Effect of bariatric surgery on the prevalence of micronutrient deficiencies and protein status

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Memòria presentada per **Violeta Moizé Arcone** per optar al títol de doctor per la Universitat de Barcelona

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ABBREVIATIONS

LIST OF ABBREVIATIONS

3-C	3 Compartment Model
AA	Amino Acids
AACE	American Association of Clinical Endocrinologists
ACD	Anemia of Chronic Disease
AGB	Adjustable Gastric Band
ASMBS	American Society for Metabolic and Bariatric Surgery
AUC	Anemia of Unknown Cause
AUC	Area Under de Curve
BC	Body Composition
BCAA	Branched Chain Amino Acids
BIA	Biolectrical Impedance Analysis
BMI	Body Mass Index
BPD	Biliopancreatoic Diversion
BS	Bariatric Surgery
BW	Body Weigh
BWL	Body Weight Loss
Ca	Calcium
CCK	Cholecystokinin
CRP	C-Reactive Protein
CRR	Clinical Research Resources
CTSA	Clinical and Translational Science
CU	Columbia University
CV	Cardiovascular
D_2O	Deuterium Oxide
DNA	Deoxyribonucleic acid
DEXA	Dual energy X-ray Absorptiometry
DIT	Diet Induced Thermogenesis
DRI	Dietary Reference Intakes
DS	Duodenal Switch
EBW	Excess of Body Weight
EE	Energy Expenditure
EWL	Excess of Weight Loss
FFM	Fat Free Mass
FID	Functional Iron Deficiency
FM	Fat Mass
GBP	Gastric Bypass
GLP-1	Glucagon-like peptide 1
Hb	Hemoglobin
HPS-G	High Protein Supplementation Group
HPT	Hyperparathyroidism

IBW	Ideal Body Weight
ID	Iron Deficiency
IR	Insulin Resistance
Kcal	Kilocalorie
LBM	Lean Body Mass
LGBP	Laparoscopic Roux-in-Y gastric Bypass
LSG	Laparoscopic Sleeve Gastrectomy
LTM	Lean Tissue Mass
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MetSd	Metabolic Syndrome
MIPS	Metabolic Impact of Protein Supplementation
N	Nitrogen
NB	Nitrogen Balance
NDSR	Nutrition Data System
P	Phosphorous
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PEG	Polyethylene Glycol
PI	Protein Intake
PRO-S	Protein Supplementation
PTH	Parathyroid Hormone
PYY	Peptide YY
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
RDI	Recommended Daily Intake
RDW	Red cell Distribution Width
REE	Resting Energy Expenditure
SD	Standard Deviation
SG	Sleeve Gastrectomy
SM	Skeletal Muscle
SPS-G	Standard Protein Supplementation Group
T2DM	Type 2 Diabetes Mellitus
TBW	Total Body Water
TOS	The Obesity Society
TSAT	Transferrin Saturation
US	United States
VAS	Visual Analog Scale
VB_{12}	VB_{12}
VD	Vitamin D
VD2	Ergocalciferol
VD3	Cholecalciferol
VG	Vertical Gastrectomy
VLCD	Very Low Calorie Diet
WHO	World Health Organization
WL	Weight loss
WM	Weight maintenance

PART I: GENERAL INTRODUCTION AND OBJECTIVES

CHAPTER 1: GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1. Obesity and bariatric surgery.

1.2. Nutritional status in severely obese subjects prior to surgery.

1.3. Potential mechanism of development of nutrient deficiencies after bariatric surgery.

1.4. Nutritional status assessment of the morbidly obese patient with special attention to iron, calcium, vitamin B_{12}, and folic acid and protein.

1.5. Impact of protein status over several systems of body functions.

1.1. OBESITY AND BARIATRIC SURGERY

In Spain, similarly to other European countries, the prevalence of overweight and obesity are 39.4 and 22.9% respectively (1, 2). Of note, in the last 3 decades, the worldwide prevalence of obesity has increased 27.5% for adults and 47.1% for children for a total of 2.1 billion individuals currently being considered overweight or obese (3). Importantly, the obesity category that has increased the most over the last years is obesity grade 3 (Body Mass Index (BMI) >40 kg/m^2) (4).

Obesity is defined by excessive adipose tissue storage and is commonly accompanied by a variety of comorbidities, mainly comprising of type 2 diabetes mellitus (T2DM), hypertension and cardiovascular (CV) disease. The exact cause of obesity is unknown; however, there appears to be a complex relationship among biologic, psychosocial, and behavioral factors, which include genetic makeup, socioeconomic status, and cultural influences. Consumption patterns, urban development, and lifestyle habits influence the prevalence of obesity (5).

Although the pathophysiology of obesity is fairly well understood, treatment and prevention have focused on the psychological and social components of the disease. According to the American Association of Clinical Endocrinologist (AACE), The Obesity Society (TOS) and the American Society for Metabolic and Bariatric Surgery (ASMBS) guidelines (6), comprehensive, multicomponent interventions are currently the treatment of choice, including lifestyle intervention, dietary restriction, pharmaceutical and surgical

management. To date, the best noninvasive interventions are dietary management and behavioral change. However, although intensive behavioral counseling can induce clinically meaningful weight loss (7), long term success is limited and the best outcomes specially in patients with severe obesity are associated with bariatric surgery.

Bariatric surgery (BS) is the most effective long-term therapy for the treatment of severe obesity. Several studies have demonstrated that BS is associated with a favorable impact on hard endpoints such as overall and CV mortality, incidence of first occurrence of fatal or non-fatal CV events, prevention and remission of T2DM and quality of life (8). A description of the bariatric procedures will be considered below. Consideration of BS as valid therapy for severe obesity should be based on the balance of benefits and complications. Current laparoscopic approach for all BS procedures has resulted in marked decrease in rates of complications (9). Indeed, laparoscopic BS associates mortality rates similar to cholecystectomy (10).

Additionally, nutritional and gastrointestinal complications can occur after bariatric surgery. The risk of nutritional deficiencies is influenced by pre-surgical and post-surgical factors including the surgical technique, postoperative weight loss, and patient compliance/adherence with the nutritional follow up (11). Nutritional complication could potentially lead to severe malnutrition or severe medical consequences if the existing nutritional problem is undiagnosed as a consequence of a poor medical follow up.

Surgical procedures

Originally bariatric surgery techniques were classified into restrictive, malabsorptive or mixed, depending on the putative mechanism responsible for weight loss. Currently this classification has become obsolete, as it is known that restriction and malabsorption are not the main mechanisms leading to significant and sustained weight loss. In fact, because of better understanding of the metabolic changes induced following different surgical interventions in the alimentary tract, BS is often referred to as metabolic surgery. This term indicates that the surgically induced change of a normal organ modifies its function and determines, at least in part, its effect on weight loss and other health related benefits.

For many years the most commonly performed BS procedure was gastric bypass (GBP) (45%), followed by vertical sleeve gastrectomy (SG) (37%), adjustable gastric band

(AGB) (10%) and, finally, biliopancreatic diversion (BD) or its variant duodenal switch (DS) (2.5%) (12). However, in recent years, the SG has gained popularity, and become the most performed surgical procedure, in US/ Canada and in the Asia/ Pacific area as well. Also in Spain, in the last 15 years the SG has increased from 0.8% to 39.6% (13).

In a shallow way, the main surgical techniques currently used are:

Roux-en-Y Gastric bypass (GBP).

It is considered the gold standard and is the most studied surgical procedure. GBP involves the division of the stomach into an upper gastric pouch, which is 15–30 ml, and a lower gastric remnant. The gastric pouch is anastomosed to the jejunum after it has been divided some 30–75 cms distal to the ligament of Treitz; this distal part is brought up as a 'Roux-limb'. The excluded biliary limb, including the gastric remnant, is connected to the bowel some 75–150 cms distal to the gastrojejunostomy (Figure 1) (14).

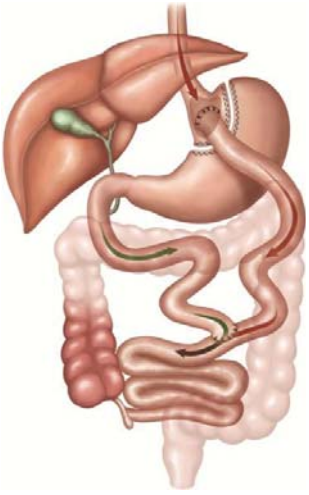


Figure 1. Roux-en-Y Gastric bypass (GBP).

Sleeve gastrectomy (SG)

In SG the stomach is transected vertically creating a high-pressure gastric tube and leaving a pouch of up to 200 ml (Figure 2). In SG, the pylorus is preserved. Thus, at variance with the RYGB, this physiological valve is placed at the connection between the reduced stomach and the gut.

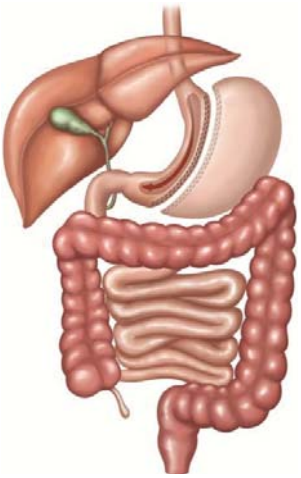


Figure 2. Sleeve gastrectomy (SG).

Adjustable gastric banding (AGB)

In AGB a silastic band is applied around the stomach just below the gastro esophageal junction, and is tightened through a subcutaneous access port by the injection or withdrawal of a saline solution (Figure 3).

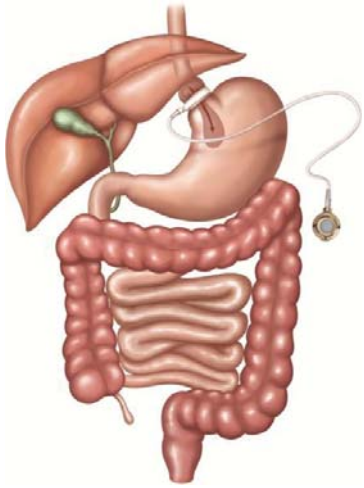


Figure 3. Adjustable gastric banding (AGB).

Bilio-pancreatic diversion (BPD) and Duodenal Switch (DS)

BPD and DS include a gastric component resulting in the reduction of gastric volume, and an intestinal component resulting in reduced nutrient absorption. The gastric component in BPD includes a partial horizontal gastrectomy with formation of a 400 ml gastric pouch (15). In DS, the gastric manipulation consists of a vertical gastrectomy similar to that in the SG described above. In both surgical procedures the small bowel is divided 250 cm proximal to the ileocaecal valve, and the alimentary limb is connected to the gastric pouch (BPD) or proximal duodenum (DS) to create a Roux-en-Y gastroenterostomy. An anastomosis is performed between the excluded biliopancreatic limb and the alimentary limb 50-100 cms proximal to the ileocaecal valve (Figure 4).

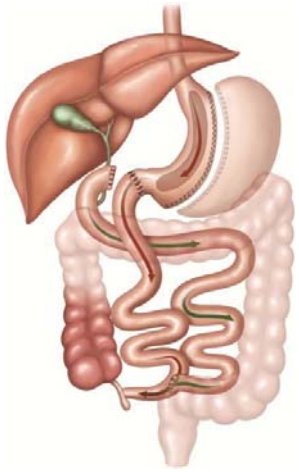


Figure 4. Bilio-pancreatic diversion (BPD) and Duodenal Switch (DS).

1.2. NUTRITIONAL STATUS IN SEVERELY OBESE SUBJECTS PRIOR TO SURGERY

Dietary food intake is repeatedly and drastically reduced over several periods during the life span in obese subjects trying to prevent further weight gain or as therapeutic approach. Thus, it could be assumed that the supply of certain essential nutrients is decreased repeatedly as consequence of intermittent dietary restrain. As the requirements of these nutrients remain unchanged, this may result in a suboptimal nutritional vitamin and mineral balance in obese

people. On the other hand, it could be argued that, during the development of obesity, these individuals increase their intake of vitamin and minerals by eating more. However, contra-intuitively, the opposite is often observed because a significant number of obese people eat inadequately or are under low-nutrient density diets. Indeed, the evaluation of dietary and nutrient intake in relation to body weight and weight control efforts has seldom been addressed. This is probably because its complicated approach given the well known problem of dietary misreporting resulting in biased estimates of self-reported intakes of energy and foods considered nutritional undesirables (16).

Diet quality, BMI, and weight control

Studies describing the diet quality in subjects with obesity are scarce and have mainly been based on the use of indexes of diet quality such as the food frequency index (FFI) or the use of biomarkers. Using the FFI index, Freisling et al (17), observed that the FFI was negatively associated with self-reported BMI. Indeed, an increment of eight units of the FFI (i.e. the difference between 'poor' and 'very good' diet) was associated with a BMI decrease of 0.8 kg/m^2. However, the cross-sectional study design underlying these observations did not allow conclusions about a causal relationship between FFI scores and BMI. The observed negative correlation could be due to an overall healthier lifestyle in subjects with higher FFI scores. An alternative explanation could be that lower FFI scores indicated more energy-dense diets, which in turn could promote weight gain. Nonetheless, a high intake of energy-dense micronutrient-poor foods is described as a convincing etiological factor that promotes unhealthy weight gain (18). Data from the Third National Health and Nutrition Examination Survey showed that participants with poor diet quality were more likely to be obese (19, 20) In this report, three different diet quality indexes were consistent as negative predictors of BMI in a sample of adults aged 20 years and over. Of note, a study from Spain with adults aged 25–74 years reported that adherence to traditional Mediterranean dietary patterns was negatively associated with BMI (21). In other studies, BMI was an independent inverse predictor of intake of sugar, fat or other low-nutrient-dense foods (22-24).

Whether or not weight control diets impact nutrient intake was approached by Kant in 2003 (25). According to his observations, with the exception of an increased risk of not meeting the weight-based standards for protein (0.8 g/kg body weight), vitamin E (in women),

and iron (in men at higher BMIs), attempting weight loss was not associated with a significantly increased risk of inadequate intake of the nutrients the examined despite lower energy intake. A trend for a lower proportion of those attempting weight loss meeting the calcium standard was noted but did not reach statistical significance. However, Ritt et al (26) identified iron and calcium as nutrients at risk in diets used for weight control. More recently, Gardner et al 2010 (27) performed a extensive dietary assessment conducted with a large number of participants in the A TO Z study to examine the vitamin and mineral intakes consumed under 4 different eight-week weight loss diets (Atkings, LEARN, Ornish, Zone). Along with significant group differences in macro nutrient intakes, many significant differences in vitamin and mineral intakes were observed —not only group differences in absolute intakes of micronutrients but also the risk of inadequate intakes. At the end of the program, a significantly higher proportion of individuals shifted to intakes at risk of inadequacy in the Atkins group for thiamine, folic acid, vitamin C, iron, and magnesium; in the LEARN group for vitamin E, thiamine, calcium, and magnesium; and in the Ornish group for vitamins E and B_{12} and zinc.

In summary, BMI seems to be associated with poor diet quality. A poor quality - low energy diet appear to affect nutrient sufficiency of some essential key nutrients, which could compromise the nutritional status of obese individuals.

Irrespective of the potential underlying mechanism, over the last years several studies in the United States (US) and in Western Europe populations have shown vitamin and micronutrient deficiencies, as assessed from plasma levels, are in a significant proportion of patients with severe obesity (28-33). The most common vitamin and mineral deficiencies reported in the severely obese patients are considered as follows:

Vitamin and mineral deficiencies

Vitamin B_1 (Thiamine)

Thiamine's is as a coenzyme in a wide variety of intricate biochemical pathways, making it vital for proper tissue and organ function. Deficiency of this essential vitamin may lead to serious metabolic imbalance causing cardiovascular and neurological manifestations. Dietary sources of thiamine include only a variety of animal and vegetable products. It is abundant only in a limited number of foods such as yeast, whole grain, beef, lean pork, and

legumes (beans and peas). It is absent from fats, oils, and refined carbohydrates. Similar to other vitamins, thiamine is rapidly destroyed in the process of food preparation and when ingested with ethanol. Thiamine plays a vital role in the metabolism of glucose. Potential mechanisms of thiamine deficiency include decreased intake or absorption due to a poor diet quality that primarily consist of simple sugars, excessive alcohol or an inadequate amount of whole grains or legumes, anorexia. Prolonged emesis could also contribute to decreased intake. Other causes as increased destruction/inactivation of vitamin B_1 (i.e. polyphenols, thiaminases), excessive losses (use of diuretics), and increased thiamine use and an altered metabolism can exacerbate thiamine deficiency. Since thiamine is absorbed primarily in the duodenum mainly through active transport, any situation that limits absorption at the duodenal level could also cause thiamin deficiency.

Obese patients have poorly controlled dietary habits that are high in calories and refined carbohydrates; they also often use/abuse diuretics (either for weight reduction or for the treatment of comorbidities), and thus they are at risk for thiamine deficiency. Although the mechanisms underlying thiamine deficiency in bariatric surgery patients postoperatively are clear, the prevalence of thiamine deficiency in morbidly obese individuals is surprising. Two studies in American populations highlight the relevance of thiamine deficiency in bariatric surgery candidates. Carrodeguas et al reported a prevalence of thiamine deficiencies of 15.5% (34). On the other hand, Flancbaum et al 2006, reported thiamine deficiency in up to 29% of the patients in their series (30), with racial differences being present (Hispanics,47,2%; African American, 31%; Caucasians, 6.8%).

Vitamin B_{12} (Cobalamin)

Vitamin B_{12} plays an important role in deoxyribonucleicacid (DNA) synthesis and neurological functions. Vitamin B_{12} is present in a variety of foods such as fish, shellfish, meats, and dairy products. Vitamin B_{12} deficiency may lead to a wide spectrum of hematologic and neuro-psychiatric disorders. Combined vitamin B_{12} and folate deficiency produces hyperhomocysteinemia, which is considered an independent risk factor for atherosclerotic disease. The true prevalence of vitamin B_{12} deficiency in the general population is unknown. The incidence, however, appears to increase with age. The relationship between BMI and vitamin B_{12} deficiency is not well established. A prospective

descriptive study evaluated whether overweight children and adolescents are at an increased risk for vitamin B_{12} deficiency (35). They found that obesity in children and adolescents was associated with an increased risk of low vitamin B_{12} concentrations. While the prevalence of B_{12} deficiency was similar between normal-weight and obese children, obesity was associated with a 4.3-fold risk for low serum levels of vitamin B_{12}, and each unit increase in BMI resulted in an increased risk of 1.24 of vitamin B_{12} deficiency. Studies evaluating the relationship between vitamin B_{12} deficiency and BMI in adults have produced mixed results. While Mahabir et al showed decreased levels of vitamin B_{12} at increased BMI (36) others have reported no differences in vitamin B_{12} deficiency in obese subjects (37, 38).

Folate

Folic acid/folate is a water-soluble vitamin, also known as vitamin B_9. Natural dietary sources of folate include leaf vegetables such as spinach and turnip greens, dried beans and peas, sunflower seeds, liver, and fruits and other vegetables. The most common manifestation of this deficiency is anemia and neural tube birth defects. Low folate levels associate an increase in serum homocysteine. Increased total plasma homocysteine concentration has been associated with increased risk for cardiovascular disease (39). The low prevalence of folate deficiency in the general population is probably related with Food and Drugs Administration's rules for fortification of common foods with folic acid. However, obese individuals showed inadequate folate levels in several studies (36, 40, 41). Ortega et al, 2009 observed that obese women are at greater risk of inadequate folate status, even with similar folate intake to that in non-obese individuals (41). Mahabir et al, 2008 observed that increased body fat percentage correlates to lower serum folate (36). In another study, BMI higher than 50 kg/m^2 correlated with greater likelihood of folate deficiency (40). Nevertheless these findings are at odds with those from two other studies that did not find differences in folate levels in obese adults as compared to non-obese individuals (37, 38). Bird et al. speculated that low serum folate may stimulate folate uptake by red blood cells or, alternatively, volumetric dilution results in lower folic acid serum levels in the obese, or that adiposity influences folate uptake by intestinal epithelium (42).

Vitamin D

Vitamin D is a fat-soluble vitamin with hormonal functions. Its major skeletal actions are complemented with a variety of extra-skeletal roles (43). Major food sources of vitamin D are fortified dairy products, fortified foods, fatty fish and eggs. Evidence supports that morbidly obese patients are at increased risk for abnormal vitamin D metabolism. Studies support that parathyroid hormone (PTH) levels correlate negatively with 25(OH)D levels, and show a positive association with increased BMI (44-46). The causes of vitamin D deficiency are multifactorial. Some authors speculated that morbidly obese people spend less time outdoors and therefore are less exposed to sunlight (47). Others have suggested excess fat in obesity results in adipose tissue sequestration of this fat soluble resulting in lower circulating vitamin D levels (48). Vitamin D deficiency could also been linked to inadequate vitamin D intake despite overall high caloric intake (47, 49). A decrease in hepatic production of 25-hydroxy vitamin D due to hepatic steatosis and a decrease in synthesis of vitamin D through the skin may also intervene (50). However the mechanisms of these abnormalities have not yet been identified.

Vitamins A and E

Vitamin A (carotenoids) and E are fat-soluble vitamins. In developed countries, 80 – 90% of the carotenoid intake comes from fruit and vegetable consumption. Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables (51). Main sources of vitamin A in Spain are vegetables (69%) fruits (16%), tomato sauce (3%), and pulses (3%). Vitamin E main food sources in Spain are: fats and oils (44%), vegetables (15%), fruits (12%), meat, poultry and fish (8%), and nuts and seeds (8%). Previous studies on associations between diet, obesity, and blood concentrations of vitamin E (alpha-tocopherol) and vitamin A (in its various forms) have been inconclusive. Serum levels of alpha-tocopherol and beta-carotene were observed to be significantly lowered in obese vs. non obese children and adolescents (52, 53). In adults, a higher prevalence of low carotenoid levels among overweight and obese adults vs. normal weight and a positive correlation between BMI and the prevalence of low vitamin E levels has also been observed (25, 54-56).

Vitamin C

Vitamin C is an antioxidant and a cofactor for various biochemical reactions, and acts as an electron donor for different enzymes. In Spain, main food sources of vitamin C consumption are fruits (51%), vegetables (32%), juices and drinks (7%) and potatoes (4%) (57). The prevalence of vitamin C deficiency in obese population has seldom been reported. Among men aged 19 to 64.9 years (but not for men aged 65 years or older) and women older than 19 years of age, with increasing BMI being associated with low levels of vitamin C (25, 54, 55).

Magnesium

Magnesium is essential to all living cells. Maintaining normal concentration of extracellular magnesium and calcium is essential for adequate neuromuscular activity. Magnesium is absorbed mainly in the jejunum and ileum. This process is stimulated by 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and can reach up to 70% of absorption in cases (58). Most dietary magnesium comes from vegetables, particularly dark green and leafy vegetables (such as spinach). Other food sources of magnesium include: legumes, fish, fresh meat, soy products (soy flour and tofu), seeds, nuts, whole grains (unrefined grains), and fruits and vegetables (such as bananas, dried apricots, and avocados) (59). Magnesium deficiency has been shown to correlate with a number of chronic cardiovascular diseases, including hypertension, diabetes mellitus, and hyperlipidemia (60, 61). Surveys on micronutrient intake in obese and non-obese youth found that in the obese group 27% had inadequate magnesium intake, in spite of energy intake being 124% of the estimated need yet 32% coming from fat (62).

Selenium

Selenium is required only in trace amounts. It is involved in anti-oxidative reactions. It is also required for normal thyroid functions and immunological reactions (63, 64). Animals that eat grains or plants that were grown in selenium-rich soil have higher levels of selenium in their muscles. Selenium also can be found in seafood. Some nuts also contain selenium, especially Brazilian nuts which are rich in this element. Abnormally low levels and deficiency of selenium were found in up to 58% of severely obese candidates for bariatric surgery (54,

65). Higher BMI was significantly associated with low selenium levels among pre menopausal women and also in men aged 19 to 64.9 years. However, the overall prevalence of low selenium levels in that study population was low (66).

Iron

Iron deficiency is the most frequently occurring micronutrient deficiency in low-income and industrialized countries (67). The human body contains about 2.5 to 4 g of elemental iron. Of this, about 70% is contained in hemoglobin. Trace amounts of iron are also associated with electron transport and several enzymes. Approximately 25% of iron is stored primarily in the liver. Smaller amounts are present in the endothelial reticulum of bone narrow cells and spleen; and in the muscle tissue. Of the stored iron, about 2/3 consists of ferritin, the soluble fraction of the non-heme iron stores. Small quantities of ferritin can be synthetized in all cells of the body. Ferritin also appears in small concentration in serum but it is not involved in iron transport. The reminder of iron storage is as insoluble hemosiderin (68). Iron transport is carried out by the transport protein transferrin.

Iron absorption is determined by the quantity and bioavailability of the ingested iron and the capacity to absorb iron. Iron absorption requires reduction from ferric- to ferrous-iron, with that being influenced by the acidic gastric environment. Nutrient inhibitors of iron absorption include phytate and poliphenols, as well as certain vegetables, protein, and calcium. In contrast, vitamin C, as well as other organic acids and animal tissues (meat, fish, and poultry) enhance non-heme iron absorption. High iron foods include liver, kidney, mussels and read meat. Foods with medium iron content include chicken, processed meat, fish and legumes (non-heme only). In many industrialized countries, cereals products are fortified with iron and provide the highest proportion of dietary iron. The bioavailability of iron in mixed diets ranges 15% to 18% (69).

Obesity may affect iron balance at several levels. Obesity may be associated not only to limited iron intake, but also to a disruption of iron metabolism because of the associated low grade systemic inflammation often times present in obese subjects. In obese adults candidate to BS, deficiency and abnormal levels of iron have been reported respectively in 14 and 16%. On the other hand, deficiency and abnormal ferritin levels have been reported in 6 and 9%, respectively. Preoperative deficiencies of iron, ferritin, and hemoglobin were also observed in

adults before bariatric surgery (30, 65). Studies conducted in children and adolescent showed a significant inverse correlation of low iron levels and BMI, but not with age or gender. The obese group accounted for 58.3% of the children with iron deficiency anemia (35). Moreover, an analysis of cross-sectional data in children 2 to 16 years from the National Health and Nutrition Examination Survey III (1988–1994) indicated that the prevalence of iron deficiency increased as BMI increased, it was particularly common among adolescents.

Zinc

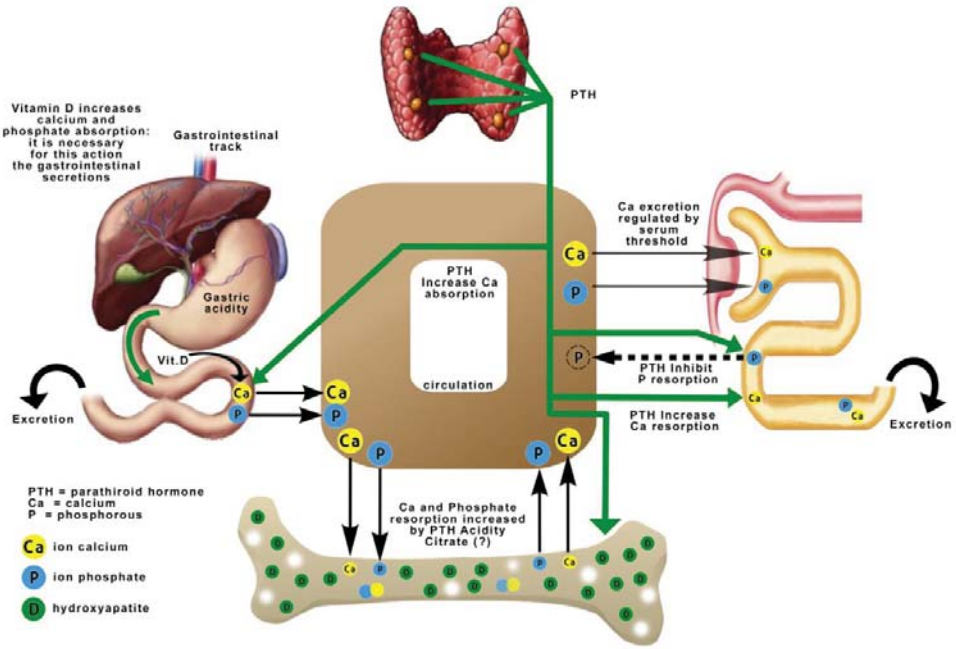
Zinc is needed for DNA synthesis, cell division, gene expression, and the activity of various enzymes in mammals, and therefore achievement of normal growth, sexual maturation and function, maintenance of a normal appetite, visual, taste and smell acuity, and normal psychocognitive function. Zinc is also important for immunological reactions, wound healing, and preservation of the integrity of epithelial surfaces. Zinc deficiency or abnormal metabolism has been suggested to play a role in the pathophysiology of the metabolic syndrome (70, 71). Zinc has an important role in adipose tissue metabolism by regulating leptin secretion and by promoting free fatty acid release and glucose uptake. It also plays a key role in the synthesis and action of insulin. Zinc is stored mainly in hair, nails and skin. Evaluation of zinc concentrations in serum and hair in obese (n=135) and non-obese patients (n=57) showed that serum and hair zinc content in obese patients was markedly lower than in control subjects (22 and 34%, respectively). Moreover, zinc content of hair and nails was inversely correlated to BMI (72). In obese patients candidates for GBP, preoperative abnormal zinc levels and deficiency were found, respectively, in 30 and 28% (65).

Calcium

Bone is the main reservoir of calcium in the body while the remainder is located in other body tissues (10g), or blood and extracellular fluids (900 mg). Calcium has a structural role in bones and teeth. It also has a regulatory role in several metabolic processes including enzyme activation, vascular contraction and vasodilatation, muscle contractibility, nerve transmission, hormone function, and membrane transport. When the levels of absorbed calcium from the diet are insufficient to balance the fecal and urinary losses, calcium is drawn from the bone, so blood calcium levels are maintained within narrow limits. The homeostatic regulation of calcium level in blood is achieved mainly by the interaction of the PTH, 1,25-

dihydroxycholecalciferol, and calcitonin (73) (Figure 5). Calcium is absorbed across the intestinal mucosa by active and passive diffusion. The active absorption is influenced, among other factors, by the calcium and vitamin D status of the individual and occurs mainly when calcium intake is decreased. Several other dietary variables affect calcium absorption, retention and, thus, status. Diets high in protein increase the urinary excretion of calcium with no calcium absorption compensation. Dietary content of oxalates and phytates can also affect the bioavailability of this mineral. In patients with renal failure, the reduced synthesis of 1,25-dihydroxyvaitamin D impairs calcium absorption. It has also been observed that obese individuals have relatively high true fractional calcium absorption before undergoing BS (74). Because of the several mechanisms that maintain calcium levels in a narrow range, abnormalities in serum calcium levels have seldom been reported. However, that calcium metabolism is altered in the obese is supported by the several reports showing morbidly obese patients are at increased risk for abnormal vitamin D metabolism, and/or present with parathyroid hormone (PTH) levels >65 pg/ml) (75).

Figure 5. Calcium homeostasis



Conclusion on vitamin and mineral deficiencies in severe obesity

It is important to consider that even when in some cases the reported deficiencies are shown as subclinical, these findings cannot be ignored. In Western countries, despite there are almost no limitation of access to a wide variety of food supplies, nutritional deficiencies can be found in the obese population. For the most part, nutritional deficiencies could be attributed to unbalanced dietary intake. Moreover, the absorption, bioavailability, distribution, metabolism, and/or excretion of these nutrients in overweight and obese individuals might be altered. Importantly, most of the data described above has been generated from cohort studies in populations from the US or Northern European countries. Although normal-weight subjects may also suffer from low vitamin levels, scientific evidence on this topic is not large and case-control studies comparing the nutritional status in obese- versus normal-weight subjects are missing.

Energy density of the diet does not imply an adequate nutrient density. The majority of studies on nutritional deficiencies in obese population have been carried out in the US. The dietary characteristics of Western countries differ from Mediterranean diet. In the context of this PhD thesis, it is important to consider that higher demands because of surgery, rapid weight loss, and loss of absorptive area after weight-reduction surgery, may facilitate that this subclinical preoperative deficiency become full-blown and potentially fatal. To date, similar studies have not been performed within the setting of a population from the Mediterranean area where the alimentary and climatic pattern is different compared to that in the populations studied so far. Whether the prevalence of nutritional deficiencies in obese individuals living in the Mediterranean area, where a high variety of healthy foods is available, is similar as that reported in Western countries remains unknown.

1.3. POTENTIAL MECHANISMS OF DEVELOPMENT OF NUTRIENT DEFICIENCIES AFTER BARIATRIC SURGERY (BS) WITH SPECIAL ATTENTION TO PROTEIN, IRON, AND CALCIUM.

There is no question that BS is the best available approach to achieve major and sustained weight loss, and improve comorbidities in severely obese subjects. Nonetheless, among the possible complications of obesity surgery, nutritional deficiencies are of note (76). As shown in Figure 6, BS results in major changes in gastrointestinal anatomy that affect gut anatomy and physiology. Regardless of the surgical procedure, the development of new eating behaviors and the changes in the anatomic conditions after BS limit total energy intake facilitating a nutrient deficit situation. The smaller size of the gastric pouch, along with gut hormonal changes, and the modification of the food channel may contribute to this phenomenon (77). Specifically, changes in food behaviors and meal patterns including reduced portion size, early satiety experienced after the meal, changes in taste preferences, and the potential for the development of persistent vomits, can contribute to a negative energy balance and limited vitamin, mineral and protein intake (78, 79). Along with these changes, impaired acid secretion because of gastric resection may further impair nutrient extraction and modifications needed for absorption. A series of successive steps can compromise nutritional status with detrimental consequences for the overall health in BS individuals (Figure 7).

Protein status

It is well known that protein is one of the main nutrients that can be affected by BS (76). It is generally accepted that not only the absolute amount of protein, but a dietary content of all of the essential amino acids (AA) are required for optimal protein synthesis and balance (80, 81). Based on limited evidence, current BS-guidelines suggest that a minimal PI of 60 g/d and up to 1.5 g/kg ideal body weight (IBW) per day could be adequate after BS as achievable and meaningful target to minimize post-surgical complications. However, limited volume of the stomach and the higher satiety experienced after a meal following BS could turn this recommendation unrealistic, consequently, dietary protein supplementation has been postulated as a useful tool to achieve daily protein needs (6). Protein digestion is initiated in the stomach by the primary proteolytic enzyme pepsin, a nonspecific protease that is maximally active at pH 2, therefore, the acidic environment favors protein denaturation (82).

Subsequently, protein degrades in the lumen of the intestine due to the activities of proteolytic enzymes secreted by the pancreas. The end products of protein digestion –free AA and small peptides- are absorbed in the mucosal cells of the small intestine. After protein is absorbed and enters the blood stream, mainly the liver takes up the free AA and one third is used for protein synthesis, energy metabolism, or gluconeogenesis. Skeletal muscle (SM) is the main site of metabolism of the branched chain amino acids (BCAA) (leucine, isoleucine and valine) (83). After BS, the exclusion of the duodenum and proximal part of the jejunum where protein is mainly absorbed, together with the anatomical changes in the gastric pouch (affecting gastric acid's secretion and pepsin), modify the enabling environment for an optimal protein uptake (84). Procedures with a high malabsortive component (jejuno ileal bypass, bilio-pancreatic diversion with or without duodenal switch, distal-GBP), normally cause diarrhea (especially of dietary fats and complex carbohydrates) (85, 86). Of note, when malabsorption occurs, fecal losses of nitrogen may be as high as 3.5 g/day (83). This is much larger when compared to the estimated amount of 0.4 g/day that is normally considered (87). A potential negative nitrogen balance (NB) status can then, be developed and sustained until the body reaches a new equilibrium by mobilizing the body protein storage (visceral tissues) affecting protein turnover system. Finally, other gastro-intestinal symptoms can be developed after BS, such as dumping syndrome and gastro-esophagic reflux and can contribute to gastro-intestinal changes (88).

Figure 6. Anatomical and functional changes of the BS procedures

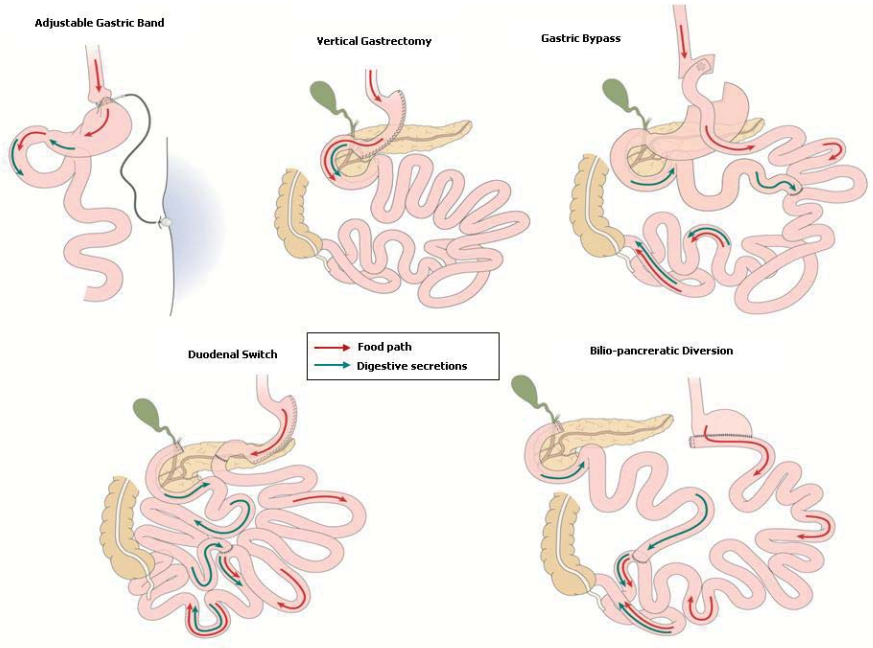
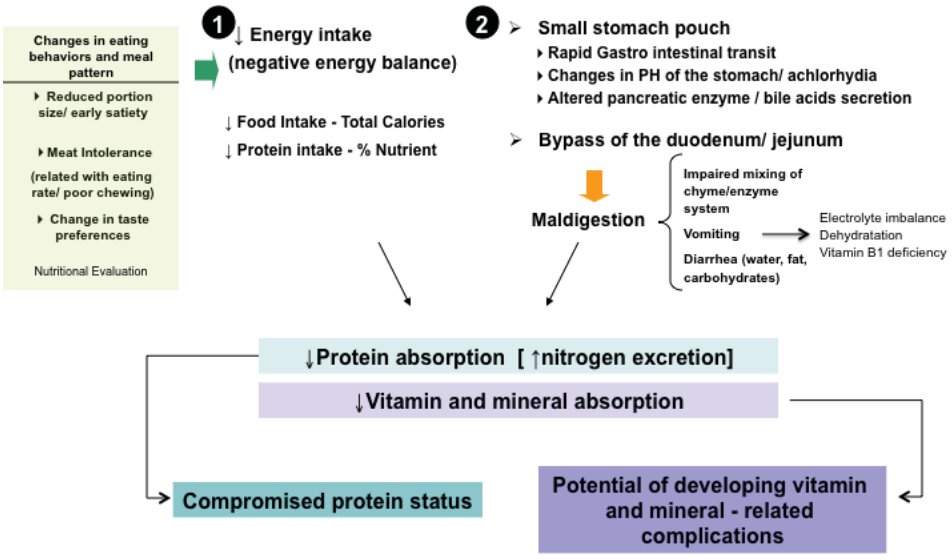


Figure 7. Compromised vitamin, mineral and protein status after bariatric surgery.



Iron

As reviewed in previous chapters of this thesis and earlier in this one, several factors can potentially affect nutrient and micronutrient balance and potentially impair nutrient storage and availability required for an optimal body function. Clinical features of iron deficiency are well defined (89). Nonetheless, several case reports shows bariatric surgery patients experiencing pica, a condition that is associated with iron deficiency and defined by unusual cravings for ice, cornstarch, clay, or other substances with deleterious health consequences (90). The mechanisms triggering iron deficiency after BS are multifactorial and could be illustrated based on the anatomical changes shown in Figure 6.

The specific mechanisms underlying the occurrence of iron deficiency are as follows:

a. **Impaired tolerance to read meat and other iron-rich foods.** Up to 50% of subjects undergoing BS report intolerance to meat, one of the major iron-rich foods. Meat intolerance has been described as late as 8 years after surgery, and is associated with diminished iron status (77, 91).

b. Decrease in the stomach capacity: limiting the dose of dietary iron potentially being absorbed.

c. Increase gastric emptying rate thus, limiting the mineral-epithelium contact.

d. Reduction of gastric acid secretion: Gastric acid is required for the absorption of iron as it transforms the ferric form ($Fe3^{+}$) to the ferrous form ($Fe2^{+}$), which is the absorbable form. Reduced gastric secretion also decreases solubilization of non-heme iron and impaires digestion of heme iron.

e. Decreasing the intestinal absorption surface: specially because of the bypass of the duodenum, which is the main site of absorption of iron (76). Although available data is limited, iron absorption could be reduced after BS. In his first study, Ruz et al (n=67 women) (92) observed that iron absorption from a standard diet and from a standard dose of ferrous ascorbate was significantly reduced 6 months after GBP (32.7%) compared to baseline values (40.3%). No further significant modifications were noted beyond this time point. Years later, in a second study (93) Ruz M et al observed that the absorption of both heme and non-heme iron is diminished 12 months after GBP and SG when assessed by isotope methods. Efficiency

of heme iron absorption was shown to be decreased from 24% before surgery to 6% one year after surgery, and that of non-heme iron from 11% to 5% regardless of the type of surgery. In this investigation estimated total body iron mass was decreased after surgery and circulating measures of iron status were not improved after surgery, which eliminates improved iron stores as an explanation for reduced absorption.

Individual factors

a. Pre-existing iron deficiencies before BS: Poor preoperative iron status has been associated with increased likelihood of post-surgical iron deficiency (ID) (94). Physiologic conditions, such as menstrual losses and pregnancy can aggravate the iron deficit in women of reproductive age.

b. Lack of adherence to iron supplementation: Oral iron has been associated with gastrointestinal side effects such as nausea, constipation and darkening of stools which can decrease adherence.

c. Low follow-up adherence to medical follow up after BS: Attrition is common after BS. A recent study found that 68–78 % of patients attended 3-, 6-, and 12-month follow up appointments, while only 41 % (95) and 33% (96) accomplish the 2 years evaluation. At long term, despite multiple contacts attempts, only 7 % attended at 10 years (96). Long-term attrition is problematic regardless of surgery type, with a meta-analysis of 28 studies revealing 83 % attrition at 3 years post surgery in laparoscopic adjustable gastric banding (LAGB) patients and 89 % attrition in GBP patients (97). The requirement for participants to cover the monetary expenses associated to follow up visits does not appear seems the only barrier. Although based on our experience as a public health care systems percentage of attrition after 10 years follow up is about 40%. It should be acknowledged this rate of attrition is higher than desired. Fortunately, since adherence to follow up is better during the perioperative stage (before and close after BS, when the risk for the development of nutritional complication is higher), nutrition evaluation allows the identification of any already existing complications and identifies patients at risk suitable for early intervention.

Table 1. Mechanisms contributing to risk for nutrient deficiencies after bariatric surgery
Adapted from Saltzman E., Karl J.P., 2013 (98)

Before surgery
▪ Obesity ▪ Poor diet quality ▪ Preoperative weight loss
After surgery
▪ Reduced food quantity ▪ Altered diet quality ▪ Vomiting ▪ Reduced gastric acid secretion ▪ Reduced intrinsic factor secretion ▪ Altered digestion ▪ Altered absorption ▪ Increase gastric emptying rate ▪ Bypass of primary sites of absorption ▪ Nonadherence to dietary recommendations ▪ Nonadherence to supplement recommendations ▪ Maladaptive or disordered eating ▪ Alcohol or substance abuse ▪ Small intestine bacterial overgrowth

Vitamin B_{12}

As it occurs with other micronutrients, multiple factors appear to contribute to vitamin B_{12} deficiency. Vitamin B_{12} is mostly found in meats. Meat is among the least-tolerated foods after GBP. Restricting stomach size results in impaired digestion and absorption of protein-bound vitamin B_{12} in food by diminishing contact of gastric acid and pepsin with food (99). Absorption of crystalline vitamin B_{12} is reduced to a lesser extent than is protein-bound B_{12}, suggesting that reduced secretion of intrinsic factor or reduced binding of intrinsic factor with vitamin B_{12} after the anastomosis of the biliopancreatic limb may also contribute to the development of deficiency (100). Symptomatic vitamin B_{12} deficiency in bariatric surgery patients may manifest as anemia and neurologic syndromes including myelopathy, neuropathy, dementia, and depression (101).

Vitamin D

As discussed before, vitamin D deficiency is highly prevalent in severely obese individuals. Absence of adequate treatment will exacerbate this condition. Factors associated with the development/worsening of vitamin D deficiency may include:

1. Inadequate vitamin D supplementation during rapid weight loss induced by bariatric surgery.
2. Bile salts deficiency associated with bariatric surgery procedures (the absorption of vitamin D requires the presence of bile salts)
3. Malabsorption of vitamin D sometimes due to intestinal bacterial overgrowth problems (102).
4. The absorption of vitamin D which basically occurs next to the jejunum and ileum can be affected by the delayed blend of nutrients ingested with bile acids and pancreatic enzymes (74, 103).

1.4. NUTRITIONAL STATUS ASSESSMENT OF THE SEVERELY OBESE PATIENT WITH SPECIAL ATTENTION TO IRON, CALCIUM, VITAMIN B_{12}, FOLIC ACID, AND PROTEIN.

Biomarkers of nutritional status

For the majority of micronutrients, there is no a gold standard method to assess the actual status and body stores. Although there are many parameters to evaluate the deposits of each micronutrient, none of them are independently sensitive, specific and predictable. These parameters might often be affected by a variety of physiological, nutritional, pathological and technical factor. Therefore, it could be necessary to involve measurements of several different parameters for each micronutrient. For instance: zinc concentration in serum and hair along with plasma metallothionein concentration will assess zinc body stores. Other combinations of these indicators will help determine the overload and toxicity for a given micronutrient. After BS there is potential risk to develop a variety of micronutrient and macronutrient deficiencies. Long term study of biomarkers of nutritional status after BS will help to identify those that warrant specific attention. To illustrate the process needed through the study of nutrient

deficiencies, a combination approach of different biomarkers that best defines the nutritional status of various vitamin and mineral are presented schematically in Table 2. Biomarkers for the most prevalent micronutrient deficiencies after BS are later considered in more detail.

