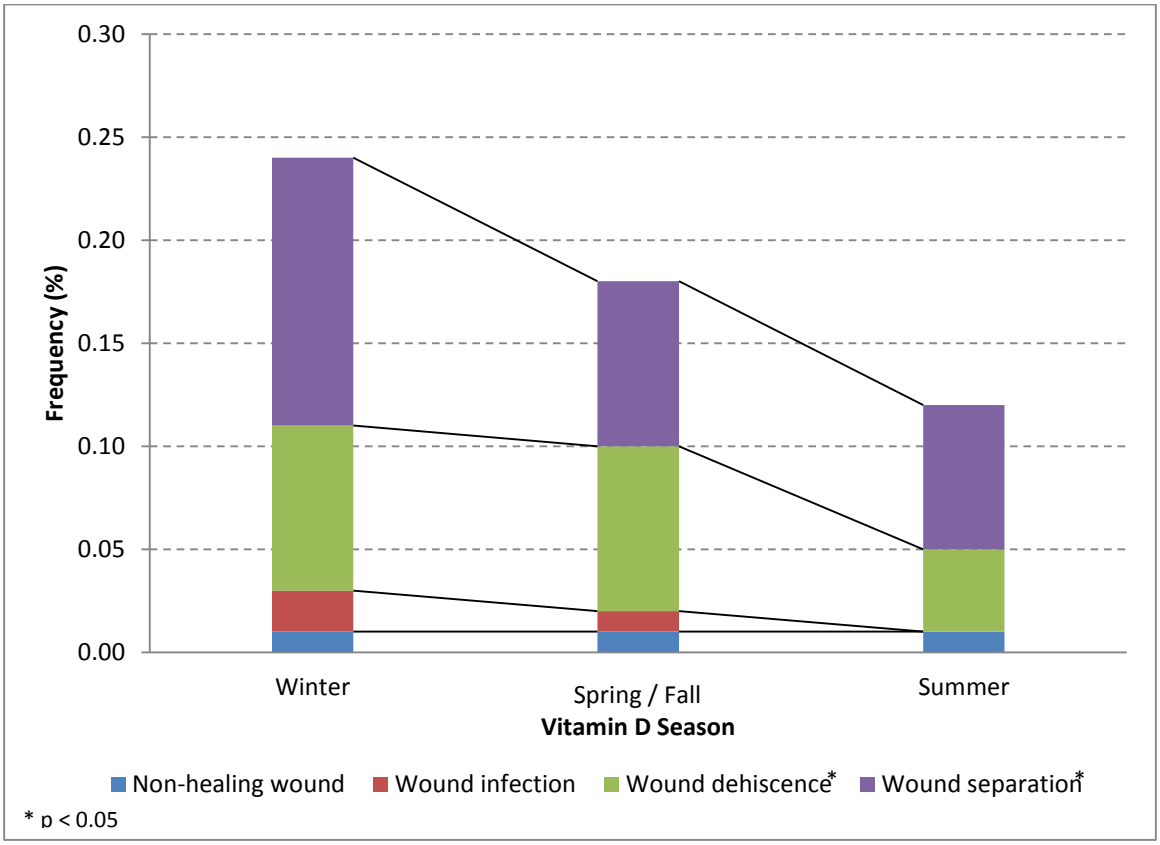

FIGURE 15 SEASONALITY OF WOUND COMPLICATIONS FOLLOWING BARIATRIC SURGERY IN THE NATIONWIDE INPATIENT SAMPLE, 2001-2010. Vitamin D Winter (January to March) is the time of lowest vitamin D status while Summer (July to September) is the highest. Spring/Fall (October to December and April to June) is the intermediate season.

Table 6 shows the frequency of adverse outcomes by latitude (North versus South). Prolonged LOS was more common in the North (p<0.001). The increased occurrence of complications in the North versus the South did not reach statistical significance (Figure 16).

TABLE 6 FREQUENCY OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY BY LATITUDE

	Total		North		South		
	n	%	n	%	n	%	p-value
Non-healing wound	72	0.01	46	0.01	26	0.01	0.895
Wound infection	76	0.01	56	0.01	20	0.01	0.477
Fascial dehiscence	666	0.07	456	0.08	210	0.06	0.416
Suprafascial dehiscence	804	0.08	577	0.10	227	0.07	0.091
Delayed wound healing	948	0.10	674	0.11	274	0.08	0.105
Any complication	1608	0.17	1124	0.19	484	0.15	0.107
Length of stay > 3 days	367,244	39.40	261,067	43.23	106,177	32.36	<0.001 *

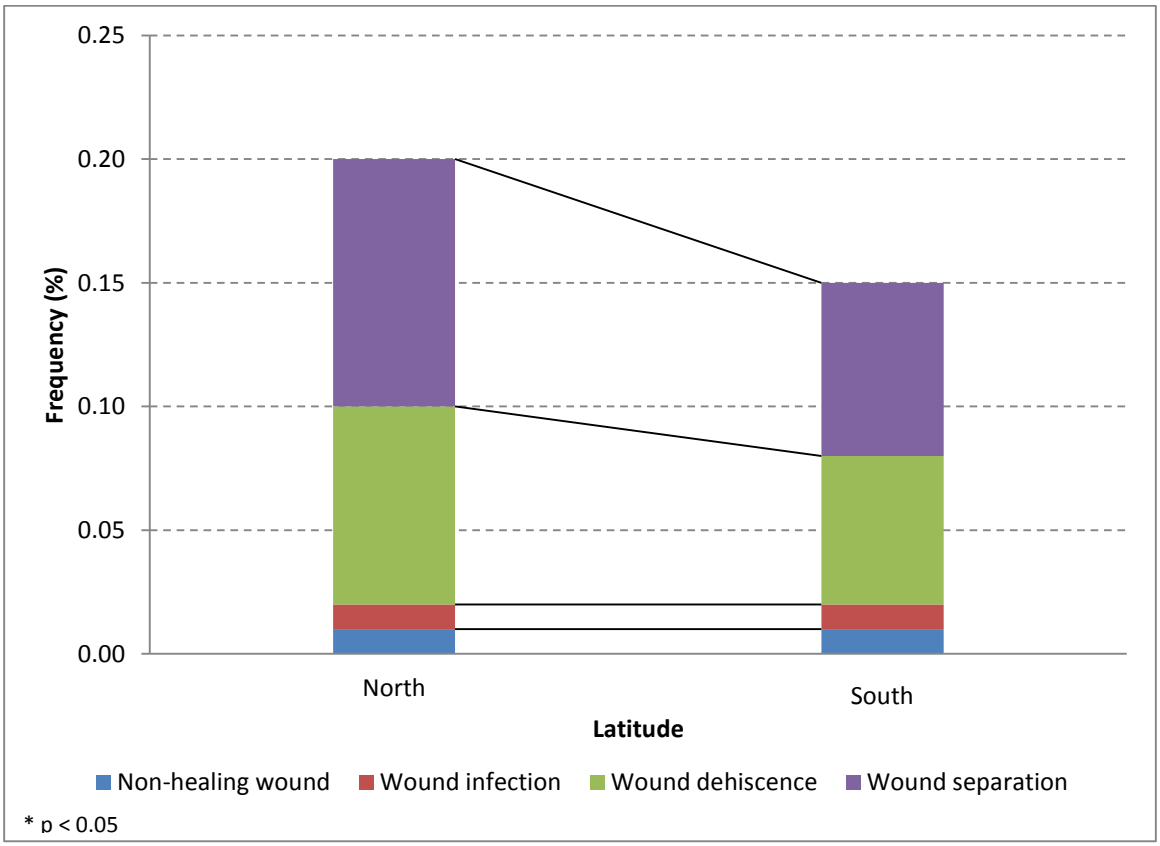

FIGURE 16 LATITUDE AND WOUND COMPLICATIONS FOLLOWING BARIATRIC SURGERY. The frequency of wound complications following bariatric surgery in the North cohort (≥37°N) versus the South cohort (<37°N) in the Nationwide Inpatient Sample, 2001-2010.

Table 7 shows logistic regression for adverse outcomes and VitD seasonality as a spectrum both unadjusted (Model 1) and adjusted for potential confounders (Models 2 and 3). VitD seasonality is significantly correlated with suprafascial dehiscence, delayed wound healing, any complication, and prolonged LOS in all models. For each season closer to VitD Winter, the odds of experiencing suprafascial dehiscence (OR 1.45-1.49), delayed wound healing (OR 1.53-1.56), any complication (OR 1.45-1.46), and LOS (OR 1.04-1.05) significantly increases. Fascial dehiscence is significantly correlated with VitD seasonality in Models 1 and 2 (OR 1.30-1.33). The strong trend observed between VitD seasonality and wound infection is significant in Model 1 (OR 2.50).

TABLE 7 ODDS OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY: SEASONALITY AS A SPECTRUM

VitD Seasonality as a Spectrum

	Model 1		Model 2		Model 3	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**	**Odds Ratio**	**p-value**
Non-healing wound	1.40	0.389	1.61	0.294	1.67	0.303
Wound infection	2.50	0.018 *	1.97	0.137	1.98	0.133
Fascial dehiscence	1.30	0.025 *	1.33	0.037 *	1.32	0.053
Suprafascial dehiscence	1.49	0.001 *	1.45	0.022 *	1.46	0.023 *
Delayed wound healing	1.56	< 0.001 *	1.53	0.006 *	1.55	0.006 *
Any complication	1.46	< 0.001 *	1.45	< 0.001 *	1.46	< 0.001 *
Length of stay > 3 days	1.05	< 0.001 *	1.04	0.001 *	1.05	< 0.001 *

Model 1: Unadjusted
Model 2: Adjusted for latitude, age, sex, and race (white vs. non-white)
Model 3: Adjusted for latitude, age, sex, race, Charlson Comobidities Index, and ZIP-code income quartile

* = significant at the $p < 0.05$ level

We also analyzed the association of adverse outcomes comparing the highest VitD season (Summer) to the lowest (Winter), see Table 8. Suprafascial dehiscence (OR 1.89-2.07), delayed wound healing (OR 2.13-2.29), any complication (OR 1.51-2.10), and prolonged LOS (OR 1.09-1.10) remained significant in all models. Fascial dehiscence (OR 1.82-1.92) was significant in Models 1 and 2. Despite strong trends, neither non-healing wound (OR 1.88-2.46) or wound infection (OR 4.38-7.56) reached statistical significance. Wound infection was borderline significant in Model 1 (OR 7.56, p=0.059).

TABLE 8 ODDS OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY: WINTER VS. SUMMER

VitD Winter versus Summer

	Model 1		Model 2		Model 3	
	Odds Ratio	**p-value**	**Odds Ratio**	**p-value**	**Odds Ratio**	**p-value**
Non-healing wound	1.88	0.390	2.46	0.303	2.44	0.305
Wound infection	7.56	0.059	4.38	0.187	4.44	0.184
Fascial dehiscence	1.82	0.034 *	1.92	0.048 *	1.85	0.063
Suprafascial dehiscence	2.07	0.001 *	1.89	0.019 *	1.89	0.019 *
Delayed wound healing	2.29	< 0.001 *	2.13	0.005 *	2.13	0.005 *
Any complication	1.51	0.007 *	2.10	< 0.001 *	2.07	< 0.001 *
Length of stay > 3 days	1.10	< 0.001 *	1.09	< 0.001 *	1.10	< 0.001 *

Model 1: Unadjusted
Model 2: Adjusted for latitude, age, sex, and race (white vs. non-white)
Model 3: Adjusted for latitude, age, sex, race, Charlson Comobidities Index, and ZIP-code income quartile

* = significant at the $p < 0.05$ level

We performed logistic analysis to determine the association of latitude with adverse outcomes (data not shown). Trends similar to seasonality were observed. The only significant relationship

with latitude was prolonged LOS (OR 1.42-159), which held after adjustment for season, age, sex, race, CCI, and ZIQ.

Finally, we analyzed differences between sex (Table 9) and race (

Table 10). Fascial dehiscence and any complication were significant in all stratums, meaning there is a significantly higher risk of complications in males compared to females and whites compared to non-whites. Suprafascial dehiscence, fascial dehiscence, delayed wound healing, and any complication were significantly more likely in males. Fascial dehiscence and any complication were significantly more likely in whites.

TABLE 9 COMPLICATIONS FOLLOWING BARIATRIC SURGERY STRATIFIED BY SEX

	Males		**Females**			
	n	**%**	**n**	**%**	**p-value**	
Total	175,259		754,346			
Non-healing wound	47	0.01	64	0.01	0.496	
Wound infection	11	0.01	66	0.01	0.647	
Wound dehiscence	253	0.14	414	0.05	<0.001	*
Wound separation	232	0.13	573	0.08	0.001	*
Delayed wound healing	251	0.14	697	0.09	0.007	*
Any complication	507	0.29	1,100	0.15	<0.001	*
Length of stay > 3 days	68,984	39.4	297,743	39.5	0.846	

* $p < 0.05$ level

TABLE 10 COMPLICATIONS FOLLOWING BARIATRIC SURGERY STRATIFIED BY RACE

	White		All Others			
	n	%	n	%	p-value	
Total	547,712		188,175			
Non-healing wound	44	0.01	< 11	0.00	0.515	
Wound infection	47	0.01	< 11	0.00	0.274	
Wound dehiscence	437	0.08	72	0.04	0.006	*
Wound separation	393	0.07	122	0.06	0.663	
Delayed wound healing	478	0.09	136	0.01	0.413	
Any complication	919	0.17	203	0.11	0.017	*
Length of stay > 3 days	205,135	37.5	66,244	35.2	0.061	

* $p < 0.05$ level

DISCUSSION

We have demonstrated a strong and graded relationship between seasonality (proxy for group VitD status) and adverse outcomes following bariatric surgery. Our results support the role of VitD in preventing complications following bariatric surgery, especially as it pertains to wound healing and infection as well as prolonged LOS in the hospital. The association was strongest for suprafascial dehiscence (p=0.001) and fascial dehiscence (p=0.034), and this relationship held after adjusting for latitude, age, sex, race, CCI, and SES. The largest estimated effect size was seen in wound infections (OR 7.56, p=0.059). Extended LOS post-operatively was also correlated with VitD season, p<0.001 in all models. The cost per case was $680.63 higher in VitD Winter than Summer and $8,961.84 in the North versus the South, some of the difference in cost likely results from longer hospital stays.

Non-healing wounds occurred in just 0.01% of bariatric surgeries overall and in each season. As with other infrequent outcomes, a relationship may be significant in future studies directly measuring individual VitD status—serum 25(OH)D concentration—instead of a proxy measurement. Furthermore, adverse outcomes appeared to be more common in the North

where solar UVB radiation is less available for VitD photoproduction with prolonged LOS reaching statistical significance ($p<0.001$).

Our analysis also revealed that suprafascial dehiscence, fascial dehiscence, delayed wound healing, and any complication were significantly more likely in males compared to females. This has been reported in other studies. [15] It is possible that wound complications occur more frequently in males due to decreased production of collagen, leading to a reduced capacity for wound healing.[15]

Fascial dehiscence and any complication were significantly more likely in whites compared to non-whites. This seems counter to the knowledge that whites are better able to photoproduce VitD given the same dose of UVB radiation. However, white skin also tends to have less efficient barrier function. Individuals of European descent are more likely to have mutations in proteins required for the barrier function of skin. One such mutation occurs in the gene for filaggrin. This mutation has tracked with low melanin production and increased ability to photoproduce VitD[15;26;27], which may explain why whites are more likely to experience wound complications in this cohort. Furthermore, VitD has been shown to impact claudins and cadherins, proteins required for skin integrity.[15]

With the obesity epidemic on the rise, bariatric surgery will become more common. It is crucial to optimize the results of these surgeries by reducing alterable risk factors. The potentially synergistic role of VitD insufficiency and obesity on chronic inflammation may lead to increased risk of poor wound healing and infection. If we can supplement patients with VitD prior to surgery it is possible we will decrease the rates of wound infection and delayed wound healing and limit the LOS required post-operatively. A study examining the effect of VitD status directly—serum 25(OH)D concentration—instead of a proxy is the logical next step. Ultimately, a

randomized, controlled trial will need to be performed to establish a causal role for VitD insufficiency in adverse outcomes.

LIMITATIONS

NIS contains data at the discharge level, not the individual level. Therefore, it is possible that some of the individuals were readmitted for complications at a later date, and we are unable to account for this. This database also lacks some variables that could affect adverse outcomes or VitD status, such as BMI or length and difficulty of the surgical procedure. Future prospective studies should include these measures if possible.

As in other bariatric surgery populations, we found smaller numbers of males and non-whites. Because of these low numbers, the results in these stratums were less significant than in females and whites, but the directionality of the relationships remained the same. This is an inherent issue to studying bariatric surgery at this time.

Furthermore, VitD season is a proxy, not the direct measure of VitD status, which is serum 25(OH)D concentration. For any individual, serum 25(OH)D could be significantly higher or lower than we would expect for the VitD season, e.g. if a patient took VitD supplements. It is also possible that there are seasonal differences in other factors that could contribute to the observed differences, such as winter being a time of increased exposure to infectious agents or that stays were extended due to snow storms. Adjusting for latitude should help account for effects such as snow storms. Future studies should be conducted measuring 25(OH)D to confirm our findings.

Strengths

The large sample size afforded by the NIS has allowed for detection of differences in adverse outcomes using proxy measures of VitD status where obtaining serum 25(OH)D concentration is not currently available. The number of surgeries has also yielded less common adverse outcomes like wound infection in sufficient number to detect a difference between VitD seasons.

By adding latitude into NIS, we strengthened our analysis. Latitude is an additional proxy measure of group VitD status, allowing us to confirm the results of our analysis utilizing seasonality. Furthermore, latitude is a potential effect modifier of the relationship between seasonality and adverse outcomes. Controlling for latitude enhanced our analysis of the primary outcome measure, season.

Conclusions and Relevance

Adverse outcomes following bariatric surgery are most common from January to March when VitD status is lowest (VitD Winter). The largest increased odds from VitD Summer to Winter were for wound infection. The strongest correlations were observed for suprafascial dehiscence, fascial dehiscence, and extended LOS. Adverse outcomes appeared to be more common in the northern latitudes where solar UVB radiation is less available for VitD photoproduction with prolonged LOS reaching statistical significance ($p<0.001$). VitD supplementation, an easy and inexpensive treatment, may mitigate these risks and reduce the number of adverse outcomes following bariatric surgery.

We have established five of Hill's Criteria for Causation: strong (1) graded (5) correlations for a coherent (7), biologically plausible pathway (6), consistent with the findings of other studies (2). Further studies are warranted to ascertain the biological gradient at the individual level (5: dose-response) and establish the temporality (4) and specificity (3) of the relationship.

CHAPTER 3: PROXY MEASURES OF VITAMIN D STATUS CORRELATE WITH ADVERSE SURGICAL OUTCOMES IN ADOLESCENTS UNDERGOING BARIATRIC SURGERY

INTRODUCTION

In the United States (US) National Health and Nutrition Examination Survey (NHANES) 2011-2012, 20.5% of 12 to 19 year olds were obese (BMI ≥ 35 kg/m^2).[21;22] This current incidence of adolescent obesity is more than quadruple that 30 years prior and reflects a global trend with the greatest increase in the top 5% of the distribution of body mass index (BMI).[30;168] Obesity was slightly more prevalent in non-Hispanic blacks (22.1%) and Hispanics (22.6%) than non-Hispanic whites (19.6%).[30] Childhood obesity increases the risk of hyperlipidemia and hypertension and in turn the risk for cardiovascular disease.[32-37] As in adults, adolescents with obesity are more likely to be pre-diabetic or diabetic.[23;24;38] In NHANES 2007-2009, 215,000 Americans under the age of 20 had diabetes.[23;24] Obesity is also associated with decreased quality of life and social exclusion.[169] Childhood obesity typically continues through adulthood with more than 3 in 4 obese children becoming obese adults.[170] Consequently, childhood obesity is likely to lead to the chronic diseases associated with adult obesity such as cardiovascular disease, metabolic syndrome, and non-alcoholic fatty liver disease (NAFLD).[171]

Several studies have demonstrated that many obese adolescents are vitamin D (VitD) insufficient (<75 nmol/L, 30 ng/ml) or deficient (<50 nmol/L, 20 ng/ml). For instance, more than 50% of Hispanic and black adolescents living in Boston were found to be deficient in VitD [171]. Another study of obese adolescents in New York City found that 65% were VitD deficient.[172] In

the Southern US, Olson et al. found VitD deficiency in 50% of children aged 6 to 16 years despite relatively plentiful sun, allowing for photoproduction of VitD.[172;173] These obese children in the Southern US had significantly lower stores of VitD compared to lean controls (49.0 versus 67.5 nmol/L), which would require an estimated 600 to 1200 IU increased intake daily to correct.[172]

The pervasiveness of poor VitD status in obese adolescents is not limited to the US. A prospective study of obese adolescents in Poland found that 86% were VitD deficient with 50% exhibiting severe deficiency (< 25 nmol/L, 10 ng/ml).[151] In this Polish cohort, VitD deficiency was more common in winter than summer, consistent with other studies.[171-176] VitD status was also correlated with body and fat mass in these Polish obese adolescent.[153;154;174] In a large study of adults, Vimaleswaran et al. found a 10% increase in Body Mass Index (BMI) was correlated with a 4% decrease in VitD status.[125;155] Similar results have been observed in other adolescent cohorts as well [156]. The inverse relationship between BMI and VitD status is hypothesized to be due to sequestration of VitD by adipose tissue, reducing VitD bioavailability.

VitD is crucial for bone calcification especially in the developing adolescent. VitD also plays a key role in re-epithelialization and thus wound healing in addition to immune function, including elimination of infection without excessive inflammation. VitD helps to improve chronic inflammation, which may prevent autoimmunity, cancer, and chronic disease.[130;157] Obesity is positively associated with such chronic inflammation[177-182], which may contribute to improper wound healing[148] and wound infection[173]. Therefore, insufficient VitD in obese patients greatly increases the risk of complications in an already at risk population.

Without successful intervention, adolescent obesity and its associated comorbidities will likely lead to premature death. Currently, bariatric surgery is the most effective long-term weight loss treatment for adults and adolescents. [158;159] As with all surgical procedures, complications are

possible; however, bariatric surgery patients may be at increased risk of complications since it is a treatment for class 3+ obesity (BMI ≥ 40 kg/m^2) and related comorbidities.

Adult pre-operative bariatric surgery patients have been found to have significantly lower VitD status compared to non-obese matched controls.[181-184] In one study of adolescents seeking bariatric surgery, VitD deficiency was found in 53% of patients while 82% were VitD insufficient.[185] Due to obesity, comorbidities, and potentially VitD deficiency, bariatric surgery patients are at greater risk of most adverse surgical outcomes. Key complications in VitD deficient patients undergoing bariatric surgery are delayed wound healing (poor re-epithelialization) and infection (weak innate immunity).[186]

Adverse surgical outcomes and clinical outcomes in adolescents undergoing bariatric surgery have been found to be similar in type and frequency as in adults.[183;187] Varela, Hinojosa, and Nguyen found significantly lower 30 day complication rates in 309 adolescents compared to 55,192 adults undergoing bariatric surgery while acute morbidity and mortality were similar.[185] The adolescents tended to have less severe comorbidities than the adults prior to surgery and fewer adolescents were sent to the intensive care unit (ICU) following their bariatric procedure.[185] In both adolescent and adult bariatric surgeries, the most prevalent procedure was gastric bypass (62% vs. 92%, laparoscopic and open combined).[185] Adjustable gastric banding (AGB) and gastroplasty were both performed laparoscopicaly and in 29% and 9% of adolescents respectively.[185] These procedures were performed more commonly in adolescents than adults (29% vs. 6% for AGB and 9% vs. 2% for gastroplasty). Since the AGB procedure has significantly lower risk of acute complications, this may contribute to the lower 30 day complication rates seen in these adolescents compared to the adults undergoing bariatric surgery. The better overall health of these adolescents is also an expected contributor.

Furthermore, Alqahtani et al. found adolescents undergoing laparoscopic sleeve gastrectomy had lower morbidity and mortality with better resolution of comorbidities than adults undergoing the same procedure.[186]

As in adults, traditional weight loss methods such as dieting or medical management are modestly effective at best in treating class 3+ obesity. Multi-disciplinary centers for childhood obesity have been formed in an attempt to augment these traditional methods. Despite these resources, many adolescents with class 3+ obesity do not successfully maintain weight loss, likely due to family and psychosocial issues.[160] Bariatric surgery offers greater weight loss and remission rates for metabolic syndrome, type 2 diabetes, and hypertension in both adults and adolescents, which is why adolescents are increasingly being referred to bariatric surgeons.[148;188] In children and adolescents, bariatric surgery has been shown to significantly decrease the risk of obesity in adulthood and obesity-related comorbidities such as NAFLD and sleep apnea.[189;190] Additionally, bariatric surgery in this younger cohort is more cost-effective in relation to the increased quality-of-life-years.[191] In the just published European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Hepatology Committee Position Statement, detailed indications and limitations were set forth for adolescents like those for adults.[189] The indications boil down to: patients with a BMI ≥ 35 kg/m_2 with severe obesity-related comorbidities such as NAFLD or a BMI ≥ 40 kg/m_2 with mild obesity-related comorbiities.[189] Defining the indications for bariatric surgery in adolescents is a major step in improving the treatment of obesity.

The classical epidemiological proxy measure for group VitD status is time of year (VitD seasonality) since photoproduction of VitD in the skin during exposure to the sun largely determines status.[162] This extensively utilized technique has been confirmed by many studies

using direct measurement of VitD status--serum 25(OH)D concentration. VitD status in the US peaks in August and troughs in February.[162] Thus, the time of highest VitD status (VitD Summer) is July to September. VitD Winter, the time of lowest VitD status, is January to March. These are somewhat delayed from traditional seasons (summer: June to August, winter: December to February). To include the full spectrum of group VitD status, we used VitD Spring (April to June) and VitD Fall (October to December) as moderate VitD season.

Pre-operative bariatric surgery patients have been shown to have improved VitD status during summer compared to winter.[162] We have also investigated VitD seasonality in adult bariatric patients. Using the Nationwide Inpatient Sample (NIS), we showed that adverse outcomes following bariatric surgery are most common from January to March when VitD status is lowest (VitD Winter), see Chapter 2. The largest increased odds from VitD Summer to Winter were for wound infection. The strongest correlations were observed for dehiscence and extended LOS. We also found that a case would cost $680.63 more in Winter compared to Summer and $8,961.84 in the North versus the South (Chapter 2). A likely contributor to this increased cost is longer hospital stays.

Another classic epidemiological proxy measure of group VitD status is latitude since individuals farther from the Equator have less opportunity to photoproduce VitD. Skin exposed to sunlight on a cloudless day in Boston (42.2°N) from November to February did not photoproduce VitD.[18] The farther north, the longer that photoproduction cannot occur. In Edmonton (52°N), no photoproduction occurred from October to March. [19] VitD can be photoproduced all year long in more southern latitudes such as 34°N (Columbia, South Carolina) and 18°N (US Virgin Islands) [29]. Previously, we investigated latitude in adult bariatric patients. Adverse outcomes appeared to be more common in the northern latitudes where solar UVB radiation is less available for VitD

photoproduction with prolonged LOS reaching statistical significance ($p<0.001$). We have chosen the cut-point of 37°N (Virginia Beach, Virginia). At and above 37°N, VitD photoproduction is limited during winter and thus VitD deficiency is more likely. We grouped hospitals located at or above 37°N into the North cohort, the low VitD status group, and those located below 37°N into the South cohort, the high VitD status group.

The aim of our study was to investigate the association between proxy measures of group VitD status (seasonality and latitude) and the risk of adverse outcomes following bariatric surgery in adolescents.

METHODS

We conducted a retrospective cohort study using the Nationwide Inpatient Sample (NIS), a database established as part of the Healthcare Cost and Utilization Project and sponsored by the Agency for Healthcare Research and Quality. NIS contains all-payer data on inpatient stays from over 1,000 hospitals in the US each year. NIS approximates a 20% stratified sample annually, meaning every US hospital will be sampled at least once in any 10 year period. Our analysis was performed using inpatient stays from 2001 to 2010, one 10 year period.

We restricted our analysis to bariatric surgery patients aged 13 to 21 years using International Classification of Diseases, 9th Revision, (ICD-9) codes. The codes 446.8 and 449.5 are sufficient to indicate bariatric surgery alone. The code for class 3 obesity (278.01) must be used in conjunction with the following codes to indicate bariatric surgery: 438.9, 443.1, 443.8, and 443.9. Furthermore, we excluded stays including cancer (150, 151, 152, 157, and 199), ulcer (531 and 533), or revision codes (442.1-2, 437, 445, 446.9, 449.6-7).

Primary Exposure and Outcomes

We indirectly assessed group VitD status in bariatric surgery cases using two classical epidemiological proxy measures. The first measure, the primary exposure, is time of year (season). Season utilizes the principle that the primary determinant of VitD status is sun exposure (photoproduction). VitD season was defined by the following groups: VitD Summer (July to September), Winter (January to March), and Fall/Spring (October to December and April to June). VitD status is highest in VitD Summer (July to September) and lowest in VitD Winter (January to March). VitD status is moderate in VitD Fall (October to December) and VitD Spring (April to June).

The second proxy measure, latitude, is based on the principle that individuals farther from the equator have less opportunity to photoproduce VitD and thus have lower VitD status as a group. For all analysis, we used the latitude of the sampled hospital. Latitude was computed based on the hospital ZIP-code from the NIS. SAS version 9.3 PROC GEOCODE (SAS Institute, Cary, NC) was used to convert ZIP-codes to latitudes. We created a dichotomous variable to distinguish between locations at or above 37°N (North) and those below 37°N (South).

The primary outcomes were adverse surgical outcomes: non-healing wounds, wound infection, fascial dehiscence, suprafascial dehiscence, delayed wound healing, any complication, and prolonged length of hospital stay. Outcomes were determined using ICD-9 codes, grouped as in

Table 11 Suprafascial dehiscence is defined as the early stage of dehiscence where wound separation is limited to skin and subcutaneous tissue while fascial dehiscence occurs once wound separation has deepened to include fascia and possibly muscle. Delayed wound healing is defined as the occurrence of non-healing wound, wound infection, or suprafacial dehiscence.

We also used the LOS variable in NIS to create the dichotomous variable extended LOS (> 3 days versus ≤ 3 days).

TABLE 11 DEFINING ADVERSE OUTCOMES FOR BARIATRIC SURGERY IN THE NATIONWIDE INPATIENT SAMPLE

Outcome Variable	ICD-9 Codes	Description
Non-healing wound	998.83	Non-healing surgical wound
Wound infection	998.51	Infected postoperative seroma
Fascial dehiscence	998.31	Dehiscence of surgical wound involving the fascia and/or muscle
Suprafascial dehiscence	998.3, 998.32	Dehiscence of surgical wound involving external tissues, i.e. mucosa, skin, subcutaneous tissues
Delayed wound healing	998.3, 998.32, 998.51, 998.83	All above except fascial dehiscence
Any complication	998.3, 998.31, 998.32, 998.51, 998.83	All above

ICD-9 = International Classification of Diseases, 9th Revision

STATISTICAL ANALYSIS

Statistical analysis was performed using Stata 12.1 (StataCorp, College Station, TX). We compared the occurrence of adverse outcomes over the spectrum of VitD seasonality. Since our outcome is dichotomous (outcome / no outcome) and our independent variables are a mix of continuous and categorical, X^2 tests and logistic regression models were used. In our logistic regression models, we utilized Charlson Comorbity Index (CCI) to adjust for comorbidities. CCI interprets multiple pieces of data to predict the 10 year mortality for an individual, allowing comparison between myriad of diseases in a cohort with multiple comorbidities. We utilized ZIP-

code income quartile (ZIQ) to adjust for socioeconomic status (SES). ZIQ is an approximation of the SES of the area in which an individual lives, which is predictive of the SES of an individual.

Due to the differences among groups, we utilized multiple methods of analysis. Missing data was treated as missing at random and imputed where possible. We set α=0.05 and β = 0.20 for all analysis and used two-sided tests. Power calculations were performed for the primary outcomes (Table 12). There is insufficient power to detect a difference between VitD Winter and Summer for all adverse outcomes. We had sufficient power for the latitude analysis with prolonged LOS only.

TABLE 12 POWER FOR PRIMARY OUTCOMES IN OBSERVED PROPORTIONS

	Season	**Latitude**	
Non-healing wound	0.0%	0.0%	
Wound infection	0.0%	0.0%	
Wound dehiscence	6.4%	5.4%	
Wound separation	0.0%	0.0%	
Delayed wound healing	0.0%	0.0%	
Any complications	6.4%	5.4%	
Length of stay > 3 days	11.6%	100.0%	*

* β < 0.20

RESULTS

We identified 15,510 adolescent bariatric surgeries in the weighted NIS dataset from 2001-2010 (

Table 13). Most of these surgeries (52%) occurred during the moderate VitD season (Spring/Fall) while 28% occurred during high VitD season (Summer) and 20% occurred during low VitD season (Winter). The hospitals where these surgeries occurred were mostly urban (86.4%), which was

unchanged throughout the seasons. The median ZIP code income quartile (ZIQ), a rough approximation of socio-economic status (SES), was 2.68 with no variation between the seasons. More surgical procedures were performed in northern latitudes (66.0% in the North). The majority of procedures were performed in women (79.3%) and in whites (68.3%). The median age was 19.2 years in all seasons.

TABLE 13 NATIONWIDE INPATIENT SAMPLE BARIATRIC SURGERY DEMOGRAPHICS BY SEASON

	Total	Winter	Spring/Fall	Summer	p-value
Surgeries	15,510	3,156 (20%)	8,083 (52%)	4,270 (28%)	0.000 *
Urban	86.4%	83.9%	85.8%	89.1%	0.148
ZIP code Income (Quartile)	2.68 ± 0.06	2.67 ± 0.12	2.74 ± 0.09	2.73 ± 0.11	0.628
North (≥37°N)	66.0%	67.1%	66.2%	65.0%	0.729
Female	79.3%	78.4%	79.6%	79.4%	0.822
Age (years)	19.2 ± 0.12	19.4 ± 0.14	19.2 ± 0.12	19.1 ± 0.20	0.000 *
Race/ethnicity					0.573
White	68.3%	65.6%	69.5%	68.1%	
Black	12.9%	13.2%	12.7%	12.9%	
Hispanic	12.6%	14.0%	11.9%	13.0%	
Asian/PI	0.7%	1.0%	0.8%	0.4%	
Native Amer.	0.8%	0.8%	1.0%	0.3%	
Other	4.7%	5.4%	4.2%	5.2%	

* = significant at the $p < 0.05$ level

Table 14 shows the demographics displayed by latitude (North versus South). We found the North to be wealthier ($p<0.001$) and perhaps more rural ($p=0.077$). Both geographic regions were mostly females (79%) with median ages of 19.2 years. The North cohort is more prominently white (69.8% versus 65.8%, $p = 0.021$). This was also observed in our analysis of

adult bariatric surgeries in the NIS, but the adult cohort overall was even more predominantly white (74.4% versus 68.3%). The difference in race composition between the North and South is mostly due to fewer Hispanic adolescents (10.2% vs. 16.8%). In our analysis of adult bariatric surgeries in the NIS, the overall difference in race between the North and South cohort was similar, but there were more blacks (13.6% vs. 12.6%) and fewer Hispanics (11.3% vs. 16.8%) in the South than in this adolescent cohort.

TABLE 14 NATIONWIDE INPATIENT SAMPLE BARIATRIC SURGERY DEMOGRAPHICS BY SEASON & LATITUDE

	Total	North (≥37°N)	South (<37°N)	p-value
Surgeries	15,510	10,243 (66%)	5,267 (34%)	0.000 *
Urban	86.4%	84.8%	89.1%	0.077
ZIPcode Income (Quartile)	2.54 ± 0.07	2.80 ± 0.11	2.57 ± 0.10	0.000 *
Female	79.3%	80.5%	76.9%	0.023 *
Age (years)	19.2 ± 0.10	19.4 ± 0.17	18.9 ± 0.16	0.497
Race/ethnicity				0.021 *
White	68.3%	69.8%	65.8%	
Black	12.9%	13.1%	12.6%	
Hispanic	12.6%	10.2%	16.8%	
Asian/PI	0.7%	0.6%	1.0%	
Native Amer.	0.8%	0.8%	0.7%	
Other	4.7%	5.6%	3.2%	

* = significant at the $p < 0.05$ level

PI = Pacific Islander

Table 15 shows the frequency of adverse outcomes by season, ranging from n=15 for fascial dehiscence to n=5,201 for prolonged LOS. Only prolonged LOS might be different by season. There is a non-significant trend towards decreasing frequency with increasing VitD status (seasonality) for fascial dehiscence and any complication. Extended LOS was less frequent in VitD Summer than Winter, but was not significant.

TABLE 15 FREQUENCY OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY BY SEASON

	Total		Winter		Spring / Fall		Summer		
	n	%	n	%	n	%	n	%	p-value
Non-healing wound	0	0.00	0	0.00	0	0.00	0	0.00	-
Wound infection	0	0.00	0	0.00	0	0.00	0	0.00	-
Fascial dehiscence	15	0.10	< 11	0.15	< 11	0.13	0	0.00	0.556
Suprafascial dehiscence	0	0.00	0	0.00	0	0.00	0	0.00	-
Delayed wound healing	0	0.00	0	0.00	0	0.00	0	0.00	-
Any complication	15	0.10	< 11	0.15	< 11	0.13	0	0.00	0.556
Length of stay > 3 days	5,201	33.54	1,053	33.38	2,789	34.50	1,359	31.83	0.420

* $p < 0.05$ level

Table 16 shows the frequency of adverse outcomes by latitude (North versus South). Prolonged LOS was more common in the North (p=0.012). The increased occurrence of complications in the North versus the South did not reach statistical significance.

TABLE 16 FREQUENCY OF ADVERSE OUTCOMES FOLLOWING BARIATRIC SURGERY BY LATITUDE

	Total		North		South			
	n	%	n	%	n	%	p-value	
Non-healing wound	0	0.00	0	0.00	0	0.00	-	
Wound infection	0	0.00	0	0.00	0	0.00	-	
Fascial dehiscence	15	0.10	11	0.10	< 11	0.09	0.907	
Suprafascial dehiscence	0	0.00	0	0.00	0	0.00	-	
Delayed wound healing	0	0.00	0	0.00	0	0.00	-	
Any complication	15	0.10	11	0.10	< 11	0.09	0.907	
Length of stay > 3 days	5,201	33.54	3,763	36.73	1,439	27.32	0.012	*

We performed logistic regression for adverse outcomes and VitD seasonality as a spectrum both unadjusted (Model 1) and adjusted for potential confounders (Models 2 and 3). Model 2 adjusted for latitude, age, sex, and race (white vs. non-white). Model 3 adjusted for the potential confounders in Model 2 as well as Charlson Comorbidities Index, and ZIQ. We had insufficient numbers to produce models for all adverse surgical outcomes except prolonged LOS.

VitD seasonality is non-significantly correlated with prolonged LOS in all models (OR 1.00 to 1.07).

We also performed logistic analysis to determine the association of latitude with adverse surgical outcomes. Model 1 showed a significant relationship with living in the North and prolonged LOS (OR 1.54, p=0.012), but the significance did not hold after adjustment for confounders (Models 2 and 3).

We analyzed differences between sex and race (white versus non-white). Prolonged LOS appeared more prevalent in non-whites compared to whites (34.0% vs. 29.9%, p=0.110). Extended LOS had approximately the same prevalence in females compared to males (33.6% vs. 33.4%, p=0.931).

DISCUSSION

We have previously demonstrated a strong and graded relationship between seasonality (proxy for group VitD status) and adverse outcomes following bariatric surgery in adults. This study is an attempt to replicate these results in adolescents. Our results are in line with VitD status potentially playing a role in preventing complications following bariatric surgery, especially in relation to prolonged LOS in the hospital. We were only sufficiently powered to detect a difference in LOS by latitude (North vs. South), which did yield a significant negative correlation between VitD status and prolonged LOS (p=0.012). Our other results showed a non-significant trend towards a negative correlation of VitD status (seasonality or latitude) and adverse surgical outcomes (dehiscence).

Bariatric surgery in adolescents is sparse during the period studied (2001 to 2010), though becoming more established. The lower complication rate in adolescents compared to adults

yielded few cases of adverse surgical outcomes. Fewer complications may be due to adolescents simply being younger and thus healthier. It is also likely that this is the first abdominal surgery for these patients, which leads to a less technically difficult surgical procedure and one that is less likely to be converted to an open procedure. Open procedures are more likely to lead to complications, particularly wound complications.