Table 2. Combination of biomarkers to evaluate the nutritional status for certain vitamin and minerals

Thiamin
▪ Thiamin diphosphate, in whole blood specimens. ▪ Plasma thiamin concentration reflects recent intake rather than body stores. ▪ TPP ▪ ETK-AC ▪ Thiamin carried by albumin will be decreased with concomitant hypoalbuminemia.
Vitamin B6
▪ PLP-plasmatico ▪ Urinary pyridoxic acid ▪ AST erythrocyte ▪ Tryptophan overload test ▪ Activation coefficient of erythrocyte AST
Folate
-Deficiency ↓RBC folate ↑serum homocysteine and normal MMA
Fat-soluble vitamins (A, E. K)
- Deficiency and toxicity indicative parameters ▪ Vitamin A: Retinol binding protein and plasma retinol ▪ Vitamin E deficiency: plasma α-tocopherol ▪ Vitamin K deficiency: ↑DCP in deficiency state
Magnesio
-Deficiency $[Mg]_T$ en suero + [Mg] en orina de 24 horas $[Mg]_T$ en suero + prueba de carga de Mg -Toxicity [Mg2+] en CS + [Mg en orina de 24 horas]
Manganese
-Deficiency Mn SOD in lymphocyte + [Mn] serum or blood -Toxicity [Mn] serum or blood + brain MR
Zinc
-Deficiency due to stress or low intake [Zn] plasma + [MT] plasma -Light and severe deficiencies Serum [Zn] + Hair [Zn]
Selenium
-Short-term deficiency Eritrocyte GSH-Px activity + Plasma [Se] -Long-term deficiency [Selenium] in nails
Copper
-Nutritional Status EEA ceruloplasmin + Cu-Zn SOD erithrocyte activity EEA ceruloplasmin + citocrome-c oxidase
CA- ETK: Coefficient of activation of erythrocytic transketolase, TPP: Thiaminpyrophosphate, AST: aspartate aminotransferase, PLP: pyridoxal phosphate, R-Tf: soluble transferrin receptor; HB: hemoglobin; ZPP: erythrocyte zinc-protoporphyrin, [ ]: concentration; TIBC: total iron union capacity; ST: transferrin saturation; $[Mg]_T$: [Mg] total; $[Mg^{2+}]$: [Mg ionized]; CS: Blood cells; Mn SOD: Mn superoxide dismutase; MRI: magnetic resonance; MT: metallothionein; GSH-PX: glutathione peroxidase; AEE ceruloplasmin: specific enzyme activity of ceruloplasmin (ceruloplasmin / ceruloplasmin activity). DCP: Des-gamma carboxy prothrombin, MMA: methyl malonic acid,

Table adapted from (104)

Methods to assess iron status after BS

Limitation in the diagnosis of anemia and iron deficiency (ID)

As discussed in the previous chapter of this thesis, anemia with or without ID is more prevalent in obese individuals. Inflammation-induced disturbances of iron homeostasis (functional iron deficiency or decrease iron availability, due to high hepcidin levels induced by the low grade chronic inflammation associated with obesity). Lower bioavailable iron among obese adults might also potentially be related to the greater adipose hepcidin. Although hepcidin expression is more than 100-fold higher in hepatocytes than in adipocytes, secreted hepcidin from both tissues may have relevance for humans because in obesity, adipose tissue mass may be 20-fold greater than liver mass.

The study of iron status could lead to a five different scenarios based mainly in transferrin saturation and iron stores levels.

Iron deficiency without anemia

ID can occur while hemoglobin is within the normal range. The iron storage depletion requires time before hemoglobin (Hb) falls below the laboratory definition of anemia (Hb < 12 g/dL for women, Hb < 13 g/dL for men according to WHO criteria). At that point ID manifest with symptom of chronic fatigue, a normal Hb level with a low mean corpuscular haemoglobin (MCH), or in the lower limit of normality (normal range: 28-35 pg), or an increased red cell distribution width (RDW, normal range: 11-15) indicate mild ID without anemia. A ferritin level <30 ng/mL in the absence of inflammation (e.g., serum concentrations of C-reactive protein [CRP] < 0.5 mg/dL) reflects true ID.

Iron deficiency anemia (IDA)

It occurs when Hb, TSAT (< 20%), and ferritin concentrations (<30 ng/mL) are low regardless of inflammation) (105). The mean corpuscular hemoglobin (MCH) rather than mean corpuscular volume (MCV) became the most important red-cell marker for detecting ID in circulating red blood cells. MCV is a reliable and widely available measurement but is a relatively late indicator in absence of bleeding. The mean corpuscular hemoglobin (MCH) rather than mean corpuscular volume (MCV) became the most important red-cell marker for detecting ID in circulating red blood cells. MCV is a reliable and widely available

measurement but is a relatively late indicator in absence of bleeding.

Anemia of chronic disease (ACD)

ACD will be considered when:1) There is evidence of chronic inflammation (e.g., high CRP level), 2) Low Hb concentration <13g/dL for men and <12g/dL for women and 3) Low transferrin saturation (TSAT< 20%), but normal or increased serum ferritin concentration (> 100 ng/ml) (106).

Anemia of chronic disease with functional iron deficiency

In presence of chronic inflammation, low hemoglobin concentration and low transferrin saturation (TSAT < 20%), a serum ferritin concentration > 30 and < 100 ng/ml (107).

Non-iron deficiency anemia

Patients presenting with anaemia and TSAT > 20%, vitamin B_{12} and folate levels should be investigated. If vitamin B_{12} and folate levels are low and accompanied of a MCV > 100 fL, macrocytic anemia should be suspected and patient referred to the hematologist for further evaluation. If folate and vitamin B_{12} levels are normal, the diagnosis of anemia of unknown cause (AUC) should be considered and further studied by the specialist.

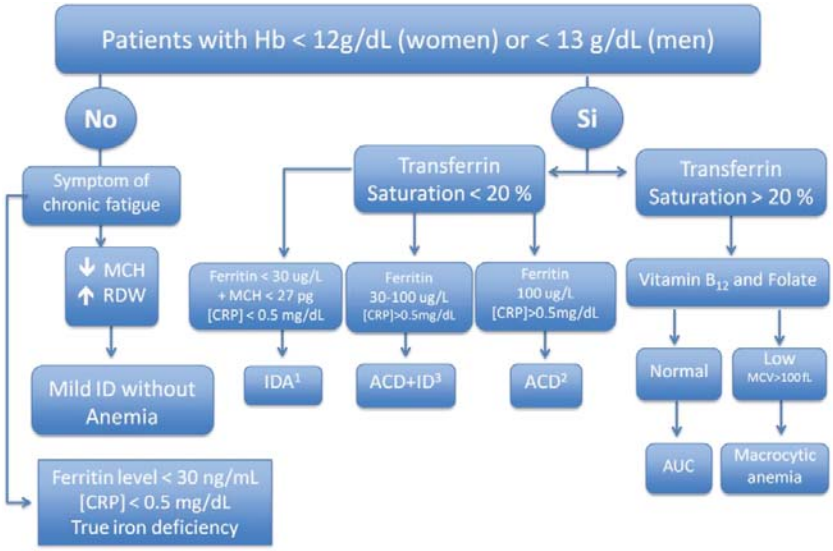
Table 3: Main laboratory test for the diagnosis of anemia and hematinic deficiency

Laboratory test	*Normal values, units*	*Conversion to SI units*
A. Iron deficient red cell production		
• Haemoglobin (Hb)	12-16 g/dL 13-17 g/dL	x 0.6206 mmol/L
• Mean corpuscular volume (MCV)	80-100 fL	
• Red cell distribution width (RDW)	11-15	
• Mean corpuscular Hb (MCH)	28-35 pg	
• Hypochromic red cells (HYPO)	< 5%	
• Reticulocyte Hb content (CHr)	28-35 pg	
B. Iron depletion in the body		
• Serum iron	50-180 mg/dL	x 0.179 mmol/L
• Transferrin (Tf)	200-360 mg/dL	x 0.01 g/L
• Transferrin saturation (TSAT)	20-50%	
• Ferritin (Ft)	30-300 ng/mL	x 2.247 pmol/L
• Soluble transferrin receptors (sTfR)	0.76-1.76 mg/L	6.4-25.7 nmol/L
• Ratio of sTfR to serum Ft (sTfR/log Ft)	< 1	
C. Other parameters		
• Serum vitamin B_{12}	□270 pg/mL	x 0.738 pmol/L
• Serum folate	□3 ng/mL	x 2.266 nmol/L
• C-reactive protein (CRP)	< 0.5 mg/dL	x 10 mg/L
• Creatinine	0.5-1.3 mg/dL	x 88.4 □mol/L

Adapted from Muñoz et al. 2009

In summary, diagnosis of iron deficiency in obese individuals may be missed if clinicians rely primarily on the falsely normal ferritin concentrations, which are likely increased by chronic inflammation rather than by iron overload. Based on previous discussion, preoperative assessment of patients should include a complete hematological work-up, including measurement of iron stores and status of inflammatory parameters (serum iron, ferritin, transferrin saturation index, C-reactive protein) and vitamin (B_{12} and folic acid) status tested preferably 30 days before the scheduled surgical procedure. Of note, the assessment of iron status in many reports especially in preoperative surveys of patients awaiting surgery has been inconsistent. For example, iron status has been variably assessed by serum iron, transferrin saturation, and ferritin concentration. Incorporation of markers of inflammation, such as C-reactive protein, in interpretation has seldom been included. Figure 8 shows the algorithm to evaluate iron deficiency anemia after bariatric surgery Main laboratory test for the diagnosis of anemia and hematinic deficiency are summarized in Table 3.

Figure 8. Algorithm to evaluate iron deficiency anemia after bariatric surgery



Adapted from Muñoz et al. 2009

Methods to assess calcium status after bariatric surgery

Dietary calcium intake and absorption can be compromise after BS. As consequence, calcium deficiency and metabolic bone disease can potentially occur within this population. Possible causes contributing to this phenomenon could be associated with:

- Low dietary calcium or vitamin D: Daily products that are high in calcium and vitamin D (whole milk and milk products) are poorly tolerated after BS.
- Low vitamin D endogenous synthesis by a deficient sun light exposure
- High dietary phosphorous and phytates may interfere with calcium absorption

In the state of reduced consumption of calcium, or if the amount of calcium that reaches the circulation is inadequate, (normally as consequence of deficient vitamin D status) the level of calcium in the serum tends to decrease, producing a secondary parathyroid stimulation, (state known as secondary physiological hyperparathyroidism). Secondary hyperparathyroidism allows compensating for hypocalcemia through two main mechanisms. First, increased PTH stimulation leads to an increase in osteoclast activity at bone level. Increased osteclast activity turns into acceleration of calcium and phosphate resorption from the skeleton. Second, effect of increased PTH activity at renal level causes inhibition of

phosphate resorption and increased calcium reuptake by the renal system. Initially, there is an increase in phosphate in the urine, although it is eventually reduced, as is the level of calcium excretion. Conversely, calcium and phosphorus fecal losses can remain at excessive levels. The increased osteoclast activity on bone leads to skeletal instability. Increased osteoclast activity and increased bone turnover could be accompanied by increased serum alkaline phosphatase levels. Rapid and extreme weight loss is also associated with bone loss even in the presence of normal vitamin D and PTH levels (6) and contributes to the above mentioned complication. An increase in serum intact PTH is indicative of a negative calcium balance or vitamin D deficiency or both. Secondary hyperparatiroidism promotes bone loss while increasing the risk of osteopenia and osteoporosis. Elevated levels of bone specific alkaline phosphatase and osteocalcin levels, indicative of increased osteoblastic activity and bone formation, are often the initial abnormalities (6). Biomarkers or other procedures that can help to assess calcium status after BS are listed in Table 4.

Table 4. Biomarkers or other procedures that can help to assess calcium status after BS

Laboratory test of plasma concentrations/other investigations	Status reflecting metabolic bone disease
▪ 1.25 (OH) 2D	↓
▪ PTH	↑
▪ Alkaline phosphatase	↑
▪ Osteocalcin	↑
▪ Calcaemia	↓
▪ DEXA	↓ (bone density)
▪ Quotient: calcium/ creatinine (urine)	↑ (low calcium intake)

Methods to assess vitamin B_{12} and folic acid status after BS

It has been suggested whether megaloblastic anemia results from folate deficiencies or vitamin B_{12} deficiency could be evaluated by measuring homocysteine in plasma for folate, and mathylmalonic acid in plasma for vitamin B_{12}.

Indices to assess folate status include folate levels in serum and in erythrocytes, although homocysteine in serum or plasma is used as a functional test of folate deficiency. As a coexisting deficiency of vitamin B_{12} may interfere with the diagnosis of folate deficiency, test for folate deficiency should be performed together with an assessment of serum vitamin B_{12} concentrations. Is important to highlight that approximately two-thirds of the folate in

serum is bound to protein, of which about 50% is albumin. Albumin levels should then also be considered when evaluating folate status. Serum folate concentrations fluctuate with recent changes in folate intake, or temporary changes in folate metabolism even when body storages remain stable. Hence, serum folate levels reflect acute folate status but provide no information on the size of the folate storage, and cannot be used to distinguish between a transitory reduction of folate intake and chronic folate deficiency associated with depletion of tissue folate. On the other hand, erythrocytes incorporate folate as they are formed in the bone marrow, and levels remain constant throughout the life span of the corpuscle (a total of 120 days). Hence, erythrocyte folate concentrations are less sensitive than serum folate levels to short term fluctuations in folate status and status and decrease more slowly than serum or plasma folate during folate deprivation. Also erythrocyte folate correlates with liver folate levels (108), and thus better reflects folate stores. Consequently, erythrocyte folate concentration is a more reliable measure of folate status than serum folate and should be used in the evaluation of the nutritional status after BS.

Total serum vitamin B_{12} is the biochemical test used for routine screening for vitamin B_{12} deficiency. B_{12} concentration reflects both the vitamin B_{12} intake and body stores. However, it has low sensibility. Some persons, notably the elderly with serum vitamin B_{12} concentrations apparently within low to normal range have abnormalities in biochemical function (109). As a result, measurement of serum methylmalonic acid or serum homocysteine concentrations is recommended if serum vitamin B_{12} is <225pmol/L. These two additional tests are more sensitive and specific indicators of functional vitamin B_{12} deficiency. Table 5 shows the most frequent parameters used in the evaluation of nutritional status for vitamin B_{12}.

Figure 9. The four stages towards a negative vitamin B_{12} balance triggered by decreased intake.

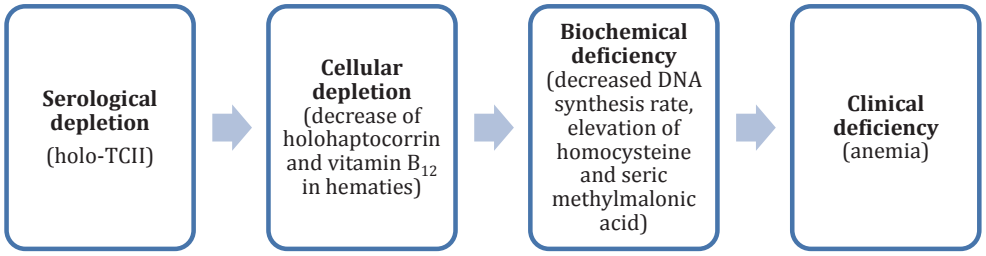


Table 5. Most frequent parameters used in the evaluation of nutritional status for vitamin B_{12}

Method	Observations
▪ Serum Vitamin B_{12} (cobalamins) ▪ TCII bound to vitamin B_{12} (holo-TCII) ▪ Hematological indices (MCV, Hb) ▪ Methylmalonic acid ▪ Total Homocysteine	▪ Well accepted ▪ Reflects tissue repletion ▪ Unspecific ▪ Better functional test ▪ Unspecific

Methods to assess protein status after BS

As multiple factors could potentially contribute to the development of protein deficiencies after BS, the approach to assess protein status after BS should also be multifactorial. Since any assessment method has its limitations, a combination of different approaches will help us better appraise protein status in the BS patient. Unfortunately, only few of these methods are applicable to everyday clinical practice whereas the vast majority should be seen mainly as research tools. The available methods are briefly summarized in Table 6.

Nutritional assessment:

The evaluation of dietary intake is considered an indirect measure of the nutritional status. Since clinical signs can appear late in the development of the deficiency, this approach could serve as an orientation of the patient's situation. A variety of dietary assessment methods could be used to estimate the new dietary habits, energy and protein intake (PI) after BS. A brief description of the most frequent dietary assessments methods successfully implemented as part of the nutritional interview is summarized in Table 6. Its application can be either for clinical practice or in research (110). This method allows one to obtain shallow

qualitative and quantitative information about PI and thereby identify targets that require further approach or more specific dietetic intervention. However, specifically in the area of research, the inherent limitations of the interviewed-based dietary evaluation that rely on self reported dietary information should not be ignored (111). For this reason, it is important to include possible biomarkers of dietary exposure to validate self-reported intakes when examining nutritional health in relation to body weight. Hence, when evaluating protein intake, would be possible to collect urinary-nitrogen (N) over 24h repeatedly, and determine PI from urinary N.

Over the years, it has been observed that protein-containing foods are poorly tolerated after BS (77). Meat intolerance was observed in over 50% of a cohort of 200 patients that were followed up to 8 years after surgery (91). To determine adequacy of dietary protein a careful and detailed dietary interview should be performed including the patient's-participant's food preferences, intolerances, rate of eating, number of meals day and presence of vomiting, since the development of new eating behaviors have been identified after BS (78). Meat intolerance was observed in over 50% of a cohort of 200 patients that were followed up to 7 years after surgery (91). To determine adequacy of dietary protein a careful and detailed dietary interview should be performed including the patient's-participant's food preferences, intolerances, rate of eating, number of meals day and presence of vomiting, since the development of new eating behaviors have been identified after BS (78).

Clinical Assessment: Serum protein and AA concentrations

Clinical assessment of the protein status after BS might include, but is not limited to the determination of the serum levels of proteins of hepatic origin listed in Table 6. Several authors have shown that serum albumin can accurately reflect body protein stores and that albumin synthesis by the liver is a sensitive index of dietary protein intake (112). To further assist the interpretation of blood protein measurements is necessary to include a measure of an acute-phase reactant -C-reactive protein- since serum proteins are affected by inflammation and infection in addition to a nutritional status per se (113). Plasma free AA can be affected after acute alterations in the dietary protein and AA intake. Its determination is frequently limited to research studies.

Nitrogen balance:

Nitrogen balance (NB) methodology is a measure of the net status of protein metabolism. It does not provide information on the size of the protein stores or about nutritional status (83). However, it provides a holistic assessment of protein balance, allowing insight on the relationship between energy status, dietary protein, and fat free mass (FFM). Several factors may precipitate a negative NB in the BS setting. These include inadequate protein or energy intakes, imbalance in non-essential:essential AA ratio, accelerated protein catabolism, and excessive diarrhea. A negative energy balance may have a negative and direct effect on the protein synthesis rate and, consequently, over the skeletal muscle (SM) mass.

Whole body muscle protein turnover

Decreases in SM mass in response to negative energy balance are due to imbalanced rates of muscle protein synthesis and degradation (114). It has been shown that PI stimulates muscle protein synthesis (115). Limited in the area of metabolism and nutritional research, tracer AA techniques made possible to directly measure both, whole body and specific tissue, protein turnover (116). Leucine is the most frequent isotope being used. It is predominantly metabolized in muscle and act as an intracellular marker. Stable isotopes can be used to measure flux across a tissue (i.e SM) and the fractional synthesis rate of individual proteins, such as albumin, in interpreting of whether AA intake is adequate (117). Research in this specific area is needed to further our understanding of muscle protein turnover in BS patients.

Muscle Function Test

An easy and available tool useful to assess muscle function is by voluntary handgrip strength. It can be measure with a handgrip dynamometer, which test grip strength to 90kg. Other tests of muscle function include pulmonary function test and electrical stimulation, which are normally used in critically ill patients. Others functional tests may also include isometric knee extension, sit-to-stand, and in older individuals the Senior Fitness Test.

Although post-surgical changes in FFM after BS have been reported in several studies, reports on changes in muscular strength and physical performance after BS are scarce. To the best of our knowledge, evaluation of handgrip strength in BS subjects has been reported only in two studies. In a series of 25 middle-aged (mean age 37y) subjects, Otto et al did not find

significant changes in handgrip strength at short term after GBP (117) but was observed at long term by Cole et al (118). Interestingly, in both studies an association between FFM and handgrip strength was found. On the other hand, Handrigan evaluated isometric knee extension in a series of 10 middle-aged subjects (mean age 46y) that underwent duodenal switch (119). Lower limb maximum force at 12-m decreased approximately 33% relative to baseline. A series of handgrip measurements combined with isometric knee extension test or sit-to-stand test would successfully assess changes in muscle function after BS.

Table 6. Clinical and research methods to assess protein status after bariatric surgery.

1. Nutritional and dietary intake assessment methods
Self reported methods of dietay intake
3 to 7-days dietary history
24hs-dietary recalls
Weighing method
Food frequency questionnaire
Laboratory assessment of dietary protein intake
24hs. urinary nitrogen excretion
2. Laboratory assessment
Albumin
Transferrin, retinol-binding protein, prealbumin
Urinary creatinine excretion
Total lymphocyte count
3. In vivo measurement of muscle mass
Antropometry
Bioelectrical impedance analysis
Dual-energy X-ray absorptiometry
Magnetic resonance imaging
Computed tomography
Isotopic methods ^{15}N-creatinine
In-vivo neuron activation analysis
Nuclear magnetic resonance
4. Nitrogen balance
5. Protein turnover
Isotope Kinetic study: L-[^{13}C] leucine as a tracer
6. Functional Test
Handgrip dynamometer
Functional strength (isometric knee extension and sit-to-stand test)

1.5. IMPACT OF PROTEIN STATUS OVER SEVERAL SYSTEMS OF BODY FUNCTIONS.

Protein in the body is widely distributed throughout the different tissues and is a major component of all living cells. Protein represents 17% of the weight of an average healthy adult, making it the second largest component after water (120). SM represents about 43% of total protein, followed by the visceral protein pool, and together, embrace the metabolically available protein known as "body cell mass" (83). Protein (composed by macromolecules made up of linear chains of AA that contain carbon, hydrogen, oxygen, nitrogen, sulphur and, in some cases, selenium) perform vital functions. Unlike fat and carbohydrate, there are no dispensable stores in humans. Hence, loss of body protein in response to a reduced or inadequate supply of dietary nitrogen and or of specific indispensable AA, results in the loss of essential structural elements as well as impaired functions. The four major biochemical systems responsible for maintaining body protein and aminoacids homeostasis are 1) AA transport and uptake, 2) AA oxidation and catabolism, 3) protein synthesis, and 4) protein breakdown (121).

The first response to a reduced protein and AA intake is the reduction in the rate of AA oxidation, associated with a decline in the rate of specific organ and tissue protein synthesis. Protein and AA metabolism in muscle and liver is greatly affected when dietary PI is restricted, with reduced rates of muscle protein synthesis and of the synthesis of export proteins from liver occurring at early period (122) resulting in a significant and negative effect on the SM mass (123).

As shown in Figure 10, due to the behavioral and anatomical changes as attributed to BS there is an increasing risk to compromise protein status. Consequently, the equilibrium and status quo of various key systems of the body can be altered and need to be carefully monitored in order to avoid complications. The actual knowledge of the potential complications associated with changes in the protein status after BS will be discussed below.

Weight loss

The consequences of negative energy and protein balance on total body and SM mass are well established (124). It has been demonstrated during weight loss there is close to 75% reduction of adipose tissue and 25% of fat-free mass (125). Apart of the beneficial reduction of adipose tissue in obese individuals during weight loss, the decrease in lean body mass

(LBM) may down-regulate metabolic processes, such protein turnover and basal metabolic rate, thus compromising long-term healthy weight management (126). Weight loss induced by BS can reach about 60% excess weight loss (EWL) or about 30% total weight loss at one year (30-50kg) with changes in body composition being of comparable proportions of conventional treatment (127).

Nitrogen balance, skeletal mass loss: effect of PI

Long-term negative NB can be associated with loss of lean and fat tissue (128). An increase in absolute grams of PI would improve NB and thus defend the amount of FFM loss during weight loss. Some studies have investigated the effect of different levels of PI on body composition changes during weight loss. Uncontrolled studies carried out by Bistrian et al. (129) showed that under severe caloric restriction, PI at the recommended dietary allowances (RDA) levels (0.8g/ IBW/ day) does not allow for nitrogen equilibrium. Rather, increasing PI up to 1.5g/ IBW/ day would result in mean nitrogen equilibrium. In an attempt to establish the adequate amount of dietary protein needed to FFM preservation, Krieger performed a meta-analysis of the literature available in the field (130). From this meta-analysis it was suggested that at short term (less than 12 weeks), protein intakes >1.05 g/kg/day were associated with an extra 0.60 kg of FFM retention when compared with intakes <1.05g/kg/day. In studies lasting more than 12 weeks, FFM retention was increased up to 1.22 kg when PI was >1.05 g/kg/day. Studies performed in obese individuals undergoing very low calorie diet (VLCD) (VLCD contains <800kcal/ day) can help us understand the effect of PI on protein balance while losing weight in the BS patient. As a matter of fact, a similar situation (regarding kcal intake) occurs during the first trimester of BS when energy intake is drastically and spontaneously reduced ranging between 400-800 kcal/ day and the rate of weight loss is very significant (131). To understand PI adequacy under this condition Hoffer et al compared the metabolic effect of 2 levels of PI during VLCD: 0.8g prot/ kg IBW/ day versus 1.5g/ kg IBW/ day during 8 weeks intervention (132). They observed that in the higher PI group, NB was closer to zero reaching NB after three weeks of the dietary intervention while the low protein group remained in negative NB during the entire 8 weeks. A more recent RCT (133) studied the effect of high-protein low calorie diets on fat-free mass and muscle protein synthesis during 31-day weight loss intervention. Participants were allocated to different groups providing protein at 3 levels: 0.82g/kg/day (Recommended Dietary Allowance (RDA)), 1.63g/kg/day

(2xRDA) and 2.43g/kg/day (3xRDA). Changes in body mass (kg) were significantly different between RDA and 3xRDA groups. The RDA group lost more weight (0.8), but also significantly higher FFM% compared with the other groups. During the first 2 weeks of intervention all groups were in negative NB, but by the end of the study (day 30) the RDA group remained in negative balance while the 2x RDA group reached NB and the 3x RDA group improved their NB and was not significantly different from 2X RDA. In summary, based on the studies discussed above it could be concluded during active weight loss FFM is better preserved with higher levels of PI.

Effect of PI on resting energy expenditure (REE) after weight loss (WL)

LBM is the main determinant of REE, explaining 75% of the REE variance (134) with REE being the largest component of 24-h energy expenditure (EE). The impact of daily PI not only on changes in body composition but also on REE were studied in a 6 month randomized parallel study that compared 2 low energy diets containing the required protein level (0.8g protein/kg BW/day) and above (1.2g protein/Kg BW/day). During the 6 months energy restriction, sustaining PI at the level of requirement appeared to be sufficient to induce body weight loss while preserving FFM. However, PI above requirements resulted in a greater decrease in fat mass and greater preservation of FFM and REE with similar effect on body weight loss (135). Of note, reduced REE may trigger weight regain in BS population (136). Diet high in protein may increase REE while preventing lean body mass (LBM) loss (134) during weight loss. It has also been suggested that increased EE from dietary protein is attributed to an enhanced thermic effect (15±4%) compared to carbohydrates (6±2%) or lipids (7±3%) (137).

Dietary PI and satiety

A reduction in PI can also affect satiety. High protein diet has been shown to increase satiety even in the context of energy restriction (138). Underlying proposed mechanisms are as follows: a ketogenic state, relatively elevated plasma AA levels, an increase of anorexigenic hormones such as neuropeptide YY, glucagon-like peptide 1 and cholecystokinin, and a decreased of the orexigenic hormone ghrelin produced in response to peripheral and central detection of AA and feedback to the central nervous system to prolong the duration of

satiation (139, 140). Support has been found for a high PI promoting a negative energy balance by enhancing satiety, decreasing hunger and decreasing energy intake (140).

Roles of dietary PI on circulating levels of BCAA

It has long been recognized that circulating levels of AA, including BCAA, are elevated in persons with obesity, insulin resistance, or T2DM, compared to healthy controls (141). More recently, using targeted metabolomic analysis, BCAA including leucine, phenylalanine, tyrosine, and products of BCAA catabolism, were shown to be associated with insulin resistance (142). Infusion of AA in humans resulted in insulin resistance (143). A recent epidemiological study reported that elevations in plasma levels of essential AA, including BCAA, phenylalanine and tyrosine in healthy individuals, predicted a five-fold increase in the risk of developing T2DM (144). This indicates that elevated BCAAs may be a marker of the disease process, or contribute to the development of insulin resistance, and sensitive to therapeutic interventions, including BS which is associated with reduced concentrations of plasma BCAAs (145) and therefore, with improved insulin sensitivity (146, 147).

Protein turnover

Altered intakes of protein and AA modulate rates of the major systems (synthesis, degradation and oxidation) responsible for the maintenance of organ and whole body protein and AA homeostasis (122). The body tends to adapt to inadequate nutritional conditions by reducing the whole body protein turnover (↓synthesis and ↑ breakdown) (148) resulting in SM loss, indeed, simultaneously impaired by surgery induced weight loss. This adaptation can compromise the host‘s capacity to resist a stressful stimuli and have deleterious effect on overall health (122).

Bone health

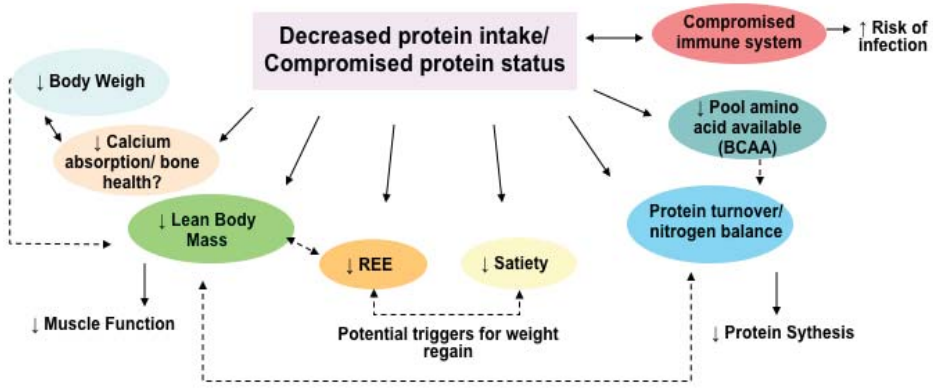
Protein malnutrition or insufficiency compromise bone health. There is evidence that increased essential amino acid or protein availability can enhance muscle protein synthesis and anabolism, as well as improve bone homeostasis (114). Hence, optimal PI (higher than the recommended levels) is recommended for bone health, particularly in the elderly (149). It was suggested that low protein diet (0.7g/kg body weight) compared to a high protein diet (2.1

g/kg) induced a lower calcium intestinal absorption (150). Also calcium and VD deficiency and hyperparathyroidism are prevalent after BS (151).

Immune system

The immune system can be impaired with protein malnutrition (152). Over nutrition and obesity also reduce immunity. Nutrition is a critical determinant of immune responses and malnutrition the most common cause of immunodeficiency worldwide. Protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations, and cytokine production (153). Apparently both the T-dependent (cell-mediated immunity) and B-dependent (humoral immunity) systems, particularly the immunity to primary antigens, are suppressed in malnourished adult patients (154). A relatively short period of nutritional repletion can result in improvement of immune indexes. Although the mechanisms responsible for suppression of the immune response in patients with protein-calorie malnutrition and for its improvement after hyper alimentation are not entirely clear, the data add to our understanding of the nutritional-immunological relation in man.

Figure 10. Complications associated with a compromised protein status after bariatric surgery



REE: resting energy expenditure

BCAA: Branched chain amino acids

CHAPTER 2: OBJECTIVES, HYPOTHESIS AND DESIGN

2. OBJECTIVES, HYPOTHESIS AND DESIGN

2.1. RESEARCH OBJECTIVES

The overall aim of this project was to better understand the increased risk for nutritional deficiencies in subjects undergoing bariatric surgery. As the review of the literature suggests subjects presenting for bariatric surgery are at risk nutritional deficiencies, we evaluated de nutritional status of obese individuals prior to bariatric surgery. Additionally, we evaluated the nutritional consequences associated with the drastic reduction of daily energy intake (limiting micronutrient and macronutrient incorporation in the body), the anatomical changes associated to BS, and the massive weight loss induced by BS associated with the two most currently performed surgical techniques (namely, gastric bypass and sleeve gastrectomy). We deem this knowledge may contribute to optimize the nutritional management as well as the prevention of nutritional complications in the BS patient and to contribute to the development of evidence-based nutritional guidelines.

2.2. HYPOTHESIS:

Based on our overall research aim, we defined the following hypothesis:

Hypothesis 1. Nutritional deficiencies are already prevalent in obese patients who are candidates for bariatric surgery in a Mediterranean population

Hypothesis 2. Patient that had undergone GBP will show a higher prevalence of vitamin, mineral and protein deficiencies compared to SG patients, despite dietary intake after GBP and SG being comparable.

Hypothesis 3. The therapeutic approach of vitamin D deficiency, one of the most prevalent after BS, needs to be individualized.

Hypothesis 4. As obesity is considered an inflammatory disease, the prevalence of iron deficiency is highly affected by the measurement of inflammatory markers.

Hypothesis 5. Protein supplementation will help achieve protein intake goals. Higher levels of protein intake will be related to a better protein status and with FFM during weight loss induced by BS.

Hypothesis 6. PI will be significantly and negatively related to FFM loss as a percent of total weight loss at 4- and 12-months after surgery following two commonly performed BS procedures, namely GBP and SG.

Hypothesis 7. The results of this integrative protocol will contribute to the development of evidence-based data regarding the safe and optimal dietary PI and protein supplementation (PS) after BS.

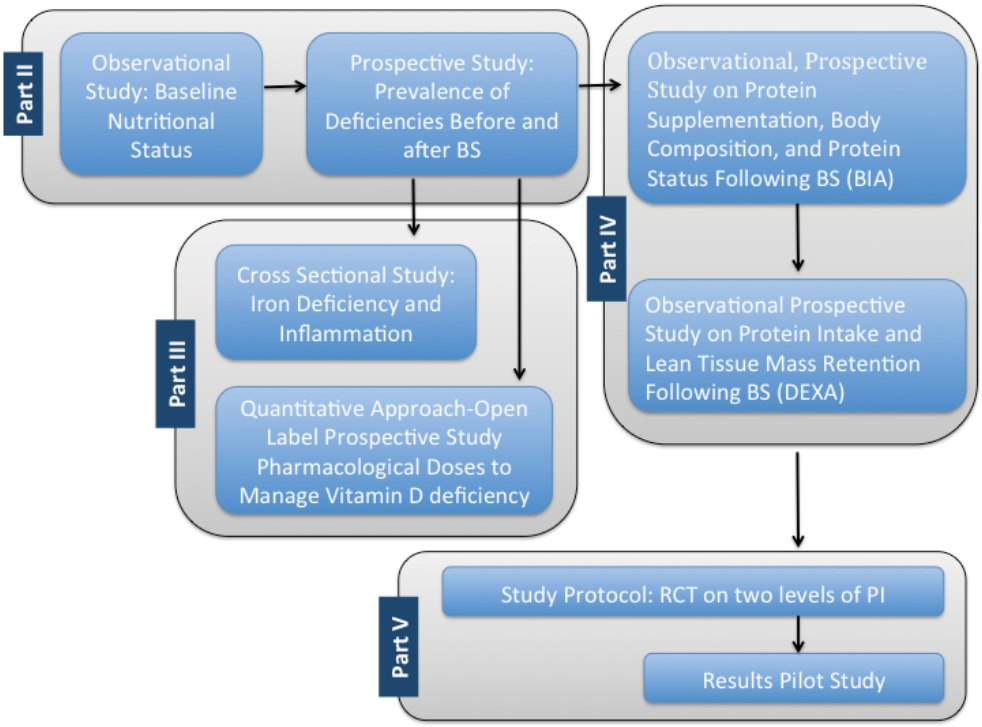
Hypothesis 8. For each aim of the pilot study:

- **Hypothesis 8.1**: High PS groups will be in NB (zero or close to zero), whereas standard PS groups will be in negative NB.
- **Hypothesis 8.2**: Patients in High PS group will maintain greater FFM and REE after surgical weight loss compared to Standard PS.
- **Hypothesis 8.3:** Patients in the High PS group will experience higher levels of perceived satiety compared with patients in the standard PS group.
- **Hypothesis 8.4:** High-PS individuals will present a higher BCAA levels compared with GBP

2.3. DESIGN

To achieve the thesis objectives and test the hypothesis, different studies were conducted. Figure 11 provides an overview of the different parts of the project.

Figure 11. General design of the research project.



A big part of the PhD project consisted of a prospective study in which patients were nutritionally evaluated before bariatric surgery and during long term follow up. Data was collected during the nutritional appointments regarding food intake, laboratory assessment, vitamin and mineral supplements use, protein supplements and body composition changes during weight loss. (Part II)

In view of the high prevalence of abnormal levels of vitamin D and vitamin D deficiency, we decided to better understand the pharmacological treatment and whether or not it could be optimized in clinical practice. To that effect, we performed two open-label, prospective studies in patients undergoing BS that aimed to evaluate the efficacy and safety of

achieving 25-hydroxy vitamin D (25(OH)D) levels ≥75 nmol/L with two different doses of vitamin D supplementation during the follow-up. In the same context, since our previous studies identified that iron deficiency was highly prevalent we were interested in further explore this abnormality and better characterize indices of iron status in bariatric surgery candidates with systemic inflammation as well. In this study, the clinical characteristics of BS candidates associated with iron deficiency and anemia with iron deficiency were also described (Part III).

To get insight on the effect of dietary protein intake on body composition we performed two observational prospective studies to help elucidate the influence of protein intake (PI) on fat free mass (FFM) and protein status changes during weight loss induced by BS. Body composition analyses were performed throughout two different methodological approaches (Part IV).

As a further step in this research path, a randomized control trial proposal was designed to determine the effect of two different levels of dietary protein intake (standard versus high) after bariatric surgery on the nitrogen balance, body composition, energy expenditure, hormonal and perceived satiety, plasma levels of BCAA and insulin sensitivity, as well as the feasibility of protein supplementation up to one year after bariatric surgery (BS). This translational protocol integrates mechanistic, metabolic, and energy homeostasis outcomes to critically evaluate the adequacy of protein recommendations for severely obese individuals after BS. There are still many questions that need to be addressed in the field before protein recommendations for this population can rely on evidence based science. This is a novel research approach, and we are confident that the results of this study will help answer the gaps in the literature. Finally, preliminary results of a pilot study are presented to show the feasibility of the study protocol as part of the development of this thesis project (Part V).

PART II: NUTRITIONAL INTAKE AND PREVALENCE OF NUTRITIONAL DEFICIENCIES IN OBESITY: EFFECT OF BARIATRIC SURGERY

CHAPTER 3: NUTRITIONAL INTAKE AND PREVALENCE OF NUTRITIONAL DEFICIENCIES PRIOR TO SURGERY IN A SPANISH MORBIDLY OBESE POPULATION

This chapter is based on:

Moizé V, Deulofeu R, Torres F, Martinez de Osaba J, Vidal J.

Nutritional Intake and Prevalence of Nutritional Deficiencies Prior to Surgery in a Spanish Morbidly Obese Population

Obesity Surgery (2011) 21:1382–1388.

3. NUTRITIONAL INTAKE AND PREVALENCE OF NUTRITIONAL DEFICIENCIES PRIOR TO SURGERY IN A SPANISH MORBIDLY OBESE POPULATION
    - 3.1. Introduction
    - 3.2. Methods
        - 3.2.1. Participants
        - 3.2.2. Study protocol
        - 3.2.3. Analytical methods
        - 3.2.4. Statistical analysis
    - 3.3. Results
    - 3.4. Discussion
    - 3.5. Conclusions
    - 3.6. References

3 NUTRITIONAL INTAKE AND PREVALENCE OF NUTRITIONAL DEFICIENCIES PRIOR TO SURGERY IN A SPANISH MORBIDLY OBESE POPULATION

Background: The prevalence of obesity in Spain is on the rise with the consequent increase in bariatric surgery (BS). Studies in non-Mediterranean populations have shown that micronutrient deficits are present before surgery. However, there is no data on this topic in a Spanish population.

Methods: We evaluated food intake and the prevalence of nutritional deficiencies in 231 obese patient (72.3% women, 45.6 ± 9.9 years, BMI 48.2 ± 7.8 kg/m^2) candidates for BS. Forty-six normal weight individuals with similar demographic variables except BMI were included for comparison of deficiencies.

Results: In obese subjects, the mean estimated energy intake was 2,584 ± 987 kcal/day in males and 2,094 ± 669 kcal/day in females ($p<0.05$). After adjusting for kilocalorie intake, carbohydrate intake was of 38.7% [CI 36.2 to 41.1] and 39.9% [CI 37.8 to 40.8] (n.s.), lipid intake was 41.9% [CI 39.6 to 44.2] and 43.0% [CI 41.7 to 44.8] (n.s.) and protein intake was 19.1% [CI 17.7 to 20.5] and 17.3% [CI 16.4 to 18.1] (n.s.) for men and women, respectively. The most prevalent deficiency was vitamin D25(OH): obese 94%, control 24%; ($p < 0.0001$). Above normal PTH levels were observed in 41.0% and 20.0% of obese and normal weight subjects, respectively ($p < 0.0497$). Increased prevalence of deficiencies in obese patients included magnesium, vitamin B6 and anemia ($p < 0.05$). Other vitamin deficiencies were observed although did not reach statistical significance.

Conclusions: Nutritional deficiencies are commonly found in the Spanish obese population undergoing BS and are significantly more prevalent than in normal weight individuals.

3.1. Introduction

The prevalence of obesity in Spain is increasing [1]. In parallel with this rise in the number of patients with obesity, the numbers of bariatric surgery interventions and centers performing this procedure have also increased in our country [2]. Bariatric surgery is an effective technique in the treatment of obesity for a carefully selected group of patients [3]. This procedure allows important weight loss (around 60–70% of excess weight [EW] at 1 year after surgery) as well as a significant improvement in the metabolic and social complications associated with obesity [4].

Among the possible complications of obesity surgery, nutritional deficiencies are of note [5, 6]. Several studies in the USA and in Western Europe populations have shown that the vitamin and micronutrient deficits are present in a significant proportion of patients with morbid obesity even before surgery [7–13]. Accordingly, it has been suggested that deficiencies should be identified prior to surgical treatment to minimize their worsening after the gastrointestinal anatomy has been modified and food intake reduced [13]. However, to date similar studies have not been performed within the setting of a population from the Mediterranean area where the alimentary and climatic pattern is different from that in the other populations described.

Against this background, the aim of the present study was to evaluate the dietetic intake and the prevalence of nutritional deficiencies in obese patients who are candidates for bariatric surgery in a Spanish university hospital.

3.2. Methods

3.2.1. Participants

A total of 231 obese patients undergoing bariatric surgery were consecutively evaluated from January 2003 to December 2005. A control group of 46 normal weight subjects with similar age and gender distribution was also studied to compare the prevalence of nutritional deficiencies.

3.2.2. Study protocol

Evaluation of food intake was based on 4-day food records (one of which was a non-working day). Instructions on how to fill the 4-day food record were explained by a registered dietician (VM) during the clinical evaluations. Patients were instructed to complete the dietary registers the week prior to the nutritional interview. A 24-h food recall, tridimensional models (Nasco International, Modesto, CA, USA; http://www.enasco.com/) and a photographic album [14] of food portions sizes were used to increase the precision of the food intake estimates. Quantification of daily caloric, macronutrients contents, calcium,

phosphorus, magnesium and iron intake were determined by using Dietsource 2.0® (Novartis) database. Data were then compared with the recommended daily intake (RDI) [15]. The basal metabolic rate was estimated by the Harris and Benedict equation [16]. Low energy reporters (LERs) [17] were identified and excluded from the study based on a reported energy intake of less than 1.2 times the basal metabolic rate [18].

Analytical parameters included blood cell count, hemoglobin and standard hematological indexes as well as biochemical test including glucose, creatinine, urea, ionogram, visceral protein markers (albumin, prealbumin), iron, ferritin, transferrin, calcium, phosphorous, magnesium, zinc, folates and vitamins A, B_1, B_6, B_{12}, 25-hydroxy vitamin D and PTH. Calcium concentration was corrected by the actual albumin concentration [corrected calcium (mg/dL) = (4.0 serum albumin [g/dl]) × 0.8 + Ca total (mg/ dL)] in which 4.0 represents the mean albumin level. According to Beutler and Waalen [19], anemia was defined as hemoglobin below 137 g/L for men and 122 g/L for women.