Due to insufficient numbers of surgeries as well as cases of adverse outcomes, we were unable to detect relationships between proxy measures of VitD status (seasonality and latitude) and adverse outcomes except in the case of latitude and LOS. Infrequent outcomes, such as wound infection, may show a significant relationship in future studies directly measuring individual VitD status—serum 25(OH)D concentration—instead of proxies. Furthermore, as bariatric surgery in adolescents becomes more prevalent and the number of surgeries increases, the number of cases of adverse outcomes will also increase, allowing for the detection of relationships not possible in the current version of the NIS dataset.

LIMITATIONS

Since the NIS data is amassed at the discharge level, not at the level of the individual, it is possible that some of the individuals were readmitted for complications at a later date. We are unable to account for this. This database also lacks some variables that could affect adverse outcomes or VitD status, such as BMI or length and difficulty of the surgical procedure. Future prospective studies should endeavor to include these measures.

Fewer males and non-whites were represented in this database, which is commonly seen in bariatric surgery populations. Modest representation of males and non-whites is an inherent

issue in bariatric surgery at this time. This adolescent cohort contains a larger proportion of Hispanics than we have seen in adults using the NIS.

While we utilized two different classical epidemiological proxy measure for VitD status, they are not direct measures of VitD status, which is serum 25(OH)D concentration. At this time, serum 25(OH)D concentration is not available in the NIS or a similar cohort of this size. While the status of the group as a whole is likely being estimated well by these proxy measures, the status of any individual could be significantly higher or lower than we would expect for the VitD season or latitude, e.g. if a patient took VitD supplements. It is possible that there are additional seasonal differences that could contribute to the observed differences, such as winter being a time of increased exposure to infectious agents or that stays were extended due to snow storms. Adjusting for latitude should help account for effects such as snow storms. Future studies would benefit from determining serum 25(OH)D concentration.

STRENGTHS

Using a national database like the NIS provides a large sample of bariatric surgery cases. This may allow for detection of differences in adverse outcomes using proxy measures of VitD status where serum 25(OH)D concentration is at present unavailable. Replication of this study with additional data from future years may yield significant relationships between VitD proxy measures and adverse surgical outcomes.

We strengthened our analysis by adding latitude, the second proxy measure for VitD status, into the NIS dataset. Adding a second proxy measure of group VitD status potentially allowed us to confirm the results of our analysis utilizing seasonal. Latitude is also a potential effect modifier

of the relationship between VitD season and adverse outcomes; therefore, controlling for latitude improved our analysis of season, the primary outcome measure.

Conclusions and Relevance

Low VitD status may be a risk factor for prolonged LOS as well as adverse surgical outcomes, such as dehiscence. In this study, we were only sufficiently powered to detect a difference in LOS by latitude (North vs. South), yielding a significant negative correlation between VitD status and prolonged LOS ($p=0.012$). Improving VitD status prior to surgery could prove to be a simple and cost-effective measure to decrease the risk of prolonged LOS and negative outcomes in adolescents as well as adults. Therefore, further study into this relationship is merited for both age cohorts.

CHAPTER 4: BASELINE NUTRITIONAL STATUS IN A SAMPLE OF PATIENTS APPROVED TO UNDERGO BARIATRIC SURGERY AT THE JOHNS HOPKINS CENTER FOR BARIATRIC SURGERY

INTRODUCTION

More than 78 million American adults have obesity [192] (BMI ≥35 kg/m^2), costing an estimated $147 billion annually due to medical care alone [193-195]. Non-Hispanic blacks and Hispanics are disproportionately affected with obesity rates of 47.8% and 42.5% respectively compared to 32.6% for non-Hispanic whites [196].

Currently, sustained long-term weight loss is most successfully obtained with bariatric surgery. Since the inception of modern weight loss surgery in 1977 with the development of the Roux-en Y gastric bypass procedure, bariatric surgeons have attempted to optimize patient care by improving techniques and post-operative care, including nutritional counseling [196]. Pre-operative nutrition, on the other hand, is largely uncultivated.

Nutritional deficiencies after surgery are commonly treated, particularly following malabsorptive procedures like the Roux-en Y gastric bypass. If deficiencies are present prior to surgery, it stands to reason that remedying them prior to surgery could be beneficial and certainly easier than after a malabsorptive procedure. Furthermore, malnutrition has been linked to adverse surgical outcomes in many studies [196].

In this study, we assessed the baseline nutritional status of our patients who were approved and scheduled to undergo Roux-en Y gastric bypass. Our aim was to determine the incidence of micronutrient deficiencies in our patient population prior to surgery. We have focused on

vitamins A, B-12, D, E-α, E-β/γ, thiamine, folate, and iron as these are the micronutrient deficiencies we see most commonly in our post-operative patient population.

METHODS

We determined the nutritional status in 58 consecutive patients in this prospective, cross-sectional study. Patients were included if they were aged 18 to 65 years and were approved to undergo the Roux-en Y gastric bypass procedure. As part of the standard of care and preparation for surgery, all patients underwent routine history and physical examination. On the day of surgery in the pre-operative holding area, we collected blood specimens to determine the nutritional status of vitamins A, B-12, D, E-α, E-β/γ, thiamine, folate, and iron (iron, total iron binding capacity (TIBC), and iron saturation). High pressure liquid chromatography was used for vitamins A, E-α, and E-β/γ, liquid chromatography with tandem mass spectrometry for thiamine, and immunoassays for vitamins B-12, D, and folate. Spectrophotemetry was used for iron and turbidity for transferrin.

Statistical analysis was performed using Stata 12.1 (StataCorp, College Station, TX). Missing data was treated as missing at random. We set $\alpha=0.05$ and $\beta = 0.20$ for all analysis and used two-sided tests. Descriptive univariate and bivariate analyses were utilized to describe the nutritional status and comorbidities of our patient cohort. Results are expressed as median ± standard deviation.

This research study was approved by our Institutional Review Board. All patients underwent informed consent prior to participation.

RESULTS

This cohort was 77.6% female and 63.8% white (

Table 17). The median age was 42.5 years (range: 24 to 65 years) with no significant difference by sex or race/ethnicity. The males were heavier and taller. Median BMI was 46.3 kg/m^2 (range: 33.5 to 64.6 kg/m^2), which varied only by race/ethnicity. Hispanics and blacks tended to present with higher BMIs than whites. Comorbidities were highly prevalent in this cohort with multiple comorbidities present in 41.4% of patients: 54% had hypertension, 42.0% were diabetic, and 34.0% had sleep apnea. The highest rate of multiple comorbidities was seen in males, which was largely driven by a higher rate of diabetes and sleep apnea.

Group status for each nutrient is reported in Table 18 (see Tables 19 and 20 for definitions of clinical and frank deficiency, respectively, for each nutrient). Median group status indicates deficiency in vitamin D (clinical: < 30 ng/ml, frank: < 20 ng/ml) in total and in all stratums. Folate (clinical: < 7.2 ug/L, frank: < 4 ug/L) and iron saturation (clinical: < 20%, frank < 14%) were significantly higher in males than females. Nutritional status was poorer in black and Hispanics with vitamin A (clinical: < 20 ug/dL, frank: < 10 ug/dL), vitamin D, vitamin E-α (clinical: < 5.5 mg/L, frank: < 3 mg/L), and thiamine (clinical: < 78 nmol/L, frank: < 70 nmol/L) being significantly lower than in whites. Vitamin D was strongly correlated with BMI (p=0.003) and age (p=0.030). Vitamin A strongly correlated with age (p=0.001) and number of comorbidities (p=0.003).

TABLE 17 PRE-OPERATIVE CHARACTERISTICS OF PATIENTS PRESENTING FOR BARIATRIC SURGERY

Cohort Demographics by Sex

	Total	Female	Male	
Demographics				
Age (years)	42.5 ± 10.9	41.9 ± 11.0	43.0 ± 11.1	
Female	77.6%	n = 45	n = 13	
White	63.8%	60.0%	76.9%	
Weight (lbs)	288.9 ± 46.6	281.5 ± 43.1	316.6 ± 50.5	*
Height (in.)	66.3 ± 3.4	65.2 ± 2.8	70.3 ± 2.4	*
BMI (kg/m^2)	46.3 ± 6.9	46.63 ± 7.0	45.0 ± 6.8	
Medical History				
Hypertension	54.0%	48.7%	72.7%	
Diabetes	42.0%	30.8%	81.8%	*
High cholesterol	32.7%	29.0%	45.5%	
Gastroesophageal reflux	32.0%	30.8%	36.4%	
Sleep apnea	34.0%	25.6%	63.6%	*
Cancer	4.0%	0.0%	18.2%	*
Irritable bowel syndrome	4.0%	0.0%	18.2%	*
Cholecystectomy	14.0%	10.3%	27.3%	
Multiple comorbidities	41.4%	33.3%	69.2%	*

Cohort Demographics by Race/Ethnicity

	White	Black	Hispanic	
Demographics				
Age (years)	43.8 ± 11.7	40.4 ± 9.2	32.7 ± 12.5	
Female	73.0%	83.3%	100%	
White	n = 32	n = 14	n = 3	
Weight (lbs)	283.4 ± 42.5	298.4 ± 56.6	302.7 ± 33.1	
Height (in.)	66.8 ± 3.2	65.8 ± 3.7	62.3 ± 1.5	
BMI (kg/m^2)	44.6 ± 6.3	48.3 ± 6.8	55.0 ± 8.5	*
Medical History				
Hypertension	51.5%	57.1%	66.7%	
Diabetes	45.5%	28.6%	66.7%	
High cholesterol	37.5%	21.4%	33.3%	
Gastroesophageal reflux	45.5%	0.0%	33.3%	*
Sleep apnea	39.4%	21.4%	33.3%	
Cancer	6.1%	0.0%	0.0%	
Irritable bowel syndrome	6.1%	0.0%	0.0%	
Cholecystectomy	15.2%	7.1%	33.3%	
Multiple comorbidities	43.2%	38.9%	33.3%	

* $p < 0.05$

TABLE 18 PRE-OPERATIVE NUTRITIONAL STATUS OF PATIENTS PRESENTING FOR BARIATRIC SURGERY

Nutritional Status by Sex

	Total			Female			Male			
Vitamin A (μg/dL)	55.5	±	21.1	54.4	±	22.1	59.5	±	17.4	
Vitamin B-12 (pg/ml)	451.9	±	235.6	444.0	±	240.6	481.3	±	223.2	
Vitamin D (ng/mL)	16.8	±	6.6	16.7	±	7.1	17.3	±	4.3	
Vitamin E-α (mg/L)	11.4	±	4.8	11.0	±	4.9	12.7	±	4.6	
Vitamin E-β/γ (mg/L)	2.1	±	0.9	2.2	±	0.9	1.7	±	0.7	
Thiamine (nmol/L)	133.7	±	47.0	132.1	±	51.5	139.6	±	24.5	
Folate (ng/mL)	15.3	±	6.2	14.3	±	5.7	19.2	±	6.7	*
Iron (μg/dL)	75.3	±	47.9	70.4	±	36.0	92.6	±	75.5	
TIBC (μg/dL)	360.0	±	80.1	369.9	±	79.8	325.7	±	74.0	
Iron saturation (%)	22.5	±	16.8	19.4	±	9.6	33.4	±	28.9	*

Nutritional Status by Race/Ethnicity

	White			Black			Hispanic			
Vitamin A (μg/dL)	62.9	±	21.1	42.9	±	15.0	40.0	±	2.6	*
Vitamin B-12 (pg/ml)	434.9	±	224.5	464.4	±	185.0	591.0	±	580.9	
Vitamin D (ng/mL)	18.2	±	5.6	14.6	±	7.8	10.7	±	7.4	*
Vitamin E-α (mg/L)	12.7	±	5.3	9.0	±	2.8	9.9	±	1.9	*
Vitamin E-β/γ (mg/L)	2.2	±	1.0	1.9	±	0.7	2.2	±	0.1	
Thiamine (nmol/L)	150.6	±	47.6	105.9	±	30.3	103.0	±	32.2	*
Folate (ng/mL)	15.9	±	6.1	14.3	±	6.8	14.1	±	0.9	
Iron (μg/dL)	83.6	±	56.1	59.2	±	23.3	70.0	±	20.8	
TIBC (μg/dL)	356.4	±	78.7	367.7	±	90.5	358.7	±	33.1	
Iron saturation (%)	25.4	±	20.0	17.0	±	6.6	19.7	±	7.2	

* $p < 0.05$

To further explore the differences in this cohort, we dichotomized nutritional status into normal versus clinical deficiency (Table 19) and frank deficiency cut points (Table 20). We found significant malnutrition, particularly in vitamin D and iron. Deficiency was more prominent in blacks. Multiple micronutrient deficiency (MMND) was more common in blacks, 50.0% versus 39.7% overall. Number of comorbidities did not significantly correlate with MMND.

TABLE 19 FREQUENCY OF CLINICAL DEFICIENCY PRIOR TO BARIATRIC SURGERY BY SEX AND RACE

	Cut-point	Total	Female	Male	White	Black	Hispanic	
Vitamin A	< 20 ug/dL	1.7%	2.2%	0%	0%	5.6%	0%	
Vitamin B-12	< 180 ng/L	3.5%	2.2%	7.7%	2.7%	5.6%	0%	
Vitamin D	< 30 ng/mL	92.9%	90.9%	100%	91.9%	93.8%	100%	
Vitamin E-α	< 5.5 mg/L	5.2%	6.7%	0%	0%	16.7%	0%	*
Thiamine	< 78 nmol/L	1.8%	2.3%	0%	0%	0%	33.3%	*
Folate	< 7.2 ug/L	5.3%	6.7%	0%	2.7%	11.8%	0%	
Iron	Female: < 50 ug/dL Male: < 65 ug/dL	36.2%	35.4%	38.5%	37.8%	38.9%	0%	
TIBC	> 400 ug/dL	22.4%	26.6%	7.7%	24.3%	22.2%	0%	
Iron saturation	< 20%	56.9%	62.2%	38.5%	51.4%	66.7%	66.7%	
Multiple micronutrient deficiencies	**≥ 3**	**39.7%**	**40.0%**	**38.5%**	**35.1%**	**50.0%**	**33.3%**	

* $p < 0.05$

TABLE 20 FREQUENCY OF FRANK DEFICIENCY PRIOR TO BARIATRIC SURGERY BY SEX AND RACE

	Cut-point	Total	Female	Male	White	Black	Hispanic	
Vitamin A	< 10 ug/dL	0%	0%	0%	0%	0%	0%	
Vitamin B-12	< 150 ng/L	1.8%	2.2%	0%	2.7%	0%	0%	
Vitamin D	< 20 ng/mL	71.4%	72.7%	66.7%	64.9%	81.3%	100%	
Vitamin E-α	< 3 mg/L	0%	0%	0%	0%	0%	0%	
Thiamine	< 70 nmol/L	1.8%	2.3%	0%	0%	0%	33.3%	*
Folate	< 4 ug/L	0%	0%	0%	0%	0%	0%	
Iron	Female: < 35 ug/dL Male: < 50 ug/dL	36.2%	35.6%	38.5%	37.8%	38.9%	0%	
TIBC	> 400 ug/dL	22.4%	26.6%	7.7%	24.3%	22.2%	0%	
Iron saturation	< 14%	19.0%	22.2%	7.7%	13.5%	33.3%	0%	
Multiple micronutrient deficiencies	**≥ 3**	**20.7%**	**22.2%**	**15.4%**	**21.6%**	**22.2%**	**0.0%**	

* $p < 0.05$

DISCUSSION

The American Society for Metabolic & Bariatric Surgery (ASMBS) reports that 80% of bariatric surgery patients are female [196], similar to this cohort (77.6% female). Patients are typically between the ages of 40 and 64 years of age [197-201]; our median age was 42.5 years (IQR: 33 to 50 years of age). The majority of surgeries are performed on whites despite higher burden of obesity in blacks and Hispanics. We observed this as well (63.8% white).

The highest risk of morbidity and mortality in obesity is class 3+ obesity (BMI ≥ 40 kg/m^2). Class 3+ obesity was present in 81.0% of this pre-operative bariatric surgery cohort and was more frequent in blacks and Hispanics. Multiple comorbidities were less common in class 3+ obesity (37.5% versus 63.6% in BMI < 40 kg/m^2). This is likely due to the approval process for the surgery itself. Patients with a BMI under 40 kg/m^2 are considered candidates for bariatric surgery if they have "high-risk comorbid conditions such as life-threatening cardiopulmonary problems (for example, severe sleep apnea, pickwickian syndrome, or obesity-related cardiomyopathy) or uncontrolled type 2 diabetes mellitus" or "obesity-induced physical problems interfering with lifestyle (for example, joint disease treatable but for the obesity, or body size problems precluding or severely interfering with employment, family function, and ambulation)" [202-206]. Such considerations may also explain the high rate of multiple comorbidities (41.4%) as well as for hypertension (54%), diabetes (42.0%), and sleep apnea (34.0%) since these likely improve the chance a patient will be approved for bariatric surgery.

Males presented with the highest rate of multiple comorbidities, driven by a higher rate of diabetes and sleep apnea. This is despite the fact that class 3+ obesity was present in 69.2% of males compared to 84.4% of females. This also may be due to the approval process for bariatric surgery. It may be due to the differing motivations for males versus females. Females

undergoing bariatric surgery more frequently cite physical appearance as a motivator [207;208]. Additionally, males have a higher rate of sleep apnea largely due to greater central adiposity [150;151;176;209-212]. Sleep apnea also adds to the metabolic syndrome, e.g. insulin resistance, hypertension, and dyslipidemia [213].

While most comorbidities present in this cohort did not vary by race, the rate of gastroesophageal reflux disease (GERD) was significantly lower in blacks. None of the 14 blacks reported GERD while 45.5% of whites and 1 out of 3 Hispanics reported GER. Caucasians are known to be at greater risk of GERD [70].

Racial disparities also exist in this cohort in nutritional status with blacks and Hispanics having significantly lower status in vitamin A, D, E-α, and thiamine. The primary deficiency in blacks and Hispanics is in vitamin D, which was present in all Hispanics (n=3) in this cohort. Slightly fewer blacks (81.3%) presented with frank vitamin D deficiency and even fewer whites (64.9%) fell into this category. Race is indicative of melanin concentration, and increased melanin concentration in the skin is a risk factor for vitamin D deficiency. Since melanin prevents ultraviolet (UV)-B solar radiation from photoproducing vitamin D in that skin, darker skin tones (blacks and Hispanics) are less able to photoproduce vitamin D given identical sun exposure. In addition to frank deficiency, clinical MMND was more frequent in blacks (50.0% versus 39.7% for the cohort).

Overall, vitamin D and iron were the chief nutritional deficiencies found in pre-operative bariatric surgery patients. Vitamin D was strongly correlated with BMI ($p=0.003$) and age ($p=0.030$), a common finding in the literature [149-152;209-212]. Vitamin A strongly correlated with age ($p=0.001$) and number of comorbidities ($p=0.003$). Since vitamin A is an acute phase reactant, one would expect illness and chronic inflammation to correlate with vitamin A status.

The National Health and Nutrition Examination Survey (NHANES) 2003-2006 was used to develop the body iron model, which predicts that 9% of females will have iron deficiency in the US population [149;152]. The rate of iron deficiency in our cohort is higher than this prediction. We found 22.4% to 56.9% deficiency in all patients. This breaks down to 26.6% to 62.2 % females and 7.7% to 38.5% males. 36.2% of our cohort was deficient with serum iron concentration while TIBC shows 22.4% were deficient. An interesting finding is that more males were deficient than females, according to serum iron concentration (38.5% vs. 35.6%).

The median vitamin D status in this cohort indicates frank deficiency as a group and in each subgroup (stratified by sex and race). According to NHANES 2005–2006 survey, the mean serum 25(OH)D in the United States is 22.4 ng/ml (56 nmol/L) [147-152], higher than the 16.8 ng/ml (42 nmol/L) mean in this cohort of pre-operative bariatric surgery patients . Obesity is a known risk factor for vitamin D deficiency due to an inherent need for more VitD to exhibit the same serum 25(OH)D concentration [196]. This greater need is due to sequestration of this fat soluble hormone in adipose tissue [104;214]. As fat mass increases, an individual will require greater amounts of VitD (via photoproduction from sun exposure, dietary intake, and/or supplementation).

LIMITATIONS

Limited numbers of males and non-Caucasians were included in this study. A larger study would be able to include more subjects from these groups, which currently undergo fewer bariatric surgeries.

STRENGTHS

To our knowledge, this is the most extensive study of pre-operative nutritional status in patients undergoing Roux-en-Y gastric bypass. Our population is generalizable to most academic Centers of Excellence with the majority of patients being female and Caucasian. Additionally, our study assessed a broad range of micronutrients and identified two chief deficiencies for future study: iron and vitamin D. This is consistent with other studies.

CONCLUSIONS

Malnutrition in one or multiple micronutrients is pervasive in this pre-operative bariatric cohort, chiefly in vitamin D and iron. Minorities tended to have greater malnutrition. Further studies should be carried out to determine whether these deficiencies increase the risk of adverse surgical outcomes. Furthermore, the effect of pre-operative supplementation, especially vitamin D and iron, should be explored.

CHAPTER 5: THE STATE OF VITAMIN D TREATMENT IN BARIATRIC SURGERY PATIENTS: A PROSPECTIVE CHART REVIEW OF PRE-OPERATIVE BARIATRIC SURGERY PATIENTS AT THE JOHNS HOPKINS CENTER FOR BARIATRIC SURGERY

INTRODUCTION

Obesity is a known risk factor for vitamin D (VitD) deficiency and fat mass correlates with VitD status [150;175;215]. Thus, patients with morbid obesity seeking surgical weight loss treatment are at high risk of poor VitD status. In patients approved to undergo bariatric surgery, reports of VitD deficiency (< 20 ng/ml, 50 nmol/L) range from 40 to 68.1% according to the 2009 American Society for Metabolic & Bariatric Surgery Medical Guidelines [130;147;156;157]. Risks of VitD deficiency include increased susceptibility to infection, autoimmunity, cancer, chronic disease, and even mental disorder [112]. We have found VitD deficiency in 71.4% of a sample of our patients at the Johns Hopkins Center for Bariatric Surgery (JHCfBS).

In addition to frank deficiency, VitD insufficiency (< 30 ng/ml, 75 nmol/L) is also prevalent in pre-operative bariatric surgery patients. VitD insufficiency increases the risk of many complications and diseases already highly prevalent in obesity such as metabolic disorder [216] and adverse surgical outcomes [104]. According to a previous study, the vast majority (92.9%) of our patients at JHCfBS have VitD insufficiency prior to undergoing bariatric surgery (Chapter 4).

Once a patient has been identified as being VitD deficient or insufficient, they can be treated with VitD supplementation. The best regime for supplementation has changed over time and is still under debate. Cholecalciferol ($VitD_3$) is more effective at increasing serum 25(OH)D

concentration than ergocalciferol ($VitD_2$). A systematic review by Zittermann et al. showed that supplementation with $VitD_3$ resulted in an 8.08 ng/ml (20.19 nmol/L) greater increase compared to the same dose of $VitD_2$.[20] Despite the reduced efficacy of $VitD_2$ most pharmaceutical preparations of VitD in the United States (US) are $VitD_2$. $VitD_2$ is the plant version of VitD and it is not found in significant concentration in the human body without supplementation. Pharmaceutical preparation of $VitD_2$ are often used for high dose, medically monitored, VitD supplementation in VitD deficient and insufficient patients. Contrastingly, most over-the-counter VitD supplements contain $VitD_3$, the form naturally produced in the human body during sun exposure.

Our practice requests serum 25(OH)D testing from the Primary Cary Physician (PCP) of each bariatric surgery candidate if testing has been run pre-operatively. The aim of our study is to assess the extent to which our bariatric surgery candidates, a high risk population for VitD deficiency and insufficiency, have their VitD status tested by their PCP prior to bariatric surgery. Our secondary aim is to assess whether and how our patients are being treated for VitD deficiency and insufficiency pre-operatively.

METHODS

We reviewed the electronic medical records of all pre-operative bariatric surgery patients at our center with upcoming appointments from November 2014 through January 2015 (3 months). In order to assess whether VitD testing was performed by PCPs, we collected serum 25(OH)D concentration and test date or test order date if the testing was still pending. In order to assess treatment, we collected supplementation information including multivitamin and calcium supplements containing VitD. We collected information about type of VitD ($VitD_3$ or $VitD_2$) as well as dosing and frequency.

RESULTS

The majority of our pre-operative patients were female (83.0%, $p<0.001$) with an almost 50/50 split between whites (48.7%) and blacks (44.5%) with a small number of other races (6.4%), $p<0.001$ (Table 21 and

Table 22). The median age was 43 years with males tending to be older than females (49 ± 11 years vs. 42 ± 13 years, p=0.002). Age did not vary significantly by race. Median BMI was 46.3 kg/m^2; BMI did not significantly differ by sex or race. Male surgical candidates were more likely to be white (71.1%, p<0.001) and black candidates were most likely to be female (90.7%, p=0.001-0.004).

TABLE 21 DEMOGRAPHICS OF BARIATRIC SURGERY CANDIDATES BY SEX

	Total	Female	Male	p-value	
Candidates	265	220 (83.0%)	45 (17.0%)	< 0.001	*
Age (years)	43 ± 13	42 ± 13	49 ± 11	0.002	*
BMI (kg/m^2)	46.3 ± 10.5	47.3 ± 10.3	43.5 ± 11.0	0.417	
Procedure				0.175	
GBP	96 (35.6%)	80 (36.4%)	16 (35.6%)	0.870	
VSG	148 (55.8%)	123 (55.9%)	25 (55.6%)	0.941	
Undecided	20 (7.6%)	17 (7.7%)	3 (6.7%)	0.858	
AGB	1 (< 1%)	0	1 (2.2%)	--	
Approach				0.952	
Laparoscopic	207 (78.1%)	172 (78.2%)	35 (77.8%)	0.890	
Open	58 (21.9%)	48 (21.8%)	10 (22.2%)	0.942	
Race				0.004	*
White	129 (48.9%)	97 (44.3%)	32 (71.1%)	< 0.001	*
Black	118 (44.7%)	107 (48.9%)	11 (24.4%)	< 0.001	*
Other	17 (6.4%)	15 (6.8%)	2 (4.4 %)	0.630	

* = significant at the p < 0.05 level

BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band

TABLE 22 DEMOGRAPHICS OF BARIATRIC SURGERY CANDIDATES FROM BY RACE

	Total	White	Black	p-value		Other	p-value	
Candidates	265	129 (48.7%)	118 (44.5%)	0.166		17 (6.4%)	< 0.001	*
Age (years)	43 ± 13	45 ± 14	43 ± 12	0.937		39 ± 10	0.425	
BMI (kg/m^2)	46.3 ± 10.5	46.0 ± 9.6	47.3 ± 11.0	0.301		42.7 ± 13.0	0.594	
Female	83.0%	75.2%	90.7%	0.001	*	88.2%	0.004	*
Procedure				0.414			0.006	*
GBP	96 (35.6%)	46 (35.7%)	45 (38.1%)	0.841		5 (29.4%)	0.176	
VSG	148 (55.8%)	75 (58.1%)	61 (51.7%)	0.016	*	11 (64.7%)	0.119	
Undecided	20 (7.6%)	8 (6.2%)	12 (10.2%)	0.068		0	0.554	
AGB	1 (< 1%)	0	0	--		1 (5.9%)	--	
Approach				0.195			0.428	
Laparoscopic	207 (78.1%)	105 (81.4%)	88 (74.6%)	0.019	*	13 (76.5%)	0.519	
Open	58 (21.9%)	24 (18.6%)	30 (25.4%)	0.099		4 (23.5%)	0.733	

* = significant at the $p < 0.05$ level

BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band

The most popular procedure was the vertical sleeve gastrectomy (VSG), 55.8%, followed by the gastric bypass (GBP), 35.6%, with 7.6% of patients still undecided on a procedure and 1 interested in the adjustable gastric band (AGB). More than 3 out of 4 of all procedures were planned to be performed laparoscopicaly (with the possibility of conversion to open) while almost 22% were to be performed using the traditional open method from the start. There was no significant difference in procedure or approach by sex or race (Figure 17Figure 18). When comparing whites to blacks, more of the VSG candidates were white (55.1% vs. 44.9%, p=0.016) as were more of the laparoscopy candidates (54.4% vs. 46.0%, p=.0192).

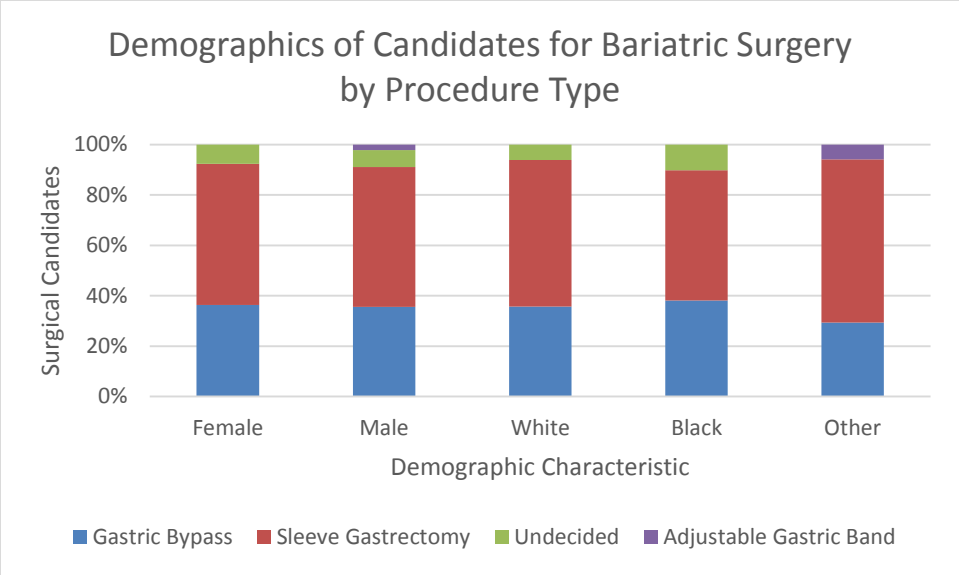

FIGURE 17 DEMOGRAPHICS OF CANDIDATES FOR BARIATRIC SURGERY BY PROCEDURE TYPE

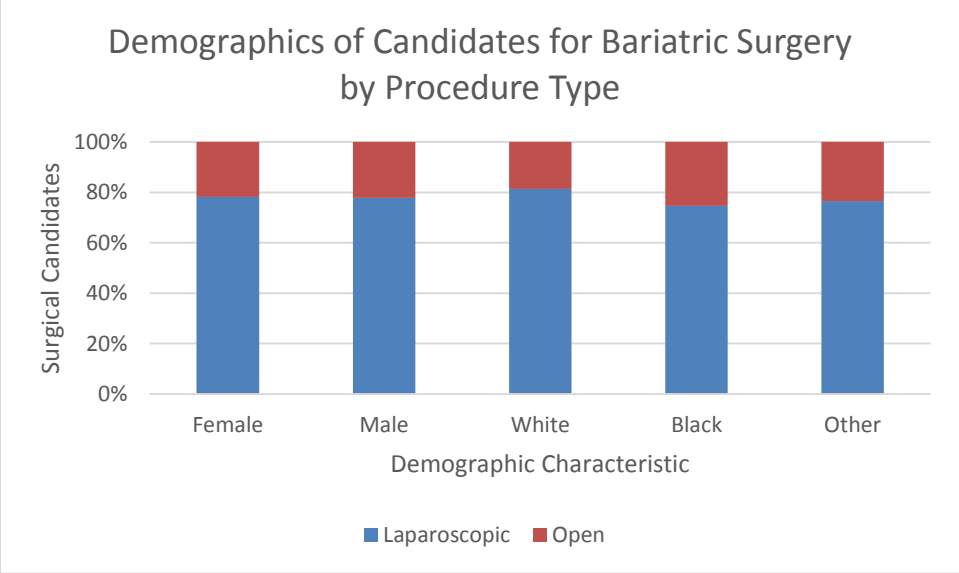

FIGURE 18 DEMOGRAPHICS OF CANDIATES FOR BARIATRIC SURGERY BY PROCEDURE TYPE

Only 18.5% of all candidates had VitD status testing results on file with orders pending for an additional 4.2% for a total of 58 out of 265 (21.9%) potentially having results prior to surgery

(Table 23 and Figure 19). Of the results on file, the median serum 25(OH)D concentration was 21.5 ± 15.3 ng/ml. Neither availability of VitD status or serum 25(OH)D concentration varied significantly by sex or race, (Table 24 and Figure 19 and Figure 20). No significant correlation was observed between any VitD related history, treatment, or supplementation with sex or race.

TABLE 23 VITAMIN D STATUS TESTING, HISTORY, AND TREATMENT BY SEX

	Total	**Female**	**Male**	**p-value**
Candidates	265	220 (83.0%)	45 (17.0%)	< 0.001 *
Laboratory Testing				
Serum 25(OH)D (ng/ml)	21.5 ± 15.3	21.9 ± 15.8	21.0 ± 10.4	0.440
25(OH)D available	49 (18.5%)	44 (20.0%)	5 (11.1%)	0.163
25(OH)D pending	9 (4.2%)	8 (4.6%)	1 (2.5%)	0.559
History & Treatment				
VitD defic. history	58 (22.0%)	52 (23.8%)	6 (13.3%)	0.121
High dose therapy	31 (11.7%)	27 (12.3%)	4 (8.9%)	0.520
Daily dose therapy	42 (15.8%)	38 (17.3%)	4 (8.9%)	0.161
Cholecalciferol ($VitD_3$)	45 (17.0%)	41 (18.6%)	4 (8.9%)	0.113
Ergocalciferol ($VitD_2$)	30 (11.3%)	27 (12.3%)	3 (6.7%)	0.279
Multivitamin	72 (27.2%)	65 (29.6%)	7 (15.6%)	0.055
Calcium with VitD	22 (8.3%)	18 (8.2%)	4 (8.9%)	0.876

* = significant at the $p < 0.05$ level

defic. = deficiency, VitD = vitamin D

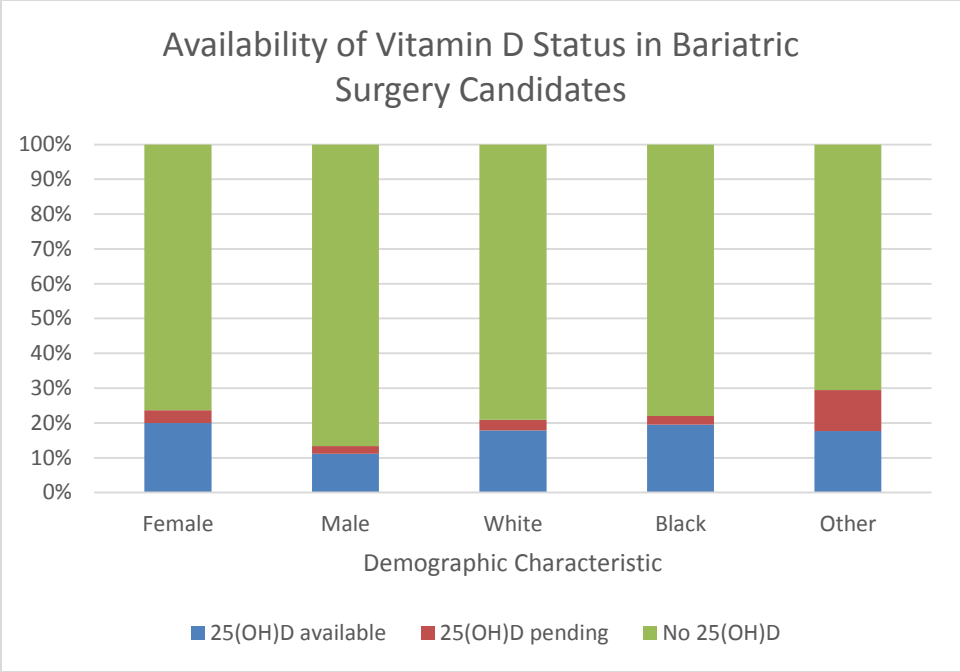

FIGURE 19 AVAILABILITY OF VITAMIN D STATUS IN BARIATRIC SURGRY CANIDATES

TABLE 24 VITAMIN D STATUS TESTING, HISTORY, AND TREATMENT BY RACE

	Total	White	Black	p-value	Other	p-value
Candidates	265	129 (48.7%)	118 (44.5%)	0.166	17 (6.4%)	< 0.001 *
Laboratory Testing						
Serum 25(OH)D (ng/ml)	21.5 ± 15.3	24.3 ± 15.6	21.0 ± 15.4	0.141	16.0 ± 7.0	0.242
25(OH)D available	49 (18.5%)	23 (17.8%)	23 (19.5%)	0.739	3 (17.6%)	0.941
25(OH)D pending	9 (4.2%)	4 (3.8%)	3 (3.2%)	0.812	2 (14.3%)	0.145
History & Treatment						
VitD defic. history	58 (22.0%)	29 (22.5%)	26 (22.4%)	0.990	3 (17.6%)	0.899
High dose therapy	31 (11.7%)	14 (10.8%)	16 (13.6%)	0.515	1 (5.9%)	0.595
Daily dose therapy	42 (15.8%)	24 (18.6%)	17 (14.4%)	0.376	1 (5.9%)	0.337
Cholecalciferol ($VitD_3$)	45 (17.0%)	27 (20.9%)	16 (13.6%)	0.127	2 (11.8%)	0.256
Ergocalciferol ($VitD_2$)	30 (11.3%)	13 (10.1%)	16 (13.6%)	0.396	1 (5.9%)	0.526
Multivitamin	72 (27.2%)	39 (30.2%)	29 (24.6%)	0.320	3 (17.6%)	0.408
Calcium with VitD	22 (8.3%)	13 (10.1%)	6 (5.1%)	0.141	17.6%)	0.130

* = significant at the $p < 0.05$ level

defic. = deficiency, VitD = vitamin D

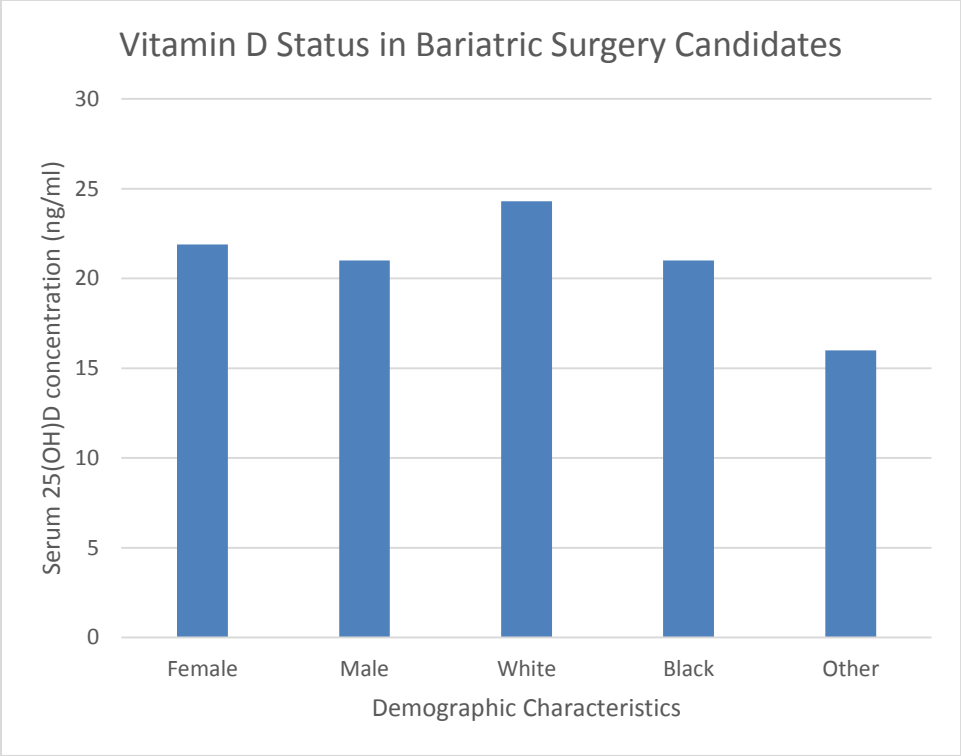

FIGURE 20 VITAMIN D STATUS IN BARIATRIC SURGERY CANIDATES

Patients who had their VitD status tested did not differ from those who were not tested by age, sex, BMI, procedure, surgical approach, or race, (

Table 25). Those who had testing on file were more likely to have a history of VitD deficiency (65.3% vs. 12.2%, p<0.001). Tested candidates were more likely to be on high dose VitD therapy (26.5% vs. 8.3%, p<0.001), and take daily VitD (28.6% vs. 13.0%, p=0.007), see Figure 21. Patients who had testing were more likely to be taking $VitD_2$ (24.5% vs. 8.3%, p=0.001) over $VitD_3$ (26.5% vs. 14.8%, p=0.049), see Figure 22. There was no significant relationship with intake of a multivitamin or calcium with VitD.