3.2.3. Analytical methods

Haematological and clinical chemistry parameters including iron, magnesium and total proteins were measured using an Advia 2400 analyzer (Siemens, Barcelona, Spain). Prealbumin was measured by nephelometry in a BNII analyzer (Siemens, Barcelona, Spain) using manufacturer's own reagents. PTH and 25-OH vitamin D were analyzed by a non-competitive quimioluminiscent automatized immunoassay (Liaison DiaSorin). Inter-assay coefficients of variation (CV) were, respectively, 4.5% and 18%. Zinc was measured by atomic absorption spectrophotometry at 213 nm, using a Perkin-Elmer 2100 AA analyser (Perkin-Elmer, Barcelona, Spain). Vitamins B1 and B6 were measured in plasma by high-performance liquid chromatography (HPLC) using the method developed by Immunochrom (Leti, Barcelona, Spain). For vitamin B1, thiamine-P is detected by its fluorescence emission at 440 nm. Intra-assay and inter- assay CV were <8.5% and <9.6%, respectively. For vitamin B6, pyridoxal-6-P is detected through its fluorescence at 418 nm. Intra-assay and inter-assay CV were <7.5% and <10.6%, respectively. Vitamin A was measured by HPLC with UV detection at 320 nm following the method recommended by National Institute of Standards and Technology (USA). Intra-assay and inter-assay CV were <8.4% and <11.0%, respectively. External quality control for these vitamins was performed at the Société Francophone Vitamines et Biofacteurs (SFVB: http://www.sfvb.org). Vitamins B12 and folic acid were measured in an automated electrochimioluminiscence immunoassay system Advia-Centaur (Siemens, Barcelona, Spain) instrument, with reagents provided by the same manufacturer. Intra-assay CV for B12 were <7.9%, and for folic acid <7.3%. Inter- assay CV

for B12 and folate were <9.8 and <10.3%, respectively. External quality control was performed follow- ing the quality assessment programme from the Sociedad Española de Quimica Clinica y patología Molecular (http:// www.seqc.es/es/Varios/4/4/Garantia_de_la_calidad/).

3.2.4. Statistical analysis

Results are expressed as means ± standard deviation (SD), baseline kilocalories adjusted least square means [95% confidence intervals (95%CI)] or frequencies and percentages (%). Continuous data were analyzed by analysis of covariance including gender and baseline caloric intake (kcal) as covariates. Categorical variables were compared using the Fisher's exact test. For correlation analysis, Pearson or Spearman coefficients were used when appropriate. The analysis was performed using SAS version 9.1.3 software (SAS Institute Inc., Cary, NC, USA) and the level of significance was established at 0.05 (two sided).

3.3. Results

Data from 231 obese patients and 46 normal weight individuals with similar demographic variables except for BMI were gathered in this study. Mean (SD) age was respectively 45.9 (10.3) and 42.5 (10.9) years, and BMI 48.4 (6.7) and 23.1 (2.5) kg/m^2. Females represented 72.3% and 78.3% in the obese and normal weight group, respectively. Demographic data on the obese patients by gender are shown in table 1. Estimated energy, macronutrient and micronutrient dietary intake for obese men and women are summarized in Tables 2 and 3. The mean estimated energy intake was 2,584 ± 987 kcal/day in males and 2,094 ± 669 kcal/day in females ($p < 0.05$). Only 13 subjects (5.6%) were categorized as LERs.

Table 1. Baseline demographic data

	Males (n=64)	Females (n=167)	*P*
Age	45.6 [43.1 to 48.0]	46.0 [44.4 to 47.6]	0.7654
BMI (kg/m^2)	47.6 [45.9 to 49.3]	48.7 [47.7 to 49.7]	0.2743
PBW (kg.)	145.7 [140.0 to 151.3]	124.2 [120.7 to 127.6]	<0.0001
IBW (kg.)	74.6 [72.9 to 76.2]	60.9 [59.9 to 61.9]	<0.0001
EW (kg.)	70.8 [66.0 to 75.71]	62.5 [59.5 to 65.4]	0.0045

Results are expressed least square means [95%CI].
All estimators are adjusted by baseline Kcal for all variables but Age.
Abbreviations: BMI= Body Mass index, PBW: Preop Body weight, IBW: Ideal Body weight, EW: Excess of weight.

As shown in Table 2, after adjusting for caloric intake, carbohydrate, lipid and protein intake were similar between male and females. Likewise, the type of ingested fats was similar with monounsaturated fats being the highest consumed. No significant differences were observed on other micronutrient intake by gender. However, as shown in Table 3, in middle-aged women iron intake (expressed as the 95% confidence interval) was below the RDI whereas men reported an iron intake greater than recommended. Phosphorous intake was higher than recommended in both men and women. These results did not differ when the 13 LER subjects were excluded from the statistical analysis (data not shown).

Table 4 summarizes the prevalence of vitamin and mineral deficits in obese men and women. Only 25% of the population studied did not present a nutritional deficiency, while 38.2% presented at least one, 22% two and 11.4% three nutritional deficiencies. A smaller percent- age of patients presented four and five (3.3% and 0.4%, respectively) nutritional alterations. The prevalence of vitamin and mineral deficiencies did not differ by gender. The most prevalent abnormality was a deficit of 25(OH) vitamin D. Only 6.0% of the obese population showed sufficient levels (≥30 ng/ml) versus 36.0% found in normal weight individuals. Insufficient levels of this vitamin (≥20 and <30 ng/mL) were observed in 26.3% of the obese patients and 40.0% of normal weight subject, while deficiency (<20 ng/mL) was observed in 67.7% of the obese individuals as compared to 24.0% for normal weight subjects ($p < 0.0001$).

Table 2. Results of energy intake estimated by 4-day food record + 24-hs recall and analyzed with 2.0 Novartis ®

	Males (n=64)	Females (n=167)	*p Value*
Energy Intake[¥] (Kcal/day)	2584 [2337 to 2830]	2093 [1991 to 2196]	0.0004
Carbohydrate			
g	193.4 [128.3 to 258.6]	243.7 [204.1to 283.3]	n.s
%	38.7 [36.2 to 41.1]	39.3 [37.8 to 40.8]	n.s
Protein			
g	105.8 [94.7 to 116.9]	94.5 [87.8 to 101.3]	n.s
%	19.1 [17.7 to 20.5]	17.3 [16.4 to 18.1]	0.0312
Lipids			
g	104.1 [98.0 to 110.1]	108.1 [104.4 to111.7]	n.s
%	41.9 [39.6 to 44.2]	43.0 [41.7 to 44.4]	n.s
Saturated fat (g)	30.4 [27.5 to 33.2]	31.5 [29.7to 33.3]	n.s
Monounsaturated fat (g)	48.6 [44.6 to 52.6]	50.4 [47.9 to 52.9]	n.s
Polyunsaturated fat (g)	9.3 [8.4 to 10.3]	10.1 [9.5 to 10.7]	n.s

Results are expressed as least square means [95%CI] adjusted by baseline Kcal. All estimators are adjusted by baseline kcal for all variables but kcal.
Kcal estimated kilocalories intake per day.

Increased PTH values were observed in 41% of the obese patients and in 20% of the normal weight individuals ($p < 0.0001$). Calcium levels were lower than normal in 4.8% of our study cohort compare with any subject in the normal weight individuals. Low magnesium levels were encountered in 29.0% and in 2.2% of the obese and normal weight individuals, respectively ($p < 0.0001$). The prevalence of vitamins A, B1 and B6 deficiency was larger in obese individuals (10.2%, 7.2% and 15.9%) as compared to normal weight subjects (8%, 0% and 0%), although comparison of proportions did not reach statistical significance. Anemia was present in 22.2% of obese and 5.9% of normal weight subjects ($p = 0.035$).

Table 3. Results of micronutrient intake estimated by 4-day food record + 24-hs recall and analyzed with 2.0 Novartis ®

Micronutrient intake (mg)	**Males (n=64)**	**Females (n=167)**	***P***	RDI	
				Men	**Women**
Calcium (mg)	911.9 [806.7 to1017.1]	928.0 [864.0 to 992.1]	n.s.	1200	1200
Phosphorous (mg)	1459.5 [1331.6 to 1587.4]	1370.8 [1293.7 to 1447.9]	n.s.	700	700
Magnesium (mg)	246.2 [227.6 to 264.9]	244.7 [233.4 to 256.16]	n.s.	420	320
Iron (mg)	16.2 [14.23 to 18.17]	*18-50 years* 12.8[12.2 to 13.4] *51 to >70 years* 13.1[12.3 to 13.9]		8 mg	*18-50 years* 18 *51 to >70 years* 8

Results are expressed as least square means [95%CI] adjusted by baseline Kcal. All estimators are adjusted by baseline kcal for all variables

RDI recommended daily intake.

Caloric intake in obese individuals was significantly correlated with weight prior to surgery only in males ($r = 0.330$; $p < 0.01$). A significant correlation was found between the BMI and PTH levels ($r = 0.364$; $p < 0.05$) and 25(OH) vitamin D ($r = -0.234$; $p < 0.05$) in females. The level of PTH was not related to calcium intake in women or men (p = n.s.). The estimated dietary intake of calcium, iron and magnesium did not show a significant correlation with the plasma levels of these micronutrients.

Table 4. Prevalence of nutritional deficiencies in obese individuals prior to surgery and in normal weight individuals.

Nutrient (units)	Cut off for deficiency	Obese individuals	Normal weight controls
Total protein (g/L)	60	3 (1.3%)	0 (0.0%)
Albumin (g/L)	37	2 (1.0%)	0 (0.0%)
Prealbumin (g/L)	0.167	12 (6.4%)	1 (2.2%)
Corrected Calcium (mg/dL)	8.5	9 (4.8%)	0 (0.0%)
Magnesium(mg/dL)	1.8	60 (29.0%)	1 (2.2%)*
Zinc (äg/dL)	56	7 (32.0%)	0 (0.0)
Iron (mcg/dL)	50	21 (26.3%)	5 (11.1%)
Ferritin (ng/mL)	15	21 (10.3%)	6 (13.0%)
Transferrin (g/L)	1.93	7 (3.5%)	3 (6.5%)
Vitamin B_1(äg/L)	35	12 (7.2%)	0 (0.0%)
Vitamin B_6 (nmol/L)	15	10 (15.9%)	0 (0.0%)*
Intraerytrocite acid folic (ng/dL)	250	6 (3.8%)	0 (0.0%)
Vitamin B_{12} (pg/mL)	200	5 (2.2%)	0 (0.0%)
Anemia	Haemoglobin Males <137(g/L) Haemoglobin Females <122 (g/L)	50 (22.2)	2(5.9)*
PTH (pg/mL)	≥70	75 (41.0%)	5 (20.0%)*
Vitamin D 25(OH) (ng/mL)	Defficiency <20 Insuficiency >20≤30 Suficiency >30	113 (67.7%) 44 (26.3%) 10 (6.0%)	6 (24.0%)* 10 (40.0%)* 9 (36%)*

Results are expressed as number of individuals (%of the studied populations). The rate of missing data was below10%for all variables except calcium, 23.5%; pth, 24.9%; Vit D, 30.7%; Vit B6, 60%; iron, 53%.
*p<0.05

3.4. Discussion

The current study shows that obese subjects from a Mediterranean population seeking bariatric surgery present a relatively elevated lipid intake and several nutritional deficiencies prior to surgery. The estimated caloric intake in our study population is similar to that reported previously in the Swedish Obese Subjects study at baseline [4], and by Valdés et al. [20] in an overweight Spanish population. The analysis of macronutrient intake in our population revealed that energy coming from fat intake was greater than the recommended 35% as suggested for a healthy lifestyle [21]. However, percentage of fat intake in our population (40– 43%) and lipid profile (45–50 g monounsaturated fatty acids, 30–32 g saturated fatty acids) were in agreement to those reported for the Spanish normal weight population in the last two decades [20, 22, 23]. On the other hand, macronutrient intake showed relatively high protein and low carbohydrate content compared to the recommendations made by the American Heart Association [21]. But again, the observed percentages were in accordance to those reported in Spanish cohorts [20, 22, 23]. Adherence

to the Mediterranean dietary pattern is significantly associated with beneficial effects on health, like reduced weight gain [24]. Nevertheless, during the past two decades, Spain has experienced a significant downward trend in adherence to the predominant dietary pattern by a Westernisation of dietary habits, experiencing an increased contribution of animal products and sugars to overall intake [25].

Surprisingly, energy intake was not greatly increased as compared to our control group (data not shown). This observation could potentially be attributed to underreporting, a well-known problem when evaluating food intake in obese populations [16, 26, 27]. Nonetheless, using the ratio between the reported energy intake and the Harris–Bennedict equation of estimated basal metabolic rate, we found that the prevalence of underreporting in our obese cohort was only 5.6%. Although this percentage was slightly lower than that encountered by other authors using the doubly labeled water method (DLW) [16, 26, 27], the under-reported energy intake of this individuals (a mean of 46.4% of their energy intake) lied within the range reported by Schoeller's (35% to 50%) using the DLW method [26].

Compared to the RDI, calcium and magnesium intake were low in both men and women. Calcium and magnesium are significantly present in dairy products, nuts, beans, whole cereals and seafood, which are important components of a Mediterranean diet [21, 28]. Thus, it could be argued that our obese Spanish cohort did not adhere to such a dietary pattern. Of note, despite an increase in the calcium intake of the general adult Spanish population between 1963 (620 mg/day) and 2003 (777 mg/day), it is still below the RDI (1,200 mg/day) [22, 23]. On the other side, the elevated phosphorous intake observed in our cohort could be attributed to the elevated protein intake.

Vitamin D status was classified based on Holick's classification [29]. According to 25-hidroxivitamin D levels three groups were established: deficient, <20 ng/ml; insufficient, >20 ≤ 30 ng/ml and sufficient, ≥30 ng/ml. Our data show that 25(OH) vitamin D deficiency was the most prevalent studied abnormality of our obese population, affecting 67.7%. Our findings are consistent with other studies in non-Mediterranean populations. In these studies vitamin D deficiency has been reported lower (57.4%) or similar (68.1%) to ours [7, 13, 30], and were higher than others (23% and 25.4%) [10, 31]. The prevalence of vitamin D deficiency in our normal weight cohort (24%) was similar to that in previously reported Spanish cohorts [32]. The mechanisms underlying vitamin D deficiency in morbidly obese subjects are not still well understood [33]. However, the prevalence of vitamin D deficiency is elevated in our environment although there is sunlight even in winter in Mediterranean countries [34]. In fact, a recent study performed in spring in 116 young healthy Spanish

subjects showed a high prevalence of vitamin D insufficiency (20 to 30 ng/mL) with no clear relationship to sun exposure or sunscreen protection [35]. The authors proposed that the low intake of food rich in vitamin D and the lack of food fortification combined with scarce effective sun exposure could account for the low serum levels of vitamin D in a healthy population [35]. These factors together with vitamin D deposition in the adipose tissue have been proposed to explain the high prevalence of 25-OH vitamin D deficiency in the obese [36].

Mirroring vitamin D deficiency, we found increased PTH levels in 41% of the obese group, twice as much as in normal weight individuals. Furthermore, the correlation found between the BMI and PTH values and 25(OH) vitamin D strongly suggest that the main cause of this increase could be attributed to the low vitamin D status, which impairs calcium absorption. Of note, in our cohort the elevated PTH levels refer to the group of patients without magnesium deficiency [37]. Thus, it could be argued that should magnesium deficiency have not been present in any obese participant, the prevalence of an elevated PTH would have been even higher.

The 22.2% prevalence of anemia found among our patients is significantly higher than that in our normal weight individuals (6%). Our findings are consistent with those in Flancbaum's study (22%) performed in US population, but were higher than others in non-Mediterranean population (5–10% and 6.4%) [7,12,13]. This finding could be explained, at least in part, by the lower iron intake in middle-aged women observed in our study. Arguably, ferritin levels were not lower in the obese as compared with the normal weight group. Ferritin levels have been associated with a mild chronic inflammatory status associated with obesity [38] Thus, this would suggest that other markers of iron status, such as the soluble transferrin receptor, could be of greater value when evaluating iron metabolism in the obese [39, 40].
Deficiencies in folic acid and vitamins A, B12 and B1 were higher among the obese patients than in the normal weight controls. Lack of statistical significance could be due to the number of evaluated subjects. However, prevalence of these deficiencies was lower than previously reported in individuals undergoing bariatric surgery in non- Mediterranean populations [7, 12]. Similar prevalence than observed in our obese cohort, vitamin A deficiency was found in 7% of non-Mediterranean obese population undergoing bariatric surgery [10]. A meta-analysis of Spanish studies conducted between 1990 and 1999 showed that the prevalence of vitamin A ranged 0–33% [22]. It has been argued that when present, vitamin A deficiencies probably resulted from inadequate dietary intake [10]. Kaidar-Person's and Toh's studies reported a folate deficiency of 6% while Ernst reported 3.4% [11–13]. Prevalence of vitamin

B12 deficiency was very variable between studies ranging from 2% to 18.1% [10, 12, 13]. Thiamine deficiency was observed in 15.5% [8] and in 29% [7]. Why these results are different from each other deserves further analysis. In contrast, deficiency in vitamin B6 in our obese population was higher than in Ernst' study (2.2%) [12]. Ultimately, vitamin deficiency seems to be prevalent between obese individuals independently on the cultural background, eating habits and geographical area. Since bariatric surgery is a risk factor for the subsequent appearance of vitamin deficiencies because of limited intake and absorption, early recognition of vitamin abnormalities will provide the opportunity to correct the nutritional status before surgery.

Finally, a deficit in prealbumin was found in 6.4% of our obese cohort. This was higher than in normal weight controls. Prealbumin deficiency in general Spanish population has not been reported. Furthermore, total protein, albumin and prealbumin alterations prior to bariatric surgery have seldom been reported [13]. Unfortunately, C reactive protein was not measured to rule out prealbumin deficiency due to the inflammatory process associated with obesity [41]. However, a nutritional origin for the low prealbumin level can be suspected since prealbumin is less sensitive to the inflammatory process than albumin [42]. Thus, special attention should be paid to prealbumin levels prior to surgery in order to maintain an adequate protein status after surgery.

Studies focusing on impaired nutritional status prior to bariatric surgery are increasing in the scientific literature [7–13]. These studies have been performed in countries with different nutritional habits. These deficiencies may also be a consequence of poor dietetic-nutritional education, social factors and the presence of eating disorders. Related to this later aspect, in our study, we did not found any relationship between binge eating disorder and prevalence of deficiencies (data not shown). The importance of the deficiencies found in our study is strengthened by recent evidence indicating that nutritional status is likely to worsen after surgery. A retrospective study of 232 individuals who underwent bariatric surgery observed that nutritional deficiencies identified at baseline were still present 1 year after surgery, and new deficiencies developed for albumin, iron, ferritin, vitamin B12 and folates [13]. Finally, several studies observed that patient's adherence to the vitamin and mineral supplements is low and plays an important role in the development of the nutritional deficits after surgery [13, 43].

3.5. Conclusions

The prevalence of nutritional deficiencies in obese subjects undergoing bariatric surgery deserves careful attention also in Mediterranean populations. The importance of ascertaining the nutritional status in those subjects prior to surgery is further emphasized because: (1) bariatric surgery is a risk factor for further deterioration, (2) surgical complications may interfere with tolerance to nutritional supplementation, (3) adherence to supplementation is low, and efficiency of such supplements has not been extensively evaluated after surgery yet.

3.6. References

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CHAPTER 4: LONG-TERM DIETARY INTAKE AND NUTRITINAL DEFICIENCIES FOLLOWING SLEEVE GASTRECTOMY OR ROUX-EN-Y GASTRIC BYPASS IN A MEDITERRANEAN POPULATION

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4 LONG-TERM DIETARY INTAKE AND NUTRITIONAL DEFICIENCIES FOLLOWING SLEEVE GASTRECTOMY OR ROUX-EN-Y GASTRIC BYPASS IN A MEDITERRANEAN POPULATION.

Background: Data on long-term dietary changes and nutritional deficiencies after sleeve gastrectomy (SG) in grade 3 obese patients are scarce.

Objective: To prospectively compare dietary changes and nutritional deficiencies in grade 3 obese patients 5 years after SG and Roux-en-y gastric bypass (GBP).

Participants/setting: Three hundred and fifty-five patients who had SG (n=61) or GBP (n=294) (May 2001-December 2006) at a Spanish university hospital.

Design: Longitudinal, prospective, observational study.

Primary outcomes/statistical analyses: Changes in energy, macronutrient, and micronutrient intake, and weight loss were analyzed using mixed models for repeated measurements.

Results: At the 5-year follow-up visit, the percentage of excess weights loss (P=0.420) and daily energy intake (P=0.826), as well as the proportion of energy from carbohydrates (P=0.303), protein (P=0.600), and fat (P=0.541) did not differ between surgical groups. Energy intake (P=0.004), baseline weight (P<0.001), and time period (P<0.001), but not the proportion of different macronutrients or the type of surgery, independently predicted the percentage excess weight loss over time. After SG or GBP, the mean daily dietary intake of calcium, magnesium, phosphorus, and iron was less than the current recommendations. Despite universal supplementation, the prevalence of nutritional deficiencies was comparable after SG or GBP, with 25-hydroxyvitamin D being the most commonly observed deficiency (SG, 93.3% to 100%; GBP, 90.9% to 85.7%, P=not significant). In an adjusted multivariate regression model, energy intake and lipid intake independently predicted plasma 25(OH)-vitamin D levels.

Conclusions Data show that SG and GBP are associated with similar long-term weight loss with no differences in terms of dietary intake. Furthermore, data demonstrate that both types of surgeries carry comparable nutritional consequences. J Acad Nutr Diet. 2013;113:400-410.

4.1. Introduction

In recent years, laparoscopic sleeve gastrectomy (SG) has gained popularity as a stand-alone operation for the treatment of morbid obesity [1-4] Compared with laparoscopic Roux-en-Y gastric bypass (GBP), SG is not only technically simpler, but also boasts weight loss levels and comorbidity resolution rates comparable to those achieved with GBP [5-7]. The health benefits associated with the different types of bariatric surgery have been well-documented [8]. However, the nutritional risks after SG have not been as frequently described as the nutritional risks after GBP have been [9-11]. GBP has been linked to changes in food preferences and in the perceived reward value of hedonically pleasing foods [12-14]. Importantly, it has been postulated that changes in food habits could act as a potential weight-loss mechanism after this type of surgery [13-15]. Worth noting is the fact that although SG has long been considered a restrictive bariatric procedure, in- creasing evidence suggests that it should be classified differently [2,16,17]. Thus, although SG and GBP yield comparable weight loss, it could be hypothesized that the former results in dietary changes comparable with those observed after GBP. Interestingly, altered food habits after SG have not yet been reported.

Vitamin and micronutrient deficiencies constitute one downside of bariatric surgery. In that respect, it has been suggested that the lower incidences of mineral and vitamin deficiencies noted in SG patients, vs those who undergo GBP, could represent an additional advantage for preferring the former [10]. However, the fundus resection that is part of the SG procedure can result in some limitations on the proportion of micronutrients overall that are likely to be absorbed, thus bringing into question claims that the risks for developing deficiencies after SG are low [10,18]. To date, there is scant documentation about micronutrient intake after SG. Further- more, long-term data on nutritional deficiencies after SG (vs GBP) are limited [9,11]. Against this background, the aims of this study were to prospectively compare both dietary intake (including energy intake, as well as macronutrient and micronutrient intake) and the most common vitamin and mineral deficiencies associated with SG and GBP during long-term follow-up.

4.2. Methods

4.2.1. Participants

A total of 355 white, grade 3 obese patients (male, n=88 [24.8%]; female, n=267 [75.2%]) participated in this prospective observational study. The study participants were

selected among those who consecutively underwent bariatric surgery at Hospital Clinic Barcelona from May 2001 to December 2006. Sixty-one patients (17%) had SG and 294 patients (83%) had GBP. SG was selected as the bariatric surgical technique based on the following criteria: a large body mass index (BMI>55) and/or an estimated increased operative risk, and/or the presence of an enlarged liver [7]. SG and GBP were done laparoscopically as previously described[7]. For detailed information about the surgical techniques done at Hospital Clinic Barcelona, please see the guidelines published in the article by Mechanick and colleagues [19]. After surgery, nutrition follow-up and dietary assessment was done at 3, 6, 12, 18, 24, 30, 36, 48, and 60 months. However, this report included data on all but the 3-, 18-, 30-, and 36-month follow-up points. The Ethics Committee of Hospital Clinic Barcelona, Spain, approved this study, and participants gave their written informed consent before examination.

Table 1. Demographics and body weight of study participants at baseline.

	SG (n=61)	**GBP (n=294)**	***p-value***
Gender (%Women)	67.2	77	0.111
Age (years), Mean (SD)	46.4 (11.6)	45.2 (10.6)	0.457
Weight (kg), Mean (SD)	137.9 (19.4)	126.1 (20.1)	<0.001
EBW (kg), Mean (SD)	70.3 (17.3)	58.6 (17.2)	<0.001
BMI (kg/m2), Mean (SD)	51.6 (6.7)	47.4 (6.0)	<0.001

SG: sleeve gastrectomy. GBP: Roux-en-Y gastric bypass. EBW: Excess body weight, BMI: Body mass index

Table 2. Percentage of patients with preoperative and postoperative deficiencies

	Time*	Baseline		**6 months**		**12 months**		**24 months**		**48 months**		**60 months**	
	Reference range	SG	GBP	SG	GBP	SG	GBP	SG	GBP	SG	GBP	SG	GBP
Total protein	63-80 g/L	5.4	1.3	0	0	3.4	1.9	0	0	4.5	2.8	0	0
Albumin	34-48 g/ L	5.4*	0.5	4.3	2.2	0	0	0	0	4.8	0	0	1
Prealbumin	0.200-0.400 g/ L	11.8	6	8.7	31.3	14.3	15.8	3.1	5.7	0	3.8	0	3.9
Hemoglobin	137 men/122 women g/ L	10*	22	12.1	20.8	11.5	19.9	11.5	17.7	15.8	17.2	14.3	25.5
Ferritin	15-200 ng/ mL	8.3	18.6	0	19	6.5	19.8	20.6	30.1	23.8	29.5	0	28.7
Transferrin	2.5-3.8 g/ L	2.8	2	0	9	7.1	5.8	9.4	6.8	0	0.9	0	0
Iron	50-170 µg/ dL	30.8	26.5	4.3	18.2	10.3	15.9	9.4	10.6	9.5	12.5	12.5	15.5
Intraerytrocite folic acid	250-1050 ng/ mL	0	1.8	13.6*	0.9	20.7	2.6	6.1	7.5	0	4.9	12.5	7.9
Zinc	59-110 µg/ dL	8.1	11.5	31.8	25.2	39.3	27.5	25	25.8	47.6	26.7	12.5	25.7
Calcium	8.5-10.5 mg/ dL	2.9	9.6	0	3.1	3.6	3.5	3.1	5.5	4.8	1.9	12.5	2.9
Magnesium	1.8-2.6 mg/ dL	37.8	29.4	12.5	19.4	10.3	14.1	6.3	7.6	4.8	5.7	12.5	5.8
Vit B_1	35-91 ng/ mL	0	5.5	4.8	8.4	9.1	6.1	25.0*	1.8	0	5.3	0	5.9
Vit B_6	15-96 nmol/ L	75*	11.3	26.3*	6	11.1	2.8	0	3.9	16.7	8.3	0	4.2
Vit B_{12}	250-1050 pg/ mL	2.7	1.8	3.7	2.9	3.2	6.2	5.9	5.5	0	5.8	12.5	5
Vit D													
Sufficiency	***≥30 ng/mL***	6.7*	9.1	22.7	40.2	22.2	26.7	13.3	12.9	33.3	18.7	0	14.3
Insufficiency	***>10-<30 ng/mL***	3.3*	30.3	54.5	38.1	40.7	40.8	20	35.6	22.2	30.7	100	38.8
Deficiency	***<10 ng/mL***	90*	60.6	22.7	21.6	37	32.5	66.7	51.5	44.4	50.7	0	46.9
PTH	**10-65 pg/ mL**	62.5*	44.1	40.9*	11.7	37	16.7	40.6	40.4	57.1	53.8	87.5	56.9

SG: Sleeve gastrectomy; GBP: Gastric Bypass
* $p<0.05$ in the Ficher's exact test

4.2.2. Statistical Analysis

Results are expressed as mean±standard deviation (SD), as baseline kilocalories adjusted to the least square means (95% confidence intervals [95% CI]), as frequencies and percentages (%), or as are otherwise specified. Continuous repeated data were analyzed by means of mixed models for repeated measurements, including the model for baseline energy intake (when specified), the time of assessment, the type of surgery, and the time by type of surgery interaction term. Although data were collected at 3, 6, 12, 18, 24, 30, 36, 48, and 60 months, and although all observation time points were included in the analysis, only data at 0, 6, 12, 24, 48, and 60 months are shown for clarity. Categorical variables were compared using the Fisher's exact test. The analysis was done using SAS version 9.2 software (SAS Institute, Inc) and the level of significance was established at 0.05 (two- sided).

4.3. Results

Three hundred fifty-five patients with a median (25th to 75th percentile) follow-up of 48 months (range 24 to 60 months) were included in this study. Dropout rates at 2, 3, and 5 years for GBP and SG were 12% vs 16%, 31% vs 41%, and 53% vs 66%, respectively. Demographics of the study participants are shown in Table 1. As expected, based on the selection criteria, patients undergoing SG had a larger BMI compared with those in the GBP group.

Dietary Intake and Weight Loss

Daily energy intake and the percentage of macronutrient intake (grams of protein/kg body weight/day) throughout the study are shown in table 2. Prior to surgery, total daily energy intake was comparable between the two groups. Within each surgical group, total daily energy intake was significantly lower for up to 5 years after surgery when compared with baseline ($P<0.001$ at all study timepoints). When adjusted for presurgical body weight, no differences were observed in energy intake at any follow-up point between the two groups. As shown in Figure 1, percentage excess weight loss was comparable between the two groups, being maximal at 18 months' follow-up and remaining at parity from 2 to 5 years after both types of surgery. Total daily energy intake ($P<0.004$), baseline weight ($P<0.001$), and time ($P<0.001$) were the only significant independent predictors for the percentage of excess weight loss over time. Accordingly, and as shown in table 2, energy intake did not significantly change in either the SG or in the GBP group from 2 to 5 years after surgery.

Prior to surgery, in the combined GBP-SG patient group the percentage of energy from protein, carbohydrates, and fats was, respectively, 18.3% (range 17.7% to 19%), 39.9% (range 38.8% to 41%), and 41.7% (range 40.6% to 42.8%). At that study time point, no differences were found between the two groups. As shown in table 2, a transient change in macronutrient distribution was observed in the two surgical groups. One year after surgery, the percentage of protein intake relative to baseline was significantly different ($P<0.001$ and $P<0.003$, respectively, for SG and GBP). Only SG individuals showed a significantly different percentage of carbohydrate intake relative to baseline ($P<0.01$), although no change was observed in terms of the percentage of lipid intake. At 5 years (last follow-up assessment), the percentages of macronutrients were not significantly different relative to baseline values in either of the two surgical groups. Although the postsurgical comparison between the SG and GBP groups revealed differences in the protein intake distribution at 18 (data not shown) and 24 months, the results proved inconsistent during the study period. Differences in the proportion of energy originating from carbohydrates and fat were only found, respectively, at 30 and 48 months. The proportion of energy from protein in the SG group was larger at 3, 6, and 12 months after surgery compared with the GBP group. The proportion of patients reporting dietary protein intake less than 60 g per day ranged from 12.7% and 11.5% before surgery for SG and GBP, respectively, to 50% and 41.2% for SG and GBP at 5 years' follow-up. The proportion of GBP patients with a protein in- take less than 60 g per day was not significantly different between the two groups throughout the study. The proportion of energy from fats in the SG group was smaller only at 30 ($P=0.053$) and 48 months ($P=0.038$) after surgery. Protein intake in g/kg current body weight/day was similar in both groups during most of the study period except at 18 ($P=0.039$; data not shown) and 24 months ($P=0.048$), proving higher in GBP patients. As expected, protein intake in g/kg current body weight/day was lower during the first 6 months.

The percentage of energy from monounsaturated fats was similar between groups over the study period, except at month 30, when the SG patients had a lower intake ($P=0.004$). The percentage of energy from saturated fat was lower in SG patients at month 30 ($P=0.04$), but larger at month 36 ($P<0.001$). The percentage of energy from polyunsaturated fats was similar between the two surgical groups over time.

Micronutrient Intake and Nutritional Deficiencies

Table 3 summarizes the dietary intake of calcium, magnesium, phosphorus, and iron.

No consistent differences between the two groups were found. As shown, when micronutrient intake from supplements was not taken into account, the mean daily dietary intake of all the evaluated micronutrients was less than the current DRIs [21]. The proportion of patients with micronutrient ingestion less than the DRIs varied throughout the study, as well as at the basal point (calcium: SG 75% to 90%, GBP 79% to 92%; magnesium: SG 87% to 100%, GBP 87% to 99%; phosphorus: SG 9% to 32%, GBP 4% to 30%; iron: SG 42% to 88%, GBP 48% to 79%), although it did not differ significantly between groups.

The prevalence of nutritional deficiencies is shown in Table 4. At baseline, the prevalence of albumin, vitamin B-6, and vitamin D deficiencies, and elevated PTH were significantly more in the SG group. In contrast, anemia was more prevalent in the GBP group ($P<0.005$). Albumin and prealbumin levels were less than the normal range, and deficiencies were observed in a similar proportion of patients in the two groups. The proportion of patients with low plasma levels of magnesium, phosphorus, red blood cell folate, and vitamins A, thiamin, vitamin B-6, and vitamin B-12 was generally less than 10% throughout the study period. This prevalence proved to be similar after the two types of surgeries, with no consistent differences in the deficiency patterns noted between the two groups.

Elevated PTH levels and low vitamin D levels were the most common biochemical abnormalities found throughout the study. Elevated PTH levels at baseline were recorded in 63% and 44%, respectively, of the SG and GBP patients ($P<0.05$).

There were no differences in PTH values between the two groups over time (treatment $P=0.287$; time-treatment interaction $P=0.233$) (Figure 2A). However, both groups showed a statistically significant reduction from month 3 to month 18 vs baseline. The increase was significant for all timepoints more than 36 months in the GBP group but not in the SG group. Normal values of PTH were observed only during the 18 months after surgery for GBP individuals and at 3 months (data not shown) and 18 months for SG patients. Vitamin D levels less than 30 ng/mL were found at baseline in 93% and 91%, respectively, of the SG and GBP cohorts. Despite universal supplementation, the proportion of patients with vitamin D levels less than normal ranged between 60% and 90% throughout the study. No differences in 25-hdroxyvitamin D were observed between the two groups over time (treatment $P=0.366$; time of intervention $P=0.656$) (Figure 2B). We conducted a multivariate regression analysis using a repeated measurement model for plasma vitamin D levels, including all factors with a $P<0.1$ in the univariate analysis and maintaining them in the multivariate model if $P<0.1$.

Time (P<0.001), weight (P<0.047), PTH serum levels (P<0.001), and lipid intake (P<0.095) were identified as independent predictors, whereas daily energy intake, carbohydrate intake, protein intake, calcium intake, age, surgery type, and sex were not predictive factors.

4.4. Discussion

The present study shows that in a Mediterranean population neither SG nor GBP are associated with long-term changes in the percentage of macronutrient intake. Furthermore, data show that after either type of surgery, daily micronutrient intake is less than current recommendations among high proportion of patients. Finally, data demonstrate a comparable incidence of nutritional deficiencies after both SG and GBP, despite the use of similar supplementation regimens. As energy intake significantly decreased, so did total grams of protein, carbohydrate, and fat, and grams of protein/kg body weight/day. The analyses of protein intake/kg body weight/day revealed that between months 12 and 36 protein intake increased to levels recommended for the general population of 0.8g/kg body weight/day. This proved true for both types of surgery except at 18 and 24 months, when GBP patients ingested higher amounts (P value=0.039 and 0.048, respectively). Although this study did not focus on assessing the food preferences of the two populations, this data could help explain why protein intake (g/kg body weight/day) was greater in GBP patients at 18 and 24 months.

It has been suggested that changes in food habits after surgery are among the mechanisms underlying weight loss after GBP [12,13,15]. Human studies have shown that, despite energy intake levels comparable to that found in post–GBP and vertical banded gastroplasty patients (VBG), the former consumed less fat-based energy at 1 and 6 years after surgery, with no differences in protein and carbohydrate intake [13]. Similarly, Ernst and colleagues reported a healthier food pat- tern, including a higher daily intake of fruits and vegetables and a lower consumption of sweet beverages, compared with patients who had gastric banding [12]. More significant weight loss levels in the setting of GBP (vs VBG or GB) have been reported by several authors[1]. As mentioned earlier, after quantifying all food and beverages that the participants from both surgical groups listed in their 3-day food records (plus the 24-hour dietary recall), we found no significant and/or consistent differences in the postsurgical dietary intake levels between those that had undergone SG or GBP. Admittedly, the analysis was based on total daily energy and macronutrient intake rather than on food preferences [13,15]. However, data is consistent with the observation that weight changes remain similar over time when comparing GBP and SG participants [5,7,9]. Importantly, we

also found no changes in dietary intake from the first postsurgical year onward as compared with the year preceding surgery. This proved true despite frequent dietary counseling. Interestingly, food habits prior to surgery and at the end of follow-up were similar to that reported in the dietary surveys recorded for the Spanish general adult population [23].

In the present study, weight loss paralleled energy intake. In fact, total daily energy intake, baseline weight, and time were the only significant independent covariates for the percentage of excess weight loss over time. Data are in agreement with that published in the Swedish Obese SubjectsStudy, in which the postsurgical reduction in energy intake mirrored weight loss for up to 2 years of follow-up [27]. Similarly, Bobbioni-Harsch and colleagues found that a reduction in energy intake after GBP was a significant determinant of weight loss, whereas qualitative dietary modifications did not play a significant role [28]. In contrast, Faria and colleagues found a significant association between glycemic load and total daily carbohydrate intake, with a relatively average monthly weight loss in those patients who had GBP [15]. However, their study was restricted to the first year after surgery, and therefore its conclusions cannot be applied to the type of long-term follow-up evaluated in this study.

The present study showed that a significant percentage of participants experienced biochemical abnormalities after SG. Moreover, these abnormalities (ie, in certain vitamins and minerals) occurred at a rate similar to that of GBP. However, the incidence of vitamin and mineral deficiencies after SG, and comparisons with those after GBP, has seldom been reported [9-11]. Nonetheless, any comparisons between the two studies must be made cautiously. There are differences in study design, follow-up schedule, the prescribed mineral and vitamin supplementation, and the criteria used to diagnose a particular deficiency [9-11]. In the randomized clinical trial conducted by Kehagias and colleagues, participants who had SG were prescribed multivitamins and minerals only for the first 6 months after surgery, whereas GBP patients were advised to remain on such supplements for the entire 12-month study period [9]. In contrast, in the SG series of patients reported by Aarts and colleagues, patients were prescribed a daily multivitamin preparation containing 150% of the RDA plus 1,000 mg of calcium and 880 IU of vitamin D [10]. Finally, in the non- randomized prospective study published by Gehrer and colleagues, a different dose of vitamins and minerals was used with no additional supplements being prescribed except when deficiencies occurred [11]. In the study by Gehrer and colleagues, and at variance with the present findings, SG patients presented lower rates of iron, vitamin B12, and vitamin D deficiencies

[11]. In contrast, a study by Kehagias and colleagues found no differences after the two surgical techniques [9]. Again, the reasons for these differences among the various studies remain elusive, although they could be attributed, at least in part, to methodological factors. Importantly, however, the reported prevalence of deficiencies after SG ranged between 0% and 43%, depending on the analyzed parameter [9-11]. Thus, the present data, as well as those from other groups, underline the potential nutritional consequences of SG [18].

As described in a previous publication by this group [29], as well as in another report [30], the prevalence of vitamin D and PTH abnormalities is elevated in the local environment, al- though in Mediterranean countries there is sunlight even in winter. In the present study, vitamin D insufficiency or deficiency was the most common deficit observed after SG or GBP (>60% at all study timepoints). Correspondingly, a high proportion of patients had elevated PTH throughout the study after either type of surgery. In the study by Gehrer and colleagues, a lower proportion of patients presented vitamin D deficiency after SG or GBP after a mean follow-up of 24 months (32% and 52%, respectively) [11]. However, the criteria used to diagnose vitamin D deficiency in that study differed from that used here. Interestingly, in the present study plasma vitamin D levels improved slightly during the first months after surgery, but then decreased toward baseline values. Whether this transient early postsurgical increase in vitamin D stems from better adherence to calcium and vita- min supplementation in the first months after surgery, or to vitamin D release from adipose tissue storage during the weight-loss phase, is currently unknown [31]. Nonetheless, multivariate regression analysis clearly shows that dietary intake is an independent contributor to postsurgical 25-hydroxyvitamin D levels.

Regarding the higher prevalence of hypoalbuminemia in SG patients, we believe that this is most likely related to the degree of obesity rather than to protein malnutrition because prealbumin, which is considered a parameter indicative of adequacy of protein intake, was similar in both groups. Nor do we know that vitamin B-6 deficiency was higher in SG as has been previously reported [24]. In fact, we believe that this also may be related to the degree of obesity.

Decreased intake and absorption have been implicated as potential contributors to vitamin and mineral deficiencies after bariatric surgery [18,32]. In the present study, micronutrient intake was less than the DRIs in a high proportion of patients after SG and GBP. Thus, data supports the view that universal micronutrient and vitamin supplementation

should be required after either type of surgery [18]. Nonetheless, although all of the study participants were advised to adhere to micronutrient and mineral supplementation for the entire follow-up period, a substantial proportion still presented at least one deficiency. Admittedly, this could be attributed to low adherence to multivitamin and mineral supplementation. However, other mechanisms may play a role not only after GBP, but also after SG. The latter has been associated with rapid gastric emptying [32]. Furthermore, reduced production of intrinsic factor, as well as decreased hydrochloric acid secretion, may also mediate nutritional deficiencies after this type of surgery [18]. Finally, lower sunlight exposure (compared with the nonobese population) due to psychological or environmental factors could be a contributing mechanism for vitamin D deficiency after SG and GBP [11].

In general, and as stated by the American College of Surgeons in a recent paper [33], laparoscopic sleeve gastrectomy has morbidity and effectiveness levels that positions it between gastric banding and laparoscopic Roux-en-Y Gastric Bypass/Open Roux-en-Y Gastric Bypass for up to 1 year after surgery. Because gastric banding is not done at Hospital Clinic Barcelona, an assessment regarding its relative morbidity/effectiveness head-to-head with other procedures could not be done. However, when compared with the GBP, it is clear both in the short- and long-term that the prevalence of nutritional deficits and weight loss are comparable.

The present study has some limitations and strengths. It must be acknowledged that a randomized study would have provided a more powerful comparison between the two surgical procedures. This lack of random assignment was addressed by adjusting the outcome variables by the baseline weight and by the energy intake. Furthermore, food-intake underreporting and the lack of authentifiable adherence to mineral and vitamin supplementation are potential concerns. However, the present study included a large cohort of consecutive patients who were undergoing surgery at a single Institution and who were being followed up over a long period of time. Furthermore, the ascertainment of food intake was done by only two registered dietitians (V.M. and A.A.), thus reducing variability. Moreover, patients in the two surgical groups were advised in equal fashion regarding the supplementation regimen throughout the entire study period. It must be admitted that by relying on circulating levels of vitamins for the diagnosis of deficiency there is the possibility that only biochemical abnormalities, rather than true vitamin deficiencies, were properly considered. However, it could be argued that functional assays could be better indicators of

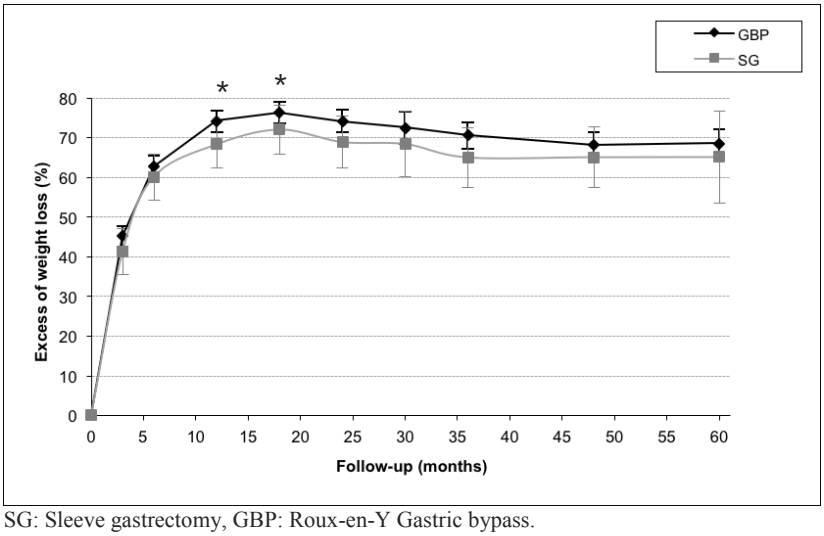
true vitamin deficiencies. However, because there are so few functional tests as well as few sensitive and specific indexes of nutrient status, the term *deficiency* has been used for all of the assessed vitamins, based on what is readily available for routine clinical practice.

Similarly, it could be argued that the lack of an estimation of vitamin dietary intake is a limitation. The current study was primarily planned to evaluate energy and macronutrient intake, as well as the dietary intake of the most commonly reported mineral deficiencies after bariatric surgery (namely iron and calcium metabolism). Although the occurrences of vitamin deficiencies were also included, the influence of nutrient intake on such biochemical abnormalities warrants further investigation. Finally, the high dropout rate at longer follow-up timepoints in our study is of concern. However, the dropout rate in our cohort is lower than previously reported in cohorts with shorter follow-up [34,35].

4.5. Conclusions

In summary, data reported herein demonstrate that in a Mediterranean population SG and GBP patients experience similar long-term changes in their dietary intake. Data also suggests that decreased energy intake, rather than modifications to the proportion of macronutrient intake, is an important factor in weight loss after either type of surgery. Finally, the present study stresses the importance of assessing a patient's adherence to routine mineral and vitamin supplementation after GBP, but also SG.

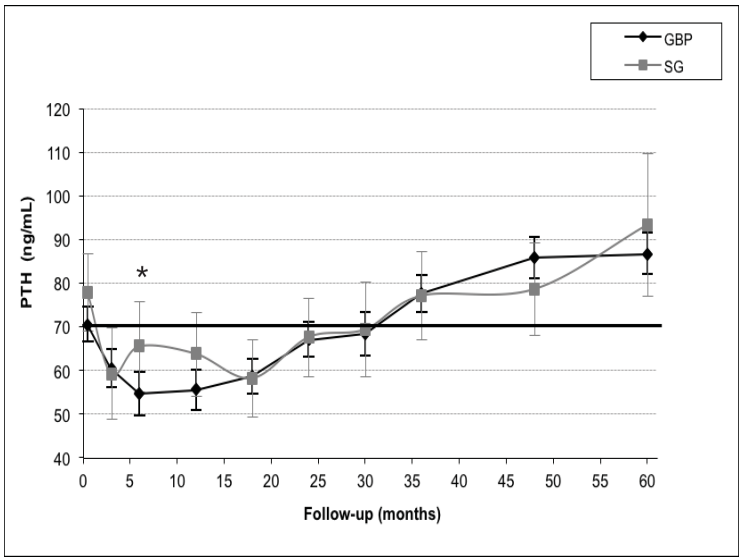
Figure 1. Percentage of excess weight loss over time



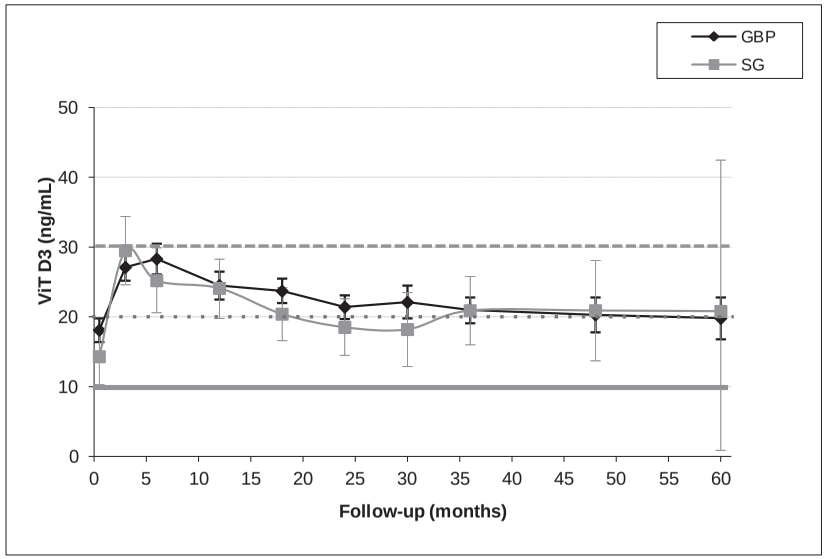
SG: Sleeve gastrectomy, GBP: Roux-en-Y Gastric bypass.
Mixed Models for Repeated Measurement (MMRM) least square means for percentage of excess weight loss by time and type of surgery, adjusted for baseline Kcal intake.
* **$p<0.05$**= significantly different between groups.

Figure 2. Time course of the post-surgical changes in plasma PTH (A) and 25 (OH) vitamin D (B) levels

A



B



Mixed Models for Repeated Measurement (MMRM) least square means PTH by time and type of surgery.
SG: Sleeve gastrectomy; GBP: Roux-en-Y Gastric Bypass. Number of patients at baseline was 294 for GBP and 61 for SG.
***: p<0.05=** significantly different between groups.

Table 3. Micronutrient intake as estimated from 3-day food records plus 24-hour recall for the two surgical groups[ab]

		Time[c]					
	Treatment	**Baseline**	**6 mo**	**12 mo**	**24 mo**	**48 mo**	**60 mo**
Calcium intake (mg)	SG[d]	884.9 (53.9) [779.3; 990.6]	741.1 (53.0) [637.1; 845.2]	798.3 (55.2) [690.1; 906.6]	887.2 (63.3) [763.0; 1,011]	924.2 (81.2) [765.0; 1,083]	854.9 (131.3) [597.5; 1,112]
	GBP[e]	891.7 (25.1) [842.6; 940.9]	707.7 (25.3) [658.1; 757.2]	756.3 (25.2) [706.9; 805.7]	801.2 (27.8) [746.7; 855.8]	791.2 (34.4) [723.8; 858.6]	757.3 (37.6) [683.5; 831.1]
	Differences	6.8 (59.4) [−110; 123.3]	−33.5 (58.8) [−149; 81.8]	−42.0 (60.7) [−161; 77.0]	−85.9 (69.2) [−222; 49.7]	−133 (88.2) [−306; 39.9]	−97.6 (136.5) [−365; 170.2]
		P=0.909	*P*=0.569	*P*=0.489	*P*=0.214	*P*=0.132	*P*=0.475
Iron intake (mg)	SG	14.3 (1.7) [11.0; 17.7]	8.4 (1.7) [5.1; 11.8]	16.9 (1.8) [13.4; 20.4]	9.0 (2.1) [4.9; 13.1]	13.5 (2.7) [8.1; 18.9]	7.5 (4.5) [−1.4; 16.4]
	GBP	13.8 (0.8) [12.2; 15.4]	8.8 (0.8) [7.2; 10.4]	9.1 (0.8) [7.5; 10.7]	9.5 (0.9) [7.7; 11.2]	10.4 (1.2) [8.1;12.6]	8.8 (1.3) [6.3;11.3]
	Differences	−0.5 (1.9) [−4.3; 3.2]	0.4 (1.9) [−3.3; 4.1]	−7.9 (2.0) [−11.7; −4.0]	0.5 (2.3) [−4.0; 4.9]	−3.1 (3.0) [−8.9; 2.7]	1.3 (4.7) [−8.0; 10.5]
		P=0.777	*P*=0.839	*P*=<.001	*P*=0.840	*P*=0.291	*P*=0.787
Magnesium intake (mg)	SG	234.9 (12.9) [209.6; 260.3]	139.6 (12.7) [114.7; 164.5]	153.3 (13.3) [127.3; 179.3]	176.7 (15.4) [146.5; 206.9]	171.2 (20.0) [131.9; 210.4]	157.1 (32.8) [92.7; 221.5]
	GBP	248.5 (6.0) [236.7; 260.3]	142.8 (6.1) [130.9; 154.7]	165.6 (6.0) [153.8; 177.5]	178.3 (6.7) [165.1; 191.5]	185.6 (8.4) [169.0; 202.2]	169.9 (9.3) [151.7; 188.1]
	Differences	13.6 (14.2) [−14.3; 41.5]	3.2 (14.1) [−24.4; 30.8]	12.3 (14.6) [−16.3; 40.9]	1.6 (16.8) [−31.3; 34.5]	14.4 (21.7) [−28.2; 57.0]	12.7 (34.1) [−54.2; 79.7]
		P=0.340	*P*=0.819	*P*=0.398	*P*=0.924	*P*=0.506	*P*=0.709
Phosphorous intake (mg)	SG	1,434 (67.8) [1,301; 1,567]	919.4 (67.1) [787.7; 1,051]	1,042 (69.0) [907.0; 1,178]	1,118 (79.2) [962.4; 1,273]	1,070 (102.8) [868.6; 1,272]	968.1 (168.2) [638.1; 1,298]
	GBP	1,413 (34.9) [1,345; 1,482]	897.6 (34.3) [830.3; 965.0]	976.6 (33.4) [911.0; 1042]	1,022 (34.7) [954.3; 1,090]	1,080 (43.5) [995.2; 1,166]	970.0 (47.8) [876.3; 10,64]
	Differences	−20.4 (76.2) [−170; 129.1]	−21.7 (75.4) [−170; 126.2]	−65.7 (76.6) [−216; 84.6]	−95.4 (86.5) [−265; 74.2]	10.3 (111.6) [−209; 229.2]	1.9 (174.9) [−341; 344.9]
		P=0.789	*P*=0.773	*P*=0.392	*P*=0.270	*P*=0.927	*P*=0.991

[a]Results are expressed as the least square means (standard error of mean –SEM—) [95%CI], estimated from the mixed models for repeated measurements analysis.
[b]All estimates are adjusted by baseline caloric intake for all variables.
[c]Timeline nutritional evaluations through the study. After surgery, data were collected every 6 months and all observation time-points were included in the analysis; however, for simplicity purposes, only data at 0, 6, 12, 24, 48, and 60 months are shown in this table.
[d]SG=sleeve gastrectomy. Number of patients at baseline was 61 for SG.
[e]GBP=Roux-en-Y gastric bypass. Number of patients at baseline was 294 for GBP.

Table 4. Percentage of patients with preoperative and postoperative deficiencies

	Reference range	Baseline		6 mo		12 mo		24 mo		48 mo		60 mo	
		SG	**GBP**	**SG**	**GBP**	**SG**	**GBP**	**SG**	**GBP**	**SG**	**GBP**	**SG**	**GBP**
Total protein	63-80 g/L	5.4	1.3	0	0	3.4	1.9	0	0	4.5	2.8	0	0
Albumin	34-48 g/L	5.4*	0.5	4.3	2.2	0	0	0	0	4.8	0	0	1
Prealbumin	0.200-0.400 g/L	11.8	6	8.7	31.3	14.3	15.8	3.1	5.7	0	3.8	0	3.9
Hemoglobin	137 men/122 women g/L	10*	22	12.1	20.8	11.5	19.9	11.5	17.7	15.8	17.2	14.3	25.5
Ferritin	15-200 ng/mL	8.3	18.6	0	19	6.5	19.8	20.6	30.1	23.8	29.5	0	28.7
Transferrin	2.5-3.8 g/L	2.8	2	0	9	7.1	5.8	9.4	6.8	0	0.9	0	0
Iron	50-170 1-g/dL	30.8	26.5	4.3	18.2	10.3	15.9	9.4	10.6	9.5	12.5	12.5	15.5
Intraerytrocite folic acid	250-1,050 ng/mL	0	1.8	13.6*	0.9	20.7	2.6	6.1	7.5	0	4.9	12.5	7.9
Zinc	59-110 1-g/dL	8.1	11.5	31.8	25.2	39.3	27.5	25	25.8	47.6	26.7	12.5	25.7
Calcium	8.5-10.5 mg/dL	2.9	9.6	0	3.1	3.6	3.5	3.1	5.5	4.8	1.9	12.5	2.9
Magnesium	1.8-2.6 mg/dL	37.8	29.4	12.5	19.4	10.3	14.1	6.3	7.6	4.8	5.7	12.5	5.8
Thiamin	35-91 ng/mL	0	5.5	4.8	8.4	9.1	6.1	25.0*	1.8	0	5.3	0	5.9
Vitamin B-6	15-96 nmol/L	75*	11.3	26.3*	6	11.1	2.8	0	3.9	16.7	8.3	0	4.2
Vitamin B-12	250-1,050 pg/mL	2.7	1.8	3.7	2.9	3.2	6.2	5.9	5.5	0	5.8	12.5	5
Vitamin D		6.7*	9.1	22.7	40.2	22.2	26.7	13.3	12.9	33.3	18.7	0	14.3
Sufficiency	>30 ng/mL	3.3*	30.3	54.5	38.1	40.7	40.8	20	35.6	22.2	30.7	100	38.8
Insufficiency	>10 to <30 ng/mL	90*	60.6	22.7	21.6	37	32.5	66.7	51.5	44.4	50.7	0	46.9
Deficiency	<10 ng/mL												
Parathyroid hormone	10-65 pg/mL	62.5*	44.1	40.9*	11.7	37	16.7	40.6	40.4	57.1	53.8	87.5	56.9

[a]Timeline nutritional evaluations through the study. After surgery, data were collected every 6 months and all observation time-points were included in the analysis; however, for simplicity purposes, only data at 0, 6, 12, 24, 48, and 60 months are shown in this table.

[b]SG=sleeve gastrectomy.

[c]GBP=Roux-en-Y gastric bypass.

*$P<0.05$ in the Fisher's exact test

4.6. References

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PART III: VITAMIN D AND IRON DEFICIENCY MANAGEMENT AFTER BARIATRIC SURGERY

CHAPTER 5: PROSPECTIVE STUDY OF INDIVIDUALIZED OR HIGH FIXED DOSES OF VITAMIN D SUPPLEMENTATION AFTER BARIATRIC SURGERY

This chapter is based on:

Flores L, Moizé V, Ortega E, Rodríguez L, Andreu A, Filella X, Vidal J. Prospective Study of Individualized or High Fixed Doses of Vitamin D Supplementation After Bariatric Surgery

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5. PROSPECTIVE STUDY OF INDIVIDUALIZED OR HIGH FIXED DOSES OF VITAMIN D SUPPLEMENTATION AFTER BARIATRIC SURGERY

5 PROSPECTIVE STUDY OF INDIVIDUALIZED OR HIGH FIXED DOSES OF VITAMIN D SUPPLEMENTATION AFTER BARIATRIC SURGERY

Background: The degree of bariatric surgery (BS) induced vitamin D (VD) malabsorption is not well established. Objective The aim of this study is to evaluate the efficacy and safety of achieving 25-hydroxy VD (25(OH)D) levels ≥75 nmol/L with two regimens of VD supplementation after BS.

Methods: We performed two open-label, prospective studies in patients undergoing BS from 2009 to 2011. Postoperatively, all patients received Ca citrate 1,000 mg and 800 IU of VD_3/ day. In the first study, additional VD_3 was prescribed accord- ing to preoperative 25(OH)D levels—<25 nmol/L:2,800 IU/ day; 26–50 nmol/L:2,000–1,200 IU/day, 51–62 nmol/ L:1,000 IU; >63 nmol/L:0 IU/day—and we evaluated the patients at baseline and at 4 months. In the second study, an additional fixed high dose of 2,000 IU/day of VD_3 was administered, and we evaluated patients at baseline and at 4 and 12 months after BS.

Results: The first study included 176 patients [mean age 44 (11)]; 140 were females. Before BS, 171 subjects (98 %) presented 25(OH)D levels <75 nmol/L. Postoperatively, the mean 25(OH)D levels increased from 40 (17) to 77 nmol/L (29) ($p < 0.001$) with no differences in parathormone (PTH) or 25(OH)D levels between dose groups. In the second study, we enrolled 52 patients [mean age 45 (10)]; 32 were females. Postoperatively, the mean 25(OH)D levels increased from 32 (12) to 80 (22) and to 75 nmol/L (15) ($p<0.001$) at 4 and 12 months, respectively. In both studies, a high percentage of patients achieved 25(OH)D ≥75 nmol/L levels and no subject reported any serious adverse event.

Conclusions: Both schedules of daily VD_3 supplementation were effective and safe under conditions of clinical practice.

5.1 Introduction

Morbidly obese individuals undergoing bariatric surgery (BS) are at high risk of vitamin D (VD) deficiency [1, 2]. Several studies have shown an inverse relationship between the body mass index (BMI) and plasma concentrations of 25 hydroxy VD [25(OH)D] [3, 4]. Consequently, low 25(OH)D levels and compensatory secondary hyperparathyroidism (HPT) are commonly observed in obese patients prior to surgery. Our group and others have confirmed these findings in morbidly obese subjects requesting BS [1,2,4]. In fact, prior to surgery in our series, we reported a high prevalence of HPT of 36 % which was secondary to VD deficiency in 90 % of the cases [1]. On the other hand, the different BS procedures further complicate attempts to achieve adequate plasma VD levels [1]. The restricted caloric intake and the anatomical and physiological changes resulting from these procedures lead to a relative and selective malabsorptive state with diminished ability to absorb nutrients, electrolytes, and bile salts that have been implicated as potential mechanisms which may hinder the obtainment of adequate VD levels. In addition, the degree of BS-induced VD malabsorption following purely restrictive (sleeve gastrectomy), malabsorptive (biliopancreatic diversion), or mixed malabsorptive/restrictive (gastric bypass with different limb length) procedures is not well established. Thus, achieving adequate plasma VD levels is a challenge in the medical management of morbidly obese subjects after BS.

There is emerging consensus that serum 25(OH)D levels from ≥75 to 80 nmol/L are optimal for both bone health and extraskeletal benefits. This consensus is based on the serum parathormone (PTH) concentrations regarded as normal, the yield of maximal calcium absorption from the intestine, and prevention of fractures [5]. However, there are no procedure- specific guidelines on how to achieve this target in patients following BS. In 2008, D and C grade evidence-based consensus guidelines regarding calcium (Ca) and VD evaluation and supplementation to prevent and treat these deficiencies after BS were published. The recommended VD doses for postsurgical supplementation were 800 IU per day together with 1,000 mg of calcium citrate [6]. However, the results of several studies including one by our group showed that in most subjects, these doses are inadequate to achieve the recommended serum 25(OH)D levels [1,3,7]. In 2013, an up- date of these guidelines was published, recommending 1,200 to 1,500 mg of elemental calcium as a citrate supplement and at least 3,000 IU of cholecalciferol/ergocalciferol (VD_3/VD_2) titrated to 25(OH)D >30 ng/mL (75 nmol/L) [8]. Nonetheless, these recommendations continue to be C and D evidence- based. A limited number of small studies have confirmed the clinical utility

of higher doses of VD_3/VD_2 following BS [2,4,9]. Although a large dose will clearly elevate serum 25(OH)D, the degree of elevation, duration, and whether the response is linearly related to dose are un- known. Furthermore, intermittent schedules of high doses of VD, as compared with daily doses, may result in a modification of its metabolism and use. Therefore, the optimal dosage regimens for VD and the length of treatment in an obese population undergoing BS remain uncertain. Taking this background into account, the aims of our study were to evaluate the therapeutic efficacy of the administration of an individualized dose of VD3 according to preoperative 25(OH)D levels and a fixed high dose of VD3 to achieve 25(OH)D levels ≥75 nmol/L in a group of individuals under- going BS.

5.2. Methods

5.2.1. Participants

A cohort of consecutive morbidly obese patients undergoing BS in a tertiary hospital in 2009–2011 were invited to participate in this open-label, prospective study. We used the following inclusion criteria: age between 18 and 65 years, Caucasian, fulfill criteria for BS defined as BMI >40 kg/m2, or 35–40 kg/m2 with major obesity-associated comorbidities and normal renal function. We excluded subjects if they were using medications or had a disease known to influence Ca or bone metabolism, had a history of kidney stones, were taking Ca or multivitamin supplements, had known hypersensitivity to VD3, had undergone previous BS or a bariatric procedure other than laparoscopic gastric bypass (LRYGB) or sleeve gastrectomy (SG), or were lacking data on PTH and 25(OH)D levels at follow-up. LRYGB and SG were performed as previously described [10,11]. SG was mainly performed in individuals with a BMI >50 kg/m2, with a high estimated operative risk, or the presence of an enlarged liver, and the remaining patients underwent LRYGB.

5.2.2. Study protocol

The preoperative information obtained included the following: age, height (cm), weight (kg), BMI (kg/m2), sex, full medication list, medical history, and the percentage of excess body weight (EBW), which was calculated according to the ideal body weight to achieve a BMI of 25 kg/m2. In both studies, we postoperatively measured body weight and calculated the BMI, body weight loss (BWL), and excess body weight loss (EBWL) [11]. Markers for bone metabolism (serum Ca, phosphorous (P), alkaline phosphatase, intact PTH, total protein, albumin, 25(OH)D, and magnesium) and creatinine levels were measured before and 4 months after BS in the first study and before and at 4 and 12 months after BS in the

second study.

The reference range for PTH in our laboratory was 10– 70 pg/mL. HPT was defined as a PTH level ≥70 pg/mL. VD deficiency was defined as 25(OH)D <50 nmol/L, and VD insufficiency was defined as 25(OH)D from 50–74 nmol/L. Normal levels were defined as 25(OH)D ≥75 nmol/L. Safety parameters included hypercalcemia (>10.5 mg/dL limit of reference range), kidney stones, and hypercalciuria (Ca/creatinine ratio in a spot urine sample ≥210 mg/g). Adherence to recommended supplementation was evaluated using direct questions and only patients with a self-reported high level of compliance with the treatment >85 % were analyzed (6/7 days).

Vitamin D Dosing Schedule

We carried out two studies. In the first, we evaluated the effect of an individualized dose according to preoperative 25(OH)D levels at baseline and 4 months after BS, and in the second, we studied the effect of a fixed high dose of VD3 at baseline and at 4 and 12 months postoperatively. We performed the first study from 2009 to 2010, with the individualization being based on the following two assumptions. First, it has been demonstrated that in subjects with normal absorptive capacity, an increase of roughly 2.5 nmol/L (1 ng/mL) in the serum levels of 25(OH)D is observed for every 100 IU of VD taken. Thus, 800 IU would be expected to increase 25(OH)D levels by 20 nmol/L [12]. Second, data from our group have shown that this latter daily dose results in a mean increase of 11.5 nmol/L in 25(OH)D in subjects that have undergone BS. Following BS, the 800 IU per day dose would thereby be associated with a rise of only 58 % in the expected 25(OH)D levels [1]. Therefore, we estimated the daily VD3 dose to achieve 25(OH)D levels ≥75 nmol/L based on an expected 42 % lower increase in the plasma level of the hormone for every 100 IU ingested as compared to nonoperated individuals. Postoperatively, all patients received dietetic advice regarding Ca-rich foods, and calcium citrate (or calcium carbonate in the case of intolerance) 1,000 mg of elemental Ca and 800 IU of VD3 (IDEOS Unidía®) per day plus additional VD3 were prescribed according to preoperative 25(OH)D levels: <25 nmol/L:2,800 IU/day; 26–50:2000 IU/day, 51–62:1,000 IU/day; ≥63 nmol/L:0 IU/day.

We performed the second study in 2011. The dose of VD3 was chosen in order to test the applicability of a single high dose that would be safe and effective to achieve the target level of ≥75 nmol/L. In this study, a group of patients with 25(OH)D <50 nmol/L received

dietetic advice regarding Ca-rich foods and calcium citrate 1,000 mg of elemental Ca and 800 IU of VD3 (IDEOS Unidía®) per day plus a fixed dose of 2,000 IU/day of VD3.

We used a solubilized VD3 for VD supplementation in both studies. This is a watery mixture made of VD3 concentrate in oil, butylhydroxytoluene, 6-palmitoil-L-ascorbic acid, citric acid, mono and diglyceride fatty acids and propylene glycol and corn oil, s.q. which is commercially available (Vitamina D3 Kern Pharma®) in our market in 10-mL bottles with a dropper system with 20,000 IU of VD3 (1 mL = 2,000 IU). The dose was administered in combination with 125 g of unflavored yogurt.

The local Ethical Committee approved the study, and informed consent was obtained from each patient.

5.2.3. Analytical Methods

Serum Ca, P, and creatinine were determined in an ADVIA 1650 analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). Serum 25(OH)D was analyzed by automated competitive chemoluminescent immunoassay (Diasorin Inc., Stillwa- ter, MN, USA), the intra- and interassay coefficients of variation were 10 and 16 %, respectively. The sensitivity of the assay is 4 ng/mL, and reference values in our laboratory are 20–80 ng/mL. The specificity of this assay is 100 % for 25(OH)D3 and 100 % for 25(OH)D2, with negligible cross- reactivity with VD3 and VD2 (<1 %). Intact PTH levels were measured using an automated sandwich-type chemoluminescent immunoassay (ADVIA Centaur XP. Siemens Healthcare Diagnostics, Tarrytown, NY). The intra- and interassay coefficients of variation were 4.5 and 6 %, respectively. The sensitivity of the assay is 2.5 pg/mL, and the reference values of our laboratory are 10–70 pg/mL.

5.2.4. Statistical analysis

Statistical analyses were conducted with STATA version 11 software. Two-side p values <0.05 were considered to indicate statistical significance. Data are described as mean values (SD) or percentages as appropriate. The differences between time points were analyzed using paired Student's t test and repeated measures ANOVA.

5.3. Results

In the first study, we enrolled 176 [mean age 44 (11)] patients undergoing BS during 2009 and 2010. As in most BS series, over two thirds of the study population was composed of females [89 premenopausal (50 %) and 51 (30 %) postmenopausal women, and 36 men (20

%)]. The characteristics of the study population according to the dose groups assigned are summarized in Table 1. Before surgery, secondary HPT was present in 115 subjects (65 %) of the whole cohort, and 25(OH)D levels ≤75 nmol/L were observed in 172 subjects (98 %): deficiency in 126 (72 %) and insufficiency in 46 (26 %). Four months after BS, the prevalence of secondary HPT in the whole cohort was 44 % (n=77), the Ca [from 9.4 (0.5) to 9.7 mg/dL (0.7)] and P [from 3.3 (0.6) to 3.6 mg/dL (0.7)] levels significantly increased but remained within nor- mal values ($p < 0.0001$, both). Among the patients in the different dose groups, there were no differences regarding age, gender distribution, BMI, EBWL, baseline Ca, and surgery type (all, $p > 0.2$). On cholecalciferol treatment, the mean 25(OH)D levels increased from 40 (17) to 77 nmol/L (29) ($p < 0.001$), and the post supplementation 25(OH)D levels were <25, 25–50, 51–74, and ≥75 nmol/L in 0, 26 (15 %), 65 (37 %), and 85 (48 %) patients, respectively. As expected, PTH decreased and 25(OH)D levels increased compared to baseline values. At 4 months, no differences were observed in either PTH or 25(OH)D levels between dose groups (Table 2).

Table 1. Clinical characteristics of the patients in the first study (2009–2010)

	Before BS	**4 months after BS**
n	176	176
Age (years)	44 (11)	
Sex (m/f; n)	36/140	
Body weight (kg)	123 (21)	94 (15)*
BMI (kg/m2)	46 (6)	35 (4)*
EBW (kg)	56 (18)	
BWL (kg)		29 (10)
EBWL (%)	53 (12)	
25(OH)D (nmol/L)	40 (17)	77 (29)*
25(OH)D ≤50 nmol/L (%)	72	15
25(OH)D 51–74 (%)	26	37
PTH (pg/mL)	87 (38)	70 (25)*
Secondary HPT (%)	65	44*
Ca (mg/dL)	9.4 (0.5)	9.7 (0.7)*
P (mg/dL)	3.3 (0.6)	3.6 (0.7)*
LRYGB/SG %	54/46	

Data are expressed as mean (SD). To convert 25(OH)D nanomoles per liter (nmol/L) to nanograms per milliliter (ng/mL), divide by 2.5; to convert Ca milligrams per deciliter (mg/dL) to nanomoles per liter (nmol/L), multiply by 0.25; and to convert PTH picograms per milliliter (pg/mL) to picomoles per liter (pmol/L), divide by 0.105 m/f male/female, BMI body mass index, EBW excess body weight, BWL body weight loss, 25(OH)D 25-hydroxy vitamin D, PTH parathormone, HPT hyperparathyroidism, Ca calcium, P phosphorous, BS bariatric surgery, LRYGB/SG gastric bypass/sleeve gastrectomy

*$p<0.001$

In the second study, we enrolled 52 patients [mean age 45 (10), and BMI of 47 kg/m2 (5)] undergoing BS during 2011 being followed at 4 and 12 months postoperatively. Eighteen were males, 14 were postmenopausal women, 22/52 received LRYGB, and 30/52 SG. After BS, the prevalence of secondary HPT decreased from 71 to 36 % (n=16) and to 17 % (7), at 4 (100 % of the population) and 12 months (79 % of the population), respectively. The mean 25(OH)D levels in- ceased from 32 (11) to 84 (34) and 76 nmol/L (14) ($p < 0.001$) at 4 and 12 months, respectively. At 4 months, the post supplementation 25(OH)D levels were <25, 25–50, 51–74, and ≥75 nmol/L in 0, 2 (4 %), 15 (29 %), and 35 (67 %) patients, respectively, and at 12 months, the post supplementation 25(OH)D levels were 0 at <50, 18 (44 %) at 51–74, and 23 (56 %) at ≥75 nmol/L, respectively (Table 3).

In the two studies, none of the subjects reported any serious adverse event. In study 1, three patients presented mild hypercalcemia <11.0 mg/dL: one in the 1,200–2,000 IU group and 2 in the 2,800 IU group. All were women with baseline Ca values within the upper limit of normality, despite having severe VD deficiency. Hypercalciuria was observed in one patient in the 1,200–2,000 IU group (267 mg/g). The maximum 25(OH)D concentrations achieved in each group were 137, 218, 175, and 156 nmol/L in the 2,800, 1,200–2,000, 1,000, and 0 dose groups. In study 2, two women presented mild hypercalcemia <11.0 mg/dL, and hypercalciuria was observed in two patients: 221 and 296 mg/g.

5.4. Discussion

This study used a longitudinal design aimed at establishing the optimal cholecalciferol dose to be administered after BS. We have demonstrated both the utility of individualized daily doses of cholecalciferol according to the severity of VD deficiency as well as the therapeutic efficacy and safety of a single high dose (2,000 IU) of VD3 supplementation to achieve 25(OH)D levels ≥75 nmol/L in patients undergoing BS. The practical application of our findings allows postoperative prescription of an additional starting dose of a mini- mum of 2,000 IU/day in obese patients with VD deficiency. Moreover, in patients with VD insufficiency, knowledge of preoperative 25(OH)D levels allows determination of the optimal dose of VD3 to be prescribed postoperatively.

In relation to study 1, all the individuals in the different dose groups in our study showed satisfactorily increased 25(OH)D levels at 4 months. This increase largely appeared to be dose-dependent, with a smaller increase with doses of 800 IU and the largest increase with doses of 3,600 IU. These results show that individuals with greater VD deficiency can

safely receive an initial dose of up to 3,600 IU. We did not observe an association in the percentage of patients achieving 25(OH)D concentrations ≥75 nmol/L between gender, preoperative BMI, baseline 25(OH)D levels, type of surgery, and EBWL.

In the last years, several randomized controlled or nonrandomized trials have been undertaken to evaluate different VD doses to attain optimal 25(OH)D levels after BS. Goldner et al. reported that in 56 % (n=9) of the patients receiving daily VD3 supplementation with 800 IU, 25(OH)D levels remained <75 nmol/L at 12 months after gastric bypass and that higher daily doses of 5,000 IU/day (n = 10) were required to achieve adequate, safe 25(OH)D levels in 70 % of the subjects [9]. In the present two studies, the prevalence of VD deficiency decreased at 4 and 12 months after BS. Arguably, noncompliance may have been more prevalent among those who failed to reach the 25(OH)D target levels. Unfortunately, compliance in this study was determined by direct questions, which may or may not be reliable. Nonetheless, according to our results, some patients may need more than the dose proposed in this study or, as was observed at 12 months in the second study, a longer period of evaluation of supplementation may be necessary to achieve the expected response. In addition, three reports in small groups of patients have assessed large intermittent doses of VD in patients after BS. In a study by Stein et al. in which 25(OH)D levels were <62 nmol/L at baseline, the patients received either 50,000 IU of VD2 or 8,000 IU of VD3 weekly for 8 weeks. With >80 % compliance in the two treatment arms, serum 25(OH)D levels increased from 38 to 59 nmol/L in the VD3 group and from 34 to 78 nmol/L in the VD2 group [2]. In another study including GB patients with baseline serum 25(OH)D levels <80 nmol/L, Mahlay et al. administered 50,000 IU of VD2 weekly for 12 weeks followed by 800 IU/day. The 32 patients were followed at 6 months; 17 subjects responded to the intervention (94 % compliance) with 25(OH)D levels increasing from 15.9 to 42.5 ng/mL [4]. Finally, Carlin et al. studied 60 VD-depleted morbidly obese women that were randomly assigned to receive 50,000 IU of VD weekly after GB (group 1; n=30) or no additional VD after GB (group 2; n=30) in addition to daily supplement of 800 IU of VD and 1,500 mg of Ca. At 1 year after GB, VD depletion and mean 25(OH)D levels had significantly improved in group 1 (14 % and 37.8 ng/mL, respectively) compared with group 2 (85 % and 15.2 ng/mL, respectively; $p < 0.001$ for both) [13]. In contrast to our study 1, in these reports, VD doses were not individualized.

In relation to safety, the upper reference limit of 25(OH)D is between 150 and 250 nmol/L, and to achieve these levels, daily doses of 10,000 IU or more are needed over many months [14]. Moreover, the tolerable upper level of daily VD intake recently set by the Institute of Medicine is 4,000 IU [15]. The highest level observed in any individual in the present study was 218 nmol/L at 4 months, which appears to be well within the safety range. We also observed mild hypercalcemia in five patients within the more deficient groups in both studies; all were women with baseline Ca values within the upper limit of normality, despite having severe VD deficiency. We discontinued the supplements in these individuals and initiated a study to unmask primary HPT.

Taking into account the lack of knowledge regarding the safety and efficacy of high daily doses of VD in obese individuals after BS at the start of the study, our approach was prudent using rational doses of VD3 to maintain the patients within a safe margin. In 2013, an update of the guidelines for the Perioperative Nutritional, Metabolic, and Nonsurgical Support of the Bariatric Surgery Patient was published, recommending 1,200 to 1500 mg of elemental Ca as a citrate supplement and at least 3,000 IU of VD titrated to 25(OH)D >30 ng/mL (75 nmol/L). These recommendations are based on the previously mentioned study of Goldner et al. including a small sample size and on the suggestions of the Endocrine Society of Clinical Practice in regard to their position on the evaluation, treatment, and prevention of VD deficiency [5,6,9]. The results of the present prospective studies support the use of the recommended doses of VD to achieve adequate 25(OH)D levels in obese patients undergoing BS, independently of subsequently tailored upward or downward requirements of some patients to finally achieve the target level of 25(OH)D.

The present study has several strengths: the patient cohort represents a realistic clinical model of unselected, consecutively admitted patients to BS; the number of participants included was high; and the cohort design was prospective with easy application in clinical practice. The limitations of this study include no calculation of sample size; the study was designed as a pilot study since at the time of planning, no studies were available to guide the sample size. Another limitation is that it was not a randomized study. Nonetheless, based on previous studies, comparison of our treatment pro- tocol with the standard schedule of 800 IU of VD did not seem ethical because this dose has been shown to be insufficient to achieve target VD levels. Finally, our patients were recruited continuously throughout the year and were not subdivided according to season. Low concentrations of 25(OH)D at baseline may

have been affected by the season, but this should not have affected the response to supplementation. Thus, we consider that the seasonal influence is similar in patients with different supplementation dosages, and no substantial bias of season should be expected in the follow-up evaluation.

5.5. Conclusion

Both schedules of daily VD3 supplementation were effective and safe under clinical practice conditions, demonstrating that a fixed high dose of VD3 is highly effective in patients with VD deficiency. These findings may serve as a basis to develop rational and individualized guidelines for dose adjustments to overcome.

Table 2. Preoperative and postoperative weight variables, PTH, and 25(OH)D according to dose groups in the first study

Pre-BS 25(OH)D (nmol/L)	<25	26–50	51–63	>63
n	38	86	41	11
Additional cholecalciferol dose (IU)	2,800	2,000	1,000	0
Total cholecalciferol dose (IU)	3,600	2,800	1,800	800
Age (years)	46 (11)	44 (10)	44 (12)	36 (13)
Sex (m/f; n)	11/27	13/73	10/31	2/9
BMI pre-BS (kg/m^2)	47 (6)	46 (6)	45 (5)	46 (7)
BMI post-BS (kg/m^2)	36 (5)	35 (4)	34 (4)	34 (6)
EBW (kg)	61 (19)	56 (19)	54 (16)	54 (15)
BWL (kg)	31 (10)	30 (11)	28 (7)	30 (6)
EBWL (%)	53 (13)	53 (11)	53 (11)	60 (20)
Pre-BS 25(OH)D (nmol/L)**	18 (3)	36 (6)	56 (5)	75 (13)
Post-BS 25(OH)D (nmol/L)	74 (29)	76 (28)	79 (31)	87 (30)
Δ25(OH)D (nmol/L)**	56 (30)	39 (28)	23 (29)	12 (34)
Post-BS 25(OH)D ≥75 nmol/L (%)	47	47	46	73
Pre-BS PTH (pg/mL)*	106 (49)	84 (33)	79 (33)	73 (27)
Post-BS PTH (pg/mL)	74 (31)	72 (24)	66 (21)	50 (20)
ΔPTH*	33 (42)	12 (32)	13 (30)	12 (20)
LRYGB/SG (n)	15/23	51/35	23/18	5/6

Postoperatively, all patients received Ca citrate 1,000 mg and 800 IU of cholecalciferol/day. Data are expressed as mean (SD)

BS bariatric surgery, m/f male/female, BMI body mass index, EBW excess body weight, BWL body weight loss, EBWL excess body weight loss, PTH
parathormone, ΔPTH absolute change in PTH levels from baseline to 4 months, Δ25(OH)D absolute change in 25(OH)D levels from baseline to
4 months, LRYGB/SG gastric bypass/sleeve gastrectomy

*$p<0.01$, **$p<0.001$, comparison of patients according to the dose groups

Table 3. Preoperative and postoperative weight variables, PTH, and 25(OH)D in the second study

	Baseline	4 months	12 months
n	52	52	41
Additional cholecalciferol dose (IU)		2,000	2,000
Total cholecalciferol dose (IU)		2,800	2,800
Age (years)	45 (10)	45 (10)	46 (10)
Sex (m/f; n)	18/34	18/34	17/24
BMI (kg/m^2)	47 (5)	35 (4)*	30 (4)*
EBW (kg)	59 (16)		
BWL (kg)		31 (11)	47 (15)
EBWL (%)		54 (13)	80 (18)
25(OH)D (nmol/L)	32 (12)	80 (20)*	75 (15)*
25(OH)D >75 nmol/L (%)		67	56
25(OH)D >50 nmol/L (%)		96	100
PTH (pg/mL)	88 (29)	63 (35)*	52 (23)*
Secondary HPT (%)	71	36*	17*
Calcium (mg/dL)	9.4 (0.4)	9.8 (0.4)*	9.5 (0.4)*
Phosphorous (mg/dL)	3.3 (0.5)	3.6 (0.5)*	3.7 (0.4)*
LRYGB/SG (n)		22/30	17/24

Postoperatively, all patients received Ca citrate 1,000 mg and 800 IU of cholecalciferol/day. Data are expressed as mean (SD)

m/f male/female, BMI body mass index, EBW excess body weight, BWL body weight loss, EBWL excess body weight loss, PTH parathormone, HPT
hyperparathyroidism, LRYGB gastric bypass/sleeve gastrectomy

*$p<0.001$, versus baseline

5.6. References

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CHAPTER 6: INFLAMMATION AND IRON STATUS IN BARIATRIC SURGERY CANDIDATES

This chapter is based on:

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Inflammation and iron status in bariatric surgery candidates

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6. INFLAMMATION AND IRON STATUS IN BARIATRIC SURGERY CANDIDATES

6 INFLAMMATION AND IRON STATUS IN BARIATRIC SURGERY CANDIDATES

Background: Iron homeostasis is disturbed by the systemic inflammation commonly encountered in morbid obesity. However, inflammatory markers have seldom been considered in studies investigating the prevalence of iron deficiency (ID) in bariatric surgery (BS) candidates. The objective of this study was to evaluate the prevalence of ID and anemia with ID in BS candidates, accounting for inflammatory status as measured using high sensitivity C-reactive protein (hs-CRP), and to further characterize indices of iron status in BS candidates with systemic inflammation.

Methods: On the basis of ferritin, hemoglobin, and hs-CRP levels, iron status was categorized in 803 (85%) of 947 consecutive BS candidates. Ferritin <12 ng/mL in females and <15 ng/mL in males irrespective of hs-CRP level was classified as absolute-ID, whereas ferritin between those thresholds and 100 ng/mL was categorized as functional-ID (FID) if hs-CRP 43 mg/L. Anemia was defined as hemoglobin o12 or o13 g/dL in females and males, respectively. Additional iron and hematological indices were assessed in patients with FID.

Results: Prevalence of absolute- and functional-ID was 8.7 and 52.5%, respectively. Anemia was found in 11.2% of the cohort, 80% of which were associated with ID. Among patients with FID, transferrin saturation (T-Sat) o20% was common (70.0%) and associated with larger impairment of hematological indices.

Conclusion: The data show that when hs-CRP as inflammatory marker and ferritin as iron index are considered, impaired iron status could be identified in approximately two thirds of BS candidates. Furthermore, T-Sat <20%, especially along with ferritin <30 ng/mL, appears to be practical cut-offs to identify patients with FID with larger iron status impairment.

6.1 Introduction

Iron deficiency (ID) with or without anemia is amongst the most commonly reported nutritional deficiencies in bariatric surgery (BS) [1–3]. Of note, poor preoperative iron status has been associated with increased likelihood of postsurgical ID [3]. Thus, proper evaluation and management of iron status before BS has been recommended to minimize its worsening after surgery, because the procedure is known to alter iron intake and absorption [2,4,5].

Laboratory classification of iron status in the BS candidate is complicated by the low-grade systemic inflammation frequently present in morbid obesity [6–8]. Iron homeostasis is disturbed in the presence of systemic inflammation [8–11]. However, plasma inflammatory markers have seldom been considered in studies investigating the prevalence of ID in BS candidates [2,11–15]. Circulating ferritin levels, the most commonly reported measurement in the assessment of ID in the field of BS [9], have been found to be elevated in the presence of systemic inflammation [8,10,16]. In this scenario, despite apparently adequate body iron stores, insufficient incorporation of iron into erythroid precursors may occur and has been defined as functional ID [8]. Serum ferritin levels < 12 ng/mL have been established as indicative of absent iron stores irrespective of the presence of inflammation [8]. However, in non- dialyzed patients, a serum ferritin concentration up to 100 ng/mL has been associated with a high likelihood of ID in the presence of inflammation [8].

Against this background, study aims were (1) to evaluate the prevalence of ID, anemia, and anemia with ID in BS candidates when considering high-sensitivity C-reactive protein (hs-CRP) as marker of systemic inflammation, (2) to further characterize indices of iron status in BS candidates with systemic inflammation, and (3) to evaluate the clinical characteristics of BS candidates associated with ID and anemia with ID.

6.2. Methods

6.2.1. Participants and Study protocol

Participants in this cross-sectional study were selected among the 947 BS candidates evaluated at the authors' institution between 2005 and 2009. Eligibility criteria included age 18 years or older. Exclusion criteria included prior BS (n = 43), estimated glomerular filtration rate r30 mL/min/1.72 m2 (n = 7), or lack of availability of hs-CRP, ferritin, or hemoglobin (Hb) determinations obtained at the same time point and determined at the Center for Biological Diagnosis at the authors' institution during presurgical evaluation (n = 115). The local ethics committee approved this study, and written informed consent was obtained

from all participants.

6.2.2. Analytical methods

Presurgical parameters included in the current analysis encompass clinical, anthropometric, biochemical, hematological, and fibrogastroscopic findings. Weight, height, and waist circumference (WC) were measured as previously described [3]. Diagnosis of type 2 diabetes mellitus, hypertension, dyslipidemia, sleep apnea syndrome, metabolic syndrome (MetSd) [17], and tobacco use was based on medical history and laboratory data. Results on the fibrogastroscopy, routinely performed during presurgical evaluation, were reviewed and categorized as positive or negative for the presence of Helicobacter pylori and inflammatory or ulcerative lesions in the esophagus, stomach, or duodenum. High-sensitivity CRP was measured as previously reported, and values 43 mg/L were considered indicative of inflammation [18,19]. Biochemical markers of iron status included ferritin, transferrin saturation (T-Sat), and soluble transferrin receptor (sTfR), and the ratio sTfR/(log ferritin) was calculated [7,8]. Iron, ferritin, and sTfR were measured as previously reported [3,20], and transferrin was measured by immunoturbidimetry. A ferritin <12 ng/mL in females or <15 ng/mL in males was considered indicative of absolute ID irrespective of hs-CRP levels [8]. If hs-CRP 43 mg/L, a ferritin concentration between those thresholds and 100 ng/mL, was considered as functional iron deficiency (FID) [8]. Transferrin saturation (T-Sat) <20%, sTfR 41.8 mg/L, and sTfR/(log ferritin) 41.8 were considered as indicative of ID based on published literature [8,11,21]. Hematological parameters included Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and percentage of hypochromic red cells (%HRC) and were measured using an Advia 2400 analyzer (Siemens, Barcelona, Spain). Anemia was defined as Hb <12 or <14 g/L in females and males, respectively [22]. The MCV, MCH, and %HRC cut-offs indicative of impaired red cell production secondary to iron deficiency were based on published literature [11]. Serum vitamin B12 (vitB12) and folic acid were measured as previously reported [3].

6.2.3. Statistical analysis

All data are expressed as mean ± SD or percentages unless stated otherwise. Differences between groups were evaluated using parametric or nonparametric test as appropriate (chi-squared or Fisher's test for categorical variables, and ANOVA and Bonferroni statistics as post hoc analysis for quantitative variables). Sensitivity, specificity, positive and negative predictive values, and overall accuracy indices were calculated from

cross-tabs. Predictive factors of ID and anemia with ID were ascertained by logistic regression analysis. The SPSS 20.0 statistical package was used, and significance was set at a P value of 0.05.