TABLE 25 CANDIDATES TESTED FOR VITAMIN D STATUS VERSUS THOSE NOT TESTED

	Total	Tested	Not Tested	p-value	
Candidates	265	49 (18.5%)	216 (81.5%)	< 0.001	*
Age (years)	43 ± 13	46 ± 14	42 ± 13	0.637	
Female	83.0%	89.8%	81.5%	0.162	
BMI (kg/m^2)	46.3 ± 10.5	46.5 ± 9.2	46.0 ± 10.7	0.713	
Procedure				0.673	
GBP	96 (35.6%)	20 (40.8%)	76 (35.2%)	0.251	
VSG	148 (55.8%)	27 (55.1%)	121 (56.0%)	0.825	
Undecided	20 (7.6%)	2 (4.1%)	18 (8.3%)	0.496	
AGB	1 (< 1%)	0	1 (< 1%)	--	
Approach				0.782	
Laparoscopic	207 (78.1%)	39 (79.6%)	168 (77.8%)	0.533	
Open	58 (21.9%)	10 (20.4%)	48 (22.2%)	0.742	
Race				0.941	
White	129 (48.9%)	23 (46.9%)	106 (49.3%)	0.586	
Black	118 (44.7%)	23 (46.9%)	95 (44.2%)	0.555	
Other	17 (6.4%)	3 (6.1%)	14 (6.5%)	0.947	
History & Treatment					
VitD defic. history	58 (22.0%)	32 (65.3%)	26 (12.2%)	< 0.001	*
High dose therapy	31 (11.7%)	13 (26.5%)	18 (8.3%)	< 0.001	*
Daily dose therapy	42 (15.8%)	14 (28.6%)	28 (13.0%)	0.007	*
Cholecalciferol ($VitD_3$)	45 (17.0%)	13 (26.5%)	32 (14.8%)	0.049	*
Ergocalciferol ($VitD_2$)	30 (11.3%)	12 (24.5%)	18 (8.3%)	0.001	*
Multivitamin	72 (27.2%)	15 (30.6%)	57 (26.4%)	0.548	
Calcium with VitD	22 (8.3%)	4 (8.2%)	18 (8.3%)	0.969	

* = significant at the $p < 0.05$ level
BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band, defic. = deficiency, VitD = vitamin D

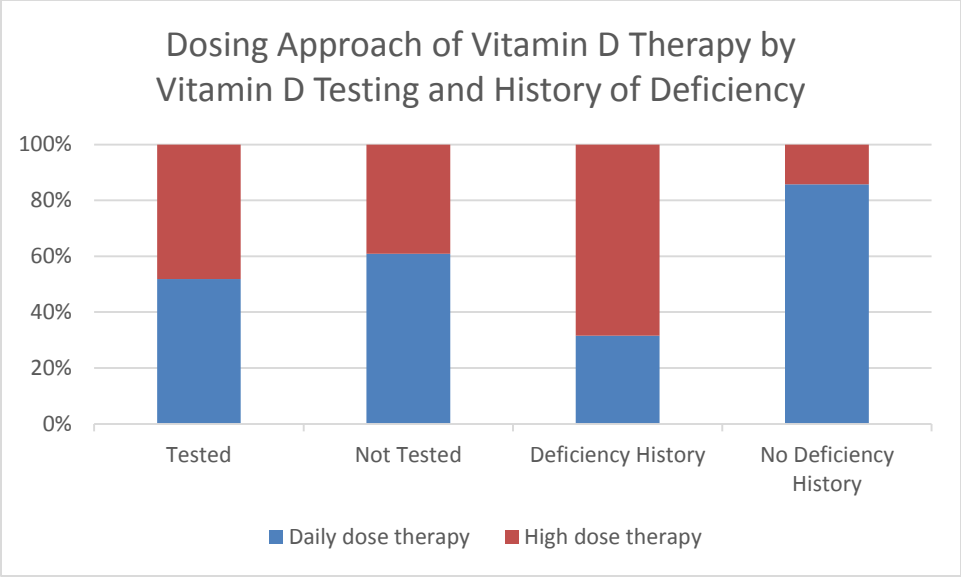

FIGURE 21 DOSING APPROACH OF VITAMIN D THERAPY B VITAMIN D TESTING AND HISTORY OF DEFICIENCY

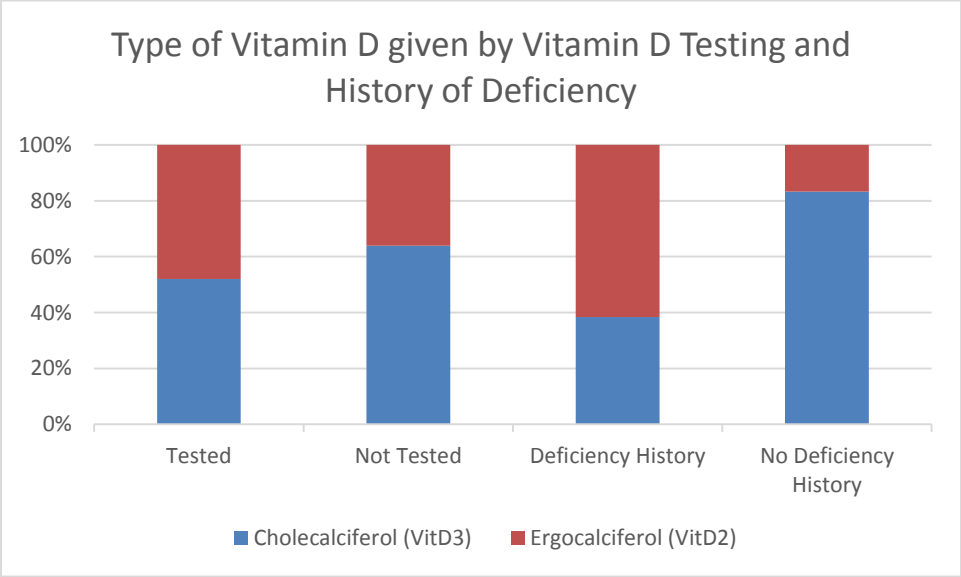

FIGURE 22 TYPE OF VITAMIN D GIVEN BY VITAMIN D TESTING AND HISTORY OF DEFICIENCY

Surgical candidates with a history of VitD deficiency were not different from those without a history or VitD deficiency by age, sex, BMI, procedure, surgical approach, or race, (Table 26).

Those with a history of VitD deficiency were more likely to be on high dose VitD therapy (44.8% vs. 2.5%, p<0.001) and were more likely to be taking $VitD_2$ (41.4% vs. 2.9%, p<0.001) over $VitD_3$ (25.9% vs. 14.6%, p=0.045), see Figure 21 Figure 22. There was no significant relationship with intake of a multivitamin or calcium with VitD. There was a borderline significant trend towards lower serum 25(OH)D concentration (poorer VitD status) in those with a history of VitD deficiency compared to those with no recorded history of VitD deficiency (21.0 ± 11.4 ng/ml vs. 27.0 ± 20.1 ng/ml, p=0.065), see Figure 23. Candidates with a history of VitD deficiency were significantly more likely to have serum 25(OH)D on file (55.2% vs. 8.3%, p<0.001) and pending (15.4% vs. 2.7%, p=0.002).

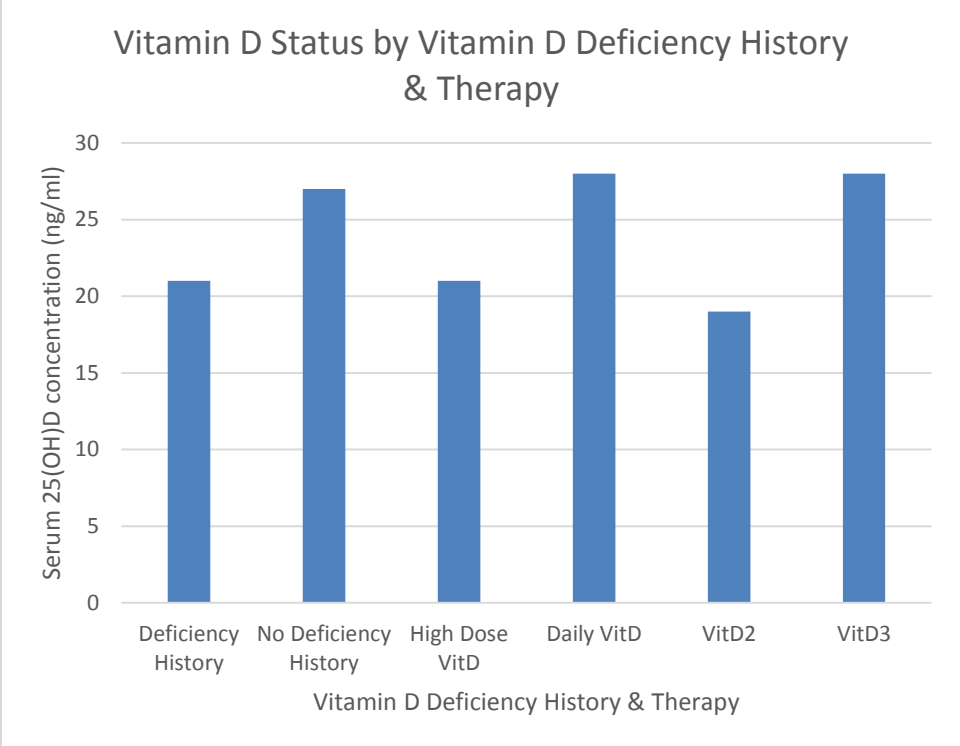

FIGURE 23 VITAMIN D STATUS BY VITAMIN D DEFICIENCY HISTORY & THERAPY

TABLE 26 CANDIDATES WITH A HISTORY OF VITAMIN D DEFICIENCY VERSUS THOSE WITHOUT A HISTORY

	Total	Deficiency History	No Deficiency History	p-value	
Candidates	265	58 (21.9%)	204 (77.0%)	< 0.001	*
Age (years)	43 ± 13	45 ± 14	43.0 ± 13	0.747	
Female	83.0%	89.7%	81.0%	0.121	
BMI (kg/m^2)	46.3 ± 10.5	47.3 ± 10.0	45.7 ± 10.6	0.534	
Procedure				0.523	
GBP	96 (35.6%)	24 (41.4%)	72 (35.1%)	0.196	
VSG	148 (55.8%)	32 (55.2%)	115 (56.1%)	0.825	
Undecided	20 (7.6%)	2 (3.4%)	17 (8.3%)	0.427	
AGB	1 (< 1%)	0	1 (< 1%)	--	
Approach				0.428	
Laparoscopic	207 (78.1%)	43 (74.1%)	162 (79.0%)	0.084	
Open	58 (21.9%)	15 (25.9%)	43 (21.0%)	0.360	
Race				0.899	
White	129 (48.9%)	29 (50.0%)	100 (49.0%)	0.820	
Black	118 (44.7%)	26 (44.8%)	90 (44.1%)	0.878	
Other	17 (6.4%)	3 (5.2%)	14 (6.9%)	0.782	
Laboratory Testing					
Serum 25(OH)D (ng/ml)	21.5 ± 15.3	21.0 ± 11.4	27.0 ± 20.1	0.065	
25(OH)D available	49 (18.5%)	32 (55.2%)	17 (8.3%)	< 0.001	*
25(OH)D pending	9 (4.2%)	4 (15.4%)	5 (2.7%)	0.002	*
History & Treatment					
High dose therapy	31 (11.7%)	26 (44.8%)	5 (2.4%)	< 0.001	*
Daily dose therapy	42 (15.8%)	12 (20.7%)	30 (14.6%)	0.266	
Cholecalciferol ($VitD_3$)	45 (17.0%)	15 (25.9%)	30 (14.6%)	0.045	*
Ergocalciferol ($VitD_2$)	30 (11.3%)	24 (41.4%)	6 (2.9%)	< 0.001	*
Multivitamin	72 (27.2%)	15 (25.9%)	57 (27.8%)	0.770	
Calcium with VitD	22 (8.3%)	3 (5.2%)	19 (9.3%)	0.320	

* = significant at the $p < 0.05$ level

BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band, defic. = deficiency, VitD = vitamin D

Candidates for bariatric surgery on high dose VitD therapy did not differ from those on daily VitD therapy by age, sex, or BMI, (Table 27). Patients on daily VitD were more likely to be white (58.5% vs. 49.2%, p=0.032) while those on high dose VitD were more likely to be black (51.6% vs. 39.0%, p=0.005). There was a trend toward more patients on high dose VitD to be seeking GBP (48.4% vs. 39.0%, p=0.059) and for those on daily VitD to be seeking VSG (56.1% vs. 45.2%, p=0.008). Surgical approach varied with VitD therapy, as a high proportion of patients on high dose VitD were to have laparoscopic surgery (80.6%) while a smaller proportion on daily VitD were slated for laparoscopy (65.8%, p<0.001). Candidates on high dose VitD therapy were more likely to have a history of VitD deficiency (83.9% vs. 26.8%, p<0.001). High dose therapy was mostly delivered as $VitD_2$ (87.1%, p<0.001) while daily dosing was almost exclusively $VitD_3$ (95.1%, p<0.001). There was no significant relationship between high dose therapy or daily dosing in terms of testing for VitD status. Serum 25(OH)D concentration was border-line significantly higher in daily versus high dose VitD (28.0 ± 20.9 ng/ml vs 21.0 ± 9.4 ng/ml, p=0.077) , see Figure 23. One patient whose main VitD therapy was high dose also took daily VitD, but no patients primarily being treated with daily dosing also took high dose therapy.

TABLE 27 CANDIDATES ON HIGH DOSE VITAMIN D THERAPY VERSUS DAILY VITAMIN D

	Total	High Dose VitD	Daily VitD	p-value	
Candidates	265	31 (11.7%)	41 (15.5%)	0.192	
Age (years)	43 ± 13	42 ± 13	47 ± 14	0.108	
Female	83.0%	87.1%	90.2%	0.674	
BMI (kg/m^2)	46.3 ± 10.5	49.6 ± 10.9	47.7 ± 9.8	0.367	
Procedure				0.654	
GBP	96 (35.6%)	15 (48.4%)	16 (39.0%)	0.059	
VSG	148 (55.8%)	14 (45.2%)	23 (56.1%)	0.008	*
Undecided	20 (7.6%)	2 (6.4%)	2 (4.9%)	0.756	
AGB	1 (< 1%)	0	0	--	
Approach				0.165	
Laparoscopic	207 (78.1%)	25 (80.6%)	27 (65.8%)	< 0.001	*
Open	58 (21.9%)	6 (19.4%)	14 (34.2%)	0.018	*
Race				0.531	
White	129 (48.9%)	14 (49.2%)	24 (58.5%)	0.032	*
Black	118 (44.7%)	16 (51.6%)	16 (39.0%)	0.005	*
Other	17 (6.4%)	1 (3.2%)	1 (2.4%)	0.829	
Laboratory Testing					
Serum 25(OH)D (ng/ml)	21.5 ± 15.3	21.0 ± 9.4	28.0 ± 20.9	0.077	
25(OH)D available	49 (18.5%)	13 (41.9%)	13 (31.7%)	0.378	
25(OH)D pending	9 (4.2%)	1 (5.6%)	3 (10.7%)	0.545	
History & Treatment					
VitD defic. history	58 (22.0%)	26 (83.9%)	11 (26.8%)	< 0.001	*
High dose therapy	31 (11.7%)	31 (100%)	0	< 0.001	*
Daily dose therapy	42 (15.8%)	1 (3.2%)	41 (100%)	< 0.001	*
Cholecalciferol ($VitD_3$)	45 (17.0%)	5 (16.1%)	39 (95.1%)	< 0.001	*
Ergocalciferol ($VitD_2$)	30 (11.3%)	27 (87.1%)	2 (4.9%)	< 0.001	*
Multivitamin	72 (27.2%)	9 (29.0%)	21 (51.2%)	0.059	
Calcium with VitD	22 (8.3%)	2 (6.4%)	8 (19.5%)	0.113	

* = significant at the $p < 0.05$ level

BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band, defic. = deficiency, VitD = vitamin D

In patients receiving $VitD_2$ versus $VitD_3$, there was no significant variation in age, sex, BMI, or surgical approach, (Table 28). The group receiving $VitD_2$ had border-line significantly higher BMI (50.6 ± 11.0 kg/m^2 vs. 46.6 ± 8.9 kg/m^2, p=0.059), see Figure 23. Those taking $VitD_2$ were more likely to be black (53.3% vs. 35.7%, p<0.001) and those taking $VitD_3$ were more likely to be white (59.5% vs. 43.3%, p<0.001). Patients on $VitD_2$ were mostly seeking GBP (53.3% vs. 38.1%, p=0.002) while those on $VitD_3$ were mostly seeking VSG (57.1% vs. 40.0%, p<0.001). Those on $VitD_2$ were more likely to have a history of VitD deficiency in their record (80.0% vs. 31.0%, p<0.001) and be on high dose VitD therapy (90.0% vs. 4.8%, p<0.001). Those on $VitD_3$ were less likely to have a history of VitD deficiency in their record (31.0% vs. 80.0%, p<0.001) and more likely to be on daily VitD therapy (92.9% vs. 10.0%, p<0.001).

TABLE 28 CANDIDATES ON ERGOCALCIFEROL (VITAMIN D_2) VERSUS CHOLECALCIFEROL (VITAMIN D_3)

	Total	**VitD_2**	**VitD_3**	**p-value**	
Candidates	265	30 (11.3%)	42 (15.8%)	0.071	
Age (years)	43 ± 13	42 ± 13	46 ± 14	0.245	
Female	83.0%	90.0%	92.9%	0.665	
BMI (kg/m^2)	46.3 ± 10.5	50.6 ± 11.0	46.6 ± 8.9	0.059	
Procedure				0.358	
GBP	96 (35.6%)	16 (53.3%)	16 (38.1)	0.002	*
VSG	148 (55.8%)	12 (40.0%)	24 (57.1%)	< 0.001	*
Undecided	20 (7.6%)	2 (6.7%)	2 (4.8%)	0.691	
AGB	1 (< 1%)	0	0	--	
Approach				0.619	
Laparoscopic	207 (78.1%)	23 (76.7%)	30 (71.4%)	0.092	
Open	58 (21.9%)	7 (23.3%)	12 (28.6%)	0.372	
Race				0.330	
White	129 (48.9%)	13 (43.3%)	25 (59.5%)	< 0.001	*
Black	118 (44.7%)	16 (53.3%)	15 (35.7%)	< 0.001	*
Other	17 (6.4%)	1 (3.3%)	2 (4.8%)	0.772	
Laboratory Testing					
Serum 25(OH)D (ng/ml)	21.5 ± 15.3	19.0 ± 10.2	31.5 ± 19.7	0.027	*
25(OH)D available	49 (18.5%)	12 (40.0%)	12 (28.6%)	0.317	
25(OH)D pending	9 (4.2%)	1 (5.6%)	3 (10.0%)	0.590	
History & Treatment					
VitD defic. history	58 (22.0%)	24 (80.0%)	13 (31.0%)	< 0.001	*
High dose therapy	31 (11.7%)	27 (90.0%)	2 (4.8%)	< 0.001	*
Daily dose therapy	42 (15.8%)	3 (10.0%)	39 (92.9%)	< 0.001	*
Cholecalciferol (VitD_3)	45 (17.0%)	3 (10.0%)	42 (100%)	< 0.001	*
Ergocalciferol (VitD_2)	30 (11.3%)	30 (100%)	0	< 0.001	*
Multivitamin	72 (27.2%)	10 (33.3%)	21 (50.0%)	0.159	
Calcium with VitD	22 (8.3%)	2 (6.7%)	8 (19.0%)	0.134	

* = significant at the $p < 0.05$ level

BMI = body mass index, GBP = gastric bypass, VSG = vertical sleeve gastrectomy, AGB = adjustable gastric band, defic. = deficiency, VitD = vitamin D

DISCUSSION

This pre-operative bariatric surgery cohort is more predominantly female (83.0%) than reports of many post-operative cohorts including our own center. Within that last 6 months, 75% of the bariatric surgeries performed at our center have been in women. This may be due to attrition of female patients prior to surgery. The median age of patients undergoing bariatric surgery at our center during this same 6 month time period was 45 years, which is very similar to the median age in this pre-operative cohort (43 ± 13 years). Median BMI (46.3 ± 10.5 kg/m^2) in these pre-operative patients is also in line with patients who undergo surgery at our center (46 kg/m^2). While the distribution of race is not dissimilar to what we typically observe at our center, there are fewer whites with this cohort being about 50% white and our post-operative cases being about 65% white. This may mean that some black candidates do not make it through the process to undergo bariatric surgery.