6.3. Results

The main clinical characteristics of the 803 study participants are shown in Table 1. A hs-CRP < 3 mg/L was found in 96 (12.0%) patients, of whom 8 (8.3%) presented with absolute ID (ferritin <12 or <15 ng/dL, respectively in females and males). Of 707 (88%) patients with hs-CRP > 3 mg/L, a ferritin level below these thresholds was found in 62 (8.8%), and a ferritin level between these cut-offs and 100 ng/dL was found in 423 (59.8%). Thus, on the basis of set criteria, absolute ID was identified in 8.7% of the study participants, but an additional 52.5% of the cohort presented with FID. Comparison of patients with ID or FID combined versus those with normal iron stores showed a greater frequency of ID in females and in individuals who had slightly younger age, a smaller WC, and absence of hypertension, dyslipidemia, sleep apnea syndrome, or MetSd (Table 1). Indeed, logistic regression analysis with gender, age, and diagnosis of MetSd as covariates confirmed these were independent predictors of ID before surgery: female gender: odds ratio (OR) 10.362

Table 1. Clinical and biochemical characteristics of the study participants

	All Patients	ID		Anemia	
		Yes	No	With ID	Without ID
Number	803	493	310	70	20
Gender (% female)	71.4	88.4	44.0*	88.6	40.0†
Age (years)	44.0 ± .4	43.2 ± .5	45.3 ± .6‡	45.7 ± 1.1	49.4 ± 2.6
BMI (kg/m^2)	47.4 ± .2	47.5 ± .3	47.2 ± .4	46.2 ± .7	49.7± 24
WC (cm)	133.3 ± .5	131.2 ± .6	136.3 ± .9*	128.1 ± 1.6	139.8 ± 4.3§
Smoker (%)	25.4	26.7	23.5	19.4	10.0
T2 DM (%)	30.4	28.0	34.5	34.8	50.0
HTN (%)	40.9	35.8	48.9*	47.8	32.7¶
Dyslipidemia (%)	25.3	21.3	31.9‡	27.5	52.6
MetSd (%)	76.1	71.0	84.5*	78.6	90.0
SAHS (%)	23.9	17.9	33.3*	20.3	40.0
H. pylori Þ (%)	47.7	48.1	47.0	43.1	31.3
Altered-FGS (%)	26.2	23.7	30.0	17.1	30.0
GFR (mL/min)	81.5 ± .7	80.3 ± .3	83.5 ±1.3‖	81.3 ± 2.2	79.0 ± 6.2
hs-CRP (mg/L)	12.4 ± .4	13.6 ± .5	10.4 ± .7*	14.2 ± 1.8	20.9 ± 6.5
Ferritin (ng/mL)	98.4 ± 4.0	40.2 ± 1.2	190.9 ± 7.8*	20.4 ± 2.2	154.0 ± 26.7†
Hemoglobin (g/dL)	13.6 ± .5	13.1 ± .6	14.3 ± .8*	11.2 ± .2	12.2 ± 2.8†
T-Sat (%)	18.5 ± .4	15.6 ± .4	23.1 ± .1*	9.4 ± .6	18.0 ± 1.6†
sTfR (mg/L)	1.52 ± .0	1.61 ± .03	1.38 ± .02*	2.03 ± .11	1.36 ± .14†
MCV (fl)	86.9 ± .2	85.8 ± .2	88.6 ± .3*	80.6 ± .7	87.1 ± 1.3†
MCH (pg)	28.8 ± .1	28.3 ± .1	29.7 ± .1*	25.6 ± .3	28.5 ± .5†
%HRC (%)	3.4 ± .3	4.5 ± .4	1.6 ± .1*	13.7 ± 1.8	3.5 ± .9†
Folic acid (ng/mL)	420.8 ± 5.0	437.5 ± 6.8	394.3 ± 6.8*	531.2 ± 23.9	443.0 ± 29.8
Vitamin B12 (pg/mL)	410.5 ± 5.9	399.9 ± 6.5	427.9 ± 11.1‖	387.2 ± 17.9	427.8 ± 51.5

ID ¼ iron deficiency; BMI ¼ body mass index; WC ¼ waist circumference; T2 DM ¼ type 2 diabetes mellitus; HTN ¼ hypertension; MetSd ¼ metabolic syndrome; SAHS ¼ sleep apnea hypopnea syndrome; H. pylori Þ ¼ presence of Helicobacter pylori on fibrogastroscopy; Altered-FGS ¼ presence of eritematous or ulcerative lesions in esophagus, stomach, and/or duodenum on fibrogastroscopy; GFR ¼ glomerular filtration rate; hs-CRP ¼ high-sensitivity C-reactive protein; T-Sat ¼ transferrin saturation; sTfR ¼ soluble transferrin receptor; MCV ¼ mean corpuscular volume; MCH ¼ mean corpuscular hemoglobin; %HRC ¼ percentage hypochromic red cells.

Data are expressed as mean ± SE.
*P <0.001, for the comparison between patients with and without iron deficiency.
†P <0.001, for the comparison between patients with anemia associated with iron deficiency or not.
‡P <0.01, for the comparison between patients with and without iron deficiency.
§P <0.01, for the comparison between patients with anemia associated with iron deficiency or not.
¶P <0.05, for the comparison between patients with anemia associated with iron deficiency or not.
‖P <0.05, for the comparison between patients with and without iron deficiency.

(95% confidence interval (CI) 6.977–15.346), P <.001; age: OR .981 (95% CI .965–.977), P =.019; absence of MetSd: OR .580 (95% CI .379–.888), P =.012. Of note, when the analysis was restricted to females and menopausal status was considered, menopause was associated with lower likelihood of ID (OR .540; 95% CI .307–.9952; P = .033).

Anemia was found in 90 (11.2%) of the cohort and was associated with abnormal ferritin levels in 70 (77.8%) patients (with hs-CRP 43 mg/L in 50%). Among those with anemia not associated with ID, only 2 patients presented with low-vitB12 levels, whereas none presented low folic acid. Thus, based on ferritin, vit B12, and folic acid levels, the micronutrient deficiency associated with anemia could not be identified in 18 (20.0%) patients. Decreased MCV, decreased MCH, or increased %HRC was found in 46%, 76%, and 47%, respectively, of the study patients with anemia and ID, suggesting presence of mixed anemia (anemia of chronic disease plus ID) in the remainder. Of the patients with anemia, ID was associated with female gender, a smaller WC, and absence of hypertension (Table 1). Logistic regression analysis showed female gender as the only independent predictor of presurgical anemia with ID (OR 8.157; 95% CI 2.068–32.184; P <.005). Menopause was not an independent predictor when the analysis was restricted to females although the number of patients was small (OR .240; 95% CI .052–1.100; P = .066).

Next, biochemical markers and hematological indices of iron status were characterized in patients with hs-CRP 43 mg/L and normal vit B12 and folic acid. T-Sat (73.4%), sTfR and sTfR/(log ferritin) (88%), MCV (100%), MCH (100%), and %HRC (65%) were available in most but not all patients in this group. Paired comparison of patients with FID versus patients with ferritin 4100 ng/mL (no-ID) within this group showed T-Sat o20% (FID: 69.4%, no- ID: 39.7%; P <.001), MCH o27 pg (FID: 16.7%, no-ID: 7.1%; P 1⁄4 .002), and sTfR/(log ferritin) 41 (FID: 9.4%, no-ID: .0% ; P < .001) were more commonly associated with FID. As shown in Table 2, overall T-Sat o20% was best in discriminating patients with FID from patients with no ID as defined from ferritin levels. On the other hand, elevated sTfR/(log ferritin) yielded the highest specificity and positive predictive values, but was associated with poor

sensitivity, negative predictive value, and overall accuracy. Within the FID sub-group, a low T-Sat was present in 70.2% and was associated with differences in mean values as well as proportions of patients with altered hematological indices values suggestive of ID (Table 3). As shown in Table 3, ferritin levels in patients with T-Sat <20% were lower compared with patients with normal T-Sat, although ranged from 13 to 99 ng/mL. Elevated sTfR/(log ferritin) was found in 12.7% study patients with FID and T-Sat <20%, but only in 2.7% of those with FID but T-Sat 420%. The concurrence of T-Sat <20% and sTfR/ (log ferritin) >1.8 was associated with even larger impairments of iron indices, and all patients in this group presented with ferritin <30 ng/mL (Table 3).

Table 2. Accuracy of different markers of iron status in identifying patients with functional iron deficiency (ferritin 12 [females] or 15 [males] ng/mL to 100 ng/mL) or no-iron deficiency (ferritin ≻100 ng/mL) in patients with high- sensitivity C-reactive protein ≻3 mg/L

	T-Sat (<20%)	sTfR (>1.8mg/L)	sTFR/(log ferritin) (>1)	MCV (<80fl)	MCH (<27pg)	%HRC (>5%)
Sensitivity (%)	69.4	21.1	9.4	9.2	16.7	13.1
Specificity (%)	60.3	85.4	100	93.5	92.9	91.1
Positive predictive value (%)	75.4	72.7	100	74.4	81.7	72.5
Negative predictive value (%)	52.8	36.9	35.3	35.4	37.2	36.7

T-Sat ¼ transferrin saturation; sTfR ¼ soluble transferrin receptor; sTfR/ (log ferritin) ¼ ratio between transferrin soluble receptor and logarithm of ferritin; MCV ¼ mean corpuscular volume; MCH ¼ mean corpuscular hemoglobin; %HRC ¼ percentage hypochromic red cells.
Cut-off values defining impaired iron status is shown between parentheses for each iron index.

Table 3. Indices of iron status in patients with high-sensitive C-reactive protein ≻3 mg/L and functional iron deficiency (ferritin 12 [females] or 15 [males] ng/mL to 100 ng/mL), according to the presence of abnormalities in T-Sat and the concomitant presence of low T-Sat and elevated sTfR/logF.

		T-Sat		T-Sat< 20%	
		< 20%	>20%	sTfR /logF<1.8	sTfR /logF>1.8
Number		174	74	152	22
hs-CRP	mg/L	14.1 ± .8	10.6 ± .8*	14.2 ± .9	13.6 ± 2.3
Ferritin	ng/mL	37.3 ± 1.9	53.7 ± 2.8†	40.2 ± 2.0	17.2 ± 1.3‡
	(>30 ng/mL)	(51.7%)	(20.3%)†	(44.4%)	(100%)‡
Hb	g/dL	13.2 ± .1	13.9 ± .1†	13.3 ± .1	12.6 ± .1‡
MCV	fL	85.1 ± .3	89.2 ± .4†	85.6 ± .3	81.5 ± 1.0§
	(<80 fl)	(15.5%)	(0%)*	(12.6%)	(36.4%)§
MCH	Pg	28.3 ± .1	29.9 ± .2†	28.4 ± .1	27.2 ± .5§
	(<27 pg)	(22.4%)	(4.1%)¶	(17.9%)	(54.5%)‡
HRC	%	3.4 ±.3	1.5 ± .2†	3.3 ± .3	6.5 ± 1.6‡
	(>5%)	(16.0%)	(2.3%)*	(10.5%)	(58.5%)‡

T-Sat ¼ transferring saturation; sTfR/logF ¼ ratio between transferrin soluble receptor and logarithm of ferritin; hs-CRP ¼ high sensitive C-reactive protein; Hb ¼ hemoglobin; F ¼ female; M ¼ male; MCV ¼ mean corpuscular volume; MCH ¼ mean corpuscular hemoglobin concentration.

Data are expressed as mean :!: SE and proportion of patients with an altered value according to the displayed prespecified criterion.
*P< .01, for the comparison between group T-Sat > 20% versus < 20%.
†P< .001, for the comparison between group T-Sat > 20% versus < 20%.
‡P< .001, for the comparison between group with T-Sat < 20% and sTfR/logF < 1.8 versus T-Sat < 20% and sTfR/logF > 1.8.
§P< .01, for the comparison between group with T-Sat < 20% and sTfR/logF < 1.8 versus T-Sat < 20% and sTfR/logF > 1.8.
¶P< .05, for the comparison between group T-Sat > 20% versus < 20%.

6.4. Discussion

The present data show that when CRP as an inflammatory marker and ferritin as iron index are considered, impaired iron status could be identified in approximately two thirds of BS candidates. The data suggest that assessment of additional iron indices may help to better delineate iron status in patients with elevated CRP. A T-Sat <20% is a pragmatic cut off to identify patients within this group with larger impairment of iron metabolism, with those within this group with ferritin < 30 ng/mL presenting even larger impairment of iron status. Finally, the present data underscore females of reproductive age as a high-risk group for ID and anemia with ID before BS.

A wide range of prevalence of ID has been reported before BS [9,11,12]. Although the association between increasing obesity and systemic inflammation is well established, inflammatory markers such as CRP have seldom been reported in studies evaluating iron status in BS candidates [2,11–15]. In 4 of 6 of those studies, despite being measured, CRP was not considered in the categorization of iron status [2,13–15]. The proportion of study participants with elevated CRP was reported neither in the study by Muñoz et al. [11] nor in that by Salgado et al. [12]. In both studies, ID was defined as ferritin < 30 ng/mL in the absence of elevated CRP and FID as T-Sat o20% in the presence of inflammation regardless of ferritin level. Muñoz et al. identified ID and FID in 10.3% and 20.7%, respectively, BS candidates (n 1⁄4 67) [11]. On the other hand, either ID or FID was present in 20% of the study participants (n 1⁄4 102) in the study by Salgado et al. [12]. The present finding of elevated hs-CRP in most of the present larger cohort underscores the importance of considering inflammatory markers when evaluating iron status in BS candidates [9,10]. Differences in criteria used may help explain differences in the prevalence of ID and FID in the present study compared with those discussed above. Despite using stricter ferritin criteria for ID, up to 8.7% patients in the present study presented with absolute ID. The initial assessment of iron status was based on ferritin levels, because this has become the standard test for assessing iron stores and has been the iron index most commonly used in the literature on BS candidates [8,9]. On the basis of accepted ferritin criteria [8], prevalence of ID in the present cohort falls in the upper limit of the range used in previous studies in BS candidates

[9]. Nonetheless, as discussed below, other iron markers were analyzed in patients with FID rather than absolute ID.

The present findings from additional indices of bodily iron depletion indicate low T-Sat and elevated sTfR/(log ferritin) is more common in patients with ferritin <100 ng/mL compared with those with ferritin > 100 ng/mL. This supports the notion of impaired iron metabolism in those with seemingly normal ferritin levels in the presence of inflammation in the present population. Although T-Sat has been used as an alternative to ferritin in the identification of patients with FID, T-Sat values are also affected by inflammation [8,10,23]. Alternatively, sTfR, which is less affected than ferritin and T-Sat by the inflammatory response, has been considered as a useful clinical marker to assess iron status in inflamed individuals [23]. Nonetheless, cost, availability, and limited external quality assessment issues have so far precluded wider use of this index in this population [8]. Of note, in patients with elevated hs- CRP in the present study, presence of low T-Sat especially along with elevated sTfR/(log ferritin) was associated with higher frequency of hematological abnormalities indicative of impaired red cell production secondary to iron deficiency. Thus, impairment of these biochemical markers within this population identified a subgroup of patients at a more advanced stage of ID [10]. Interestingly, a ferritin < 30 ng/mL was encountered in 100% of patients with concurrent impairment of T-Sat and sTfR/(log ferritin). Thus, the present data suggest that T-Sat and ferritin would suffice as parameters in routine clinical practice to identify patients with more advanced impairment of iron stores in the presence of inflammation.

The high risk for ID and anemia with ID before BS associated with female gender, especially of reproductive age, was not unexpected [8]. To the authors' knowledge, previous studies in the field have not analyzed a set of clinical factors associated with ID or anemia with ID as broad as reported herein [8]. Interestingly, the present analyses excluded gastrointestinal blood losses as independent determinant of ID in BS candidates. It has been proposed that inadequate micronutrient intake, because of poor dietary habits or as a result of reduced energy diets in which iron is typically a limiting nutrient, could be implicated in ID in BS candidates [1,2,4]. Unfortunately, iron intake was not assessed in the present study. Nonetheless, insufficient compensation of menstrual blood losses because of impaired iron absorption and mobilization of iron stores resulting from the inflammatory state associated with obesity could also play a role in the female participants. Absence of the MetSd was

independently associated with the presence of ID in the present cohort. Again this was not unanticipated, because elevated serum ferritin levels suggestive of dysregulated iron homeostasis have been shown previously in patients with the MetSd [24]. Impaired iron metabolism in patients with the MetSd has been linked to low-grade systemic inflammation and increased production of hepcidin, a key homeostatic regulation of systemic iron metabolism [16,17]. Of note, the relationship between absence of the MetSd and ID in the present cohort was maintained when hs-CRP was considered in the model (data not shown).

The present study has several limitations and strengths. Admittedly, hepcidin, was not assessed in the present cohort [10]. Moreover, hs-CRP was assessed at a single time point, and thus, the assessment of systemic inflammation could have been influenced by biological variability or preanalytical factors influencing hs-CRP plasma levels [25].

Nonetheless, the present study represents the largest cohort reported so far pondering the role of inflammation, a factor known to increase hepcidin production, on iron status in BS candidates. Unfortunately, a complete data set of all the evaluated indices of iron metabolism was available in most but not all patients in this study. The authors acknowledge that other factors potentially influencing iron balance, such as dietary iron intake and the use of proton pump inhibitors, oral contraceptives, or iron supplements, were not evaluated. However, unlike previous studies in the field, the influence of co-morbidities and of upper-gastrointestinal tract lesions potentially associated with blood losses was assessed [9].

6.5. Conclusion

The present data show inflammation and impaired iron status is commonly observed in BS candidates. The data suggest that female gender and reproductive age are independently associated with ID and anemia with ID before surgery. Furthermore, the data suggest that T-Sat < 20%, especially along with ferritin < 30 ng/mL, could be a practical cut-off for the identification of BS candidates with systemic inflammation and larger impairment of iron metabolism. Whether the latter group of patients should be a specific therapeutic target for the prevention of ID after BS warrants further investigation.

6.6. References

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PART IV: EFFECT OF PROTEIN STATUS AFTER BARIATRIC SURGERY

CHAPTER 7: PROTEIN INTAKE, BODY COMPOSITION, AND PROTEIN STATUS FOLLOWING BARIATRIC SURGERY

This chapter is based on:

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Protein Intake, Body Composition, and Protein Status Following Bariatric Surgery.

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7. PROTEIN INTAKE, BODY COMPOSITION, AND PROTEIN STATUS FOLLOWING BARIATRIC SURGERY

Background: Daily protein intake recommendations have recently been proposed for the bariatric patient. We aimed to evaluate the accomplishment of these recommendations, and the influence of protein intake (PI) on fat free mass (FFM) and protein status changes following bariatric surgery.

Methods: We examined 101 consecutive patients undergoing laparoscopic Roux-in-Y gastric gypass (GBP) or laparoscopic sleeve gastrectomy (LSG). Based on 3-day food records, PI from food and supplements were quantified at 4, 8, and 12 months after surgery. The association between PI and body composition (bioelectrical impedance), plasma albumin and pre-albumin was evaluated at all study time points.

Results: A PI <60 g/day was present respectively in 45%, 35%, and 37% of the cohort at 4, 8, and 12 months after surgery ($p<0.001$ relative to baseline). Despite our universal recommendation of protein supplementation, supplements were taken only by 63.4, 50.5, and 33.7% of the participants at 4, 8, and 12 months. However, protein supplementation was effective in helping patients to achieve the daily protein intake goal. In linear regression analysis, male gender and weight loss, but not PI, were significantly associated with loss of FFM ($p < 0.001$). No significant correlation between PI and plasma albumin or pre-albumin was found.

Conclusions: Our study underscores the value of protein supplementation for the achievement of the recommended daily protein intake in the bariatric patient. However, our data does not help to define a PI goal as critical in determining the FFM and protein status changes following GBP or LSG.

7.1 Introduction

Bariatric surgery is currently the most successful weight loss strategy for morbid obesity [1]. Despite the goal of weight loss in morbid obesity being loss of fat mass (FM), weight loss interventions are systematically accompanied by some degree of fat free mass (FFM) loss [2]. Importantly, not only a greater caloric restriction, but also certain types of bariatric surgery techniques such as Roux-in-Y Gastric Bypass (GBP) and Bilopancreatic Diversion (BPD) are accompanied by a larger loss of FFM [2]. In contrast, exercise [2], and a higher protein intake [3] have been demonstrated to influence body composition changes following weight loss in a way that spares FFM. Likewise, a higher protein intake has been shown to positively influence plasma protein status following GBP [4].

The current guidelines for nutritional support of the bariatric surgery patient state that protein intake with meals, including supplementation, should be in the range of 60– 120 g/day [5]. Specifically for those with GBP, a daily protein intake ≥60 g is recommended. Nonetheless, this recommendation is not without limitations. First, as acknowledged by the guidelines writing committee, there is no conclusive evidence to support this recommendation [5]. Second, protein intakes in the range of 70–100 g/day or 0.8–1.5 g/kg ideal body weight (IBW)/day are typically advised in forms of caloric restriction comparable to that after bariatric surgery such as in the very low calorie diets (VLCD) [6]. Importantly, VLCD are designed to produce rapid weight loss while preserving lean body mass [2, 6]. Third, to our knowledge there is no data on the impact of supplementation on daily protein intake after GBP. Finally, it should be taken into account that no matter the daily protein intake goal, protein-deficient meals are common after GBP. This phenomenon has been largely attributed to the common development of intolerance to protein-rich foods occurring more often in the first year after surgery [7, 8]. Against this background, the purpose of the present study was to examine the accomplishment of the recommended protein intake, and the influence of protein intake on FFM and protein status following bariatric surgery.

7.2. Methods

7.2.1. Participants

During the period October 2006 to April 2007, 101 consecutive patients scheduled to undergo laparoscopic Roux-in-Y gastric bypass [9] or laparoscopic sleeve gastrectomy (LSG) [9], and without evidence of clinical edema, were recruited to participate.

7.2.2. Study protocol

This is an observational study. Indications for surgery followed the National Institutes of Health criteria, i.e., a body mass index (BMI) >40 kg/m2 or a BMI >35 kg/ m2 with obesity-related morbidity [5]. All patients signed informed consent before surgery, and our institution ethics committee approved the protocol.

7.2.2.1. Protein intake counseling

Prior to surgery, all subjects attended group and individual sessions including nutritional counseling according to the current guidelines for the bariatric patient [5]. Dietary advice was systematically given to the patients during hospitalization, and at 2 and 6 weeks after surgery, and then at 4, 8, and 12 months, to sustain a hypocaloric and protein- rich diet. Following hospital discharge, patients were universally advised to supplement dietary protein with 15 g of protein powder (Resource Protein, Nestlé, Vevey, Switzerland). Based on 3-day dietary food records, protein intake from food and supplements was quantified at each follow-up visit. Patients were categorized based on the accomplishment of two daily protein intake goals: ≥60 g/ day or ≥1.2 g/kg IBW/day. The ≥1.2 g/kg IBW/day goal was based on the recommendations in VLCD [5, 9]. At each study visit, dietary and supplementation protein were advised to achieve the daily protein intake goals.

7.2.2.2. Body composition analysis

Anthropometry and body composition were evaluated prior to surgery, and a 4, 8, and 12 months after surgery. Patients were weighed and body composition was obtained using bioelectrical impedance analysis (BIA; Tanita BC-418 MA, Body Composition Analyzer). Patients were evaluated after an overnight fast, and according to the specifications from the manufacturer. BIA is based on the fact that lean tissue has more electrolyte and water content than fat. This difference in electrolyte content permits an estimate of fat free mass (FFM) measuring the amount of electricity that flows through the body from the source, to the sink electrodes. FFM estimates obtained from the body composition analysis, were used to derive total fat mass (FM), and the percent of body weight (BW) loss consisting of FFM at any particular study time point [$100^{*}(FFMt_t - FFMt_0)/(BWt_t - BWt_0)$].

7.2.2.3. Biochemistry

Prior to surgery, and a 4, 8, and 12 months thereafter, blood samples were drawn following an overnight fast for the determination of albumin, prealbumin, and total leukocyte count were measured using an Advia 2400 analyzer (Bayer Diagnostics, Tarrytown, NY, USA), high-sensitive C-reactive protein (hs-CRP) was determined by inmunonefelometry

(Boehring Nephelometer analyzer; Dade Boehring, Marburg, Germany). Based on plasma protein levels, subjects were categorized as hypoalbuminemic (<35 g/L), or with mild pre-albumin deficiency (<0.170 g/L) [10].

7.2.3. Statistical analysis

Values are given as mean±SEM unless otherwise specified. Paired or unpaired non-parametric tests were used for comparisons between groups. Partial correlation analysis was used to evaluate the association between variables, while controlling for the pre-surgical BMI was performed. A one-way between-groups analysis of covariance was used to compare FFM loss between groups while adjusting for the pre-surgical BMI. P values<0.05 were considered significant. For linear regression analysis, variables were Ln-transformed. Data were analyzed using the Statistical Package for Social Science (SPSS 16.0; SPSS Inc, Chicago, IL, USA).

7.3. Results

The study cohort was composed by 76 females and 25 males with an age of 43.2 ± 1.0 years, and a pre-surgical BMI of 47.7±0.7 kg/m^2. GBP and LSG were performed respectively in 66.3% and 33.7% of the cohort. The retention rate in the study was 96% or greater at all study time points.

7.3.1. Daily caloric and protein intake

Data on daily caloric intake and protein intake are shown in Table 1. Total daily caloric intake was markedly reduced at 4 months after surgery and gradually increased over time. Similarly, daily protein intake was markedly reduced at 4, 8, and 12 months when compared to the pre-surgical situation ($p < 0.001$). Despite our universal recommendation of protein supplementation, only 63.4, 50.5, and 33.7% of the study participants at 4, 8, and 12 months took supplements, respectively. The amount of protein from supplements varied widely among individuals (2.5–40.0 g/ day). At 4, 8, and 12 months protein from supplements represented respectively 15.6 ± 3.1, 13.9 ± 0.9, and 12.2 ± 0.9% of the daily total protein intake.

At baseline, 5% and 32% of the study cohort reported, respectively, <60 g/day and <1.2 g/kg IBW/day of total protein consumption. The percentage of subjects consuming <60 g of protein per day was 45%, 35%, and 37%, respectively, at 4, 8, and 12 months after surgery ($p < 0.001$ for all comparisons relative to baseline). Likewise, the percentage of subjects consuming <1.2 g/kg IBW per day was 87%, 75%, and 68%, respectively, at 4, 8, and 12

months after surgery ($p < 0.001$ for all comparisons relative to baseline). No differences were found when the two types of surgeries were compared. As shown in Fig. 1 (panel A and B), protein supplementation was effective in helping patients to achieve the daily protein intake goals. At all post-surgical study points, a daily protein intake of ≥60 g/ day (Fig. 1, panel A) and ≥1.2 g/kg IBW/day (Fig. 1, panel B) was attained significantly by a larger proportion of subjects consuming protein supplements when compared to those not adhering to the recommendation.

Tabla 1. Reported daily caloric and macronutrient intake data

	Pre-surgery	4 months	8 months	12 months
Caloric intake (kcal/d)	2,401±97	930±29[a]	1214±39[a]	1,418±42[a]
Carbohydrates (%)	38.5±0.8	38.8±1.1	38.0±0.9	37.1±0.9
Lipids (%)	44.0±0.8	36.2±1.2[a]	39.0±1.0[a]	42.1±0.9[a]
Proteins (%)	17.5±0.5	25.0±0.7[a]	23.1±0.6[a]	20.7±0.6[a]
Proteins (g/day)	97.9±3.5	56.9±1.9[a]	67.2±1.8[a]	71.3±2.3[a]
Proteins (g/IBW/d)	1.48±0.05	0.86±0.03[a]	1.01±0.26[a]	1.08±0.04[a]

IBW ideal body weight data are expressed as mean ± SEM [a]$p < 0.001$

7.3.2. Body composition

Anthropometric and body composition data are shown in Table 2. On average, at 4, 8, and 12 months follow-up, patients had lost 38.2%, 57.5%, and 64.5%, respectively, of their pre-surgical excess body mass index. At all study time points, the majority of weight loss corresponded to loss of FM (4 months, 73.2 ± 8.9%; 8 months, 77.3 ± 7.1%; 12 months, 77.1±8.4%). FFM loss as a percent of total body weight loss was larger at 4 months (26.3 ± 0.8%) compared to 8 months (22.2 ± 0.6%; $p < 0.001$), and 12 months (22.1 ± 0.8%; $p < 0.001$). A large inter individual variability in the amount of FFM loss relative to the total weight loss was observed (4 months, 6.9–56.8%, 8 months, 2.2–33.1%, 12 months, 3.3–51.5%). On partial correlation analysis, FFM loss was significantly correlated with total body weight loss at all study time points independently of the initial BMI (4 months, rho = 0.508, $p < 0.001$; 8 months, rho = 0.711, $p < 0.001$; 12 months, rho = 0.644, $p < 0.001$).

We did not find a significant correlation between the protein intake (expressed as grams of ingested protein per kilogram of ideal body weight) and FFM loss relative to total weight loss even when controlling for the pre-surgical BMI (4 months, rho =−0.06, $p = 0.546$; 8 months, rho = 0.07, $p = 0.506$, 12 months, rho = 0.02, $p = 0.836$). As shown in Table 3, using one-way ANCOVA analysis, the FFM loss relative to total weight loss adjusted for the

pre-surgical BMI was not significantly different at any time point when subjects achieving or not the 60 g/day and 1.2 g/kg IBW/ day protein intake goals were compared. Similarly, we did not find a significant correlation between protein intake and FFM loss relative to the pre-surgical FFM. The type of bariatric surgery did not influence the observed results.

Male gender was associated with a greater proportion of weight loss as FFM (4 months, male 28.9 ± 2.1%, female 23.8 ± 1.2%, $p < 0.05$; 8 months, male 26.3 ± 1.1%, female 20.9 ± 0.6%, $p < 0.001$; 12 months, male 26.7 ± 1.4%, female 20.6 ± 0.8%, $p < 0.001$). Moreover, male gender and weight loss were the only factors significantly associated with loss of FFM ($p < 0.001$) at all study time points in a linear regression analysis including also pre-surgical BMI, type of surgery, caloric intake, and protein intake as independent variables. This was despite daily protein intake adjusted per IBW being similar between the two gender groups (4 months, male 0.87±0.28 g, female 0.85±0.29 g, $p= 0.767$; 8 months, male 1.05 ± 0.29 g, female 1.00 ± 0.25 g (<60 g/day 31.5 ± 1.4%, ≥60 g/day 25.1 ± 1.8%; $p < 0.05$) but not <1.2 g/ideal body weight ($p = 0.614$) was associated with a larger FFM loss at 4 months after surgery. Significant associations were not found between protein intake and FFM loss at any other time points neither in males nor in females.

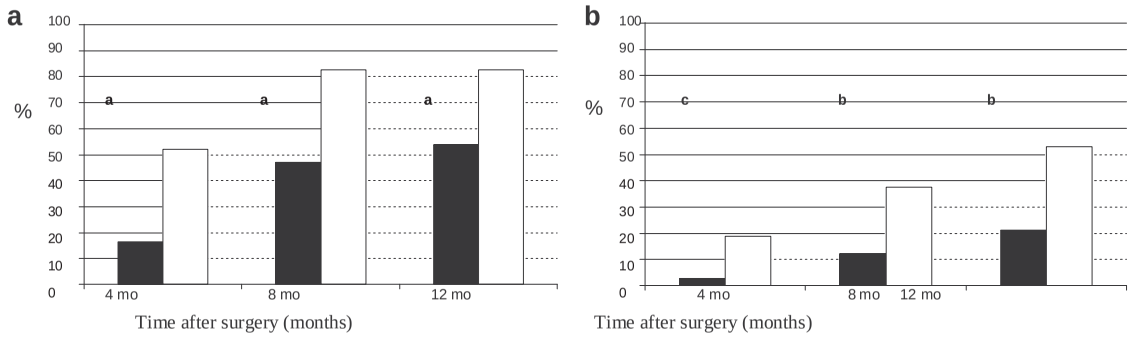

Fig. 1 Percentage of patients achieving a protein intake ≥60 g/day (a) or ≥1.2 g/IBW/day (b) throughout the study according to their adherence to protein supplementation. IBW ideal body weight. Black bars patients not adhering to protein supplementation. White bars patients adhering to protein supplementation. a $p<0.001$, b $p<0.005$, c $p<0.05$

7.3.3. Biochemistry

As shown in Table 4, serum albumin and pre-albumin levels tended to decrease over time. However, no study participant presented with an albumin plasma concentration below the 35g / L threshold. In contrast, 39%, 22%, and18% of the study subjects presented pre-albumin plasma levels below normal (0.170 g/L) at 4, 8, and 12 months, respectively. Likewise, neither the plasma albumin or pre- albumin, nor the changes in these parameters relative to baseline were significantly different when subjects achieving the 60 g/day or the 1.2 g protein/IBW/day were compared. CRP and the total leukocyte count also decreased over time (Table 4, $p < 0.001$). CRP and prealbumin levels were significantly inversely correlated at 4 months after surgery (rho = −0.233, p =0.023). A tendency toward statistical significance between the two parameters was found at 8 months (rho =−0.181, p = 0.075), but not at 12 months (p = 0.564). Albumin plasma concentration was not significantly correlated with CRP or the leukocyte count. Inflammatory parameters were not significantly associated with the changes in FFM at any study time point.

Tabla 2. Anthropometric and body composition data

	Pre-surgery	4 months	8 months	12 months
Body weight (kg)	127.1±2.0	102.0±1.7[a]	89.0±1.5[a]	83.9±1.5[a]
BMI (kg/m^2)	47.7±0.7	38.3±0.6[a]	33.4±0.5[a]	31.6±0.5[a]
Fat mass (%)	48.3±0.6	41.7±0.7[a]	32.0±0.9[a]	33.0±0.7[a]
Fat mass(kg)	61.3±1.2	42.8±1.1[a]	32.0±0.9[a]	27.9±0.9[a]
Fat free mass (kg)	65.8±1.3	59.2±1.1[a]	57.0±1.1[a]	56.0±1.1[a]
Total body water (kg)	48.0±0.9	43.3±.0.8[a]	41.8±0.8[a]	41.0±0.8[a]

Data are expressed as mean ± SEM [a]$p < 0.001$

7.4. Discussion

The data from our observational study show that protein intake is below the current recommendations (≥60 g/day) in a high proportion of subjects undergoing GBP or LSG, and that protein supplementation facilitates achieving the proposed protein intake goals. Although achieving a daily protein intake ≥60 g may be beneficial in some subgroup of bariatric surgery patients, our data does not support this protein target as a strong determinant of FFM loss following bariatric surgery. Finally, our data show that systemic inflammation rather than protein intake is a determinant of the changes in plasma protein

status in the early stages after bariatric surgery.

A low protein intake following GBP surgery has previously been described. Bavaresco et al. reported a mean total daily protein ranging from 37.1 ± 20.0 g during the third month to 46.6 ± 20.0 g/day at 12 months after surgery [11]. Moizé et al. reported a protein intake (g/day) of 45.6 ± 14.2 at 3 months and 58.5 ± 17.1 at 12 months after gastric bypass [7]. Daily protein intake in our study participants was slightly larger. However, this could be attributed at least in part to our universal recommendation of protein supplementation. In fact, in our cohort average protein intake in those not taking supplements was respectively 45.4 and 64.7 g/day at 4 and 12 months after surgery. Although a daily protein intake ≥60 g is currently recommended for bariatric patients [5], over one third of our study participants did not meet this goal. Previous studies on the bariatric patient, have not analyzed the proportion of subjects fulfilling this specific protein intake target [7, 11]. Insufficient protein consumption, possibly mediated by protein intolerance has been advocated as potential mechanism for the low protein intake following GBP [7]. Of note, in our study protein supplementation was useful to achieve the protein intake goal. Protein supplementation was recommended neither in Bavaresco's nor in Moizé's studies. In fact, Brolyn et al. [12] survey showed that protein supplements were routinely prescribed in 21% of BPD patients but not after GBP. Despite protein supplements being universally recommended in our study, data also showed a poor compliance with protein supplementation. Therefore, in the bariatric patient efforts should be made not only to universalize protein supplementation but also to increase the adherence to protein supplements in order to achieve a daily protein intake ≥60 g.

Loss of FFM may be undesirable if excessive as non- adipose tissues are responsible for the majority of resting metabolic rate and maintenance of function as the body ages. As shown with other weight loss interventions [2, 13], we found a large inter-individual FFM loss variability following surgery. We aimed to evaluate the relative contribution of protein intake on that variance in FFM loss. It has been proposed that following energy restricted diets, lesser FFM loss as a percentage of total weight loss is achieved with larger daily protein intakes [3, 14, 15]. In contrast to these findings, none of the protein intake estimates (total daily protein intake, daily protein intake/ IBW, protein intake above or below 60 g/day or 1.2 g/IBW/ day) was significantly correlated with FFM loss relative to total weight loss when all patients in our cohort were combined.

In fact, only in males a protein intake of <60 g/ day was significantly associated with a larger FFM loss at 4 months after surgery. A larger average FFM loss in males compared to females following different type of weight loss interventions has previously been reported [2, 16]. To our knowledge, no previous study has looked at the impact of protein intake on the changes in body composition following GBP or LSG. Contradictory findings among different studies on the effects of protein intake on body composition may relate to the small sample sizes, different durations of follow-up, and differing methods of assessing body composition. Nonetheless, it should be taken into account that the factors influencing body composition changes associated with weight loss may differ depending on the weight-loss strategy [2].

Several mechanisms, including insufficient protein in- take [4], may potentially lead to protein deficiency after bariatric surgery [17]. As shown by others [18, 19], in our cohort albumin and prealbumin levels decreased after bariatric surgery compared to the presurgical period. No individual in our cohort presented with a plasma albumin level <35 g/L. Despite deficits in albumin have been reported in as much as 13% of patients following the distal GBP, albumin deficiency has seldom been reported when the limb length does not exceed 150 cm as in our study [17–19]. The proportion of subjects in our cohort with plasma prealbumin <0.170 g/L varied between 18 and 39%. This is larger than previously reported by Coupaye et al. [19]. Nonetheless, in that study a prealbumin cut-off level of 0.110 g/L was considered. In fact, only 1% of our study cohort presented with prealbumin <0.110 g/L. We did not find a significant relationship between protein status and protein intake. This is in contrast with data reported by Rinaldi Schinkel et al. [4], who found a positive linear relationship between protein intake and serum albumin in patients undergoing GBP. However, subjects in that study were selected on the basis of the presence of postoperative complications requiring nutrition support services. Our finding on the significant correlation between CRP and pre-albumin levels at 4 months after surgery is in agreement with systemic inflammation being a determinant of pre-albumin plasma levels after surgery [10].

It has been shown that diversionary surgery results in greater FFM loss than purely restrictive bariatric surgery procedures such as adjustable gastric banding, independently of initial BMI and the magnitude of weight loss [2]. In our study, body composition and protein status changes were comparable between GBP and LSG.

Likewise, the type of surgical procedure was not significant in the linear regression analysis on the factors potentially influencing FFM loss in our bariatric surgery cohort. This finding could be interpreted in the context of the several similarities between GBP and LSG that support that the latter should not be considered as a purely restrictive procedure [9, 20].

We acknowledge that our study has several limitations. First, a small sample size and the observational nature may have underpowered our study to demonstrate the relationship between FFM loss and protein intake. Nonetheless, to our knowledge, no previous study has attempted to evaluate the effects of protein intake on the preservation of FFM following bariatric surgery. Second, we used BIA for body composition analysis. BIA represents a simple, inexpensive, non-invasive, and attractive method for repeated measurements of body composition assessment. However, BIA is not without limitations especially in the extremely obese population [2]. Although some authors have proposed the validity of BIA in the assessment of body composition after bariatric surgery [14, 21], future studies evaluating the importance of protein intake on FFM loss should be based on more accurate methods of body composition analysis. Second, we did not estimate physical activity. Data from rigorously supervised resistance and aerobic exercise programs has shown decreases in the loss of FFM attributable to physical activity [22, 23]. Even so, neither reported physical activity nor total or activity energy expenditure measured by doubly labeled water technique have been shown to be associated with body composition changes following GBP [14].

Table 3. Changes in fat fee mass according to daily protein intake goals

	FFM loss relative to body weight loss (%)		
	4 months	8 months	12 months
Protein intake			
<60 g/day	24.6 ± 1.4	25.7 ± 1.6	25.3 ± 1.1
≥60 g/day	21.3 ± 1.0	22.7 ± 0.7	21.9 ± 6.2
<1.2 g/IBW/day	22.1 ± 1.2	22.2 ± 1.0	22.0 ± 0.9
≥_1.2 g/IBW/day	24.1 ± 2.9	23.4 ± 1.2	22.4 ± 1.3

Data are expressed as mean ± SEM
FFM Fat free mass, IBW ideal body weight

7.5. Conclusion

Our study underscores the value of protein supplementation for the achievement of the currently recommended daily protein intake in the bariatric patient. However, our data does not help to define a protein intake goal as important in determining the body composition and protein status changes following GBP or LSG. Future studies with a larger sample size, and using more accurate methods of body composition assessment are warranted to better establish this concept.

Table 4. Protein status and inflammatory parameters

	Pre-surgery	4 months	8 months	12 months
Albumin (g/L)	45.0 ± 2.2	44.6 ± 2.5[a]	44.1 ± 2.4[b]	43.7 ± 2.3[b]
Prealbumin (g/L)	0.233 ± 0.058	0.187 ± 0.044[b]	0.196 ± 0.050[b]	0.209 ± 0.456[b]
hs-CRP (mg/dl)	1.29 ± 0.13	0.89 ± 0.10	0.60 ± 0.14	0.26 ± 0.04
Leukocytes (×1012/L)	8.41 ± 0.21	7.25 ± 0.21	7.25 ± 0.21	7.01 ± 0.25

Data are expressed as mean±SEM hs-CRP high sensitive C-reactive protein [a] $p<0.05$, [b] $p<0.001$

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CHAPTER 8: PROTEIN INTAKE AND LEAN TISSUE MASS RETENTION FOLLOWING BARIATRIC SURGERY

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8. PROTEIN INTAKE AND LEAN TISSUE MASS RETENTION FOLLOWING BARIATRIC SURGERY.

Background: Background & aim: Since current protein intake (PI) recommendations for the bariatric surgery (BS) patient are not supported by conclusive evidence, we aimed to evaluate the relationship between PI and lean tissue mass (LTM) loss following BS.

Methods: Observational study including patients undergoing gastric bypass (GBP; n = 25) or sleeve gastrectomy (SG; n = 25). Dietary advice and daily PI were assessed prior to, and at 2- and 6-weeks, 4-, 8-, and 12-months after surgery. Body composition was assessed by dual energy X-ray absorptiometry (DXA). LTM losses as percent of weight loss (%LTM loss) at 4- and 12-months after surgery were the main outcome variables.

Results: A PI ≥ 60 g/d was associated with lower %LTM loss at 4- ($p = 0.030$) and 12-months ($p = 0.013$). Similar results were obtained when a PI ≥ 1.1 g/kg of ideal body weight (IBW)/d was considered. Multilinear regression showed the only independent predictor of %LTM loss at 4-months was PI (expressed as g/kg IBW/d) (OR: -0.376, $p = 0.017$), whereas PI (OR: -0.468, $p = 0.001$) and surgical technique (OR:0.399, $p = 0.006$) predicted 12-months %LTM loss.

Conclusions: Our data provide supportive evidence for the PI goals of >60 g/d or 1.1 g/kg IBW/d as a being associated with better LTM preservation in the BS patient.

8.1. Introduction

Weight management strategies aim at reducing body fat mass (FM) while minimizing reductions in lean tissue mass (LTM). Although bariatric surgery (BS) is the most effective strategy to achieve and sustain significant weight loss in morbidly obese subjects[1], there is concern that BS may be accompanied by greater LTM loss compared to other types of weight loss interventions[2]. Systematic review of the limited available data in the literature has shown that post-surgical loss of LTM varies among different BS techniques[2]. In that respect, Roux-en-Y Gastric Bypass (GBP) has been associated with greater LTM loss as compared to adjustable gastric banding (AGB) independently of the magnitude of weight loss.

Current medical guidelines for the nutritional support of the BS patient recommend an average daily protein intake (PI) of 60-120 g in subjects undergoing GBP, and 60-80 g or 1.1 g/kg of ideal body weight (IBW) following sleeve gastrectomy (SG) to reduce an undesirable post-surgical excessive loss of LTM[3-5]. Nonetheless these recommendations are not without limitations. First, as acknowledged by the writing committees, there is no conclusive evidence[3-5]. Second, as we have previously shown, despite universal recommendation of protein supplementation a large proportion of BS patients fail to attain daily protein allowance[6]. Of note, using meta-regression analysis Krieger et al. demonstrated that low-calorie dietary interventions with PI of >1.05 g/kg were associated with larger fat-free mass retention than similarly hypocaloric diets with PI 1.05 g/kg.7 Nonetheless, there is paucity of data addressing the relationship between PI and the loss of LTM relative to total body weight loss following BS[6-8]. Available data on the influence of PI on the fate of LTM following BS has been basedon bioimpedance (BIA) rather than on more appropriate methods of body composition (BC) analysis in the severely obese subjects[9]. Against this background, the purpose of the present study wasto evaluate the relationship months after surgery following two commonly performed BS procedures, namely GBP and SG.

8.2. Subjects and methods

Fifty Caucasian bariatric surgery candidates agreed to participate in this observational study. Indications for surgery followed the National Institutes of Health (NIH) criteria (ie, BMI > 40 kg/m^2 or a BMI > 35 kg/m2 with obesity-related morbidity)[3]. The selection criteria for GBP or SG at our Institution have been previously reported and

were maintained for participants in this study[10]. SG was performed in 25 and GBP in 25 participants in our study cohort, following previously reported standardized procedure[10]. Eligible participants had to be 18 years or older, and weight stable (body weight change ±2 kg) for a period of three months prior to enrollment. The Ethics Committee at our Institution approved the studies, and written informed consent was obtained from all participants.

8.2.1. Protein intake counseling

Prior to surgery, all subjects attended group and individual sessions including nutritional counseling according to the current guidelines for the bariatric patient.3 Dietary advice was systematically given to the patients during hospitalization, and at 2 and 6 weeks after surgery, and then at 4, 8, and 12 months, to sustain a hypocaloric and protein-rich diet. Following hospital discharge, patients were universally advised to supplement dietary protein with 15 g of protein powder (Resource Protein, Nestlé, Vevey, Switzerland). Based on 3-day dietary food records, PI from food and supplements were quantified at each follow up visit. A 24-h food recall, three-dimensional models (Nasco International, Modesto, CA, USA; http://www.enasco.com), and a photographic album of food portions sizes were used to increase the precision of the protein intake estimates. PI was quantified using the software Dietsource 3.0© (Nestle). Patients were categorized based on the accomplishment of two daily PI goals: ≥60 g/day or ≥1.1 g/kg IBW/ day.[3-5] The body weight corresponding to the IBW was calculated as that corresponding to a BMI 1⁄4 25 kg/m2. At each study visit, dietary and supplementation protein were advised to achieve a daily PI of at least 60 g.

8.2.2. Body composition (BC) analysis

Anthropometry and BC were evaluated prior to surgery and a 4- and 12-months after surgery. Participants were weighted with light clothes and without shoes to the nearest 0.1 kg. Height was determined using a fixed wall stadiometer to the nearest 0.1 cm. Waist circumference was measured to the nearest 0.5 cm, at the level of the iliac crest, with a standard flexible, inelastic measuring tape. Hip circumference was measured to the nearest 0.1 cm at the maximum extension at the buttocks level. Body mass index (BMI) was calculated as weight (kg) divided per squared height (meters).

Dual energy X-ray absorptiometry (DXA) scans were performed using the whole-

body scanner GE Lunar iDXA (GE Healthcare, Madison, WI) according to the manufacturer's specifications. The iDXA is a narrow fan-beam DXA instrument with a high weight limit (204 kg) and a relatively wide scanning space (66 cm) designed to accommodate obese subjects. The subjects were positioned in the center of the table for each scan. Appropriateness of patient's position is further assessed by the DXA software by means of an automatic detection system. The instrument has three scan modes that adjust the X-ray attenuation for the thickness of each patient. For this study, scans were performed using the default scan mode automatically selected by the DXA software. The GE Lunar Body Composition Software enCORE TM 2008 GE Healthcare version 12.30.008 was used to obtain lean tissue mass (LTM), bone mineral content, and FM measurements.

For the current analysis the percent of body weight (BW) loss consisting of LTM was used as the main outcome variable. The LTM loss as a percent of weight loss (%LTM loss) at any particular study time point (tx) was calculated as [100*($LTM_{baseline}$-LTMtx)/($BW_{baseline}$-BWtx)]. Furthermore, we estimated the adequacy of the LTM attained at 12-months in our surgical cohort as compared to that in a non-operated control population. To do so, we used multilinear regression to derive a predictive equation for LTM on the basis of gender, age, height, and body weight in a control population (n=185) with similar age and gender distribution as our surgical cohort, and a wide range of BMI (21.3-45.8 kg/m2). Predictors in the equation accounted for 91.2% (adjusted R^2 1⁄4 0.912, $p < 0.001$) of the variability of the DXA measured LTM in this control population.

8.2.3. Statistical analysis

Values are given as mean±standard error or proportions unless otherwise specified. Paired (Wilcoxon Rank Test, McNemar Test) or unpaired (ManneWhitney U) non-parametric tests were used for comparisons between groups. Chi-squared was used to compare frequency distributions between groups. Non-parametric bivariate correlation analysis was used to evaluate the association between PI and %LTM loss. Multilinear regression analysis with % LTM loss as dependent variable was used to evaluate the inde-pendent contribution of PI to the post-surgical variability in LTM loss. We anticipated an effect size of 30% derived from the inclusion of daily protein intake, age, gender, lean tissue mass at baseline, and the type of surgery as predictors of LTM at 4 and 12 months after surgery. Thus, a minimum sample size of 50 subjects was required for our study

(statistical power: 80%, alpha- error: 0.05). A one-way between-groups analysis of covariance (ANCOVA) was used to compare LTM loss between surgical groups or groups differing in the attainment of daily PI goals. p value <0.05 was considered statistically significant. Data were analyzed using the Statistical Package for Social Science (SPSS 16.0; SPSS Inc, Chicago, IL, USA).

8.3. Results

The clinical characteristics of the study participants at baseline are shown in Table 1. 41 females and 9 males composed the study cohort. Male gender tended to be over-represented in the SG group as compared to the GBP group albeit not significantly (p = 0.138). SG patients were heavier (p = 0.019) and had a larger waist circumference (p = 0.013) as compared to their GBP counterparts. Fat mass (FM), expressed as percent of total body mass, was comparable between groups. However, differences in body weight between the SG and GBP groups resulted in significantly larger FM (kg) and LTM (kg) in SG patients (both p < 0.05). Daily caloric and macronutrient intake pattern prior to surgery was comparable between the two surgical groups.

Table 1. Characteristics of the study participants at baseline.

	All ($n = 50$)	Gastric bypass ($n = 25$)	Sleeve gastrectomy ($n = 25$)	p
Age (years)	44.0 ± 1.7	43.0 ± 2.0	45.0 ± 2.9	0.415
Gender [M/F, n]	9/41	2/23	7/18	0.138
Body weight (kg)	122.9 ± 2.6	116.8 ± 3.2	129.0 ± 3.9	0.015
BMI (kg/m^2)	46.3 ± 0.6	45.5 ± 0.6	47.2 ± 1.0	0.123
WC (cm)	130.9 ± 1.6	127.0 ± 1.6	134.8 ± 2.6	0.021
FM (%)	52.5 ± 0.6	52.8 ± 0.7	52.2 ± 1.0	0.831
FM (kg)	62.4 ± 1.5	59.9 ± 2.0	64.9 ± 2.2	0.026
LTM (kg)	56.5 ± 1.4	53.5 ± 1.6	59.6 ± 2.2	0.028
Daily CI (kcal/d)	2246 ± 108	2058 ± 127	2426 ± 170	0.116
Carbohydrate intake (% CI)	37.3 ± 1.1	37.1 ± 1.3	37.6 ± 1.8	0.208
Lipid intake (% CI)	45.4 ± 1.1	44.8 ± 1.7	46.0 ± 1.5	0.816
Protein intake (% CI)	17.3 ± 0.6	18.1 ± 0.9	16.6 ± 0.7	0.377
Protein intake (g/day)	91.0 ± 3.4	89.2 ± 4.5	92.8 ± 5.1	0.124
Protein intake (g/kg IBW/day)	1.38 ± 0.05	1.39 ± 0.06	1.36 ± 0.07	0.808

M: male. F: female. BMI: body mass index. WC: waist circumference. FM: fat mass. LTM: lean tissue mass. CI: caloric intake. IBW: ideal body weight.

8.3.1. Weight loss and changes in BC

At 4- and 12- months after surgery study participants had lost respectively 21.1±0.6% (range 8.0-32.6%; $p < 0.05$) and 32.8 ± 1.4% (range 3.5-53.7%; $p < 0.01$) of their baseline body weight. No differences in weight loss were found between the two surgical groups throughout follow up (4-months: GBP 21.2 ± 0.8% vs SG 21.0 ± 1.0%, $p = 0.375$; 12-months: GBP 32.1 ± 1.6% vs SG 33.5 ± 2.2%, $p = 0.503$). The changes in BC at 4- and 12-months after surgery are shown in Table 2. Weight loss during the 12 months observation period was mainly at the expense of FM, as FM represented a lower percentage of total body weight both at 4- ($p < 0.001$) and 12-month ($p < 0.001$) study time points compared to baseline. %LTM loss was larger at 4 months 24.9 ± 1.0% (range 12.0-45.9) compared to 12 months 18.9 ± 0.9% (range 3.5-31.1) either when the whole cohort was analyzed ($p < 0.001$) or each surgical group was assessed separately (GBP: 4-months 22.0 ± 1.4% vs 12-months 16.8 ± 1.2%, $p = 0.001$; SG: 4-months 26.7 ± 1.7% vs 12-months 21.1 ± 1.2%, p 1⁄4 0.002). Interestingly, ANCOVA analysis with LTM at baseline as covariate (kg) showed that %LTM loss in the SG group tended to be larger as compared to the GBP group at 4- months ($p = 0.061$), and was significantly larger on the 12-month evaluation ($p = 0.027$).

8.3.2. Protein intake throughout the study period

Total daily caloric intake was markedly reduced at 4 months after surgery, and increased at 12-months although it remained lower as compared to baseline (Table 2). The percent of daily caloric intake coming from protein was larger at 4 months ($p < 0.001$) and 12 months ($p < 0.001$) compared to baseline. However, when expressed as grams per day or grams per kg of IBW per day, protein was markedly reduced at both post-surgical examination time points relative to baseline (Table 2).

Table 2. Body composition and dietary intake changes throughout follow-up.

	All ($n = 50$)	Gastric bypass ($n = 25$)	Sleeve gastrectomy ($n = 25$)	p^*
4 months after surgery				
FM (%)	46.9 ± 0.8^{A}	46.1 ± 1.0^{A}	47.6 ± 1.3^{A}	0.146
FM (kg)	40.4 ± 1.4^{A}	41.1 ± 1.4^{A}	47.7 ± 2.4^{A}	0.011
LTM (kg)	50.1 ± 1.3^{A}	48.0 ± 1.5^{A}	52.1 ± 2.2^{A}	0.174
Daily CI (kcal/d)	886 ± 31^{A}	899 ± 41^{A}	871 ± 47^{A}	0.353
Carbohydrate intake (% CI)	37.3 ± 1.1	37.1 ± 1.6	37.5 ± 1.5	0.992
Lipid intake (% CI)	36.5 ± 1.1^{A}	35.8 ± 1.8^{B}	37.2 ± 1.1^{B}	0.702
Protein intake (% CI)	26.2 ± 0.9^{A}	27.3 ± 1.3^{A}	25.1 ± 1.2^{A}	0.219
Protein intake (g/day)	57.3 ± 2.5^{A}	60.8 ± 4.0^{B}	92.8 ± 5.1^{A}	0.197
Protein intake (g/kg IBW/day)	0.87 ± 0.04^{A}	0.95 ± 0.06^{B}	0.78 ± 0.04^{A}	0.054
12 months after surgery				
FM (%)	$53.1 \pm 1.1^{A,a}$	$37.6 \pm 1.4^{A,a}$	$38.7 \pm 1.6^{A,a}$	0.287
FM (kg)	$30.8 \pm 1.5^{A,a}$	$28.6 \pm 1.4^{A,a}$	$33.0 \pm 2.7^{A,a}$	0.299
LTM (kg)	$48.8 \pm 1.4^{A,a}$	47.3 ± 1.5^{A}	$50.3 \pm 2.3^{A,a}$	0.498
Daily CI (kcal/d)	$1253 \pm 56^{A,a}$	$1194 \pm 78^{A,a}$	$1312 \pm 81^{A,a}$	0.255
Carbohydrate intake (% CI)	37.5 ± 1.3	36.4 ± 2.0	38.4 ± 1.7	0.299
Lipid intake (% CI)	$41.4 \pm 1.2^{C,b}$	42.9 ± 1.8^{b}	39.9 ± 1.7^{C}	0.322
Protein intake (% CI)	$21.2 \pm 0.8^{A,a}$	20.6 ± 1.2^{b}	$21.6 \pm 1.1^{B,b}$	0.483
Protein intake (g/day)	64.1 ± 2.9^{A}	59.5 ± 3.9^{A}	$68.7 \pm 4.0^{A,b}$	0.114
Protein intake (g/kg IBW/day)	0.96 ± 0.4^{A}	0.92 ± 0.05^{A}	$1.00 \pm 0.05^{A,b}$	0.240

BMI: body mass index. WL: weight loss. FM: fat mass. LTM: lean tissue mass. CI: caloric intake. IBW: ideal body weight. p*: comparison between the gastric bypass and sleeve gastrectomy groups. A: $p < 0.001$; B: $p < 0.01$; C: $p < 0.05$ for the comparison within the same group between the baseline and the follow up (4- or 12-month) evaluation. a: $p < 0.001$; b: $p < 0.01$ for the comparison within the same group between the 4- and 12-months evaluation.

The percentage of subjects reporting a daily protein consumption of less than 60 g was 58.3% and 46.0% respectively at 4- and 12- months after surgery [both $p < 0.001$ relative to baseline (8%)]. At 4 months after surgery, the proportion of subjects with a daily PI < 60 g was comparable between the two surgical groups (GBP: 52.0% vs SG: 65.2%; $p = 0.263$). However, at 12 months the proportion of subjects with daily PI < 60 g was larger in the GBP group (GBP: 60.0% vs SG: 32.0%; $p = 0.044$). A daily PI in the upper range of the currently recommended protein allowance (100 - 120 g/d) was reported only by 1 and 3 patients respectively at 4- and 12-months follow up. Only 10 (20.8%) and 17 (34.0%) subjects of the whole cohort reported a daily PI ≥1.1 g/kg IBW/d at 4, and 12 months after surgery. In our cohort, a daily protein intake of 60 g and 120 g corresponded to a PI 0.92 ± 0.01 and 1.83 ± 0.03 g/kg IBW/d respectively. Conversely, a daily PI of 1.1 g/kg IBW corresponded to 72.92 ± 1.23 g.

Table 3. Multilinear regression analysis of the determinants of lean tissue mass loss relative to weight loss (as a percent), at 4- and 12-months after surgery.

	OR	95% CI	*p*
4 months after surgery			
Protein intake (g/kg IBW/d)	−0.376	(−20.071), (−2.113)	0.017
Gender (Male = 1, Female = 2)	0.120	(−6.234), (11.163)	0.570
Age (years)	−0.071	(−1.245), (0.154)	0.645
Lean tissue mass at baseline (kg)	0.068	(−0.298), (0.405)	0.798
Type of surgery (GBP = 0, SG = 1)	0.197	(−1.823), (8.011)	0.211
12 months after surgery			
Protein intake (g/kg IBW/d)	−0.468	(−16.764), (−4.753)	0.001
Gender (Male = 1, Female = 2)	0.221	(−2.791), (9.953)	0.263
Age (years)	0.148	(−0.067), (0.217)	0.290
Lean tissue mass at baseline (kg)	0.215	(−0.124), (0.389)	0.303
Type of surgery (GBP = 0, SG = 1)	0.399	(1.538), (8.382)	0.006

OR: odds ratio. CI: confidence interval. IBW: ideal body weight. GBP: gastric bypass. SG: sleeve gastrectomy.

8.3.3. Relationship between protein intake and changes in BC

Simple correlation analysis showed a significant inverse association between daily PI (expressed as g/kg IBW) and %LTM loss at 4 months (Spearman rho = - 0.516, $p < 0.001$), and 12 months (Spearman rho = - 0.315, $p < 0.031$). Thus, at both study time points a larger daily PI was associated with better retention of LTM. This association hold statistically significant in multiple linear regression analysis with %LTM loss as dependent variable (Table 3). At 4 months, a multilinear regression analysis with gender,

age, type of surgery, and daily PI (g/kg IBW) accounted for 17.2% (Adjusted $R^2 = 0.153$, $p = 0.038$) of the variability of %LTM loss. In that model, daily PI was the only significant predictor of %LTM loss ($p = 0.017$) (Table 3). However, significance was lost ($p = 0.220$) when daily caloric intake was taken into account. An analog multilinear regression analysis was constructed to evaluate the relationship between daily PI and %LTM loss at 12 months after surgery. Variables in the model [gender, age, type of surgery, lean tissue mass prior to surgery (kg), daily protein intake (g/kg IBW)] accounted for 26.4% (Adjusted R2 1⁄4 0.264) of the variability of %LTM loss, with the type of surgery ($p = 0.003$) and PI (p 1⁄4 0.027) being the only significant independent predictors of %LTM loss (Table 3). At 12 months, the addition of daily caloric intake into the model did not attenuate the significant association between daily PI and %LTM loss (data not shown). As shown in Fig. 1, in our study cohort, SG appeared to be associated with larger %LTM loss as compared to GBP surgery at all observed PI.

Finally, we compared %LTM loss between subjects that achieved or did not achieve the pre-defined PI goals of 60 g/day or 1.1 g/kg IBW/day. ANCOVA analysis, with type of surgery and LTM at baseline (kg) as covariates, showed that a daily protein intake above 60 g was associated with lower %LTM loss both at 4- (PI ≥ 60 g/d: 16.5 ± 1.4% vs PI < 60 g/d 20.5 ± 1.1%; $p = 0.030$) and 12-months (PI = 60 g/d: 21.7 ± 1.5% vs PI < 60 g/d 27.5 ± 1.6%; $p = 0.013$). Similarly, a daily protein intake above 1.1 g/kg IBW was associated with a lower %LTM loss at 4- and (PI ≥ 1.1 g/kg IBW/d: 19.3 ± 2.6% vs PI < 1.1 g/kg IBW/d 25.5 ± 1.3%; $p = 0.044$) 12-months (PI ≥ 1.1 g/kg IBW/d: 15.5 ± 1.4% vs PI < 1.1 g/kg IBW/d 20.8 ± 1.0%; $p = 0.004$).

8.3.4. Estimation of the adequacy of LTM at 12-months after surgery as compared to a control population

Age, gender, height, and body weight accurately predicted the DXA measured LTM in our control population ($n = 185$; adjusted $R^2 = 0.912$, $p < 0.001$). The difference between the predicted-LTM based on this equation and the DXA-measured LTM in our control population was -0.063 ± 0.134 kg (mean ± standard error) ($p = 0.875$). Using this equation, the predicted-LTM of our surgical participants at 12 months after surgery was estimated based on the corresponding age, gender, height, and attained body weight. As shown in Fig. 2, in our surgical cohort the LTM measured by DXA was very similar to that predicted based on the equation derived from our control population. The difference

between the predicted- LTM based and the DXA-measured LTM in our surgical group was -3.311 ± 0.658 kg (mean ± standard error) ($p < 0.001$).

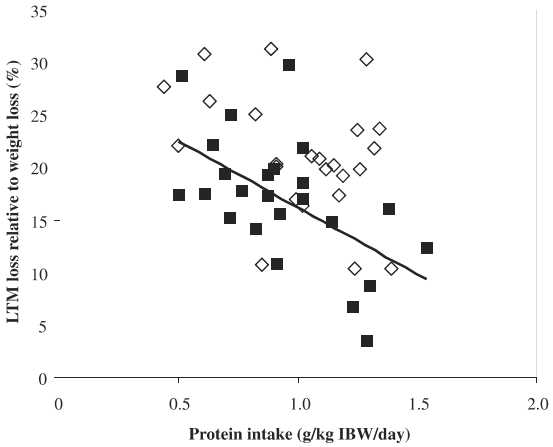

Fig. 1. Relationship between daily protein intake (expressed as g of protein per kg of ideal body weight) and lean tissue mass loss as a percentage of weight loss at 12 months after Roux-en Y gastric bypass or sleeve gastrectomy. Black squares: Roux-en-Y gastric bypass subjects. Open diamonds: sleeve gastrectomy subjects. Solid line: Roux- en-Y gastric bypass group regression line (n = 24, R^2=0.309; p = 0.005). Regression line for the sleeve gastrectomy group is not shown as was not statistically significant (n = 24, R^2=0.154; p = 0.064).

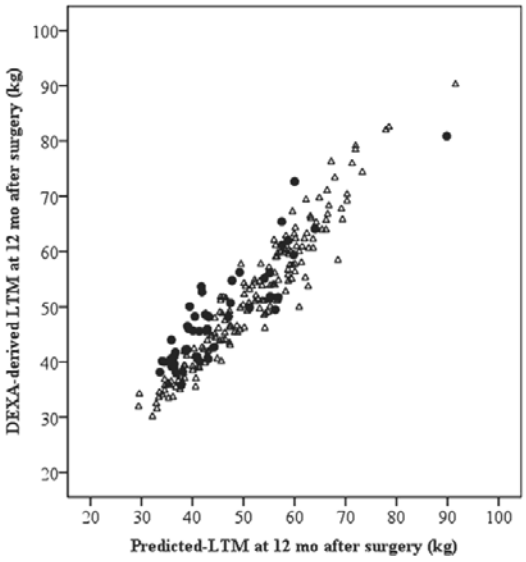

Fig. 2. DXA-measured lean tissue mass versus predicted-lean tissue mass in non- operated controls (n = 185) and bariatric surgery patients (n= 50). Open triangles: non-operated controls. Black circles: bariatric surgery patients.

8.4. Discussion

Our study shows that a larger PI is associated with a better preservation of LTM following two commonly performed BS procedures, namely GBP and SG. Despite a large proportion of study participants not achieving the currently recommended daily protein intake allowance, our data supports the currently recommended PI goal of at least 60 g/d or 1.1 g/kg IBW/d as significantly associated with better preservation of LTM. Finally, our data suggest that factors specifically related to SG are relevant for the proportion of LTM loss relative to total weight loss after this BS technique.

Our findings on LTM loss following GBP are consistent with previous studies in which DXA has been used as method for BC analysis. Percent LTM loss at 12 months after GBP has been reported in the range of 12.0%-28.0%, with larger %LTM loss at shorter intervals after surgery[11-17]. To our knowledge, no previous studies have been published on the changes in BC following SG using DXA as reference. Using BIA, Damms-Machado et al. reported a 46.1% and 25.4% fat free mass (FFM) loss relative to total weight loss at 3 and 12 months, respectively, after SG[18]. The systematic review by Chaston et al. nicely demonstrated that LTM loss varies among different BS procedures, with larger %LTM being associated with procedures classified by the authors as malabsorptive (biliopancreatic diversion and GBP) as compared to AGB[2]. Interestingly in a recent randomized clinical trial in which two BS techniques were compared, a larger %FFM loss (assessed either by BIA or total body potassium) was found following duodenal switch as compared to GBP[19]. The finding of a larger %LTM loss following SG as compared to GBP is puzzling and needs further confirmation. SG is not associated with the bypass of the duodenum and proximal jejunum, and thus malabsorption should not be a contributing factor to LTM-loss. Admittedly, it could be argued that technical problems associated with the use of DXA as BC method could have affected our results. However, to our knowledge, the relative accuracy of DXA use following GBP as compared to after SG has not been reported. Studying the mechanism accounting for our finding of a larger LTM loss following SG is beyond the scope of our study. Because of the partial removal of the stomach, plasma ghrelin levels have been reported lower in subjects undergoing SG as compared to GBP[20]. Ghrelin mimetics have been found to prevent the decline in fat free mass (FFM) in healthy older adults[21]. However, the potential relationship between ghrelin and BC changes following BS has not been evaluated. We deem our findings and

those from others highlight an important area for additional investigation, as FFM is a determinant of resting energy expenditure (REE) and REE has been associated with weight maintenance after BS[22].

Studies addressing the relationship between PI and the changes in BC following BS are scarce. Using underwater weighing as BC method, Carey et al. reported a mean daily PI of 68.9 g (corre- sponding to 0.95 g/kg IBW) with a %LTM loss of 33.0% in a cohort of patients evaluated at 6 months after GBP[23]. Likewise, Carrasco et al. reported a PI of 56.6 g/d (0.9 g/kg IBW) and 45.4 g/d (0.7 g/kg IBW) respectively at 6- and 12-months after GBP, with the corresponding %LTM loss being 33.4% and 27.1% as assessed by DXA[17]. However, in these two studies the relationship between PI and the changes in BC was not reported. By using BIA, in a previous study from our group, we failed to find a significant association between FFM loss and PI[6]. In contrast, using BIA Raftopoulos et al. reported a significant association between post-surgical PI and %FFM as assessed by BIA[8]. In that study, compliance with a daily PI goal of 1.1 g per kg of current body weight was associated with greater excess weight loss, larger %FFM, and lower %FM at 6- and 12-months after GBP. Interestingly, the mean daily PI at 12 months was higher (96.8 ± 36.4 g) than that in our cohort (64.1 ± 2.9 g). Should have we considered the PI goal of 1.1 g per kg of current body weight, the corresponding total daily PI goal in our series would have been 106.8 ± 2.5 g and 90.7 ± 2.7 g respectively at 4- and 12-months after surgery. Of note, only 1 patient (2.0%) at 4 months and 4 patients (8.0%) at 12 months in our cohort would have accom- plished such PI goal despite pre- and post-surgical nutritional surveillance. This is in sharp contrast with the reported accomplishment of the PI goal of 72.5% and 71.3% at the 6- and 12-months post-surgical evaluations reported in the study by Raftopoulos et al[8]. Although the reasons for discordance between the two studies remain uncertain, we consider the data reported herein support current PI recommendations in the bariatric surgery patient as it pertains to LTM-loss following BS[3-5]. In our study, a PI larger than 60 g/d and PI larger than 1.1 g/kg IBW/d were associated respectively with a %LTM loss of 17.1 ± 1.1% and 15.5 ± 1.3% at 4 and 12 months after surgery. Admittedly, a good definition of what is the appropriate LTM loss in weight loss interventions is lacking[2]. However, that a post-surgical %LTM loss in that range is similar to that expected from the attained BMI is suggested by our comparison with a control group of the same geographical background. In our surgical cohort the DXA-measured LTM at 12 months after surgery was larger

than the predicted-LTM based on our non- operated control population.

We acknowledge our study has several limitations. First, the observed size effect of our predictive factors on %LTM at 4- and 12- months was smaller than predicted. Thus, a larger sample size would have been required to draw definite conclusions. Second, although DXA has been widely used for BC in severe obesity it has been suggested that DXA use for the longitudinal assessment of BC analysis in subjects undergoing BS is not without concerns[15]. When compared to total body water, DXA resulted in overestimation of LTM prior to surgery with no significant difference between the two methods of assessment at 6 and 12 months after surgery. Thus, it could be argued that by using DXA as the method for BC analysis we may have overestimated the LTM loss as a percent of weight loss. This limitation should have had no meaningful impact on the analysis of the relationship between PI or the type of surgery and the post-surgical changes in BC. However, our data should be replicated using other BC methods. Third, we used 3-day rather than 7-day food records to estimate daily PI. Admittedly, 7-day food records should provide a more representative estimate of daily PI. However, to increase precision of our estimates, we used 24-h dietary recall, three-dimensional models, and a photographic album of food portions sizes in addition to 3-day food records. Fourth, in our study we did not assess physical activity. A higher leisure time physical activity, and a shorter time of television viewing have been associated with an LTM sparing effect following GBP[16]. Furthermore, an association has been shown between physical activity and daily PI compliance in GBP patients[9]. Finally, our study was not designed nor powered to compare the changes in BC following GBP or SG. Thus, our data on the differential effect of the type of surgery on LTM loss should be replicated and interpreted cautiously.

8.5. Conclussion

In summary, our data emphasize the importance of PI as independent predictor of the changes BC following BS. Furthermore, our data provide supportive evidence for the currently recommended PI goals of ≥ 60 g/d or ≥ 1.1 g/kg IBW/d as being associated with better LTM preservation in the bariatric surgery patient. How these PI goals relate to other outcomes following BS (i.e. insulin sensitivity or protein malnutrition) is an open question that was beyond the scope of our study. Finally, further studies are warranted to establish a potentially differential effect of SG on LTM retention as compared to GBP.

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Conflict of interest

The authors have no competing financial interests in relation to the work described herein.

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VM, AA, AJ, JV, designed research (project conception, devel- opment of overall research plan, and study oversight). VM, AA, LR, LF, and JV conducted research. AI, and AL, performed surgery in study participants. VM, AA, AJ, and JV analyzed data or performed statistical analysis. VM, AA, LF, JV made major contribution in writing the manuscript. VM, AA, and JV had primarily responsibility for final content of the manuscript. We acknowledge María J Coves and Judith Viaplana for technical assistance. The present study was developed without specific funding.

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PART V: GAPS ON THE LITTERATURE AND NEW STUDY PROTOCOL

CHAPTER 9: PROTOCOL OF A RANDOMIZED CONTROLLED TRIAL ON DIETARY PROTEIN DURING SURGICAL WEIGHT LOSS: EFFECT ON NITROGEN BALANCE, THERMOGENESIS, BODY COMPOSITION, SATIETY AND CIRCULATING BRANCHED CHAIN AMINO ACID LEVELS UP TO ONE YEAR AFTER SURGERY

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PART V: GAPS IN THE LITTERATURE AND NEW STUDY PROTOCOL

9. PROTOCOL OF A RANDOMIZED CONTROLLED TRIAL ON DIETARY PROTEIN DURING SURGICAL WEIGHT LOSS: EFFECT ON NITROGEN BALANCE, THERMOGENESIS, BODY COMPOSITION, SATIETY AND CIRCULATING BRANCHED CHAIN AMINO ACID LEVELS UP TO ONE YEAR AFTER SURGERY.
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9.10. References

9 EFFECT ON NITROGEN BALANCE, THERMOGENESIS, BODY COMPOSITION, SATIETY, AND CIRCULATING BRANCHED CHAIN AMINO ACID LEVELS UP TO ONE YEAR AFTER SURGERY: PROTOCOL OF A RANDOMIZED CONTROLLED TRIAL ON DIETARY PROTEIN DURING SURGICAL WEIGHT LOSS

Background: Bariatric surgery (BS), the most effective treatment for severe obesity, typically results in 40-50 kg weight loss in the year following the surgery. Beyond its action on protein metabolism, dietary protein intake (PI) affects satiety, thermogenesis, energy efficiency, and body composition (BC). However, the required amount of PI after surgical weight loss is not known. The current daily PI recommendation for diet-induced weight loss is 0.8 g/kg ideal body weight (IBW) per day, but whether this amount is sufficient to preserve fat-free mass during active surgical weight loss is unknown.

Objective: To evaluate the effect of a 3-month dietary protein supplementation (PS) on nitrogen balance (NB), BC, energy expenditure, and satiety in women undergoing either gastric bypass or vertical sleeve gastrectomy.

Methods: In this randomized prospective study, participants will be randomized to a high protein supplementation group (1.2 g/kg IBW per day) or standard protein supplementation group (0.8 g/kg IBW per day) based on current guidelines. Outcome measures including NB, BC, circulating branched chain amino acids, and satiety, which will be assessed presurgery, and at 3-months and 12-months postsurgery.

Results: To date, no studies have examined the effect of dietary PS after BS. Current guidelines for PI after surgery are based on weak evidence.

Conclusions: The results of this study will contribute to the development of evidence-based data regarding the safe and optimal dietary PI and supplementation after BS.

Trial Registration: Clinicaltrials.gov NCT02269410; http://clinicaltrials.gov/ct2/show/NCT02269410 (Archived by WebCite at http://www.webcitation.org/6m2f2QLeg).

9.1. Introduction

Bariatric surgery (BS) is the most effective long-term therapy for the treatment of severe obesity. BS is associated with a favorable impact on overall and cardiovascular mortality, incidence of first occurrence of fatal or nonfatal cardiovascular events, prevention and remission of type 2 diabetes mellitus (T2DM), and quality of life [1]. Short-term studies showed no apparent difference between gastric bypass (GBP) and vertical sleeve gastrectomy (SG) on T2DM remission and weight loss [2]. GBP and SG are the most accepted procedures currently being performed, with SG increasing in prevalence since its inception in 2003 [3]. Although the surgeries differ widely (GBP is considered a malabsorptive and restrictive procedure while SG is solely restrictive), the prevalence of nutrient deficiencies seems to be comparable [4]. However, the metabolic impact of dietary protein intake (PI) in the early phase of active weight loss has not been studied.

PI during diet-induced weight loss and weight maintenance has been associated with retention of fat-free mass (FFM) [5,6], better satiety [7], and, if insufficient, with malnutrition [8]. Surgical weight loss is associated with decreased circulating levels of branched chain amino acids (BCAAs) [9].

Caloric intake decreases significantly during the first 3-6 months after surgery and may be frequently associated with vitamin, mineral [4,10], and protein deficiencies [4,8-11]. Prospective studies observed that low albumin levels, a clinical marker of protein deficiency [12], can occur up to 2 years after GBP [8,13] with a prevalence ranging from 3 to 18% [9,13-15]. Protein deficiency is more commonly observed after malabsorptive procedures, such as the biliopancreatic diversion [16]. Changes in taste and food preferences, and some degree of stomach discomfort during meals (with or without dumping syndrome), contribute to a poor dietary protein tolerance, thereby affecting the net PI [17]. The potential macronutrient maldigestion and/or malabsorption observed after BS [18] may also contribute to a compromised protein status.

It is generally accepted that diets containing all indispensable amino acids (AAs) are required for optimal protein synthesis and balance [19,20], and optimal intake of dietary protein should even be increased in vulnerable populations [21]. Nitrogen balance (NB), the difference between nitrogen intake and loss, is often compromised with trauma or infection, even with attempted nutritional interventions [22,23]. BS compromises NB

via lower PI and an early maintained (or generally higher) protein demand following surgery, so high protein diets are recommended by various guidelines [24-26]. The most updated American Association of Clinical Endocrinologists guidelines suggest that a minimal PI of 60 g/day (and up to 1.5 g/kg ideal body weight [IBW] per day) after BS should be *adequate,* although these recommendations are only supported by a low level of scientific evidence (grade C or D). However, as the clinical tolerance of protein-containing foods is low after BS, recommendations are rarely followed and patients often do not reach their PI goal [17]. Our group and others have shown that daily consumption of 60 g of protein can be challenging during the first 4 months after surgery, even when protein supplements are recommended and supplied at no cost [27]. PI of 1.5 g/kg IBW per day would represent (when considering IBW for a body mass index [BMI] of 25 kg/m2) 105 g/day for a woman with an IBW of 70 kg. The low protein tolerance mentioned above makes this recommendation difficult to follow, even with the most motivated patients. Therefore, finding an acceptable amount of PI would ensure optimal FFM retention, limit muscle breakdown, maintain resting energy expenditure (REE), and contribute to the development of a healthier diet that supports weight loss maintenance without interfering with glucose homeostasis.

9.2. Background

9.2.1. Risk of Decreased Lean Body Mass and Resting Energy Expenditure With Surgical Weight Loss: Effect of Dietary Protein

The consequences of negative energy and protein balance on visceral mass and skeletal muscle mass are well established [28]. Surgical weight loss results in both fat mass (FM) and lean body mass (LBM) loss: 75.2% and 24.8%, respectively [29]. LBM is the main determinant of REE, and explains 70% of the REE variance [30], with REE being the largest component of 24-hour energy expenditure (EE). Thus, reduced EE after weight loss is a factor of resistance for weight loss, and it may trigger the regaining of weight in the BS population [31]. The impact of daily PI on REE after BS has not been previously addressed, while PI is known to impact postprandial thermogenesis. Although the reduction of FM in obese individuals during weight loss is beneficial, the decrease in LBM may down-regulate the metabolic process, including protein turnover and basal metabolic rate, thus compromising long-term healthy weight management [32]. Studies on the impact of PI on body composition (BC) after BS are scarce and inconclusive. While some studies failed to find a significant correlation between absolute PI and FFM

loss relative to total weight loss after BS [5,33-35], others found that higher levels of PI improved BC changes by enhancing the loss of FM and reducing FFM loss after BS [36]. High protein diets may increase EE while preventing LBM loss [30] during weight loss. Increased EE from dietary protein is attributed to an enhanced thermic effect of food (15%, standard deviation [SD] 4) compared to carbohydrates (6%, SD 2) or lipids (7%, SD 3) [37]. Studies related to the effect of high protein versus standard protein diets on the prevention of LBM loss, which in turn lead to a lesser reduction in REE, are often inconclusive [8,34,38]. In addition, comparisons between GBP and SG have not been completed.

9.2.2. Nitrogen Balance Study in the Bariatric Surgery Setting

NB is classically used to determine adequate PI with regards to daily nitrogen loss, and to estimate whole body protein balance in response to nutritional interventions [39]. Sustained negative NB can be associated with loss of lean and fat tissue [40]. Thus, ideally the goal of PI after BS should aim at preventing and/or limiting negative NB, even under energy restriction.

Occurrence of malabsorption should be considered when assessing NB in BS subjects [11]. In a malabsorptive state, fecal losses of nitrogen may be as high as 3.5 g/day [12]. Thus, in addition to the other components of the NB equation, fecal nitrogen losses should be measured after BS, and not simply estimated at 0.4 g/day, as recommended by the Joint Food and Agriculture Organization/World Health Organization expert committee, under nonmalabsorptive conditions [41,42]. Of note, Odstrcil et al [18] studied the contribution of malabsorption on the reduction in net energy absorption 5 and 14 months after long-limb GBP. Net absorption of protein was significantly reduced after BS, and malabsorption accounted for 13% of the total reduction in protein absorption at both study time points [18]. However, a protein kinetic study using stable isotopes demonstrated that protein digestion and absorption were not impaired, and even accelerated, 3 months after GBP [43].

9.2.3. Roles of Protein Supplementation on Circulating Levels of Amino Acids

It has long been recognized that circulating levels of AAs, including BCAAs, are elevated in persons with obesity, insulin resistance (IR), or T2DM, compared to healthy controls [44,45]. BS is associated with reduced concentrations of plasma BCAAs [46] and

decreased IR [8,46]. To date, the protein sparing effect of long-term protein supplementation (PS) has not been studied.

9.2.4. Dietary Protein Intake and Satiety

High PI has been shown to increase satiety in the context of energy restriction [47,48]. Proposed factors that may enhance satiety include: a ketogenic state; relatively elevated plasma AA levels [49]; an increase of the satiety peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and cholecystokinin (CCK) [50]; and/or a decrease of the orexigenic hormone ghrelin [13,51]. We aim to further explore the relationship between PI and satiety after BS procedures.

9.3. Study aims

Considering the complexity of metabolic and behavioral changes after BS, the overall aim of our research study will be to establish adequate PI after BS. To achieve this goal, we will compare the effect of 2 levels of PI (high protein supplementation group, HPS-G; and standard protein supplementation group, SPS-G) after BS (GBP and SG) on (1) NB, (2) BC, (3) REE and diet-induced thermogenesis (DIT), (4) satiety, (5) the release of gut hormones, (6) circulating levels of BCAAs in relation to insulin sensitivity, and (7) adherence to protein supplements. Five specific objectives will address our aims.

Objective 1: Nitrogen Balance

Total body NB will be measured to assess the levels of PI and protein absorption. The measure of NB will be performed during an inpatient stay before surgery, after 3 months of controlled PS, and 12 months after BS. PI will be established at 1.2 g protein/IBW per day for all participants in the month before surgery. After surgery, participants will be randomized to either 1.2 g protein/IBW per day (HPS-G) or 0.8 g protein/IBW per day (SPS-G). Participants will receive PS for 3 months after BS, up to the second inpatient study time point. During the inpatient stay, all foods and drinks will be strictly controlled, and 24-hour urine and stool samples will be collected. Nitrogen content of food from aliquots, urine, and stool will be measured, as explained in the *Methods* section.

Objective 2: Effect of Protein Supplementation on Body Composition

We will compare the effect of HPS and SPS on LBM. Changes in BC will be

assessed before surgery, at 3 months, and 12 months after surgery in the 2 PS study groups using anthropometry, total body water (TBW), and plethysmography (BOD POD).

Objective 3: Effect of Protein Supplementation on Resting Energy Expenditure and Diet-Induced Thermogenesis

We will compare the effect of HPS and SPS on EE, measured by indirect calorimetry at rest (REE) and 4 hours after a high protein liquid test meal (DIT), before surgery, at 3 months, and 12 months after BS.

Objective 4: Branched Chain Amino Acid Levels

We will characterize changes in circulating BCAA levels in relation to insulin sensitivity and PI adequacy after GBP and SG. Circulating BCAA levels will be measured by targeted metabolomics and compared to insulin sensitivity (calculated by the homeostatic model assessment-insulin resistance or Matsuda index) before surgery, at 3 months, and 12 months postsurgery.

Objective 5: Effect of Surgical Procedures on Nitrogen Balance and Satiety

We will compare the effect of HPS and SPS, the hormonal response after a meal, and NB between GBP and SG. Satiety and hunger will be measured by visual analog scales (VASs) while fasting and in response to a high protein meal, before surgery, at 3 months, and 12 months after BS. Blood samples will be obtained before and after the meal test to measure the satiety-related gut hormones CCK, PYY, GLP-1, ghrelin, along with insulin and glucose levels. NB will also be compared between the surgical procedures.

9.4. Methods

9.4.1. Participants

All subjects will be recruited from the Bariatric Surgery Institute at Mount Sinai St Luke's Hospital (New York, NY), and will be required to sign an institutional review board -approved consent form prior to enrollment. A total of 112 volunteers scheduled to undergo either GBP or SG will be recruited. Based on our experience, we anticipate a conservative 30% attrition rate. Therefore, approximately 80 patients are expected to remain in the study at completion.

Inclusion and Exclusion Criteria

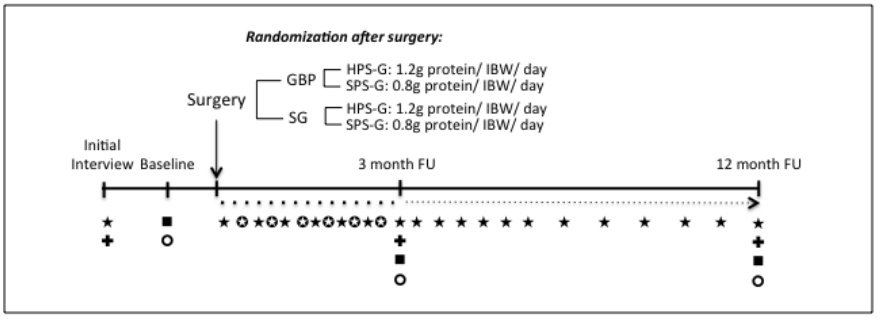
BC differences exist between women and men, and the BS patient population is 75% women, so only premenopausal women (18-40 years of age, BMI <50 kg/m2) will be included in this study. Other inclusion criteria include: any race/ethnicity; patients scheduled to undergo either GBP or SG; treated or untreated resting systolic/diastolic blood pressure less than 160/100 mmHg; fasting triglyceride concentration less than 600 mg/dl, without regard for diagnosis or prescription for other dyslipidemias; absence of diabetes, or diet-controlled diabetes (taking no medications).

Exclusion criteria include: presence of nitrogen retention disease (eg, renal or hepatic disease); abnormal thyroid function; known malabsorption syndrome; cardiovascular disease; current mucosal (gastrointestinal, respiratory, urogenital) or skin (cellulitis) infection; any psychiatric disorder; and any other condition which, in the opinion of the investigators, may make the candidate unsuitable for participation in this study.

9.4.2. Study Design

This study is a prospective randomized controlled trial (RCT), in which 112 obese participants (with no major comorbidities) scheduled to undergo GBP or SG will be randomly allocated to SPS-G or HPS-G cohorts. Participants will undergo three 5-day inpatient stays: presurgery, 3 months postsurgery, and 12 months postsurgery. The inpatient stays will be in the Clinical Research Resource (CRR) in the Irving Institute of Clinical and Translational Research at Columbia University Medical Center. The overall study design is displayed in Figure 1.

Table 1. Overall study design of protein supplementation after bariatric surgery.



• • • • 3 months protein supplements provided.
..... 9 months free living diet.

★ In person nutritional interview/ Dietary evaluation: 24hs Recall + 4-d food record
✪ Phone contact
✚ 7-day Physical Activity monitoring (Accelerometer)
■ Anthropometry/ Body Composition: TBW, BodPod
○ 5-day Impatient Stay
- Nitrogen Balance : feces and urine collection
- Meal test Satiety/ Resting Energy Expenditure/ Diet Induced thermogenesis with Indirect Calorimetry

GBP: gastric bypass, SG: Sleeve gastrectomy, High protein supplementation group. SPS-G: Standard protein supplementation group, HPS-G:

9.4.3. Protein Supplementation Protocol

PS regimens will be supplied during the 3 months immediately following surgery. The HPS-G group will receive 1.2 g protein/kg IBW per day, and the SPS-G group will receive 0.8 g protein/IBW per day, with IBW established as a BMI of 25 kg/m2 [4]. PS goals will be achieved partly by providing subjects with an instant high quality protein-isolate whey protein powder-supplement (Unjury; ProSynthesis Laboratories, Sterling, VA) [52]. Whey protein is the richest source of BCAAs and leucine, which has been demonstrated to stimulate muscle protein synthesis in elderly populations [53,54]. The SPS-G group will require approximately 1.5 servings per day (21 g protein/serving) while the HPS-G group will require 3 servings per day to meet their individually prescribed protein requirement.

Preceding Diet at Baseline

The NB study will be preceded by an outpatient diet stabilization period lasting 7 days. During this time, a meal plan will be tailored to each participant and PS will be provided, based on the individual's preferences, to promote adherence. Participants will be asked to complete a 7-day food record during this outpatient period, and will be contacted by phone one time during this period in order to evaluate adherence to, and tolerance of, the prescribed diet. This diet stabilization period is designed to allow each participant to adapt to the required level of PS [55]. Energy and protein requirements will be based on 35 kilocalorie (kcal)/IBW per day and 1.2 g protein/IBW per day, respectively [56], and will be calculated using the Nutrition Data System for Research (NDSR), 2011 [57]. Energy intake will be adjusted to minimize weight changes (within 0.75 kg of body weight) during the period of controlled diet, prior to admission, and during the inpatient stay [58].

Inpatient Study Period Before Surgery

During the initial 5-day inpatient study period all foods and drinks will be provided, and carefully monitored and controlled, under the supervision of the research dietician (VM) and the staff of the Bionutrition Unit in the CRR. All foods, drinks, and protein supplements will be aliquoted and weighed on a 0.1-gram precision scale before consumption. To minimize variability and accurately control the intake of nitrogen,

participants will eat the same diet every day. Participants will not be allowed to eat or drink any other food except for tap-filtered water. Diets will be based on regular/natural foods that require minimum manipulation. To meet the dietary PI goals, Unjury isolate whey protein products will be included in the diet as the main source of protein during the entire 5-day period. Vitamin and mineral supplementation, if any, will be continued during the inpatient study. Participants will be asked to eat the entire food portion that is served. Supervision will be ensured during each meal, and after each serving the trays will be examined by staff members, and the volume of any unconsumed beverage/soup will be measured in a graduated cylinder. All existing uneaten food will be weighed and recorded for day-to-day dietary adjustments. Meals for 1 complete day during the stay will be prepared and cooked in duplicate, and will be homogenized in a heavy-duty commercial blender to obtain 24-hour diet aliquots. Two aliquots will be frozen at –70° Celsius for later analysis of total nitrogen and energy content.

Post-Bariatric Surgery Dietary Protein Supplementation Intervention

After BS, participants will be randomized using the technique of permuted block randomization to ensure that equal numbers of patients are assigned to each treatment arm (HPS-G or SPS-G). In accordance with post-BS practice guidelines, the meal plan will consist of 6 small meals per day (breakfast, morning snack, lunch, afternoon snack, dinner, and late snack), plus powdered PS that will be distributed during either 3 (SPS-G) or 6 (HPS-G) meals. Phone and in-person contacts will be provided on alternating weeks during the 12-week PS intervention. At each contact time point, dietary and PS adherence, food tolerance, and hydration will be evaluated by reviewing the food records specifically designed to quantify the number of scoops of the PS used in each meal. Urinary nitrogen excretion will be used as a biomarker for PI, and to measure compliance. Subjects will be asked to collect their 24-hour urine at 5 different time points during the 12-week period. PS containers will be supplied during the in-person participant-dietitian biweekly interviews. Participants will be asked to bring the empty PS containers for review and quantification at each appointment.