Most procedures were slated to be performed laparoscopicaly, 78.1%. The majority of patients were interested in undergoing the VSG (55.8%), the vast majority of which would likely be performed laparoscopicaly (91.2%). In comparison, only 58.3% of all GBPs were intended to be performed laparoscopicaly. VSG shows the highest frequency of laparoscopy in any procedure when excluding the lone AGB, which is expected to be laparoscopic. VSG is predominant in this pre-operative cohort (55.8% of all procedures), but not as predominant as we would expect for our center. Over 70% of all procedures in the last 6 months at our center have been VSG. This could be due to attrition of patients seeking GBP or perhaps these patients decide to undergo VSG instead. More surgeries in the past 6 months were actually performed laparoscopicaly than this pre-operative cohort would indicate (96% vs.65.2% in VSG and 86% vs. 27.0% in GBP). This may also be due to attrition, perhaps in those patients who are told they are only candidates for traditional open surgery.

Despite the relatively similar proportions of whites and blacks in this cohort as a whole, VSG and laparoscopy represented significantly more white candidates than black (55.1% vs. 44.9%, p=0.016 and 54.4% vs. 46.0%, p=.0192 respectively). The difference in procedure may be due to a larger proportion of undecided candidates, which was border-line significant (10.2% vs. 6.2%, p=0.068). As for fewer black candidates undergoing laparoscopy, one would anticipate this to be due to risk factors associated with the need for traditional open surgery, such as higher BMI and scarring from previous surgery (preventing placement of the ports). While the median BMI is higher in black candidates, this difference is not statistically significant (47.3 ± 11.0 kg/m^2 vs. 46.0 ± 9.6 kg/m^2, p=0.301). Perhaps these patients have had previous surgeries, which require the bariatric procedure to be performed in the traditional open approach.

The median serum 25(OH)D concentration (VitD status) in this bariatric pre-operative cohort is 21.5 ± 15.3 ng/ml (53.8 ± 38. 2 nmol). VitD status did not vary by sex or race. To further explore differences by race, we compared whites to blacks, but found the relationship was still non-significantly different (Δ=6.8 ng/ml, p=0.141). Despite darker skin pigmentation (higher concentration of melanin) being a risk factor for VitD deficiency, there was no difference by race or when comparing blacks to whites in the availability of VitD status test, whether testing had been ordered, history of VitD deficiency, treatment for VitD deficiency, or use of a multivitamin or calcium with VitD. There was also no relationship with these factors and sex with the exception of multivitamin intake. Women were border-line significantly more likely to take a multivitamin (29.6% vs. 15.6%, p=0.055).

We attempted to determine factors that may lead to a candidate being tested for VitD deficiency by their PCP, (

Table 25). There were no differences in age, sex, BMI, procedure, approach or race. The proportion of whites versus blacks who were tested was also comparable (17.8% vs. 19.5%, p=0.500). One might expect a higher proportion of blacks to have their VitD status tested given the additional risk factor (beyond obesity), but this was not seen in this cohort. Perhaps this is an issue of access to care, but it is also possible that more education is necessary for PCPs relating to the need for VitD testing in this population, especially in patients with multiple risk factors (e.g. obesity and high melanin concentration). Where we did see distinction in the likelihood of testing was with history and treatment of VitD deficiency. Those who had VitD status testing in their medical record at our center were much more likely to have a history of VitD deficiency (65.3% vs. 12.2%, p<0.001). This may be because those with a history of VitD deficiency are more likely to undergo periodic VitD testing. Candidates who were tested were also more likely to be on high dose VitD therapy (26.5% vs. 8.3%, p<0.001) and $VitD_2$ (24.5% vs. 8.3%, p=0.001); another indicator of medical management of VitD deficiency. Candidates for whom we had 25(OH)D were also more likely to be taking $VitD_3$ (26.5% vs. 14.8%, p=0.049). This may mean that their physicians have transitioned them from repletion therapy (high dose $VitD_2$) to maintenance therapy (daily $VitD_3$).

VitD status was tested in 18.5% of this cohort, and 34.8% were VitD deficient (< 20 ng/ml, 50 nmol/L) while 71.7% were VitD insufficient (< 30 ng/ml, 75 nmol/L), see Chapter 4. Previous studies in bariatric candidates have reported much higher rates of VitD deficiency and insufficiency. In a previous study at our center, we found 71.4% of our pre-operative patients were VitD deficient and 92.9% met clinical criteria for treatment (< 32 ng/ml, 80 nmol/L), see Chapter 4. This means a significant proportion of those who were not tested are likely to be VitD deficient and/or insufficient. One would anticipate that patients undergoing a malabsorptive procedure like GBP might be more likely to be tested for deficiency in fat soluble vitamins, a side

effect of the procedure, but that is not clear from these results. VitD status was tested in 20.8% of candidates for GBP and 18.2% in candidates for VSG, not a statistically significant difference (p=0.292).

In 87.1% of all cases of high dose, medically monitored, VitD supplementation in this cohort, $VitD_2$ was administered instead of $VitD_3$, $p < 0.001$, likely due to the fact that most pharmaceutical preparations in the US are $VitD_2$. Furthermore, there was a conception that $VitD_2$ is safer than $VitD_3$ and many physicians were trained to administer $VitD_2$ in high doses. The opposite is true of daily VitD supplementation in this cohort. $VitD_3$ was used in 95.1% of all cases of daily VitD therapy, $p < 0.001$. Daily VitD supplementation was positively associated with intake of a multivitamin (51.1% vs. 22.3% in non-daily users, $p<0.001$) and calcium with VitD (20.0% vs. 5.9% in non-daily users, p=0.002). A similar link is observed between those taking a multivitamin (30.6% vs. 10.4%, $p < 0.001$) and calcium with VitD (36.4% vs. 14.0%, p=0.006) with daily VitD therapy.

Whites were more likely to use VitD daily instead of high dose VitD therapy (58.5% vs. 49.2%, p=0.032), while blacks were more likely to be prescribed high dose VitD therapy rather than using VitD daily (51.6% vs. 39.0%, p=0.005). This racial difference may be explained if blacks are less likely to take VitD supplementation unless found to be VitD deficient, in which case they are likely to be prescribed high dose VitD therapy. In fact, those on high dose VitD therapy were more likely to have a history of VitD deficiency (83.9% vs. 26.8%, $p<0.001$). It is also possible that white candidates may have a larger proportion of individuals who are more proactive with their health and thus take daily VitD to prevent deficiency.

Candidates hoping to undergo the VSG procedure were more likely to take VitD daily rather than high dose VitD therapy (56.1% vs. 45.2%, p=0.008) and to be taking $VitD_3$ versus $VitD_2$ (57.1% vs.

40.0%, $p<0.001$), likely because over-the-counter formulations are typically $VitD_3$. Since VSG is not a malabsorptive procedure, the risk of VitD deficiency after surgery is less than with GBP, which may mean that PCPs are less likely to treat their patients with medically monitored high dose VitD therapy if they are VSG candidates instead of GBP candidates.

LIMITATIONS

The data for this study were gathered from our electronic medical record (EMR). There is the possibility of human error in terms of data entry. It is also possible that there is a lag in updates appearing in the EMR. This lag may be due to data transfer from one program to another or to delay from the time of hard copy receipt to entry into the EMR. This also affects clinical care.

There is a potential for reporting bias in our patients. If a patient has not seen the surgeon or nutritionist, they may not fully comprehend the importance of reporting all supplements and any nutritional testing. However, if we were to implement a pre-operative treatment program, we would face similar data limitations.

STRENGTHS

We have analyzed the EMR of 265 consecutive patients with appointments at our center, an academic Center of Excellence, over a period of 3 months. The large sample size as well as the sampling technique strengthens the validity of our analysis. We have established a baseline to assess future interventions at our center. Our results are also generalizable to most academic Centers of Excellence.

CONCLUSION

We found testing for VitD status to be lower than would be clinically indicated in pre-bariatric surgery patients. While all patients in this cohort have obesity (BMI 46.3 ± 10.5 kg/m^2), which is a risk factor for VitD deficiency, at least 44.5% have more than one risk factor, since these patients have high melanin concentration (darker skin pigmentation). Despite this, only 18.5% of bariatric candidates had serum 25(OH)D concentration on file in their medical records at our center. Of that group, 34.8% were VitD deficient (< 20 ng/ml, 50 nmol/L) and 71.7% were VitD insufficient (< 30 ng/ml, 75 nmol/L). Much higher rates of VitD deficiency and insufficiency have been reported for pre-operative bariatric surgery patients including a previous study at our center where we found VitD deficiency in 71.4% of patients prior to surgery. A significant proportion of bariatric surgery candidates have not had their VitD status tested but yet are likely to be VitD deficient and/or insufficient. The consequences of this deficiency on surgical and clinical outcomes have not been fully elucidated. Furthermore, those patients who are being treated for VitD deficiency are not being treated with the most effective formulation, $VitD_3$ (cholecalciferol). Since pharmaceutical preparations are largely $VitD_2$ (ergocalciferol) the likelihood of a patient being prescribed $VitD_2$ over $VitD_3$ is great; however, education, particularly targeting PCPs, could overcome this and lead to improved treatment of VitD deficiency and insufficiency.

CHAPTER 6: FUTURE PROJECTS

We have many future projects on the horizon. Clinically, we would like to improve the proportion of our patients undergoing testing for serum 25(OH)D concentration. This information will be crucial for The VISTA trial: The effect of VItamin D supplementation on nutritional STatus and Adverse outcomes in bariatric surgery. The VISTA trial is a randomized, double-blinded controlled trial that has been approved by the John Hopkins Institutional Review Board (IRB00044789). We are actively recruiting participants into this study.

An additional study which is currently underway involves a collaboration between the Wong Laboratory in the Johns Hopkins Center for Metabolism and Obesity Research and the Johns Hopkins Center for Bariatric Surgery: Altered Vitamin D and Adiposity in Obesity. We have developed a translational research project to study the relationship between vitamin D status and adipokines in human adipose tissue in the abnormal metabolic state of obesity. We hypothesize that vitamin D status and adipokine expression varies depending on body weight, and aim to study this in the morbidly obese patient pre- and post-bariatric surgery.

THE VISTA TRIAL: THE EFFECT OF VITAMIN D SUPPLEMENTATION ON NUTRITIONAL STATUS AND ADVERSE OUTCOMES IN BARIATRIC SURGERY

OBJECTIVES

Primary Objective: To determine whether 10,000 IU of vitamin D_3 (cholecalciferol) daily for 30 days prior to bariatric surgery (RYGB or VSG) will significantly increase the vitamin D status, as measured by serum 25(OH)D concentration.

Secondary Objective: To determine whether 10,000 IU of vitamin D_3 (cholecalciferol) daily for 30 days prior to bariatric surgery (RYGB or VSG) leads to decreased risk of adverse surgical outcomes.

BACKGROUND

An estimated 2 in 3 American adults are either overweight (BMI 25-29.9 kg/m^2) or obese (BMI 30-39.9 kg/m^2). The prevalence of obesity, class 3 obesity (BMI 40-49.9 kg/m^2), and even class 4 (BMI 50-59.9 kg/m^2) and 5 (BMI ≥ 60 kg/m^2) obesity are increasing [217;218]. Obesity is a leading contributor to global mortality and contributes to the burden of disease associated with diabetes, cardiovascular disease, musculoskeletal disorders such as osteoarthritis, and some cancers [104]. Morbid obesity reduces life expectancy by 8 to 10 years, similar to the effect of being a regular cigarette smoker [219].

Several studies have demonstrated that most obese adults are vitamin D (VitD) insufficient (<75 nmol/L, 30 ng/ml) or deficient (<50 nmol/L, 20 ng/ml). The inverse relationship between body mass index (BMI) or body fat mass and VitD status is hypothesized to be due to sequestration of VitD by adipose tissue, reducing the bioavailability of VitD [220]. The classical role of VitD is in the maintenance of bone calcification, but more recent research has elucidated a more varied role for this hormone. Lack of VitD has been associated with increased susceptibility to infection, autoimmunity, cancer, and chronic disease [221].

Bariatric surgery is currently the most successful means of long-term weight loss. Since deficiency in fat-soluble vitamins, such as VitD, is considered a metabolic complication of bariatric surgery, determining the VitD status of these individuals and perhaps correcting it prior to surgery may prove greatly beneficial. Potential complications relating to VitD insufficiency and deficiency include adverse surgical outcomes such as improper wound healing, infection of the surgical incision, and atrial fibrillation. Since the indications for bariatric surgery are obesity and obesity-related comorbidities, bariatric surgery patients are at an increased risk of having an adverse surgical outcome.

The Johns Hopkins Center for Bariatric Surgery (JHCBS) is designated as a Center of Excellence by the American College of Surgeons. To comply with this designation, our center must maintain a certain standard of care (SoC) and minimize complication rates. Given the potential relationship between VitD status and adverse surgical outcomes, we are currently reviewing pre-operative VitD status (serum 25(OH)D concentration) collected as routine SoC and investigating the relationship with surgical outcomes under an Institutional Review Board (IRB) approved protocol (NA_00087502). Our findings reveal that most of our patients are VitD insufficient and deficient pre-operatively. To date, there is no standard regarding treating these deficiencies pre-operatively and as such our center does not intervene prior to surgery. A randomized, double-blinded, placebo-controlled pilot trial is needed to assess the causality of the relationship between pre-operative VitD status and adverse surgical outcomes in the bariatric surgical patient.

We plan to identify new patients approved for bariatric surgery at the JHCBS. These patients will be randomized consecutively in a 1:1 ratio to either 30 days of 10,000 International Units (IU) of $VitD_3$ plus SoC or 30 days of placebo plus SoC immediately prior to surgery. We will monitor

these patients for any adverse surgical outcomes, including wound infection, dehiscence, and prolonged length of hospital stay. We will also monitor their long-term clinical outcomes such as malnutrition, weight loss, and resolution of comorbidities at their routine clinic care visit: 2 weeks, 6 weeks, 3 months, 6 months, and 12 months post-operatively. At these visits the patients are assessed for obesity related comorbidities, wound healing, weight loss, nutritional status, and other key markers of health, such as vital signs.

We aim to determine if 10,000 IU of $VitD_3$ daily for 30 days prior to bariatric surgery (Roux-en Y Gastric Bypass (RYGB) or Vertical Sleeve Gastrectomy (VSG)) will significantly increase the VitD status (serum 25(OH)D concentration) and whether the associated change in VitD status leads to decrease risk of adverse surgical outcomes and/or improved clinical outcomes.

STUDY DESIGN

All patients who present to the JHCBS have pre-operative blood work, which includes VitD status (serum 25(OH)D concentration), which is used to inform the treatment of each patient with VitD supplementation post-operatively. We propose to screen this data in order to identify patients who have insufficient VitD stores (< 75 nmol/L, 30 ng/ml), and thus may be at risk for adverse surgical outcomes. Once these patients are identified, we will approach them to participate in this research study (see Figure 24). If the patient chooses to participate, they will receive a 30 day supply of either placebo or 10,000 IU of $VitD_3$ for research purposes in addition to receiving standard of care (SoC). We will ask that they take one of these supplements (placebo or VitD) daily with their largest meal of the day until their surgery. On the day of their surgery, we will administer a questionnaire designed to assess compliance (what dose was actually delivered) and satisfaction (how easy the experience was for them).

VISTA Trial Diagram

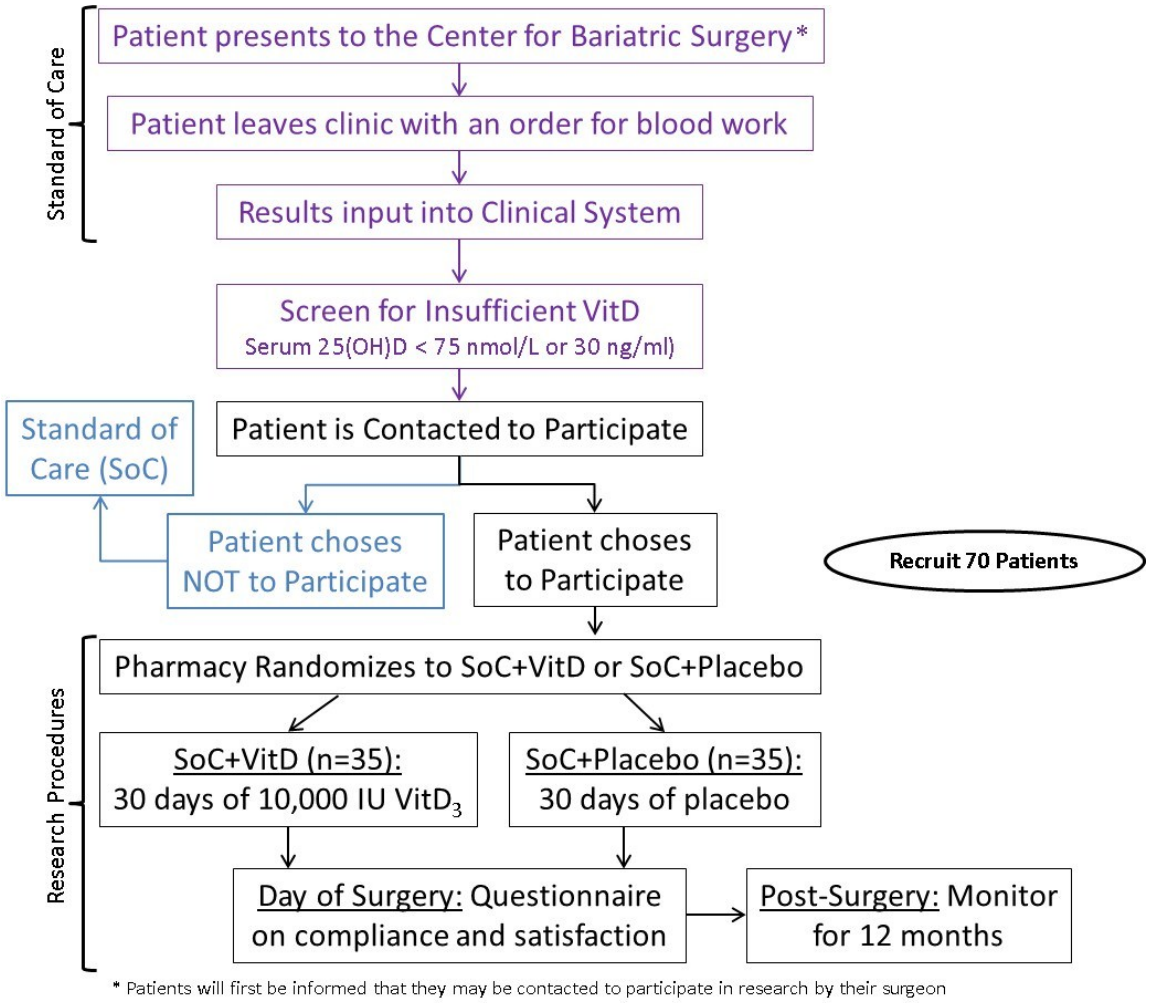

FIGURE 24 DIAGRAM OF THE VISTA TRIAL

After initiating informed consent and obtaining written consent, consecutive patients will be randomly assigned in a 1:1 ratio to either the treatment arm (SoC plus 10,000 IU of $VitD_3$) or the control arm (SOC plus placebo). Randomization will be performed by the Johns Hopkins Bayview Research Pharmacy. Using a computer-generated randomization scheme, uniform distribution will be utilized. Variables will be drawn with equal probability from all values in the range of 0 to 1. Each block of 4 will contain equal numbers of treatment (VitD) and placebo

assignments. Each successive study participant will be randomized by selecting the next available treatment assignment in the random code. The research study team will be blinded to the contents of the kits, knowing only for which study identification number they are intended.

The research study team will be given kits containing either VitD (treatment) or placebo (control) to give to the study participants. The placebo—produced by the Research Pharmacy—will consist of gummy buttons in blister packs. This preparation will appear very similar to the VitD supplements in the treatment arm.

The VitD supplements will be provided by Bariatric Advantage at no cost. This product is a chewable gel that delivers 10,000 IU of $VitD_3$ (cholecalciferol) per dose (1 gel). The patented gel-emulsion technology is designed to support greater bioavailability. Each gel contains 5 calories from 0.5 g of sugar alcohol. They also contain a natural lemon flavor. The packaging is specially engineered to protect each individual gel from light and oxygen to maintain optimal stability (blister packs). Our center recommends this product to our bariatric patients for VitD supplementation post-operatively.

Both the gummy buttons and the VitD gels are not vegetarian. They are made of beef gelatin (not encapsulated). We will inform all subjects that beef gelatin is used in these supplements during the informed consent process.

Subjects will be provided a 30 day supply of either the Bariatric Advantage VitD chewable gels or placebo (gummy buttons in blister pack) about 1 month from surgery. They will be instructed to take 1 gel each day with their largest meal. The fat typically present in the largest meal of the day will aid in the absorption of the VitD, which is fat soluble, if the patient has been randomized to the VitD arm.

On the day of surgery, we will ask them to complete the Post-Supplementation Survey to assess their compliance and experience taking the supplement. As we do in our other approved protocol, we will monitor these patients for any adverse surgical outcomes (wound infection, separation, dehiscence, and prolonged length of hospital stay) in addition to their long-term clinical outcomes such as malnutrition, weight loss, and resolution of comorbidities.

Our center participates in the American College of Surgeons Surgical Quality Improvement Program (ACS NSQIP®). ACS NSQIP® requires that we report mortality, surgical site infection, and other adverse events for 30 days following surgery. The routine SoC at our center is to see patients at 2 weeks, 6 weeks, 3 months, 6 months, 12 months, and annually thereafter, as required by the ACS to maintain our Center of Excellence designation. We will monitor the outcomes of these patients through the 12 month visit. This will include information about adverse events, wound healing, weight loss, vital signs, nutritional status, resolution of comorbidities, etc.

STUDY DURATION AND NUMBER OF STUDY VISITS

Our center performs 380 surgeries each year or roughly 7 surgeries per week. Preliminary data shows that 71% of our patients are VitD deficient (< 50nmol/L, 20 ng/ml) and 93% are VitD insufficient (< 75 nmol/L, 30 ng/ml) prior to surgery. We will likely have about 6 surgeries per week performed on patients with VitD insufficiency, making them potentially eligible to participate in this study. If we expect 75% to be willing to participate (based on experience with our previous studies), we should be able to recruit 4 patients per week. At this rate we should be able to recruit all 70 participants necessary in about 18 weeks. Thus, we expect this study to actively recruit participants for approximately 5 months. Following each patient requires an additional year, so we anticipate that this study will last for 1.5 years.

We will see each participant for 2 study visits. The first visit will occur at their routine pre-operative clinic visit, at which we will provide them with their 30 day supply of supplement (placebo or VitD at no cost) and a parking pass. The second visit will occur on the day of their surgery in the Ambulatory Surgery Unit. At this final visit, we will administer the questionnaire on compliance and satisfaction and provide them with an additional parking pass. We will not interact with the participants after their surgery, but instead will monitor them through the clinical system.

Blinding

This is a double-blinded, placebo controlled pilot study. The blinding prevents bias from the investigator or the participant, and the placebo allows us to account for the placebo effect. Blinding, randomization and preparation of the study drug will be carried out at the Johns Hopkins Bayview Inpatient Research Pharmacy.

Justification for inclusion of a placebo group

At present, the routine SoC at our center is not to administer VitD supplements prior to surgery regardless of nutritional status (serum 25(OH)D concentration). Supplementation currently only occurs after surgery, when patients are instructed to take 3,000 IU of $VitD_3$ daily in addition to a calcium supplement that must contain VitD. This means each patient should be taking up to 4,200 IU of $VitD_3$ daily. They are then monitored for their VitD status at 3, 6, and 12 months and annually thereafter or more frequently if clinically indicated. High dose treatment is frequently administered post-operatively in addition to daily supplementation. This high dose treatment ranges from 50,000 IU of VitD weekly to twice weekly for 8 to 16 weeks.

The placebo group in this study, thus, receives the routine SoC at our center—what they would receive if they decline to participate in this study.

Definition of treatment failure or participant removal criteria

We will perform an intention to treat analysis, but will also assess compliance via a questionnaire—the Post-Supplementation Survey. If a participant took their supplement "Occasionally (10-15 of 30)", "Rarely (< 10 of 30)", or "Never (0 of 30);" we will remove them for the purposes of a subset analysis.

Inclusion/Exclusion Criteria

Inclusion Criteria:

- Patients approved for and undergoing clinically indicated bariatric surgery –Roux-en-Y Gastric Bypass (RYGBP) or Vertical Sleeve Gastrectomy (VSG)
- 18 to 64 years of age
- BMI of 35 to 49.9 kg/m^2
- VitD insufficient pre-operatively: serum 25(OH)D concentration < 75 nmol/L or 30 ng/ml

Exclusion Criteria:

- Any patient who does not want to participate in the study
- Any patient who has dietary restrictions/proscriptions prohibiting ingestion of beef gelatin
- Expected poor compliance with the medical regimen
- Any active medical conditions that could, in the opinion of the investigators, jeopardize the safety of the subject or the integrity of the study
- The elective bariatric surgery is cancelled prior to incision by a surgeon for any reason
- Pregnancy: The routine standard of care is to determine whether a female patient is pregnant either by history and/or urinary pregnancy test on the day of surgery. No additional testing specifically for this study is planned beyond the standard of care

RATIONALE FOR CHOOSING THE DRUG AND DOSE

In order to improve VitD status (raise serum 25(OH)D concentrations) in patients, supplementation with cholecalciferol ($VitD_3$) is recommended. While supplementation with the plant version of VitD, ergocalciferol ($VitD_2$), has been used in the past it is becoming clear that there are advantages to $VitD_3$ and that it is more effective than $VitD_2$ at remedying deficiency (repletion). $VitD_3$ is safe even at very high doses such as 600,000 IU annually or 40,000 IU daily. While large monthly (100,000 IU) or weekly dosing (50,000 IU) has been used to correct VitD deficiency the pharmacokinetics of VitD favor daily dosing, as declining 25(OH)D (regardless of total amount) leads to declining activation into $1,25(OH)_2D$. A more appropriate dosage would thus be 7,000 to 10,000 IU daily, which is why we have selected a 10,000 IU dose of $VitD_3$ to be taken daily. The Institute of Medicine has deemed VitD to be safe in doses up to 10,000 IU per day for prolonged periods of time in the general population. Since this population will be VitD insufficient, any potential risks of VitD supplementation will be greatly diminished and certainly outweighed by the potential benefits. In addition, daily supplement intake creates a habit, which increases the likelihood of compliance, assuring dosage delivery.

STUDY STATISTICS

Primary outcome variable: VitD status as measured by serum 25(OH)D concentration.

Secondary outcome variables:

- Adverse surgical outcomes: surgical site infection, wound separation and dehiscence, anastomotic leak, prolonged length of hospital stay (> 3 days), re-admittance to the hospital within 30 days post-operatively.
- Clinical outcomes: wound healing, weight loss, nutritional status, resolution of comorbidities, and other key markers of health, such as vital signs (fever, blood pressure, heart rate, pain, etc.) and return of a regular menstrual cycle.

STATISTICAL PLAN

We expect the average serum 25(OH)D concentration (VitD status) in our patients to be 37.5 nmol/L or 15.0 ng/ml from preliminary data. We hope to raise 25(OH)D in the VitD arm to above 50 nmol/L or 20 ng/ml with only 35% of participants remaining VitD deficient after treatment—a roughly 50% decrease. To detect this difference in the proportion of patients who are VitD deficient (71.4% versus 35%), we will need to recruit 28 patients for each group for a total of 56. Given a 20% loss to follow up we will need to recruit 34 patients for each group (68 total). We will round that number up and recruit 35 participants per group for a grand total of 70 participants in this study.

We will perform an intention to treat analysis, but will also assess compliance via a questionnaire—the Post-Supplementation Survey. If a participant took their supplement "Occasionally (10-15 of 30)", "Rarely (< 10 of 30)", or "Never (0 of 30);" we will remove them for the purposes of a subset analysis. We will also perform analysis stratified by procedure type to assess for potential differences between the malabsorptive RYGB compared to the mildly malabsorptive VSG.

VitD status is a continuous variable, so for most analysis we will use it as such. However, it may also be divided into biologically or population relevant categories. To classify VitD deficiency, we will use guidelines from the Institute of Medicine, < 50 nmol/L or 20 ng/mL. Additionally, we will use 75 nmol/L or 30 ng/mL as the cut-off to determine sufficiency, which may be necessary for the immune bolstering effects of VitD. Within our cohort, it may also be informative to divide VitD status by tertiles or quartiles as well as to focus on the highest and lowest VitD status of tertiles or quartiles.

Early stopping rules

If we find that 10,000 IU of $VitD_3$ daily does not increase serum 25(OH)D concentration (VitD status), in the VitD arm during our interim analysis, we will discontinue this version of the study and re-design it with a higher dose, using this data to inform that higher dose. We will perform our interim analysis after collecting post-operative VitD status for the 18^{th} subject into the VitD arm.

Risks

Medical risks

The Institute of Medicine has deemed VitD to be safe in doses up to 10,000 IU per day for prolonged periods of time in the general population. Since this population will be VitD insufficient (< 75 nmol/L, 30ng/ml), any potential risks of VitD supplementation will be greatly diminished and certainly outweighed by the potential benefits in this population. Toxicity is not possible in this population of VitD insufficient individuals due to the dose (10,000 IU of $VitD_3$ daily) and duration (30 days) of supplementation. The serum 25(OH)D concentrations in these patients would need to be increased an order of magnitude in order to become at risk of VitD toxicity (> 500 nmol/L, 200 ng/ml). The potential risks of excessive VitD are primarily hypercalcemia and hypercalciuria. These are uncommon and have been found at similar rates in the control and treatment groups of numerous other VitD supplementation studies.

Steps taken to minimize the risks

We have chosen a dose (10,000 IU of $VitD_3$ daily) at which we should see a profound improvement in VitD status with minimal risk. In addition, the short duration of supplementation (30 days) will limit the possibility of side effects, though rare even at higher doses and durations or in the general population. It is possible that this dose/duration

combination will not be sufficient to replete this population, which is why we will perform an interim analysis to assess the efficacy of this supplementation in this study population.

Plan for reporting unanticipated problems or study deviations

All events will be reported to the Johns Hopkins Medicine (JHM) IRB as required by the JHM IRB reporting guidelines posted on the JHM IRB website.

Legal risks such as the risks that would be associated with breach of confidentiality

There should be no legal risks by participating in this study beyond those associated with routine care.

Financial risks to the participants

There should be no financial risks by participating in this study beyond those associated with routine care.

Benefits

No direct benefits for study subjects, but the results of this work may improve care of future bariatric patients.

Payment and Remuneration

Since there are no extra visits and little extra time required of the participants in this study, we will not compensate them monetarily. Participants will receive 2 parking passes (1 in clinic when they receive their supplements and 1 on the day of surgery when they complete the questionnaire) as compensation for their time and efforts.

Costs

There are no costs to participate in this study. Participants (or their insurance) are responsible for paying the costs of routine standard of care and follow-up clinical examination visits associated with bariatric surgery.

ALTERED VITAMIN D AND ADIPOSITY IN OBESITY

SPECIFIC AIMS

An estimated two-thirds of American adults are either overweight or obese, and the prevalence of obesity is increasing.[216] Several studies have demonstrated that most obese adults are vitamin D (VitD) insufficient (<80 nmol/L, 32 ng/L) or deficient (<50 nmolL, 20 ng/L).[104;147;150;210] The inverse relationship between BMI or body fat mass and VitD status [147;151;210] is hypothesized to be due to sequestration of VitD by adipose tissue, reducing the bioavailability of VitD.[217;218]

Adipose tissue is now recognized as a metabolically active endocrine organ. Adipose tissue expresses the VitD receptor (VDR) and activates the circulating form of VitD, 25(OH)D, into the active form, 1,25(OH)D, which binds to the VDR [147]. Adipose tissue may both regulate and be regulated by VitD [217]. Adipose tissue as an endocrine organ also secretes adipokines, which are proteins involved in inflammation, metabolism and energy homeostasis. Adiponectin is an adipokine exclusively secreted by adipose tissue that modulates glucose metabolism and fatty acid oxidation, with an inverse correlation with body weight and metabolic syndromes. The C1q/TNF-related proteins (CTRP) family of proteins are adiponectin paralogs that play an important role in regulating glucose and lipid metabolism. Understanding the link between VitD status and energy homeostasis may be one of the vital links in ameliorating the obesity epidemic.

In a strong collaboration between the Wong Laboratory in the Johns Hopkins Center for Metabolism and Obesity Research and the Johns Hopkins Center for Bariatric Surgery, we have developed a translational research project to study the relationship between VitD status and adipokines in human adipose tissue in the abnormal metabolic state of obesity. **We hypothesize that vitamin D status and adipokine expression varies depending on body weight, and aim to study this in the morbidly obese patient pre- and post-bariatric surgery.**

Specifically, we aim:

1. To determine vitamin D status—serum 25(OH)D concentration—pre- and post-bariatric surgery (immediately prior to surgery and 3, 6, and 12 months after surgery).
2. To evaluate circulating adipokines pre- and post-bariatric surgery (immediately prior to surgery and 3, 6, and 12 months after surgery).
3. To assess vitamin D metabolites and receptor expression and adipokines in obese adipose tissue and compare to normal weight control tissue (healthy controls).
4. To elucidate the relationship between vitamin D and adipokines, and their impact on metabolism in the obese patient.

BACKGROUND

VITAMIN D AND OBESITY

According to the National Health and Nutrition Examination Survey (NHANES) 2001-2004, 1 in 3 Americans have deficient VitD stores (<50 nmol/L, 20 ng/ml) and 3 in 4 have sub-optimal or insufficient VitD stores (<75 nmol/L, 30 ng/ml) [154]. Risk factors for VitD deficiency include living in the northern latitudes, greater melanin concentration in the skin, sun avoidance practices such as daily sunscreen use or veiling, working the night shift, absence of fatty fish intake, liver or kidney disease, exclusive breast feeding for infants, inability to digest/absorb fats (i.e. after gastric bypass), polymorphisms, as well as obesity. A large study by Vimaleswaran et al. found a 10% increase in body mass index (BMI) was correlated with a 4% decrease in VitD status [151]. This decrease is most likely due to sequestration of VitD in the fat, leading to lower VitD bioavailability.

The classical role of VitD is in the maintenance of bone calcification, but more recent research has elucidated a more varied role for this hormone. Lack of VitD has been associated with increased susceptibility to infection, autoimmunity, cancer, and chronic disease [222]. VitD plays a

role in regulating the inflammation that contributes to many chronic diseases, and has shown the potential to prevent diseases such as type 2 diabetes mellitus [153;154]. Obesity is also associated with chronic inflammation and an increased risk of chronic diseases. Hence, obese individuals with insufficient stores of VitD may have extraordinary risk of chronic inflammation and associated comorbidities.

VITAMIN D AND BARIATRIC SURGERY

Currently, sustained long-term weight loss is most successfully obtained with bariatric surgery. During bariatric surgery, the digestive tract is restricted in size with particular focus on the stomach, leading to weight loss. Reports suggest that 40-68.1% of bariatric surgery patients are VitD deficient [196]. Since deficiency in fat-soluble vitamins, such as VitD, is considered a metabolic complication of bariatric surgery, determining the VitD status of these individuals and perhaps correcting it prior to surgery may prove greatly beneficial [196]. Fish et al. showed that VitD insufficiency (<75 nmol/L, 30 ng/L) persisted in gastric bypass patients for 3 years following surgery despite supplementation prior to surgery (50,000 IU $VitD_3$ 3 times per week for 1 month) and

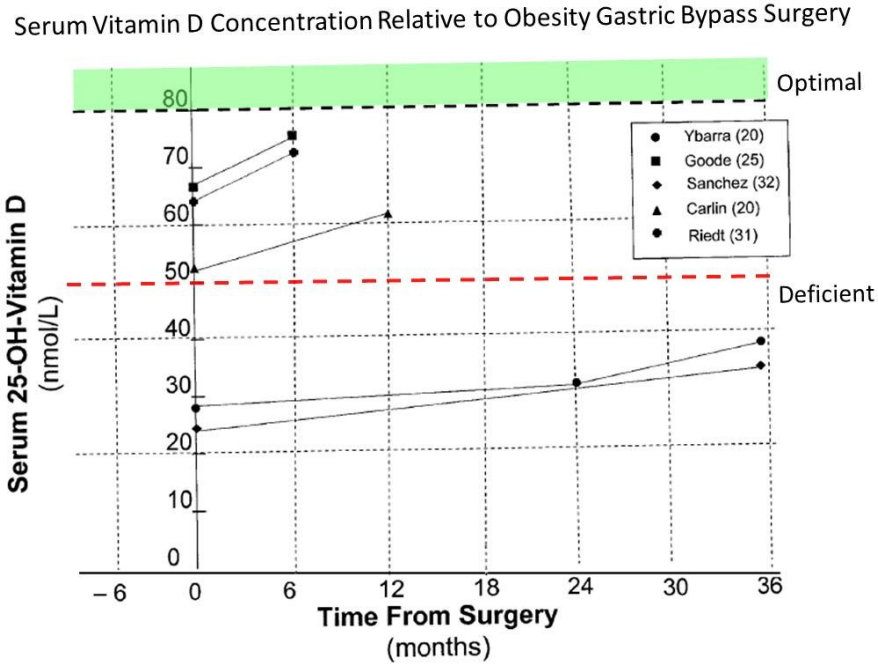

FIGURE 25 SERUM VITAMIN D CONCENTRATION RELATIVE TO OBESITY GASTRIC BYPASS SURGERY. FIGURE ADAPTED FROM COMPHER, BADELLINO, AND BOULLATA.

maintenance supplementation for life, with a daily dose of 1200 IU 25(OH)D_3 [209]. Interestingly, there appears to be an increase in serum 25(OH)D in this cohort immediately following surgery and then a slow decline in status likely due to the release of VitD from fat sequestration during weight loss [209]. Compher, Badellino, and Boullata conducted a review in 2008 and produced a graph showing serum 25(OH)D concentration over time following bariatric surgery, which we have altered to produce Figure 25[223]. Most striking about Figure 25 is that VitD deficiency lingers uncorrected in most of these patients for years.

Potential complications relating to VitD insufficiency and deficiency include adverse surgical outcomes such as improper wound healing [156], infection of the surgical incision [130;157], and atrial fibrillation [130]. Since the indications for bariatric surgery are obesity and obesity-related comorbidities, bariatric surgery patients are at an increased risk of having an adverse surgical outcome. VitD deficiency is likely a risk factor that can be easily remedied prior to surgery and therefore may prevent such adverse surgical outcomes following bariatric surgery.

Vitamin D and Adipose Tissue: The Role of Adipokines

Adipose tissue is one of the extraskeletal targets of VitD. The effects of VitD on adipogenesis *in vitro* are not completely clear, as 1,25(OH)D inhibits adipogenesis in mouse preadipocytes, while it stimulates adipogenesis in human adipocytes *in vitro* [224;225]. There is likely a role for the VDR in the regulation of adipogenesis and energy metabolism as VDR knockout mice are lean and resistant to developing obesity on a high fat diet [226]. Adipose tissue metabolizes VitD, but this function is likely altered in obesity and with weight loss.

Adipose tissue derived hormones, or adipokines, and VitD both play a role in energy homeostasis. In mouse models, 1,25(OH)D stimulates adipose leptin production in a VDR dependent manner [227], but the relationship between VitD and leptin has not been explored in humans and human adipose tissue. Further, it has been suggested by a proteomic approach that adiponectin links VitD

deficiency to obesity [228]. The Wong Laboratory focuses on a novel family of adipokines, termed CTRPs, that have anti-inflammatory properties and are involved with both glucose and lipid metabolism [229]. We hope to expand the investigation of different adipokines and their relationship to VitD in humans and human adipose tissue.

Preliminary Data

Recently, the Johns Hopkins Center for Bariatric Surgery began assessing nutritional status in their pre-operative patients as standard of care. Preliminary data shows that 98.3% of patients are VitD insufficient (<80 nmol/L, 32 ng/ml) and 72.4% are deficient (<50nmol/L, 20 ng/ml). Insufficiency and deficiency are most common in non-Caucasian patients ($p=0.015$). We have also found significant pair-wise correlations between VitD status and age ($p<0.001$) and BMI ($p=0.003$).