Preceding Diet and 5-Day Inpatient Stays at 3 Months and 12 Months After Bariatric Surgery

Daily energy intake during the preceding diet period, and during the inpatient

stays, will be significantly reduced to a 10-15 kcal/IBW per day at 3 months post-BS. This value will increase up to 20 kcal/day at 12 months [4].

9.5. Laboratory Analysis and outcome measurements

9.5.1. Nitrogen Balance

Participants will be admitted for 5 days in the CRR for complete feces and urine collections during the last 3 days of the inpatient period. These collections will take place at the 3 different study time points: (1) presurgery; (2) 3 months after BS, during the active weight loss phase; and (3) 12 months after BS to evaluate the long-term carry over effect of PS on BC changes and REE.

9.5.2. 24-Hour Complete Specimens Collection

Four 24-hour urine collections will be undertaken; 1 per day during the inpatient stay. Collection will start after the first void of the second day. Labeled urine containers will be refrigerated at 4° Celsius. Starting on the third day, and until the end of the inpatient study period, all stools will be collected with a specimen container kit placed in the toilet. Samples will be processed every day and aliquots will be obtained and stored at –70° Celsius for later analysis of 24-hour nitrogen content. To ensure a complete collection of feces and compliance with the inpatient diet, 1.5 g of polyethylene glycol (PEG) will be ingested along with other food items during the first inpatient day. PEG has been used as a fecal marker to follow time and completeness of collections, to determine when experimental diets have been eliminated, and to correct for differences in the day-to-day variation of fecal transit time [59,60]. Stool samples will be analyzed for PEG, and combustible energy from PEG will be subtracted from fecal combustible energy measured by bomb calorimetry.

Miscellaneous losses of nitrogen in sweat, sloughed skin, nails, hair, and various bodily secretions will be estimated at 8 mg/kg of body weight per day [42]. Body weight will be measured daily during the inpatient stays to estimate the daily miscellaneous nitrogen losses defined above.

NB will be calculated using the difference between daily nitrogen intake and total nitrogen losses as follows: nitrogen balance = dietary nitrogen intake–(daily urinary nitrogen excretion + daily fecal nitrogen excretion + daily miscellaneous nitrogen losses).

9.5.3. Anthropometry and Body Composition

Anthropometry will be assessed during admission at the CRR before BS, at 3 months, and 12 months after surgery. Participants will be weighed while wearing a hospital gown, and without shoes, to the nearest 0.1 kg. Height will be determined using a fixed wall stadiometer to the nearest 0.1 cm. BMI will be calculated as weight (kg) divided by the square of the height (meters).

9.5.3.1. Body Composition

BC will be estimated based on a 3-compartment model, using 3 independent measures: body weight, TBW, and body density. To determine changes in BC after BS-induced weight loss, the 3-compartment model will be used [61].

9.5.3.2. Total Body Water

TBW will be measured using the stable isotope deuterium oxide (D2 O) as the reference method. A baseline venous blood sample (approximately 7 mL) from an antecubital vein will be taken while fasting. Immediately afterwards, a known dose of deuterated water D2 O (1 g/kg) will be taken orally. Three hours following the dose, when the D2 O has equilibrated with the body water deuterium-to-hydrogen, a second fasting blood specimen will be taken (approximately 7 mL). The TBW will then be derived from the increase in plasma deuterium content in relation to the volume of D2 O ingested [62].

9.5.3.3. Air Displacement Plethysmography

Body density will be measured using the BOD POD (Cosmed, Chicago, IL; software version 2.3) [63,64]. Subjects will be clothed in a Lycra-style swim cap and tight fitting underwear. Body weight will be measured to the nearest 1 g on the BOD POD electronic weight scale. Following standard calibration procedures, the subject's body volume will be measured, with correction made for thoracic gas volume, which will be estimated using the BOD POD breathing circuit system. The final thoracic gas volume and the average of 2 body volume measurements within 0.2% will be used to calculate body density [64].

9.5.3.4. Three-Compartment Calculations

FFM and FM will be measured using a modified 3-compartment model that was developed for obese subjects in the New York Obesity Research Center Body Composition laboratory, as follows: fat (kg) = 2.122 × (body weight/density) – 0.779 ×

TBW – 1.356 × body weight [65]. Body weight is measured in kg (measured by the Weight Tronix scale), density is derived from BOD POD, and TBW is measured in kg. FFM will be calculated as body weight minus FM.

9.5.4. Energy Expenditure

EE will be measured during the last day of the 3 inpatient stays (presurgery, 3 months, and 12 months postsurgery). Height, weight, blood pressure, pulse, and temperature measurements will be taken, and an intravenous catheter will be inserted. At 8:00 a.m., subjects will be placed under the hood of the metabolic cart (Parvo Medics System, True Max 2400) [66] and rest for 30 minutes. Following the resting period, REE will be measured for 30 minutes and baseline blood samples will be taken. Subsequently, all subjects will consume an isocaloric liquid meal (Boost High Protein) over 10 minutes. Subjects will receive acetaminophen with the meal to measure gastric emptying. Following meal administration, DIT (amount of EE above the REE rate due to the cost of processing food for use and storage) will be measured for 4 hours. Blood samples and questionnaire measurements will be collected at –15, 0, 15, 30, 60, 90, 120, and 180 minutes to measure hormonal and perceived satiety. REE will be calculated as an average of the last 15-20 minutes of each measurement period if values have reached steady state, defined as <10% fluctuation in minute ventilation and oxygen consumption and <5% fluctuation in respiratory quotient. DIT will be calculated by measuring the area under the curve of postprandial metabolic rate, above extrapolated baseline REE, for each time period [67]. Baseline and postprandial CCK, GLP-1, ghrelin, and PYY gut peptide concentrations will also be measured during the meal test.

9.5.5. Satiety

Subjective and objective assessments of satiety will be collected before the surgery, at 3 months, and at 12 months postsurgery, during the meal test on the last day of the inpatient stay, using 2 different approaches: VASs, and measurements of hormonal signals of hunger and satiety.

9.5.5.1. Visual Analog Scales

Participants will rate their feelings on the following questions by means of a mark on 100-mm line VASs: “How hungry are you?”, “How full are you?”, “How much stomach discomfort do you feel?”, “How thirsty are you?”, and “How much anxiety and nervousness do you feel?” The scale will be anchored at the low end with the lowest

intensity feelings (eg, *not at all*), and with opposing terms at the high end (eg, *most imaginable*), as previously described in the literature [68].

9.5.5.2. Measurements of Hormonal Signals of Satiety

Subjects will be instructed to consume the test meal within 15 minutes. Calorie intake and nutrient distribution of the meal (Boost High Protein) will be as follows: calories, 240; protein, 15 g; carbohydrates, 33 g; fat, 6 g; sodium, 200 mg; potassium, 400 mg, fiber 0 g. Patients will be able to choose between vanilla and chocolate flavors., An intravenous catheter will be inserted at 7:00 a.m. on the day of the experiment and blood will be drawn at before the meal, and at 15, 30, 60, 90, 120, and 180 minutes after the meal to measure hormonal signals of satiety. Blood samples will be collected in ethylenediaminetetraacetic acid tubes with added aprotinin (500 kallekrein inhibitory units per mL of blood) and dipeptidyl peptidase-4 inhibitor (10 µl/mL of blood; Millipore Research), and stored at –70° Celsius. Plasma concentrations of PYY, CCK, GLP-1, ghrelin, and insulin will be determined by radioimmune assay, and glucose concentration will be determined using the glucose oxidase method with an Analox glucose analyzer (Analox Instruments, Lunenburg, MA). Serum acetaminophen levels will be measured using an enzyme-linked immunosorbent assay (Abbot Laboratories, Chicago, IL).

9.5.5.3. Measure of Food Reward

Food reward is considered a strong eating drive that could override satiety, and will be assessed by the reward-based eating drive scale [69]. This 9-question screening tool will be completed by the participant during the inpatient stay before surgery, at 3 months, and at 12 months postsurgery.

9.5.6. Metabolomics—Branched Chain Amino Acids

Fasted blood samples obtained prior to the meal test will be used to measure circulating BCAAs by mass spectrometry, as previously described [70]. BCAAs will be measured during the inpatient stay before surgery, at 3 months, and at 12 months postsurgery.

9.5.7. Insulin Sensitivity

Insulin sensitivity will be measured by the Matsuda index during the meal test as follows: 10,000/(fasting glucose × fasting insulin × mean glucose from from 0 to 180 min) × mean insulin from from 0 to 180 min)^0.5 [71].

9.5.8. Physical Activity

There will be no physical activity intervention in this study. A measure of the free-living physical activity will be obtained using the ActiGraph, a wireless activity monitor that will provide 168 continuous hours (1 week) of measurement before surgery, at 3 months, and at 12 months postsurgery.

9.5.9. Vitamins, Minerals, Prealbumin, and Albumin Levels

Laboratory assessments will be obtained as part of the regular blood tests taken before surgery, at 3 months, and at 12 months postsurgery, following the clinical practice guidelines for the evaluation of the nutritional status in the Bariatric Clinic.

9.5.10. Food Record

Dietary intake evaluation during the outpatient phase of the study will be performed as part of each nutritional follow-up. Food and beverage intake will be assessed using either a 7-day (during the preceding diet and the inpatient stay) or 4-day (regular dietary evaluations) throughout the study. As described above, the PI goal will be accomplished for each group (HPS-G and SPS-G) by using the specific PS resources provided. During the screening phase of the study, all subjects will attend 2 training sessions delivered by a Registered Dietitian, in which they will be instructed on how to record their food intake and include at least 1 weekend day when recording. This information will be analyzed with NDSR.

9.6. Sample Size Calculations

Sample size was calculated based on anticipated changes in REE during weight loss using data from published literature [72] and changes in BCAAs from our previous study [45]. Baba et al [72] studied changes in REE after high protein (n=7) versus low protein (n=6) weight loss diets. Weight loss was significantly higher in the high protein group (8.3 kg, SD 0.7) compared to the low protein (6.0 kg, SD 0.6). Change in REE was –132.3 kcal/day (SD 51.0) in the high protein group and –384.3 kcal/day (SD 84.6) in the low protein group. Assuming a Cronbach alpha of 0.05 and 0.80 (80%) power we would need to recruit 16 participants to each study group. Laferrère et al [45] showed that for a matched amount of weight loss (10 kg) in GBP (n=10) versus diet-induced weight loss (n=11), BCAA changes were –176.4 (SD 96.6) and –57.6 (SD 99.3) in surgical and nonsurgical groups, respectively. Assuming a Cronbach alpha of 0.05 and 0.80 (80%) power we would need to recruit 22 participants to each study group. Therefore,

accounting for an attrition rate of 30% after 1 year, we will enroll a total of 28 subjects per group.

9.7. Statistical Analyses

Data will be analyzed using the SPSS statistical program (IBM Software; Armonk, NY). For most study aims, a repeated measures design will be used to examine the trajectory of changes in subjects between HPS-G and SPS-G groups regarding BC, EE, NB, satiety, and circulating BCAAs, from presurgery to 3-month and 12-month postsurgery levels. Nonlinear mixed model regression (SAS PROC NLMIXED) will be used for the actual analyses since these outcomes are not likely to be normally distributed. In addition to overall tests for differences between groups (GBP and SG, and HPS-G vs SPS-G), differences over time (presurgery, 3 months, and 12 months postsurgery), and group per time interactions. Other factors will be explored as covariates to determine possible explanatory factors for significant differences between presurgery and 3-month and 12-month postsurgery levels. Significance levels will be adjusted based on the total number of comparisons being carried out, using a Bonferroni correction. Secondary outcomes will be analyzed using a similar repeated measures design with either mixed model regression or nonlinear mixed model regression where appropriate. No adjustment for the number of comparisons will be made in the case of the secondary outcomes (ie, Cronbach alpha will be 0.05 for all comparisons).

9.8. Ethics

This proposal has been approved by the Institutional Review Board of Mount Sinai St Luke's Hospital and Columbia University. Voluntary written informed consent will be obtained from each participant prior to enrollment.

9.9. Discussion

The proposed study will determine the effect of 2 different levels of dietary PI (HPS-G and SPS-G) after SG and GBP on the NB, BC, EE, hormonal and perceived satiety, plasma levels of BCAAs, and insulin sensitivity, and feasibility of PS up to 1 year after BS. In addition, the analysis of energy excreted in feces will aid in the understanding of how much malabsorption exists in the 2 procedures studied (GBP and SG). Satiety during a liquid test meal will be assessed and its possible mediators, gastrointestinal hormones released, and gastric emptying rate will also be determined.

Two nonrandomized studies demonstrated that higher levels of PI (>60 g/day) were related to higher excess weight loss 6 months after BS [18] and at 3, 6, and 12 months postsurgery [37]. Other studies have failed to observe a significant association between PI and excess weight loss [28,35,73]. PI was positively associated with LBM retention in 3 nonrandomized studies [5,17,28], although this association was not found in other studies up to 1 year after BS [35,37]. The same authors also failed to observe a relationship between PI and REE [35,37]. The relationship between PI levels and gastrointestinal hormones needs to be explored more thoroughly. One RCT failed to observe a relationship between PI and GLP-1 or ghrelin [38]. The relationship between BCAA circulation levels and glucose homeostasis after BS also needs further attention. Elevated circulating BCAAs are associated with obesity and T2DM. Comprehensive metabolic profiling of obese versus lean human subjects revealed a BCAA metabolic signature, marked by increased circulating levels of BCAAs as well as products of BCAA catabolism [73]. The reason that circulating BCAA levels are elevated in obesity is still unclear. The mechanisms responsible for the decrease in BCAA serum levels with weight loss [47] or BS [46,74,75] are still being studied. Supplementation of a high fat diet with BCAAs in rats [76], or infusion of AAs in humans [76], results in IR. A recent epidemiological study reported that elevated plasma levels of essential AAs, including BCAAs, phenylalanine, and tyrosine in healthy individuals predicted a 5-fold increase in the risk of developing T2DM [77]. To our knowledge, there are no intervention studies that address the impact of PI on BCAA serum levels after GBP or SG. Lower levels of PI seem to have a positive effect on glucose homeostasis, while sustained low circulating levels of BCAAs may have a negative impact on protein synthesis and the integrity of the skeletal muscle mass during weight loss [78]. Plasma leucine concentration has been shown to correlate with skeletal muscle protein synthesis [53], so the metabolomic study of BCAAs during the high protein meal test will contribute to the study of AA kinetics after massive weight loss induced by BS. Measuring the NB during a negative energy balance will provide an important means of understanding absorption and bioavailability of nitrogen.

Dietary guidelines, including PS after BS, are still under discussion since the levels of evidence of their recommendations are C or D [24]. As previously detailed, the relationship between dietary PI and the various outcome variables that will be measured in this proposed study are not well established, and the available literature is

contradictory. Dietary protein plays an important role in weight loss and obesity-related comorbidities, such as diabetes [79]. BS is highly popular, making the proposed work relevant, and the study will help to clarify the relationship between PI and BS outcomes by addressing some of the existing gaps in the scientific literature.

10. References

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CHAPTER 10: EFFECT OF A 3-MONTHS PROTEIN SUPPLEMENTATION INTERVENTION ON NITROGEN BALANCE, BODY COMPOSITION, ENERGY EXPENDITURE, SATIETY AND BRANCHED CHAIN AMINO ACIDS LEVELS 12 MONTHS AFTER BARIATRIC SURGERY: RESULTS OF A PILOT STUDY

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10 EFFECT OF 3-MONTHS PROTEIN SUPPLEMENTATION INTERVENTION ON NITROGEN BALANCE, BODY COMPOSITION, ENERGY EXPENDITURE, SATIETY AND BRANCHED CHAIN AMINO ACIDS 9 MONTHS AFTER BARIATRIC SURGERY: RESULTS OF A PILOT STUDY.

10. EFFECT OF A 3-MONTHS PROTEIN SUPPLEMENTATION INTERVENTION ON NITROGEN BALANCE, BODY COMPOSITION, ENERGY EXPENDITURE, SATIETY AND BRANCHED CHAIN AMINO ACIDS LEVELS 12 MONTHS AFTER BARIATRIC SURGERY: RESULTS OF A PILOT STUDY.

10.1 Introduction

Bariatric surgery (BS) is gaining popularity as the most appropriate therapeutic approach for a carefully selected group of patients with severe obesity (1). Although the rates of resolution of comorbidities are noteworthy, nutritional deficiencies are common. This is despite nutritional supplementation according to guidelines is properly advised (2). Indeed, current nutritional guidelines are under discussion since evidence on the proper dose of dietary macronutrients and micronutrients intake during the weight loss phase is unknown. From a nutritional point of view, nutrient intake should be enough to avoid nutritional complications-related deficiencies and to promote healthy weight loss in a negative energy balance situation. Protein is one of the main nutrients that can be affected after bariatric surgery (BS) (3). The anatomic changes created by the surgical procedure result in a drastic reduction of food intake and could hamper food tolerance, mainly of protein-rich foods. Low protein intake (PI) during weigh loss (WL) has been associated with a negative nitrogen balance (NB)(4). Importantly, PI has been associated with a series of events occurring after BS.

First, low PI has been associated with long-term negative NB that could favor loss of lean and fat tissue (5) during weight loss and have a negative impact on overall health (6). An increase in the absolute amount (grams) of protein intake would improve NB and thus defend the amount of fat-free mass (FFM) loss during weight loss (4). Nonetheless, a recent meta-analysis has shown that there is still limited evidence on the effect of PI on lean body mass retention during weight loss (WL) after BS.

Second, recent studies in humans (7) and animals (8, 9) suggest an increase of about 20% in resting metabolic rate (RMR) after Roux in Y gastric bypass (GBP). Faria et al. (2012) demonstrated an increase of 3.1% in the percentage of postoperative excess weight loss for each unit increase in the RMR adjusted per kg body weight (7). Admittedly, data on changes in RMR following are conflicting (10). However, to our knowledge, how PI affects energy expenditure (EE) and diet induced thermogenesis (DIT) after BS has not been explored. Third, weight loss early after BS, is associated with

reduced concentrations of plasma branched-chain amino acids (BCAAs) and improved insulin sensitivity (11). BCAAs, specifically leucine, have a major role on protein synthesis. Observations of circulating BCAA levels after BS in relation to PI are limited.

Finally, meal-related GLP-1 and PYY secretion is substantially increased after BS and may contribute to the post surgery beneficial effects on eating (by inducing satiety), body weight, and glycemic regulation. The relationship between PI, satiety, and these anorectic hormones after BS has not yet been study.

Against this background, in this pilot study we aimed at evaluating the effect of two different levels of dietary protein supplementation (standard versus high) after BS on the NB, BC, EE, satiety hormones and perceived satiety, plasma levels of BCAA and insulin sensitivity, as well as the feasibility of protein supplementation up to one year after BS. To our knowledge, no study has evaluated NB before and at long term after BS. Thus, this translational pilot study integrates mechanistic, metabolic, and energy homeostasis outcomes to critically evaluate the adequacy of protein recommendations for severely obese individuals after BS.

10.2. Methods

10.2.1. Participants

The study was conducted at Mount Sinai St. Luke´s Hospital and Columbia University Medical Center. Six female participants were selected from an eligible pool of severely obese individuals undergoing either Sleeve Gastrectomy (SG) or Gastric-by Pass (GBP). Given the small sample size and since body composition differences exist between women and men only premenopausal women (18-40 years of age, BMI <50 kg/m2) will be included in this pilot study. Other inclusion criteria include: any race/ethnicity; patients scheduled to undergo either GBP or SG; treated or untreated resting systolic/diastolic blood pressure less than 160/100 mmHg; fasting triglyceride concentration less than 600 mg/dl, without regard for diagnosis or prescription for other dyslipidemias; absence of diabetes, or diet-controlled diabetes (taking no medications). Exclusion criteria include: presence of nitrogen retention disease (eg, renal or hepatic disease); abnormal thyroid function; known malabsorption syndrome; cardiovascular disease; current mucosal (gastrointestinal, respiratory, urogenital) or skin (cellulitis) infection; any psychiatric disorder. All subjects provided written informed consent of both institutions, Mount Sinai St Luke´s Hospital (MSSLH) and Columbia University Medical Center (CUMC).

10.2.2. Study protocol

We conducted a randomized open label, pilot study, in which, 6 healthy obese women, aged 26.5 ± 5.6 years, BMI 47.16 ± 5.2 (kg/m2) undergoing bariatric surgery (GBP or VSG) were allocated to standard protein supplementation (PRO-S) (0.8g protein/kg ideal body weight (IBW)/day (n=3) or high PRO-S (1.2g protein/ kg ideal body weight (IBW)/ day) (n=3). After randomization, participants underwent to a pre-surgery 5-days inpatient stay at CUMC-CRR to conduct the pre-surgery intervention study. After surgery protein supplementation was supplied for 12 weeks based on randomization. Following the protein supplementation intervention, participants underwent to their post-surgery 5-days inpatient stay for a new study at 3 mo, and at 9 mo to evaluate the carry over effect of PRO-S.

10.2.2.1. Dietary intervention

Dietary protocol during preceding diet at home and during the inpatient stay

Preceding Diet at Home

NB study was preceded by a 7-day, outpatient-diet stabilization period. During this time, a meal plan was tailored to each participant and protein supplements were provided, meal timing and Unjury® flavors preferences were also considered in the meal plan. Participants were asked to fill a 7-day food record during this time at home, they were also contacted by phone once during this period in order to promote adherence to the prescribed diet. This diet stabilization period was designed to allow each participant to adapt to the level of protein provided during the inpatient/experimental period and to ensure gastrointestinal adaptation had occurred. Energy and nutrient intake were calculated from the nutritional database of NDSR, 2011. Energy and protein at this time was based on 35kcal/ IBW/day and 1.2gprotein/ IBW/day respectively (based on our previous observations, and included as part of this thesis). Energy intake adjustments were done when change on weight (± 0.75kg) was experienced during both the preceding diet and the inpatient stay. According to post bariatric surgery dietetic guidelines and randomization groups, meal plan was based on 6 small meals a day (breakfast, morning snack, lunch, afternoon snack, dinner and late snack), plus powder protein supplements for all dietary intervention.

Inpatient Stay Baseline

During the 5-day inpatient stay, all foods and drinks were provided and carefully monitored and controlled (VM). All foods, drinks and protein supplements were previously pre-portioned and weighed before consumption at a 0.1 precision scale. In order to ensure minimum variability and accurately control nitrogen intake, participants

ate the same diet every day. Participants were not allowed to eat and drink any food from outside. Diet was based on regular/ natural foods that required minimum manipulation. Unjury® products were included as the main protein source during all the study. Participants were trained on how to eat all the food served, spatulas were provided to help them to eat 100% of the food, they were also encouraged to rinse the containers as needed to ensure a complete consumption and to drink the water used to rinse the container. Supervision was ensured at every meal after each serving, trays were explored by the staff members and the volume of unconsumed beverage/soup were measured in a graduated cylinder, all existing uneaten food were weighted and recorded for day-to-day dietary adjustments in the computer. Meals for one complete day were cooked by duplicate and homogenized individually in a heavy duty commercial blender. Aliquots were obtained and frozen a -70C for posterior analysis of total nitrogen and energy content.

Protein supplementation

Protein supplementation was supplied during the first 3 months after surgery (protein intervention period). The dietary plan for the high PRO-S group contained 1.2g-protein/kg/ IBW/ day, whereas the dietary plan for the standard PRO-S group contained 0.8g protein/ IBW/ day. IBW was established based on BMI=25 kg/m2. PRO-S goals were achieved partly by providing subjects with an instant protein powder supplement (Unjury® isolate whey protein) until they reached their individual protein needs established for each group. The protein powder supplement contained 21g protein/ serving. The Standard PRO-S group required approximately 1.5 servings/ day while the High PRO-S group required 3 servings/ day to meet their individually prescribed protein requirement. Provided protein supplements were well tolerated by participants allowing a good level of adherence during the dietary protein intervention study phase.

Phone and in personal contacts were combined every week during the protein supplementation intervention and lasting 12 weeks. At each contact, diet progression, food tolerance, hydration, and adherence were evaluated. Protein supplements were supplied during the personal contacts.

10.2.2.2. Nitrogen balance

Nitrogen Balance study (NB):

Participants were admitted during 5 days in the Clinical Research Resources (CRR) of the Columbia University (CU) Clinical and Translational Science Award (CTSA) in 3 different periods of the study: 1) pre-surgery, 2) 3 months after BS during

the active weight loss phase and 3) at 9 months after BS during the weight maintenance phase.

Urinary and feces collection

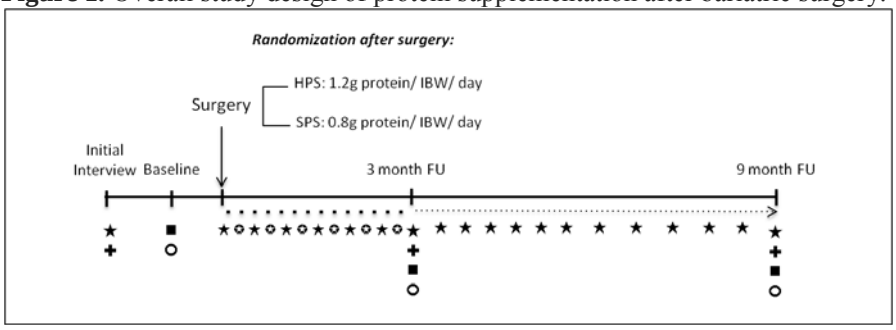
24hs complete urine and feces production was collected starting on the second and third day of the inpatient stay respectively. Stools were collected after each bowel movement by a specimen container kit that was promptly placed in the ice filled- cooler installed inside the patient's bathroom and subsequently refrigerated in one of the fridges at the CRR. Samples were processed every day and aliquots obtained and stored at -70ºC for its posterior analysis of 24hs nitrogen content. During the first day as inpatient, participants were administered Polyethylene Glycol 3350 (PEG), PEG has been used as a fecal marker to follow time and completeness of collections to determine when experimental diets have been eliminated, and to correct for differences in the day-to-day variation of fecal transit time (12).

Miscellaneous losses of nitrogen in sweat, sloughed skin, nails, hair, and various body secretions were estimated at 8mg/ kg of body weight per day, as indicated by the 1985 joint FAO/WHO/ONU report (13). Body weight was determined every day during the inpatient stay in order to estimate the daily miscellaneous nitrogen losses. NB was calculated from the difference between daily nitrogen intake and total nitrogen losses as follows:

NB = IN-(UN+FN+MN)

Where: IN= daily dietary nitrogen intake; UN=daily urinary nitrogen excretion; FN=daily fecal excretion; MN= daily miscellaneous nitrogen losses.

Figure 1. Overall study design of protein supplementation after bariatric surgery.



▪ ▪ ▪ ▪ 3 months protein supplements provided.
..... 9 months free living diet.
★ In person nutritional interview/ Dietary evaluation: 24hs Recall + 4-d food record
✪ Phone contact
✚ 7-day Physical Activity monitoring (Accelerometer)
■ Anthropometry/ Body Composition: TBW, BodPod
○ 5-day Impatient Stay
- Nitrogen Balance : feces and urine collection
- Meal test Satiety/ Resting Energy Expenditure/ Diet Induced thermogenesis with Indirect Calorimetry

HPS-G: High protein supplementation group. SPS-G: Standard protein supplementation group

10.2.2.3. Body Composition

Anthropometry was evaluated during admission at the CRR before surgery and at 3 and 9 months after surgery. Participants were weighed with light clothes and without shoes to the nearest 0.1 kg. Height was determined using a fixed wall stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided per squared height (meters). Body composition is typically estimated by dividing the body into compartments (14). A 3-compartment model (3C) is calculated from 3 independent measures: body weight, total body water, and body density. To determine changes in body composition after weight loss induced for BS a 3C was used as it is considered a gold-standard reference method in this population (15).

Total Body Water (TBW)

TBW was measured using the stable isotope deuterium oxide (D2O or 2H2O) as the reference method. A baseline venous blood sample (~7ml) from an antecubital vein was taken in fasting condition. Immediately after, a known dose of deuterated water D2O (1g/kg) was administered orally, three hours following the initial dose administration, when the D2O equilibrate with the body water deuterium-to-hydrogen (D:H), a second fasting blood specimen was taken (~7 ml). The TBW was then accurately measured from the increase in plasma deuterium content in relation to the volume of D2O ingested.

Air displacement plethysmography

Body density was measured using the BOD POD (Cosmed, Chicago, IL; software version 2.3) as described in the protocol (16, 17). Subjects were clothed in a Lycra-style swim cap and tight fitting underwear. Body weight was measured to the nearest 1g on the Bod Pod electronic weight scale. Following standard calibration procedures, the subject's body volume was measured with correction made for thoracic gas volume (VTG) estimated using the BOD POD breathing circuit system. The final VTG and the average of two body volume measurements within 0.2% were used to calculate body density.

3-Compartment Calculations

Fat-free mass and fat mass was measured using a modified 3-compartment model that was developed in obese subjects in the NYORC laboratory (15) as follows: Fat (kg) =2.122*(BW/D) – 0.779*TBW – 1.356*BW, where BW is body weight in kg (measured by the Weight Tronix scale), D is body density and will be derived from BodPod, and TBW is total body water in kg. Fat-free mass will be calculated from body weight minus FM.

10.2.2.4. Energy Expenditure

Energy expenditure was measured during the last day of the 3 inpatients periods: baseline and 3 and 9 months. Height, weight, blood pressure, pulse, and temperature measurements were taken and an i.v. catheter inserted. At 8:00 am, subjects were placed under the hood of the metabolic cart (Parvo Medics System - True Max 2400) (18) and rest for 30 min. Following the resting period, REE was measured for 30 min and baseline blood samples taken. Subsequently, a liquid meal test, isocaloric for all subjects (Boost HP, Nestle®) was consumed over a period of 10 to 15 min. At that time subjects received acetaminophen to measure gastric emptying. Following meal administration, diet-induced thermogenesis (DIT) (amount of EE above the REE rate due to the cost of processing food for use and storage) was measured for 4 hours. Blood samples and questionnaires measurements were taken at -15, 0, 15, 30, 60, 90, 120 and 180 minutes to measure hormonal and perceived satiety. Questionnaires and DIT were administered also at 240 min after the meal to ensure we reached baseline measurements and to ensure a proper collection of the complete heat production after the meal was eaten. REE was calculated as an average of the last 15-20 min of each period if values have reached steady state (defined as <10% fluctuation in minute ventilation and oxygen consumption and <5%

fluctuation in RQ). Diet-induced thermogenesis was calculated by measuring the AUC of post-prandial metabolic rate above extrapolated baseline REE for each time period and was expressed as absolute values and as % of energy intake.

10.2.2.5. Branched chain amino acid

BCAA levels from blood samples obtained during the meal test were analyzed and determined by targeted metabolomics analysis.

10.2.2.6. Satiety

Satiety will be measured at baseline and at 3 and 9 months during the inpatient stay and after a test meal by 2 different approaches: 1) questionnaires during a meal test: a self reported measure of hunger and fullness using a Visual Analog Scale (VAS) and 2) measurements of hormonal signals of satiety as PYY and GLP-1 after high protein meal. Hormone levels were analyzed during the last year of the grant as well as plasma concentrations of acetaminophen administered during the meal test as a measure of gastric emptying.

The day of the experiment, an IV catheter was inserted at 7:00AM and blood was drawn at -15, 0 and at 15, 30, 60, 90, 120 and 180 after the meal test to measure hormonal signals of satiety. Questionnaires (VAS and SLIM) were also given at the same intervals to evaluate perceived satiety. Subjects were instructed to consume the test meal within 10-15 minutes. The total amount of blood taken during each study period was 83mL. Calorie intake and nutrient distribution of the meal (Boost HP) are as follows: Calories 200, Protein (g) 26, Carbohydrate (g) 10, Fat (g) 6, Sodium (mg) 480, Potassium (mg) 800, Fiber (g) 0, Vitamins & Minerals 35% of RDI, Lactose (g) <1.

10.2.2.7. Physical activity and adherence to protein supplementation

No intervention on physical activity was done in this study. PA recommendation was given as part of the standard care provided by the bariatric unit. A measure of 24hs free living physical activity was obtained using the ActiGraph™, a wireless activity monitor that was provided for a 24 hour measure at baseline, and at 3 and 9 months after surgery.

Adherence to protein supplementation (PS) during the intervention was measured at each encounter. Participants completed a diet registry designed to evaluate PS scoops

taken. Also, the empty PS package were collected during the interview. Attending to follow up was also another way to count for adherence.

10.2.2.8. Assays

Food, urine and feces samples were digested with the Kjeldahl method, for nitrogen determination. The nitrogen content was measured with the Nitrogen Auto Analyzer from Seal Analytical (Mequon, Wisconsin) Intra-assay and interassay coefficients of variation ranged from 0.5 to 3.1%and 4.2%, respectively. Calorie content of food aliquots and feces samples was determined by Calorimeter System from Parr Instrument Company (Moline, Illinois). Intra e inter assay CVs for calorie determination 2015 was: 0.532% and 0.876% respectively. Urine creatinine levels were analyzed by calorimetric (Jaffe) cobas integral 400 plus, roche diagnostics (Indianapolis, IN) normal range (29-278mg/dL). PYY (H. PYY (3-36)) was analyzed by RIA, PYY-67HK, Millipore. Acetaminophen kits, 505-30, Sekisui Diagnostics. Inc, Canada. Plasma glucose was determined by the glucose oxidase method with a glucose analyzer (Analox, Lunenburg, MA). Total GLP-1 level was measured by radioimmunoassay after plasma ethanol extraction. Plasma insulin levels were measured by. All hormone assays were performed at the Hormonal Core Laboratory at the New York Obesity Nutrition Research Center with commercial kits (EMD Millipore).

Calculations

Total area under the curve (AUC) during the meal test was calculated using the trapezoidal method.

10.2.2.9. Statistical Analysis

Due to small sample size, analyses were mainly done without separating surgery types or PS-level. The analysis was based on a mixed effects model for repeated measures (MMRM), including time as a categorical fixed factor with the (co)variance matrix set to autoregressive first order type (AR(1)) to model the within-subject variability, and the denominator degrees of freedom for the F-test for fixed effects was estimated using the Kenward-Roger approximation. When applicable, the fixed group factor (low or high protein intake) and its interaction with time were added to the model. Nitrogen balance means were also estimated including the nitrogen food intake in the model as a covariate. The analysis was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA), and a level of significance was established at the two-sided 5% level.

10.3. Results

Recruitment and retention:

Of the 6 enrolled participants, one was lost at 3 mo follow up. NB was performed on 5 participants who completed the 3 inpatient stay.

Baseline Characteristics

Table 1 shows baseline characteristics of our homogeneous sample. Because of the pre-specified sample size of this pilot study, per inclusion criteria participants did not present comorbidities associated with obesity.

Table 1. Baseline subjects characteristics

	n=6	Mean	CI
Race (W/ AA/ H)	0/4/2		
Age (y)		26,5	21; 31
BMI (kg/m^2)		47	40,1; 53,0
Weight (kg)		125,3	110,0; 140,6
FM (kg)		64,1	50,8; 77,5
FFM (kg)		61,23	55,75; 66,7
TBW (L)		39,92	35,36; 44,48
RMR		1686	1590; 1780
Physical Activity (actigraph)			
% Sedentary		68,1	60,1; 76,1
% Light/lifestile/moderate		30	22,3; 37,7
% Vigorous		1,9	1,1; 2,7
Lipid Profile (mg/dL)			
HDL-C		45	41,8; 48,2
LDL-C		97	86,6; 107,4
TG		95	64,6; 125,4
Fasting Glucose (mg/dL)		84	72,2; 96,0
HbA1$_C$		5,5	5,2; 5,8
Sistolic BP		136	117,6; 154,4
Diastolic BP		79	68,6; 89,4

Changes after BS

Changes at 3 and 9 months in weight, body composition (FM and FFM) and total body water, is presented in table 2.

Table 2. Changes in body composition (3-C Model) and Total Body Water (deuterium). Mean [CI]

	BMI (units)	Weight (kg)	FFM (kg)	FFM (%)	Fat (kg)	Fat (%)	TBW (L)
3 months							
HPS-G	-8,3 [-12,3;-4,4]	-21,2 [-30,5;-11,9]	-3,4 [-5,79;-1,00]	-5,9 [-10,1;-1,7]	-17,3 [-26,8;-7,8]	-27,3 [-43,4;-11,3]	-0,57 [-3,4;2,2]
SPS-G	-7,5 [-10,7;-4,3]	-20,7 [-28,3;-13,1]	-2,28 [-5,21;0,66]	-4,10 [-9,3;1,1]	-18,9 [-30,6;-7,2]	-26,29 [-45,9;-6,6]	-0,45 [-3,8;3,0]
9 months							
HPS-G	-15,1 [-19,0;-11,1]	-38,3 [-47,7;-29,1]	-4,8 [-7,2;-2,4]	-8,0 [-12,3;-3,8]	-35,9 [-45,4;-26,4	-56,9 [-72,9;-40,9]	-2,63 [-5,4;0,2]
SPS-G	-14,8 [-18,0;-11,6]	-40,7 [-48,3;-33,1]	-4,1 [-7,1;-1,2]	-7,8 [-13,0;-2,6]	-34,3 [-45,9;-22,6]	-46,34 [-65,99;-26,69]	-2,85 [-6,3;0,6]

Dietary characteristics during the inpatient stay

1. Kilocalorie intake

Kilocalorie intake significantly decreases after BS for both PS groups. Kcal intake was lowest at 3 months follow up, and increased significantly between 3 and 9 months after BS. However kcal intake was significantly reduced at 3 and 9 mo after surgery relative to baseline. A complete total nutrient report for the two PS groups throughout the study is presented in table 3(a).

2. Protein intake

As part of the study design protein intake at baseline (g/day) during the inpatient was similar between the two PS groups. After surgery, PI was significantly different between randomized groups (Figure 1). PI in SPS-G decreased significantly relative to baseline while HPS-G remain similar at baseline and at 3 mo but decrease significantly at 9 months. Protein source (animal and vegetal) and quality (assessed as Protein Digestibility Corrected Amino Acid Score- PDCAAS) was the same for both groups. PDCAAS of the selected PS product is 1. Table 3(b) shows a description of amino acids intake (g/ day) for each PS group.

Table 3 (a). Estimated Kcal and macronutrient intake during the inpatient (NDSR®)

	Baseline		**3mo**		**9mo**	
	Mean	**CI**	**Mean**	**CI**	**Mean**	**CI**
Calories (Kcal)						
HPS-G	2030	1813;2248	913	695;1130	1193	975;1410
SPS-G	2294	2028;2560	990	724;1256	1155	888;1421
Total Fat (g)						
HPS-G	74,9	64,7;85,0	20,2*	10,0;30,4	31,8	21,6;42,0
SPS-G	91,8	79,4;104,3	34,0	21,4;46,4	39,6	27,1;52,1
Carbohydrates (g)						
HPS-G	264,4	206,4;322,3	104,6	46,7;162,6	153,4	95,5;211,3
SPS-G	306,8	235,9:377,8	127,5	56,5;198,4	155,7	84,7;226,6
Total protein (g)						
HPS-G	83,8	76,7;90,9	81,0*	74,0;88,1	82,9*	75,8;90,0
SPS-G	81,52	72,8;90,2	53,7	45,0;62,5	55,6	46,9;64,4
Animal protein (g)						
HPS-G	57,9	51,8;64,0	70,3*	63,5;77,1	61,5*	55,4;67,5
SPS-G	50,8	43,4;58,2	38,7	31,3;46,1	35,8	28,5;43,3
Vegetal protein (g)						
HPS-G	25,9	18,5;33,4	9,1	1,0;17,1	21,4	14,0;29,0
SPS-G	30,6	21,6;39,7	15	5,94;24,1	19,8	10,7;28,8
Percentage of Fat						
HPS-G	32,5	23,4;41,6	19,9*	10,8;29,01	23,3	14,3;32,4
SPS-G	35,4	24,3;46,5	30,4	19,3;41,5	30	18,9;41,1
Percentage of CH						
HPS-G	51,2	40,0;62,5	42,5	31,3;53,8	48,9	37,7;60,3
SPS-G	50,6	36,8;64,9	48,2	34,4;62,0	51,1	37,3;65,0
Percentage of Prot						
HPS-G	16,3	9,6;22,4	37,8*	31,0;44,4	27,7*	21,0;34,4
SPS-G	14	5,7;22,0	21,6	13,5;30,0	18,75	10,5;27,0
Nitrogen Total						
HPS-G	13,2	12,1;14,3	13*	11,8;14,1	13,15*	12,0;14,3
SPS-G	14,3	12,8;15,8	8,5	7,15;9,9	8,8	7,46;10,2
Protein (g)/ IBW/ day						
HPS-G	1,19	1,10;1,28	1,16*	1,07;1,25	0,96	0,87;1,04
SPS-G	1,22	1,11;1,33	0,78	0,68;0,89	0,69	0,58;0,80

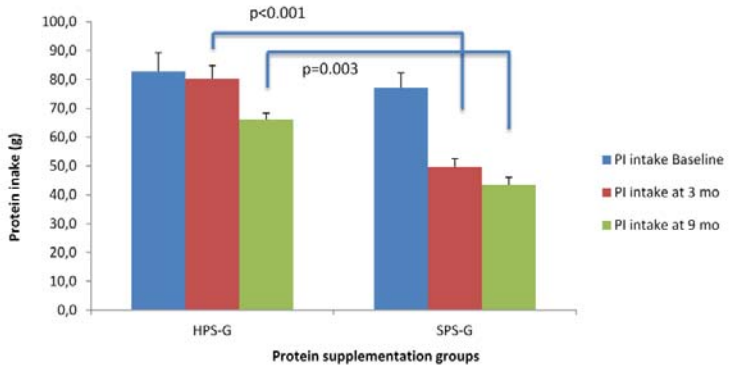
*P<0.005 between groups

Table 3 (b). Amino acids intake (g/ day) for each PS group.

	HPS-G		SPS-G		HPS-G		SPS-G		HPS-G		SPS-G	
	Baseline		Baseline		3 mo		3 mo		9 mo		9 mo	
Amino acids (g/ day)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tyr	1,52	0,10	1,40	0,15	1,57	0,09	0,98	0,02	1,53	0,10	0,96	0,00
Thr	3,86	0,25	3,60	0,30	3,87	0,21	2,45	0,00	3,82	0,22	2,45	0,01
Iso	3,83	0,25	3,62	0,24	3,87	0,22	2,46	0,05	3,85	0,20	2,55	0,07
Leu	**7,10**	**0,46**	**6,75**	**0,49**	**7,36**	**0,40**	**4,62***	**0,04**	**7,21**	**0,38**	**4,75***	**0,16**
Lys	6,16	0,46	5,70	0,34	6,58	0,36	4,09	0,01	6,32	0,32	4,06	0,03
Met	1,50	0,08	1,42	0,08	1,47	0,09	0,95	0,02	1,48	0,07	0,98	0,01
Cys	1,36	0,07	1,32	0,14	1,45	0,07	0,94	0,01	1,38	0,07	0,93	0,00
Phe	3,12	0,15	3,06	0,44	2,78	0,16	1,94	0,05	2,98	0,22	2,08	0,17
Tyr	2,26	0,14	2,18	0,34	2,08	0,13	1,41	0,02	2,18	0,15	1,42	0,04
Val	4,13	0,24	3,94	0,31	3,97	0,23	2,66	0,09	4,10	0,22	2,80	0,11
Arg	3,34	0,33	3,36	0,87	2,59	0,15	2,02	0,02	3,05	0,29	2,12	0,11
His	1,81	0,10	1,76	0,18	1,66	0,09	1,16	0,10	1,73	0,10	1,23	0,01
Ala	3,69	0,22	3,50	0,32	3,46	0,18	2,33	0,07	3,61	0,21	2,42	0,07

*** p<0.05 respect to baseline**

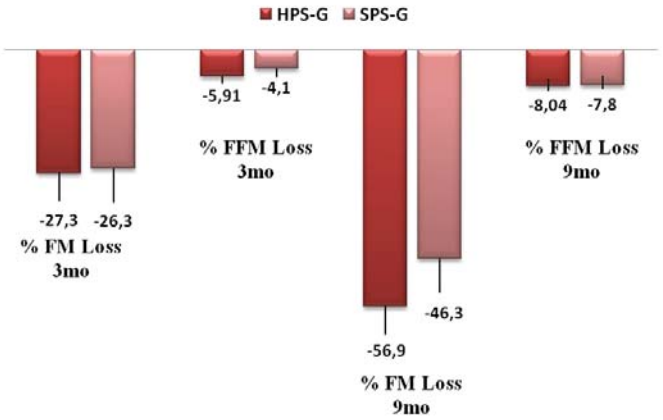
Figure 1. Protein intake (g/ day) between groups.



Body composition

Body weight, FM and FFM changes after BS were similar between dietary treatment groups and are presented in table 2. The proportion of total weight loss due to changes in FM and FFM did not differed across dietary protein levels (Fig.2).

Figure 2. Percentage of total WL due to changes in FM and FFM



Nitrogen Balance

Nitrogen balance was similar between PS groups. Figure 3, represents the NB for all participants. Data is presented as raw and after adjusting for protein intake. Although not significant, adjusted data showed a negative NB at baseline compared to 3 mo after BS (p=0.08).

Mean values and confidence intervals for nitrogen intake, and nitrogen excreted in urine and feces during the NB study are shown in table 4.

Table 4. Mean [CI] values for nitrogen intake and nitrogen excreted in urine and feces during the inpatient stay.

	Nitrogen Intake (g)		Nitrogen excreted in urine (g)		Nitrogen excreted in feces (g)	
	HPS-G	SPS-G	HPS-G	SPS-G	HPS-G	SPS-G
Baseline	13,2 [12,1; 14,3]	12,3 [11,0; 13,6]	10,4 [7,0; 13,7]	9,1 [5,0; 13,3]	1,6 [0,8; 2,5]	1,4 [0,4; 2,5]
3 months	12,8 [11,7; 13,9]	8,0 [6,6; 9,2]*	8,07 [4,7;11,45]	4,9 [0,8; 9,0]	0,7 [-0,1; 1,5]	0,5 [-0,5; 1,5]
9 months	10,6 [9,5; 11,7]	7,0 [5,6; 8,3]*	6,4 [3,0; 9,8]	5,7 [1,5; 9,8]	1,08 [0,2; 1,9]	0,4 [-0,6; 1,4]

* p<0,05

Individual values of nitrogen input (diet) and output (feces and urine) are represented in Figure 4. Figure 5 represents the NB calculated during the last 3 days of each 5-days inpatient stay during the study: before surgery, at 3 and at 9 month after BS, for each participant.