Research Design and Methods

We are conducting a translational research study as a collaboration between the Wong Laboratory in the Center for Metabolism and Obesity Research and the Center for Bariatric Surgery at the Bayview Medical Center (PI: Kimberley E. Steele, MD, PhD, FACS). Obese study patients undergoing bariatric surgery will be recruited to participate in this study (Figure 26). Blood and adipose tissue samples will be obtained, with the following aims:

1. To determine differences in vitamin D status in obese patients prior to and after undergoing bariatric surgery.
2. To assess correlations between vitamin D status, circulating adipokines, and variables such as weight, weight loss, insulin, glucose, hemoglobin A1C, cholesterol, liver function studies (LFTs), and parathyroid hormone.
3. To compare expression of the vitamin D receptor and enzyme function in obese adipose tissue and compare this to fat tissue from normal weight healthy controls.

4. To elucidate the relationship between vitamin D status and adipose tissue expression of vitamin D receptor and adipokines , and their impact on metabolism in the obese patient.

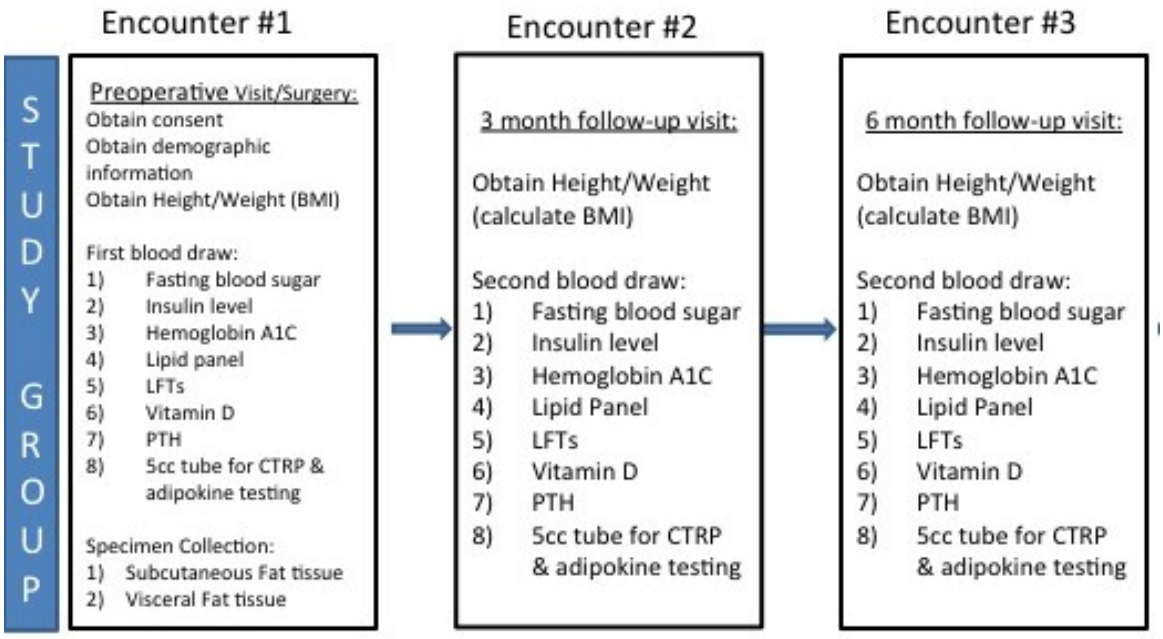

FIGURE 26 ASSOCAITION OF VITAMIN D STATUS AND ADIPOSE TISSUE FUNCTION IN OBESE PATIENTS UNDERGOING BARIATRIC SURGERY

PATIENT RECRUITMENT AND FOLLOW-UP

We will recruit 60 obese patients who are scheduled to undergo bariatric surgery at the Bayview Medical Center. Obese study patients (BMI>40) will be recruited at their pre-operative visit where demographic information, history, height and weight measurements are obtained as routine care. Pre-operative blood work (including LFTs, HbgA1C, insulin, glucose, cholesterol panel, VitD and PTH levels) and an additional blood sample will be obtained for ELISA testing of adipokines in our research laboratory. During surgery, visceral (omental) and subcutaneous adipose tissue will be procured. Obese patients will be followed at their 3, 6, and 12 month

post-surgical follow-up visits, where repeat blood work is obtained and will again be tested for adipokines. At each visit, weight and height will be measured as per their routine follow-up care.

Specimen Processing and Evaluation

Patient blood work including LFTs, HbgA1C, insulin, glucose, lipid panel, VitD, and PTH levels will be processed and performed by the core laboratory. Circulating adipokine levels will be determined using ELISA assays in the Wong Laboratory. Adipose tissue will also be processed in the Wong Laboratory. RNA extraction of the adipose tissue will be performed using the Trizol method and mRNA expression will be measured using Real-Time PCR methods to evaluate expression levels of the VDR, 1alpha hydroxylase enzyme, and adipokines, including adiponectin, leptin, and CTRPs. Healthy control tissue (n=10) will be obtained from the National Disease Research Interchange for study comparison. Protein levels of the VDR and 1α-hydroxylase enzyme will be assessed using Western Blot.

Statistical analysis

Student's t-test or Wilcoxon rank sum will be used to compare the expression of VitD and adipokines between the two groups (obese vs. lean fat). Correlations between VitD status and adipokines will be analyzed using Pearson or Spearman's correlations. Longitudinal methods will be utilized to assess VitD status and adipokine expression across different time points. VitD status and continuous variables (BMI, LFTs, HbgA1C, insulin, glucose, lipids, and PTH levels) in obese patients will be analyzed using Pearson or Spearman's correlations, and linear regression models. Stratification and subset analysis will be performed for study patients with and without diabetes, heart disease, and liver disease.

Our protocol has been approved by our institution's IRB (NA_00085888) with the exception of the analysis of VitD metabolites and PTH, which we are adding through a Change In Research

submission to our Institutional Review Board. Subjects will be consented according to our IRB policy on informed consent.

Responsibility and Role in Research Projects

Leigh Peterson is a PhD Candidate at the Johns Hopkins School of Public Health (JHSPH) in the Department of International Health in the Program on Human Nutrition and is the Senior Research Program Coordinator for The Center for Bariatric Surgery at the Bayview Medical Center. She has extensive experience in clinical studies, i.e. designing, implementing, database management, and recruiting. In addition, Mrs. Peterson holds a Master of Health Science in Molecular, Microbiology, and Immunology also from the JHSPH and has basic laboratory experience. She will be responsible for patient recruitment and follow-up, specimen procurement, database maintenance, and statistical support.

Risa Wolf is a pediatric endocrine fellow and a post-doctoral research fellow in the Wong Laboratory in the Center for Metabolism and Obesity Research at the Johns Hopkins School of Medicine. As part of the Wong Laboratory, Dr. Wolf has extensive experience in studying adipokine function in the context of metabolism. She will be responsible for performing the ELISA testing and tissue processing in the laboratory. Human specimens will be obtained, stored and studied in strict compliance with institutional guidelines.

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CURRICULUM VITAE

Leigh A. Peterson, MHS, PhD Candidate

Part I

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Program in Human Nutrition
Department of International Health
The Johns Hopkins Bloomberg School of Public Health
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BORN January 3, 1984: West Chester, Pennsylvania

EDUCATION

2010 to 2015 **Doctor of Philosophy**, Human Nutrition, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, Dissertation: *Alteration of the vitamin D endocrine system in obesity: the role in patients undergoing bariatric surgery*, Advisor: Lawrence J. Cheskin, MD

2009 to 2010 **Master of Health Science**, Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, Essay: *Vitamin D and Dermal Immunity: the Sunshine Paradox*, Advisor: Alan Scott, PhD

2006 to 2009 **Non-Degree Seeking**, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, 17 Credits Include: Molecular Biology of Disease, Biological Basis of Aging, Environmental Health, and Tissue Injury, Inflammation, & Repair

2002 to 2006 **Bachelor of Science**, Biochemistry with Distinction in the Major, Mary Baldwin College, Staunton, VA, Thesis: *Analysis of Echinacea and St. John's Wort (Supplements) Via High Performance Liquid Chromatography*, Advisor: Vladimir Garkov, PhD

PROFESSIONAL EXPERIENCE

Feb. 2014 to Present **Senior Research Program Coordinator**, The Johns Hopkins Center for Bariatric Surgery, The Johns Hopkins School of Medicine, Baltimore, MD

- Oversee 17+ simultaneous research projects in an active clinical setting
- Supervise students and volunteers: Conduct interviews, Train to execute complex research protocols, to prepare manuscripts, etc.
- Design and implement new studies from start to finish including:
    - Initial concept development and grantsmanship
    - Sponsor identification and negotiation
    - IRB approval and compliance/regulatory maintenance
    - Database design and maintenance
    - Participant screening, recruitment, and informed consent
    - Sample processing, storage, organization, and shipment
    - Data analysis and manuscript composition

June to July 2012 **Summer Intern** with Dr. Carmelita Frondoza, Nutramax Laboratories, Inc., Edgewood, MD

- Designed and validated a model of apoptosis for protection experiments
- Trouble-shot cell cultures and assays

Jan. to Feb. 2012 **Rotation Intern** with Dr. Carmelita Frondoza, Nutramax Laboratories, Inc., Edgewood, MD

- Learned sterile technique and cell culture
- Optimized a model of solar (UV) radiation damage in cell culture
- Evaluated assays for determining cell viability

July to Dec. 2011 **Research Assistant** to Dr. Hope Johnson, Johns Hopkins University International Vaccination Access Center, Baltimore, MD

- Adult Global Estimation of Disease Burden and Distribution of serotypes of serious pneumococcal and meningococcal disease (AGEDD) project
- Literature review and analysis to determine:
    - The global burden of meningococcal disease
    - Vaccine access and efficacy globally

June 2011 — **Participant**, The Mary Frances Picciano Dietary Supplement Research Practicum, National Institutes of Health, Office of Dietary Supplements, Bethesda, MD

- Selected applicant for intensive practicum on practical issues regarding dietary supplements, their ingredients, and related research
- Key participants included researchers, faculty, manufacturers, and congressional representatives

June to Nov. 2010 — **Research Assistant** to Dr. Fidel P. Zavala, Malaria Vaccine in the Mouse Model, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

- Mosquito dissection for salivary gland & sporozoite harvest
- RNA extraction of infected mouse livers
- Real Time PCR of cDNA from infected mouse livers
- Assay troubleshooting

2006 to 2010 — **Research Coordinator**, Pathogenesis and Treatment of Sleep Apnea, Johns Hopkins School of Medicine, Baltimore, MD

- Patient recruitment, enrollment, and informed consent
- Ran and analyzed experiments, including sleep studies
- Prepared abstracts, papers, and grants
- Quickly promoted from Research Assistant / Polysomnogram Technician

TEACHING EXPERIENCE

CO-INSTRUCTOR

2014 to Present — *Bariatric Medical Tutorial* with Dr. Kimberley E. Steele, The Johns Hopkins Center for Bariatric Surgery, Baltimore, MD
→ Planned and implemented this new tutorial for clinical and research experience for post-baccalaureate and undergraduate pre-medical students

TEACHING ASSISTANT

Oct. 2010 to Jan. 2014 — *Infection, Immunity and Undernutrition: Interactions and Effects*, Dr. Christian L. Coles, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
→ Planned and implemented this new course

Jan. 2012 to Jan. 2014 *Nutritional Epidemiology*, Dr. Laura Caulfield, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
→ Organized content for a new lecture on inflammation and biomarkers

Mar. 2012 to Jan. 2014 *Food Technology and Health*, Dr. Jed W. Fahey, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Sept. to Nov. 2012 *Food and Nutrition Policy*, Dr. Rolf D.W. Klemm, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
→ Designed new quizzes to assess student preparation for each session

GUEST LECTURER

2012 to Present ***Aquaculture: Health & the Environment***, *Food Technology and Health*, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

April 2006 ***Analysis of Echinacea and St. John's Wort Via High Performance Liquid Chromatography****, Experimental Biological Chemistry*, Department of Chemistry, Mary Baldwin College, Staunton, VA

- Presented my thesis as an application of this course
- Demonstrated laboratory techniques focusing on HPLC analysis of supplements

PROFESSIONAL ACTIVITIES

2011 to Present **Member**, American Society for Nutrition

2014 **Participant**, The American Society for Metabolic & Bariatric Surgery (ASMBS) and The Obesity Society (TOS) ObesityWeek, Boston, MA

2011 **Participant**, Federation of American Societies for Experimental Biology (FASEB) Summer Research Conference - Nutritional Immunology, Carefree, AZ

2008 **Participant**, Sleep: Annual Meeting of the Associated Professional Sleep Societies, Baltimore, MD

HONORS AND AWARDS

2006, **Iota Sigma Pi**, National Honor Society for Women in Chemistry

2005, **Beta Beta Beta**, National Biological Honors Society

2005, **Carpenter Society of the Quest Program**, Mary Baldwin College

2004, **International Missions on Medicine**, Beijing, Xi'an, & Chengdu

2004, **Math-Science Leadership Scholarship**, Mary Baldwin College

2004, **H. Dawbarn Endowed Scholarship for Excellence in Science & Math**, Mary Baldwin College

2003, **Cynthia H. Tyson Student Leadership Series**, Mary Baldwin College

2002, **Rufus W. Bailey Honors Scholar/Scholarship**, Mary Baldwin College

PUBLICATIONS

JOURNAL ARTICLES

2. Kirkness JP, **Peterson LA**, Squier SB, McGinley BM, Schneider H, Meyer A, Schwartz AR, Smith PL, Patil SP. Performance Characteristics of Upper Airway Critical Collapsing Pressure Measurements during Sleep. Sleep. 2011; 34 (4): 459-467.

1. Polotsky VY, Patil SP, Savransky V, Laffan A, Fonti S, **Frame LA**, Steele KE, Schweizter MA, Clark JM, Torbenson MS, Schwartz AR. Obstructive Sleep Apnea, Insulin Resistance and Steatohepatitis in Severe Obesity. American Journal of Respiratory and Critical Care Medicine. 2009 Feb 1; 79 (3): 228-34.

POSTER PRESENTATIONS

7. Wolf RM, Steele KE, **Peterson LA**, Magnuson TH, Schweitzer MA, Wong GW. C1q/TNF-related protein 3 (CTRP3) levels are significantly decreased in human obesity. Endocrine Society. 2015 March.

6. **Peterson LA**, Canner J, Cheskin LJ, Prokopowicz GP, Schweitzer M, Magnuson TH, Steele KE. Association of Seasonality with Wound Complications and Length of Stay in the Nationwide Inpatient Sample, 2001-2010. The American Society for Metabolic & Bariatric Surgery (ASMBS) and The Obesity Society (TOS) ObesityWeek. 2014 November.

5. Steele K, **Peterson LA**, Papas K, Scudder M, Kyriakides T, Magnuson TH, Lidor A, Schweitzer M. Efficacy of a chewable multivitamin/mineral supplement in preventing vitamin deficiency in

Roux en-Y gastric bypass patients: A randomized controlled clinical trial. The American Society for Metabolic & Bariatric Surgery (ASMBS) and The Obesity Society (TOS) ObesityWeek. 2014 November.

4. Bae J, **Peterson LA**, Schneider E, Prokopowicz GP, Schweitzer M, Magnuson TH, Lidor A, Steele K. Trends in Bariatric Practice 2003 to 2011. The American Society for Metabolic & Bariatric Surgery (ASMBS) and The Obesity Society (TOS) ObesityWeek. 2014 November.

3. Steele K, Prokopowicz GP, **Peterson LA**, Verde F, Magnuson TH, Schweitzer M. Magnetic Resonance Venography for the Detection of Asymptomatic Deep Venous Thrombosis in the Bariatric Surgical Patient. The American Society for Metabolic & Bariatric Surgery (ASMBS) and The Obesity Society (TOS) ObesityWeek. 2014 November.

2. **Peterson LA** and Christian P. Vitamin D Status as a Predictor of Preeclampsia Risk in Rural Nepal. Federation of American Societies for Experimental Biology (FASEB) Summer Research Conference - Nutritional Immunology: Role in Health & Disease. 2011 July.

1. Pallayova M, Schwartz AR, **Frame LA**, Laffan AM, Smith PL, Steele KE, Lidor AE, Magnuson T, Schweitzer MA, Patil SP. Effects of Bariatric Surgery on Adiposity, Sleep, and Selected Biomarkers in Severely Obese Women. Annual Johns Hopkins Bayview Research Symposium. 2009 Dec 4.

CURRICULUM VITAE

Leigh A. Peterson, MHS

Part II

CIVIC LEADERSHIP

2015 to Present — **President**, Patterson Park Neighborhood Association (PPNA), Baltimore, MD

- Platform: Increased communication
- Circulate meeting agendas prior to monthly meetings
- Annotate meeting agendas and circulate immediately following monthly meetings
- Preserve hard copy newsletter and expand digital publication
- Better utilize existing tools (bulletin board, Google Calendar, etc.)
- Increase bilingual communication
- Attract and retain new and established membership to monthly meetings

2014 to Present — **Member**, Advisory Board of Visitors, Mary Baldwin College, Staunton, VA

- Advise my alma mater on matters of significant import, such as aligning the name with its long-held university status now that the Murphy Deming College of Health Sciences has opened
- Mentor sophomore students pursuing a similar career path
- Fundraising/Institutional Advancement Sub-Committee Member

2014 to Present — **Member**, Patterson Park Master Plan Capital Improvements Committee, Baltimore, MD

- Advise Mahan Rykiel Associates on needs of the community
- Assist in revising the 1998 Master Plan to reflect current needs in the park
- Capital Improvements Sub-Committee Member

2011 to Present — **Bloom Your Block Coordinator**, PPNA Greening Partnership (formerly Greening Committee), Baltimore, MD

- Spring beautification project where neighbors plant flowers in planters
- I introduced native, pollinator/bird-friendly plants into this project
- Builds a strong sense of community through distribution & maintenance

2012 to Present — **Fall Beautification Coordinator**, PPNA Greening Partnership (formerly Greening Committee), Baltimore, MD

- Originally, neighbors decorate their stoop with fall/winter plants and a pumpkin
- I introduced a focus on sustainability and native, pollinator/bird-friendly plants
- Builds upon the spring beautification project, Bloom Your Block

2013 to 2014 — **Vice President of South Sector**, Patterson Park Neighborhood Association (PPNA), Baltimore, MD

- Liaison and representative to my sector (≈14 city blocks)
- Personal Project: Intersection bump outs with rain gardens and permeable concrete to provide traffic calming and storm water control

2012 to 2013 — **Member**, Patterson Park Planning Committee, Baltimore, MD

- Advised the Working Group for the Master Plan
- Interpreted multiple data streams into a concise recommendation

2012 to 2013 — **Secretary**, International Health Student Group, Baltimore, MD

2008 to 2012 — **Class of 2006 Gift Chair**, Mary Baldwin College, Staunton, VA

2010 to 2011 — **Captain**, Baltimore Neighborhood Energy Challenge (BNEC), Baltimore, MD

- Patterson Park was one of a handful of pilot neighborhoods for BNEC
- Spread knowledge, resources, and motivation to save energy & money
- Extension of the work by the Greening Committee of the PPNA

2005 — **Volunteer**, Resolution Recipient, City Public Works, Staunton, VA: Staunton City Hall recognized my efforts to beautify the city to continue to be competitive for the Great American Main Street Award

REFERENCES

Lawrence J. Cheskin, MD
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Relationship: MHS Advisor, PhD Oral Exam Committee Member, & PhD Dissertation Reader and Defense Committee Member

ADDITIONAL INFORMATION

PERSONAL STATEMENT OF RESEARCH

My research interests are in the intersection of nutrition and immune competence. More specifically, I am interested in the role of vitamin D as an immunomodulator in the following areas:

1. Photoproduction of vitamin D and skin cancer risk: a false paradox?
2. Supplementation vs. photoproduction
3. Delayed wound healing
4. Increase infection incidence
    a. Skin
    b. Systemic (sepsis)
5. Decreased anti-body titer following vaccination
6. Adult onset of diseases such as
    a. Chronic kidney diseases
    b. Cardiovascular disease
    c. Tuberculosis
    d. Leishmaniasis
    e. Influenza
    f. Cancer
7. Negative pregnancy outcomes
    a. Preeclampsia
    b. Spontaneous preterm birth
    c. Gestational diabetes
    d. Fetal growth restriction
    e. Fetal and infant survival
8. Maternal-child relationship
    a. Immune tolerance malformation leading to increased incidence of
        i. Autoimmunity
        ii. Food allergy
    b. Inflammation
    c. Infection

KEYWORDS

Vitamin D
Nutrition
Immunology
Bariatric surgery
Wound healing
Infection
Inflammation
Immune surveillance
Cancer
Dermatology
Neonatal health
Maternal health
Fetal imprinting
Public health
Health disparity
Supplementation