Figure 3. Whole group Nitrogen Balance: Raw data and data adjusted by protein intake.

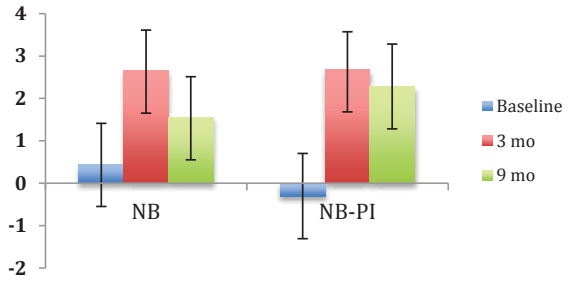


Figure 4. Nitrogen intake (diet) and output (feces and urine) individually during the 3 day inpatient stay at baseline, 3 and 9 months after BS.

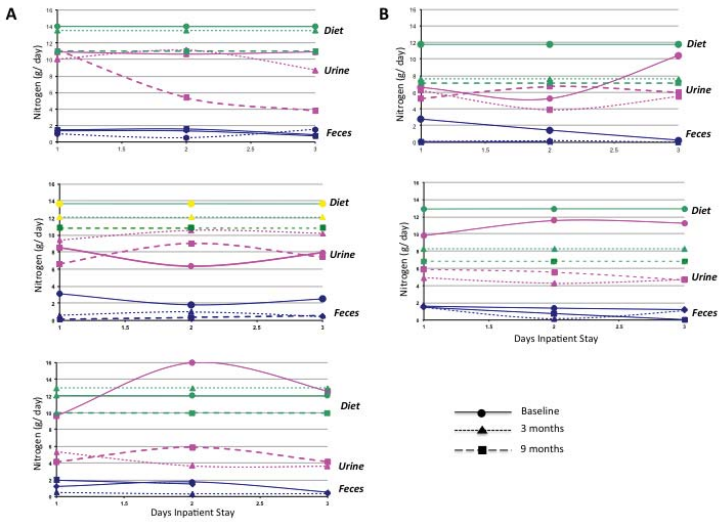
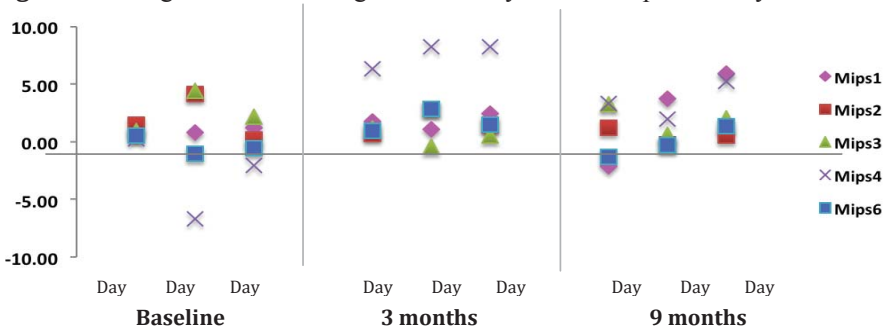


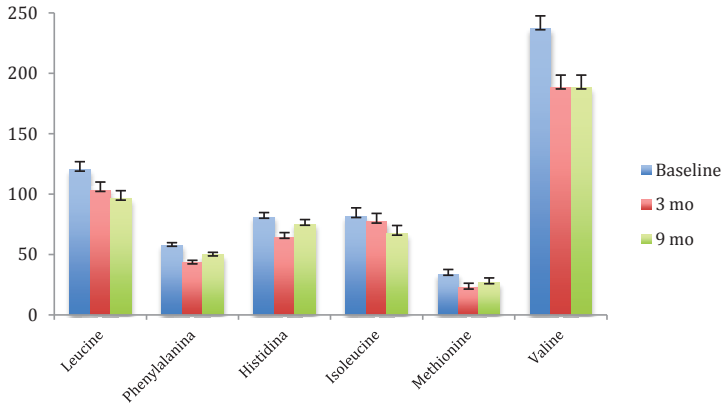
Figure 5. Nitrogen balance during the last 3 days of each inpatient stay.



Branched chain AA

Serum levels of BCAA at baseline and during the follow-up are shown in figure 6. Mollar sum of BCAA (valine, leucine and isoleucine) concentrations were reduced significantly after BS by 15.28% [-0.53; 31.08] and 19.93% (6.81) [4.22; 35.64] after 3 and 9 months respectively. No differences between protein intake groups were observed.

Figure 6. BCAA serum levels during the study.

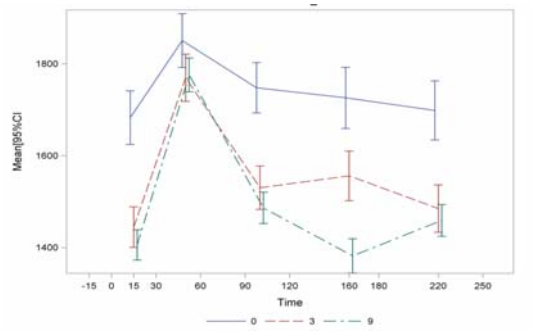


REE and Diet Induced Thermogenesis

Data of the whole group, shows that REE significantly decrease after BS (1838.6 [1677; 1999] at baseline), (1622 [1461; 1783] at 3 months $p<0.001$), (1552 [1391;1713] at 9 months $p<0.001$) respect to baseline and also between 3-9 months ($p=0.029$).

The AUC of DIT was similar for each study period (figure 7) and was 271989.0 [2336.2.0; 310375.9] at baseline, 254967.8 [216580.9; 293354] at 3 month and 245299.0 [206912.0; 283686.0] at 9mo (p=n.s). However, DIT at 20 min after the meal was significantly higher at 3 and 9 months after bariatric surgery compared to baseline (delta DIT before surgery: -191.2kcal, at 3 mo -332.5, and at 9 mo 384.5kcal $p<0.001$.

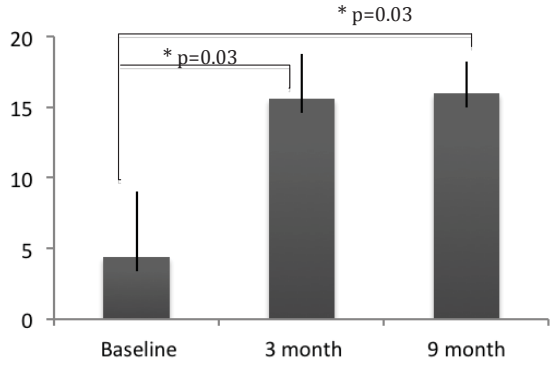
Figure 7. REE and DIT during the meal test.



Calorimetric bomb

Calorie (kcal) content in feces were 144.3 [70.9;217.6] at baseline, 113.4 [40.8; 186.7] at 3 months and 130.41 [57.0;203.7] at 9 months after BS. The percentage of calories excreted increased significantly after surgery -4.35 [-15.36; 6.6] at baseline, and 15.6 [8.2; 23.0] and 13.0 [7.7; 18.3] at 3 ($p=0.03$) and 9 ($p=0.03$) respectively Figure 8. Kcal intake obtained from food aliquots during the inpatient stay decreased significantly from 2352.3 [2209.7; 2495.0], 1228.7 [1086.1; 1371.4], 1400.6 [1258.0; 1543.3], at baseline, 3 ($p<0.001$) and 9 ($p<0.001$) months respectively.

Figure 8. Percentage of Kcal excreted in feces

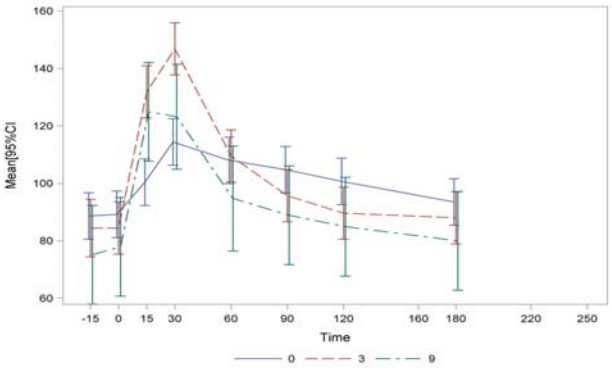


Glucose, insulin and acetaminophen.

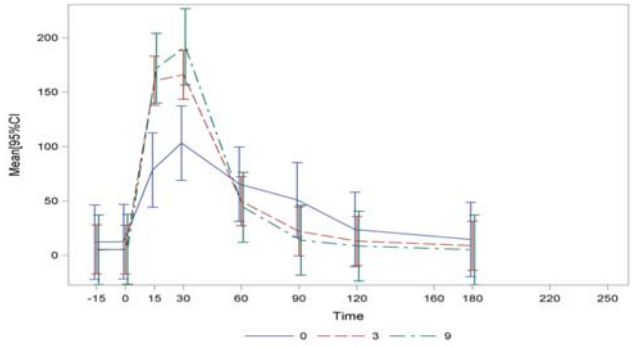
AUC for glucose, insulin and acetaminophen are shown in figure 9. The highest glucose pic was reached at 30min after the meal before surgery and at 3 months, and after 15min after 9 month. Glucose release was significantly higher after 3 and 9 months compared to baseline however, at 9 months was lower than after 3 months. Serum raise of acetaminophen occurred sooner 3 and 9 months after BS and reached its highest concentration 90 minutes after intake.

Figure 9. Glucose, Insulin and acetaminophen (AUC) during the meal test along the study

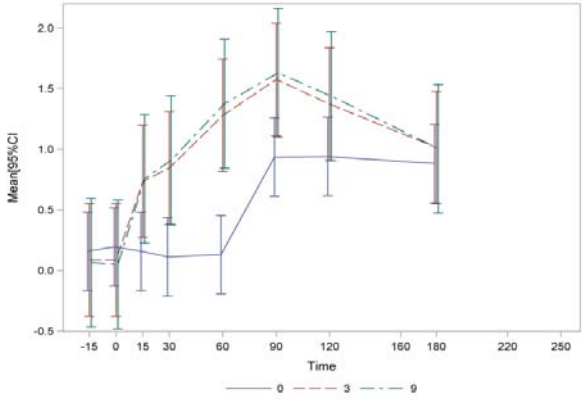
9A. Effect of a high protein meal on glucose levels before and after BS.



9B. Effect of a high protein meal in Insulin levels before and after BS.



9C. Acetaminophen levels to illustrate the rate of absorption before and after BS.



Study of satiety

Study of hormone release related to satiety: AUC for GLP-1 and PYY 3-36 is shown in figure 10A and 10B respectively.

Figure 10 A. Satiety hormone AUC: GLP-1

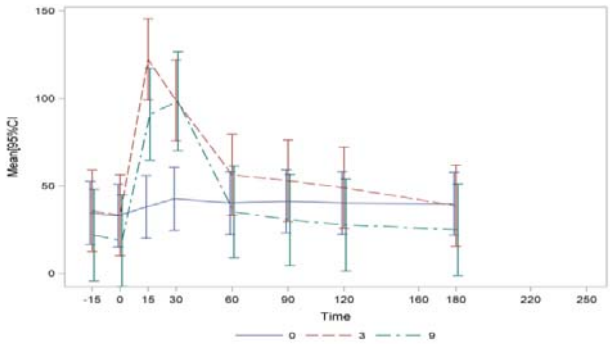
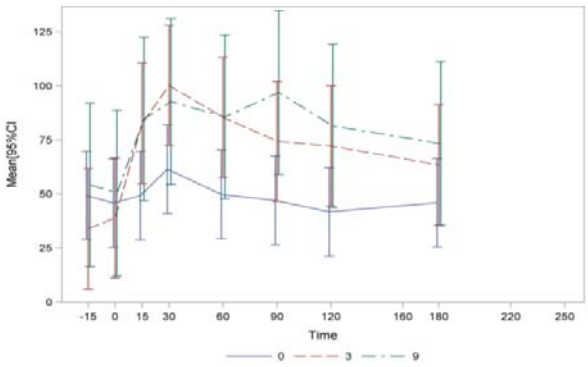


Figure 10 B. Satiety hormone AUC: PYY Total (3-36)



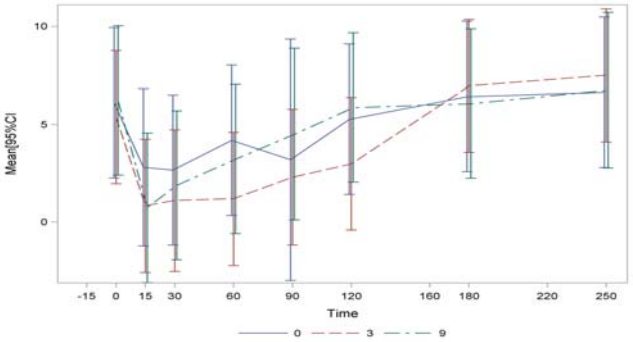
Study of perceived satiety through the study by a visual analogue scale VAS.

The feeling of hunger showed the lowest score 30 min after the meal before surgery and earlier (at 15 min after the meal) after BS (p=0.03). However, no difference was observed at 240 min after the meal (p=n.s.). Similarly, the feeling of fullness was maximum at 60 min after the meal before surgery and 15 min after the meal after surgery at 3 (p=0.006) and 9 (p=0.003) month after surgery. This difference on fullness lasted until the end of the study only at 3 mo after BS (p=0.06). At 15 min after the meal, pre and post surgery patients showed the highest scores for stomach discomfort. This difference was significant at 3 (p=0.03) and 9 (p=0.003) mo, and tent to normalized 60 min after the meal. At that time, no differences were observed before versus after BS.

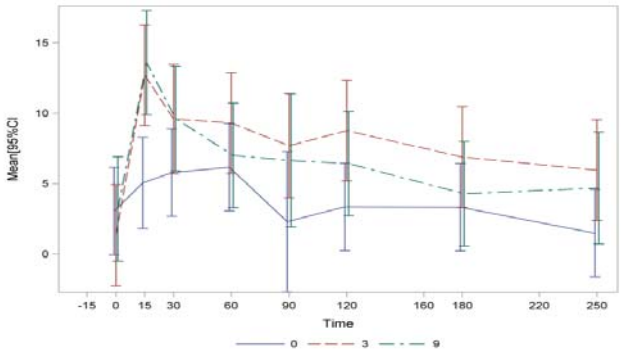
Highest levels of anxiety during the meal test were observed after surgery, especially at 9 months and at 15 min after the meal ($p<0.05$). (Figure 11)

Figure 11. AUC for hunger, fullness, stomach discomfort and anxiety during the meal test

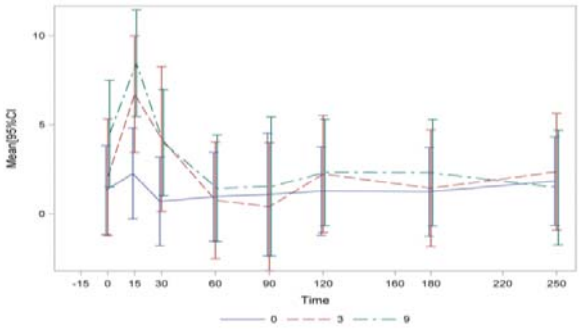
A. Hunger



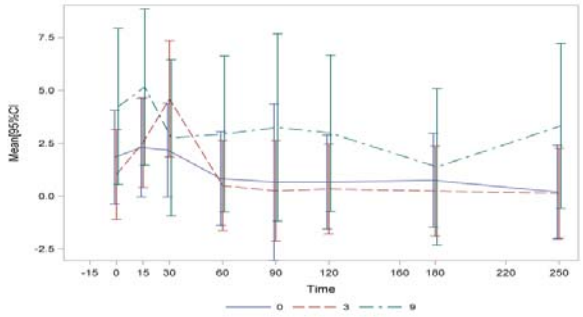
B. Fullness



C. Stomach discomfort



D. Anxiety



Physical activity (PA) and other measurements

Physical activity did not change after surgery. Participants were sedentary 68.5% of the time they wore the Actigraph monitor (p= .715), See table 2 for percentual distribution of PA intensity of participant's daily activity.

Study adherence: five important aspects related to the study were described and evaluated at each follow-up visits: a) completed dietary recalls, b) answered phone calls, c) personal contact attended, d) scoops of dietary protein taken every day during the intervention and e) completed the 5 days inpatient stays at the research clinic.

Table 4. Study Adherence

	% of completed dietary recalls		% answered phone calls		% of personal contacts completed		% of adherence to PS (Scoops)		% 5-days Inpatient Stay	
	0-3 month	9 month	0-3 month	9 month	0-3 month	9 month	0-3 month	9 month	0-3 month	9 month
Mips 1	80	50	100	33.3	82	66.6	100	100	100	100
Mips 2	44	50	35	66.6	45.4	83.3	66.6	50	100	100
Mips 3	100	75	100	100	100	100	100	100	100	100
Mips 4	54	33.3	75	33.3	73	50	92	50	100	100
Mips 5	35	0	25	66.6	27	0	66	0	50	0
Mips 6	40	40	100	66.6	82	66.6	100	33.3	100	100

10.4. Conclusions about the implementation of the pilot study

This study was a pilot study (due to budget constrictions) and was designed to address feasibility and to collect preliminary data to design a larger scale study. With pilot data we are unable to make clinical recommendations. In this randomized pilot study, we successfully obtained preliminary data on the effect of weight loss and PRO supplementation over each of our specifics study aims: (Aim1), nitrogen balance, by quantify nitrogen input (intake) and output (specimens) during the inpatient study (Aim 2), lean body mass, fat mass, total body water, also, after a meal test, we tested resting energy expenditure, and diet induced thermogenesis (Aim 3) hormone (PYY, GLP-1) and

perceived (visual analogue scale) satiety over the study; (Aim 4) serum levels of branched chain amino acids.

The main successful outcome of this study protocol is that we were able to design and validate a rigorous methodology for examining the aims described above. As a result of having successfully implementing the methods designed in this protocol, we will be able to develop of a follow-up MIPS as Randomized Control Trial with a higher number of subjects. This future proposal will significantly contribute to the development of the evidence-based nutritional guidelines for clinical practice for moderately and severely obese individuals undergoing BS at short and long term follow-up.

Over all, the implementation of the design of study was feasible and successful. The methodologies used for some of the aims of the study were not done before on this population. MIPS was extremely helpful to identify the strength and limitations of this protocol.

While de sample size is too small to take finals conclusions, this data will be key in order to support feasibility of the study management, and data analysis as well.

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PART VI: GENERAL DISCUSSION

11. DISCUSSION AND FUTURE PERSPECTIVES

The overall aim of this PhD project was to investigate the effect of bariatric surgery on the prevalence of vitamin and mineral deficiencies and the impact over the protein status. In this chapter, the main findings of the project will be discussed. In the last part, future perspectives and conclusions will be presented.

11.1 General discussion of findings

In this thesis we studied the effects of bariatric surgery (BS) on the prevalence of nutritional deficiencies and the protein status and investigated the optimum amount of dietary protein to ensure a healthy weight loss. We were also interested in clarifying the proper diagnosis and treatment of the most prevalent deficiencies in clinical practice. In this context, we first evaluated the nutritional intake and prevalence of nutritional deficiencies prior to surgery in a Spanish morbidly obese population. Thereafter, we evaluated the long-term dietary intake and the prevalence of nutritional deficiencies following sleeve gastrectomy or roux-en-y gastric bypass in a Mediterranean population. Vitamin D and iron deficiency were identified as the most prevalent nutritional complications associated to BS in our population. In respect to vitamin D deficiency we decided to better understand the pharmacological treatment and whether or not it could be optimized. Regarding iron deficiency, we studied the inflammation and iron status in BS candidates to better characterize iron status in the context of systemic inflammation. Since current protein intake (PI) recommendations for the BS patient are not supported by conclusive evidence, we studied the relationship between protein intake, body composition, and protein status following BS. In a deeper approach, we specifically investigated protein intake and lean tissue mass retention during surgically induced weight loss. Finally, we designed a protocol for a randomized controlled trial on dietary protein during surgical weight loss to better understand the effect on nitrogen balance, thermogenesis, body composition, satiety, and circulating branched chain amino acid levels up to one year after surgery. Preliminary data of this protocol is included as part of this thesis project.

Nutritional Intake and Prevalence of Nutritional Deficiency in Obesity: Effect of Bariatric Surgery.

Bariatric surgery (BS) is gaining popularity as the most appropriate therapeutic approach for a carefully selected group of patients with severe obesity. BS is currently considered the most effective treatment of severe obesity, resulting in long-term body weight loss and control or remission of comorbidities, such as type-2 diabetes, hypertension, and hyperlipidemia (8) Roux en-Y gastric bypass (GBP) and sleeve gastrectomy (SG) are the most common procedures (12) Although the rates of resolution of comorbidities are noteworthy, nutritional deficiencies can occur and are considered as long term complication. The risk of development of these deficiencies will depend on the pre-surgical levels, the alteration of its contribution and absorption, and of the body reserves. In the case of vitamin B_{12} reserves can cover periods of more than 12 months, while for vitamin B_1 reserves do not cover more than 18 days. This idea motivated our first research project. Studies in non-Mediterranean populations had shown that micronutrient deficits were present before surgery. However, until then, there was no data on this topic in a Spanish population. We identified a high prevalence of vitamin D (VD) (with only 10% of our studied population within sufficiency level), zinc, magnesium, iron, vitamin B_1 and B_6 deficiency between others. The importance of ascertaining the nutritional status in those subjects prior to surgery is further emphasized because other factors related with the surgical procedure could further contribute to compromise the nutritional status. Interestingly, a number of recently published studies have reported similar prevalence of deficiencies in individuals undergoing BS from various countries including France (155), Italy (156), China (157), Chile (158), and The Netherlands (159). We believe that, independent of their country of origin, a common factor linking obese individuals who are willing to lose weight is the poor quality of their diet (160) and the prolonged periods of kilocalories deprivation they voluntarily undergo in order to lose weight. These, along with others factors may precipitate the development of nutritional abnormalities in the obese population. In fact two studies identified preoperative deficiencies as strong predictor of nutritional deficit after BS (159, 161). However, the large number of subjects often lost to follow-up, the lack of a standardized, universal prophylaxis regimen of vitamin and mineral supplements, and the lack of adherence

to the prescribed regimen, are common limitations to identifying potential predictive factors contributing to the development of these deficiencies.

Subsequent to this analysis, we decided to evaluate and compare the long-term dietary intake and the prevalence of nutritional deficiencies following the two most popular BS procedures performed at our bariatric clinic, SG and GBP. We demonstrated that in a Mediterranean population SG and GBP patients experience similar long-term changes in their dietary intake. Our data also suggested that decreased energy intake, rather than modifications of the proportion of macronutrient intake, is an important factor in weight loss after either type of surgery. After SG or GBP, the mean daily dietary intake of calcium, magnesium, phosphorus, and iron was less than currently recommended. A significant number of vitamin and mineral abnormalities were described. Despite universal supplementation, the prevalence of nutritional deficiencies was comparable after SG or GBP. Within the group of water-soluble vitamins, the observed deficiencies of vitamin B_1 and B_6 occurred mainly during the first years after BS. Deficiency of thiamine (vitamin B_1) is especially relevant. Aasheim et al. have reported a progressive decrease in vitamin B_1 levels after GBP and CD (162). However, cases of Wernicke's encephalopathy are particularly clinically significant (163). A systematic review of 104 cases of Wernicke's encephalopathy demonstrated the presence of recurrent vomiting in 90% of cases, with a mean duration of these 21-day vomiting (163). Also of clinical relevance is the vitamin B_{12} deficiency, normally associated with neurological symptoms and anemia. With the exception of AGB, all other procedures impair gastric acid and intrinsic factor secretion. Considering our data and others, the prevalence of B_{12} deficiency increases with elapsed time after BS and is presented in a wide range from 3% and 60% at 5 years (164). Prevalence of zinc deficiency was observed between 25 and 50% along the follow up, and clinically could manifest as dermatological signs, nail dystrophy, alopecia and glossitis. Other group B vitamin deficiencies were observed albeit in lower prevalences. In spite of our findings, is important to consider the clinical relevance of deficits of riboflavin (vitamin B_2), niacin (vitamin B_3), pyridoxal phosphate (vitamin B_6), folic acid (vitamin B_9), and vitamin C are minor. However, its evaluation and follow up deserves consideration. Our observations are in line with other authors (11, 32, 76, 159, 161). However, it is important to consider that the methodology to determine the levels of deficiency, as well as the ranges of normality

are not standardized between studies, making it difficult to interpret and compare findings across studies.

Vitamin D (VD) and Iron Deficiency Management after Bariatric Surgery

VD deficiency was the most frequent nutritional complication observed after SG and GBP. From an analytical point of view insufficient plasma levels of VD coexist with elevated levels of parathyroid hormone (PTH). A normal plasma concentration of calcium will support the compensatory character of PTH elevation and therefore the diagnosis of hyperparathyroidism secondary to the VD deficit. Consistent with other authors, we have shown an inverse correlation between BMI and VD plasma levels (165, 166). It is therefore not surprising, that the prevalence of inadequate levels of VD was already high in BS candidates. A systematic review of 29 studies including approximately 4000 BS candidates, found a prevalence of VD deficiency greater than 50% (range 13-98%) (167). It has been suggested a presumptively sequestration of 25OH-VD in adipose tissue facilitated by the liposoluble nature of this vitamin (165). Since the mobilization of adipose tissue after BS is not associated with a spontaneous normalization of VD levels (168), and the importance of adequate levels of VD for calcium absorption (169, 170), it is recommended 25OH-VD levels are normalized prior to BS. Maintaining adequate VD levels is of great importance to maximize calcium absorption after surgery (169). As showed in our observational study, daily calcium intake is reduced along with caloric restriction after BS (170). On the other hand anatomical changes associated with surgical procedures such as GBP or DBP / DS imply that the food does not contact the main calcium absorption sites: duodenum and proximal jejunum. After GBP fractional calcium absorption is highly compromised even in the presence of VD levels in the sufficiency range (170). In our study, the prevalence of VD levels in the range of deficiency or insufficiency after GBP or SG was between 80% and 100% of cases over 5 years of post-surgical follow-up.

As one of the aims of this thesis we evaluated a protocol to optimize the pharmacological treatment of VD deficiency. In the most recent consensus document on the management of bariatric patients, it is recommended to supplement with 3,000 IU per day of VD to all patients undergoing BS (171) According to this protocol, the dose should be adjusted to achieve a plasma concentration of 25OHD-VD> 30 ng / ml and be accompanied by the administration of 1200 to 1500 mg of calcium. In patients with severe deficiency (levels

<10 ng/ml) doses of up to 20,000 IU/d may be required. The guidelines do not distinguish the type of VD to be used (D_2, ergocaliferol, or D_3, cholecalciferol), although it is known that in its D_3 form would be more effective. Dosing them to reach the recommended 1200 mg of calcium element, the preparations that combine calcium and vitamin D available in our environment will provide about 800 IU / d of VD. Therefore to reach the recommended doses, prescription of additional preparations of VD with higher concentrations will be necessary in routine clinical practice. In our protocol, VD dose was tailored according to VD plasma concentration. Overall, our data showed VD supplementation with doses ranging 800 to 2800 U/day based on plasma VD levels sufficed to correct the vitamin deficiency following 4 months of the tailored supplementation. Thus, our data is in line with current guidelines and provides relevant information on the lower VD requirements for those with borderline VD deficiency as compared to what it is currently recommended.

VD and calcium are key to bone health. Of note, BS is associated with a decrease in bone mineral density specifically after more malabsorptive procedures (DBP / CD and GBP) (172). Four studies have evaluated the relationship between BS and the incidence of fractures. The first one, conducted in the United Kingdom, examined 2079 patients (173). An increased incidence of fractures was not found, although it was a young population, mostly submitted to BGA, and with a mean follow-up of not more than 2 years. Contrary to the retrospective analysis of 258 patients in the Rochester Epidemiology Project cohort, they demonstrated a twofold higher risk of fracture in bariatric patients compared to the control population (174). In contrast to the British study, follow-up was longer (8.9 years) and in 75% of cases a GBP was performed. Lu and colleagues also found a 20% increase in fracture risk in a population of Taiwan (n = 2064), especially after malabsorptive procedures (175). Finally, Rousseau et al. Reported a 40% higher risk of fracture after 4.4 years of BS (n = 12676) compared to obese or non-obese nonoperative population (176). Therefore, it should be acknowledged current recommendations on calcium and VD intake after BS are based on maintenance of plasma levels in the normal range rather than on measures of bone health.

Iron deficiency

Several studies have previously reported on the prevalence of iron deficiency in bariatric surgery candidates. However, as in most studies the low-grade inflammation characteristic of obesity had been overlooked, as part of this thesis project we aimed to better characterize the diagnosis of iron deficiency. We observed that iron deficiency without anemia and iron deficiency anemia is highly prevalent in the candidates and in those who have already undergone BS. Thus, although the variability in iron deficiency frequency reported in the literature may be due to multiple causes, consideration of the low-grade inflammation parameters could be one of them (106, 177). In a state of inflammation, ferritin levels do not adequately reflect iron deposits because of the difficulty in mobilizing ferritin that is induced by inflammation. In the pre-surgical situation, the correct assessment of iron metabolism will thus require the evaluation of inflammatory parameters in addition to ferritin in order to identify patients who present with an absolute deficit or iron deficiency in the presence or absence of anemia or of parameters suggestive of ineffective erythropoiesis (for example high percentage of hypochromic hematies). The detailed assessment of iron metabolism before BS in a series of 803 patients at our center demonstrated a prevalence of 8.7% absolute deficiency and 52.5% functional iron deficiency. We found anemia in 11.2% of the subjects, being this one associated with iron deficiency in 80% of the cases. After BS, low-grade inflammation progressively improves. Twelve months after surgery the majority of patients did not present elevated C-reactive protein (178), so at that time the evaluation of iron metabolism with classic parameters could be advised. The prevalence of iron deficiency with or without anemia varies according to the series, being between 10 and 50% (106). As expected, iron deficiency anemia is more frequent in premenopausal women because of increased iron loses, in those with iron deficiency previous to surgery and with time elapsed since BS.

In the pathogenesis of iron deficiency associated with BS, insufficient iron intake, an impaired ability to release iron from food (by decreasing gastric digestion associated with anatomical modification of the stomach, with lower secretion of proteases and hypochlorhydria, and the reduction of the absorptive surface of iron (associated to the proximal duodenum and jejunum bypass) play a role (179). These changes, which vary according to the surgical technique, determine lower absorptive efficiency fails to compensate the physiological losses of iron. Illustrating the importance of the stomach in iron metabolism

after BS, it has been shown that the prevalence of iron deficiency is comparable after GBP and SG (180). It could be considered that the tendency to iron deficiency after BS would be easily avoidable with adequate iron supplementation. However, adherence to oral iron supplementation is often low since it is poorly tolerated (abdominal pain and depositional rhythm disorders) (181, 182).

To prevent of iron deficiency after BS a dose of 45-60 mg iron element is generally recommended (179). This recommendation should be adjusted according to previous iron deposits, type of BS, and the existence of losses in women of childbearing age. For the treatment of iron deficiency anemia, iron administration will be given orally except for cases with severe anemia (hemoglobin <10 g / dl) where the intravenous route is preferably (179). Factors associated with oral iron therapy (high rate of side effects, poor adherence to treatment, difficulty in absorption), as well as the ease of use of intravenous preparations means that this route is increasingly used even with hemoglobin> 10 g / dL.

Effect of protein status after Bariatric Surgery

During the rapid surgery-induced body weight loss, patients usually lose not only fat mass but also lean body mass (LBM) (183). Avoiding excessive LBM contribution to weight loss after BS is important for several reasons. Lean mass is strongly associated with resting energy expenditure (184), so its retention is desirable as it has been associated with higher rates of sustained body weight loss, lower risk of post-BS mortality and morbidities, and protection against post-BS body weight regain (6, 185, 186). Typically weight lost after BS corresponds to 75% to fat mass and 25% to LBM (125).

Bariatric surgery induced a period of negative energy balance, typically during the first 9-12 month after surgery, corresponding with the drastic weight loss phase. Energy balance and dietary protein intake are critical factors that contribute to the regulation of skeletal muscle mass by influencing whole-body and skeletal muscle protein metabolism (124, 187, 188).

Findings regarding inadequate protein intake and significant lean mass loss after GBP or SG are consistent across studies (77), despite the different surgery types, follow-up periods, patient nationalities, and methods used for measuring food intake and body composition. Nevertheless, ours studies together with the study of Rasftopoulos (189) and Schollenberger

(190) represents the literature available that assessed the association between protein intake and lean mass retention during WL. Shortly after BS, patients reduce food intake dramatically as a consequence of reduced stomach capacity and changes in gut hormone secretion (191, 192). Despite the general consensus concerning the need to guarantee adequate protein intake after BS (6), patients present low compliance with protein intake recommendations, being unable to meet them (193). Intolerance to certain foods, such as red meat or other protein sources, is normally reported (194-196) and may contribute to low protein intake. However, poor adherence to protein supplements was also reported in one of our studies (197), suggesting the need of implementing more efforts to improve adherence.

Current recommendations for PI establish a daily intake of> 60 g of protein after GBP or SG and> 120 g after BPD or DS (6). However, the scientific evidence supporting this recommendation and its relationship with lean mass preservation and protein status during BS-induced weight loss is considered weak (6). Therefore, we studied the relationship between protein intake, body composition, and protein status following BS. In a deeper approach, we specifically investigated protein intake and lean tissue mass retention during surgically induced weight loss. In our first study, we successfully showed that PS was helpful in achieving the protein intake goal. Still, we did not find a significant correlation between protein intake and MLG loss (analyzed by BIA) adjusted for weight loss in the first year after BC. However, a protein intake> 60 g / day associated greater LBM retention at 12 months compared with subjects who ingested <60 g / day. In our second study, we used the gold standard method to determine body composition during surgically induced weight loss (DEXA) and were able to provide supportive evidence for the PI goals of >60 g/d or 1.1 g/kg IBW/d as a being associated with better LBM preservation in the BS patient at one year follow up.

In short, in most patients a limited protein intake will not lead to clinically significant nutritional complications but may compromise LBM and provide the basis for further weight loss and altered physical function. Within its limitations, it should be emphasized that no subsequent study to that of ours has provided better evidence for or against our conclusions.

Nitrogen balance (NB) methodology is widely used as a holistic assessment of protein balance, allowing one to gain valuable insight regarding the relationship between energy

status, dietary protein, and skeletal muscle mass. In general, when energy intake is sufficient to meet energy demand, increasing the protein content of the diet imparts no added influence on nitrogen retention (198). However, increasing dietary protein intake may offset the increase in nitrogen excretion and negative nitrogen balance that generally occurs during periods of energy deficiency (199, 200) as occurs early after BS. A limited number of studied demonstrated that high PI (above 2 times the RDA) had a positive impact on NB during energy deficit-induced weight loss (132, 133). Of note, previous to our study, NB had not been evaluated performed in a BS population.

The last part of this thesis project was aimed to gain new knowledge integrating the obtained results of our previous studies and to address existing gaps in the literature. Thus, we designed a protocol of a randomized controlled trial on dietary protein during surgical weight loss to better understand the effect on nitrogen balance, thermogenesis, body composition, satiety, and circulating branched chain amino acid levels up to one year after surgery. This protocol, named with the acronym MIPS (Metabolic Impact of Protein Supplementation), is timely and of high significance. It will not only determine beneficial and/or detrimental metabolic and health effects of protein supplementation after surgical weight loss, but also help define evidence-based guidelines for clinical practice for moderately and severely obese individuals undergoing weight loss surgery.

Finally, we conducted a pilot study of this protocol, in order to obtain preliminary data. This pilot study investigated the effects of a 3 month-protein supplementation regimen during surgical weight loss. We tested 2 different level of protein supplementation (standard (SPS-G) and high (HPS-G)), as well as the carry-over effect of the intervention at 9 months. We investigated the safety and effectiveness of protein supplementation during weight loss on glucose homeostasis, satiety, nitrogen balance, lean body mass preservation, and total daily energy expenditure. We identified two important unresolved questions in the literature: (1) the quantity of protein supplementation that should be administered, and (2) the duration of the protein supplementation after BS. These are not trivial questions as it is difficult to get patients increase their protein intakes, particularly if levels are set unnecessarily high. There is also a lack of consensus, and of data, on the duration of protein supplementation after BS. Of note, after the immediate weight loss phase post BS, weight regain is commonly observed, specifically 24 months after surgery. In addition to inappropriate lifestyle, it is plausible yet

unproven lower resting energy expenditure (REE) and loss of fat free mass (FFM) after surgery may be risk factors for weight regain. Changes in intestinal hormones (201) and inflammatory cytokines (202) have also been considered to promote weight loss, alter body composition, and possibly modulate food intake (203). In summary, long term weight regain and low-protein intake are common after BS. Specific recommendations in terms of dietary protein supplementation need to be defined as they may have an impact on long-term health outcomes after BS.

The pilot study reported in this thesis was designed to address feasibility and to collect preliminary data to design a larger scale study. With pilot data we are unable to draw definite conclusions leading to clinical recommendations. In this randomized pilot study, we successfully obtained preliminary data on the effect of weight loss and protein supplementation over each of our specifics study aims: (Aim1), nitrogen balance, by quantify nitrogen input (intake) and output (specimens) during the inpatient study (Aim 2), lean body mass, fat mass, total body water, also, after a meal test, we tested resting energy expenditure, and diet induced thermogenesis (Aim 3) hormone (PYY, GLP-1) and perceived (visual analogue scale) satiety over the study; (Aim 4) serum levels of branched chain amino acids. In my perspective, the main outcome of our pilot study was that we were able to design and validate a rigorous methodology for examining the aims described above. As a result of having successfully implementing the methods designed in this protocol, we will be able to develop a follow-up MIPS as Randomized Control Trial with a higher number of subjects. This future proposal will significantly contribute to the development of the evidence-based nutritional guidelines for clinical practice for moderately and severely obese individuals undergoing BS at short and long term follow-up.

Nitrogen Balance

The primary observation of this investigation was that ensuring a protein intake of 0.8 g/IBW/ day was sufficient to prevent the anticipated decrease in NB at 3 months after BS because of a negative-energy balance imposed by BS over this time period. This effect likely reflects the postprandial responses to habitual feeding of higher dietary protein, as no differences were observed between the two PS groups. The link between energy intake and NB is well established. Nitrogen balance becomes negative in the face of a negative energy

balance caused by a decreased energy intake (132, 187, 204, 205). In general, acute periods of negative energy balance (i.e. fasting) result in increased whole-body proteolysis, amino acid oxidation, and nitrogen excretion, which become less pronounced and plateau over an extended period of time as the body adapts to preserve energy and protein reserves (e.g., muscle protein) (47, 206, 207). This idea might explain our observation of a slightly positive NB during a negative energy balance induced weight loss. Indeed, during the experimental three-inpatient experimental conditions, our participants (both in the HPS-G and SPS-G groups) ingested at least 0.8 g prot/IBW/day. It is well documented that high-protein diets attenuate decrements in muscle protein synthesis and protect skeletal muscle mass during energy deficit. The extent to which energy deficit modulates protein turnover and LBM is largely due to the degree and duration to which energy intake is deficient (208-210). Perhaps energy intake at 3 months after surgery is high enough to avoid a negative nitrogen balance while still under a negative energy balance to induce weight loss. This adaptive mechanism to spare protein during prolonged energy deficit was demonstrated by Ancel Keys nearly 70 years ago (211-213). Despite dramatic advances in analytical capabilities, there have been few studies that have examined actual skeletal muscle adaptation to energy deficit (133, 207, 214, 215). Areta et al. (214) also demonstrated that consuming high quality, protein-based meals (15 to 30 g whey) in combination with resistance exercise restored muscle protein synthesis (MPS) above levels observed during energy balance and did so in a dose-dependent manner. These data emphasize the link among protein turnover, total dietary protein intake, and protein consumed at each meal on the regulation of skeletal muscle mass during energy deficit. Our protein supplements where made of isolate whey protein and had a Protein digestibility-corrected amino acid score (PDCAAS) of 100 (considered the highest score). Also meal pattern during the 5 days of the experiment was designed as 6 meals a day containing between 15 to 30g per meal as stated above. Meaning that our subjects where under the recommended experimental dietary pattern that would optimize protein utilization.

The negative NB observed during the baseline inpatient stay was not expected. It is possible that 1.2gprotein/ IBW/ day was insufficient to maintain NB, even at energy balance. Data from Pikoski MA (216) and Tarnopolsky (217) support this conclusion. The 7-day preceding diet used in this pilot study as adaptation period may not have been long enough to achieve a true adaptation to the “in study” protein level A World Health Organization (1985)

report concluded that the major initial changes in nitrogen excretion occur within approximately 5–7 d in adults (218). Additionally, the level of dietary control during the adaptation period could have impacted our baseline nitrogen-balance values. Volunteers were still free-living during this adaptation period, although PS was provided; however, it is possible that they may not have adhered to the run-in diet well enough. Another possibility would be that the estimated usual protein intake at baseline was underestimated or the established PI goal at baseline was insufficient.

Effects of protein quality and branched-chain amino acids on the skeletal muscle response to negative energy balance

It is well established that the amino acid composition of dietary protein can influence the regulation of skeletal muscle protein turnover. Increasing branched-chain amino acid (BCAA) levels during energy restriction can support gluconeogenesis, maintain whole-body and muscle protein synthesis, and attenuate nitrogen excretion and whole-body and muscle proteolysis (219). Of particular importance is the BCAA leucine, a potent independent stimulator of muscle protein synthesis in cell culture and animal models through enhanced cellular regulation of mRNA translation (220). Although the recommended leucine intake is currently 14 mg/kg/d (87), the amount required to maximize the stimulation of muscle anabolic intracellular signaling may be at least 40–65 mg/kg/d (221, 222). Higher amounts, up to 7– 12 g/d have shown to contribute to the preservation of muscle mass during stressors such as energy restriction (219) and in the presence of sufficient essential amino acids (223). Leucine intake in our study was significantly higher in the HPS-G, and was within the described levels needed for muscle mass preservation. However, BCAA plasma concentrations decreased significantly after weight loss induced by BS in both PS groups. Again, in this case, the small sample size does not allow for definite conclusions. Of note, percentage of FFM loss was lower than previously reported, and may be attributed to racial differences (224). Despite its potential limitations, our pilot data show that protein intake $\geq$ 0.8g/IBW/ day was beneficial in attenuating the effects of negative energy balance, on protein metabolism.

Future perspectives

Conceptually, decreases in FFM after periods of negative energy balance may interfere in healthy weight management, as this may decrease skeletal muscle function and performance and facilitate weight regain. Although increasing dietary protein intake, and perhaps more specifically leucine, beyond the RDA has been demonstrated muscle mass sparing effects in obese non-surgical patients, a thorough understanding of the underlying molecular mechanisms is a requisite to develop nutritional countermeasures to mitigate the detrimental effects of negative energy balance.

Importantly to this thesis, despite evidence supporting an increase in dietary protein intake for LBM preservation during energy deficit (133, 225-227), no studies have established dietary protein requirements after BS. It could be argued NB studies after BS should be carried out at earlier stages than 3 months, at a time the above mentioned compensatory mechanisms have been established. Nonetheless, although pathophysiologically interesting, we deem this type of study of limited value for clinical practice. Instead, because changes in skeletal muscle mass are likely due to imbalanced rates of protein synthesis and breakdown, amino acid tracer techniques could better serve the need of assessing whole-body and skeletal muscle protein metabolic responses to varying levels of dietary protein and energy intakes (200, 207, 209, 215). Clearly, further studies are required to assess the combined effects of negative energy balance and dietary protein intake on cellular mechanisms contributing to the regulation of skeletal muscle mass after BS. Systematic, comprehensive studies that address changes in body composition, and whole body and skeletal muscle protein turnover, in combination with expression and activity patterns of intracellular regulators of muscle mass, are required to identify nutritional agents (i.e., amino acids) to counteract decreases in FFM occurring in response to negative energy balance during BS induced weight loss.

Conclusions

1. Nutritional deficiencies are commonly found in the Mediterranean obese population undergoing bariatric surgery and are significantly more prevalent than in normal weight individuals.
2. In a Mediterranean population, SG and GBP patients experience similar long-term changes in their dietary intake. When micronutrient intake from supplements was not taken into account, the mean daily dietary intake of all the evaluated micronutrients was less than the current dietary reference intakes (DRIs). Furthermore, SG and GBP carry comparable post-surgical nutritional consequences. Low vitamin D and elevated PTH levels were the most prevalent nutritional abnormalities after BS.
3. A single fixed high dose (2,000 IU) of VD supplementation is as effective and safe as an individualized daily dose of cholecalciferol in order to achieve 25(OH)D levels ≥75 nmol/L in clinical practice after BS.
4. Impaired iron status could be identified in approximately two thirds of BS candidates when considering hs-CRP as inflammatory marker and ferritin as iron index. Furthermore, ferritin<30 ng/mL especially along with T-Sat<20%, appears to be practical cut-off to identify patients with FID with larger iron status impairment.
5. Protein supplements are helpful in achieving the protein intake goal. The PI goals of >60 g/d or 1.1 g/kg IBW/d is associated with better lean tissue mass (LTM) preservation in the BS patient at one year follow up.
6. The association between PI and NB, BCAA, Body compositions changes, REE, glucose homeostasis and satiety after BS and its effect on weight loss and weight maintenance needs further studies to be elucidated. We designed and validated a rigorous methodology for examining the aims described above. As a result of having successfully implementing the methods designed in this protocol, we are in the position to develop a Follow up Randomized Control Trial with a higher number of subjects. This proposal may significantly contribute to the development of the evidence-based nutritional guidelines for clinical practice for moderately and severely obese individuals undergoing BS at short and long term follow-up.

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