AN EVALUATION OF
1) BONE CHANGES FOLLOWING BARIATRIC SURGERY
AND
2) FAT AND MUSCLE INDICES ASSESSED BY pQCT:
IMPLICATIONS FOR OSTEOPOROSIS AND TYPE-2 DIABETES RISK

By

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An Evaluation of 1) Bone Changes Following Bariatric Surgery and 2) Fat and Muscle Indices Assessed by pQCT: Implications for Osteoporosis and Type-2 Diabetes Risk

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ABSTRACT

Overview: Two separate studies involving obesity-related issues in premenopausal Caucasian females were completed: a preliminary investigation of the skeletal effects of bariatric surgery and; a cross-sectional study which explored relations among total body, central, and limb intermuscular fat, physical activity, and type-2 diabetes risk.

STUDY 1

Aim: To compare the effects of Roux-en-Y gastric bypass (RYGB) and laparoscopic adjustable gastric banding (LAGB) on changes in bone mineral density (BMD), weight loss and blood biomarkers related to bone turnover, hormonal, and nutrient status.
Subjects: Nine bariatric surgery patients.
Methods: Patients had a DXA bone scan and fasting blood draw at baseline, three, and six months following surgery.
Results: RYGB patients had greater weight loss vs. LAGB at both three (mean loss: 19 vs. 9%) and six months (26 vs. 11%), $p<0.01$. RYGB patients lost an average of 7% hip BMD at six months. Hip BMD loss at six months was correlated to decreased leptin ($r=0.88$) and increased adiponectin ($r=-0.82$), $p<0.05$. Bone turnover was indicated by elevated serum bone biomarkers after surgery.
Conclusions: Research with larger sample sizes is warranted to better evaluate potential implications for late-life osteoporosis risk following bariatric surgery.

STUDY 2

Aim: To determine repeatability for IMAT and muscle density, to evaluate the distribution of foreleg muscle and fat indices measured by pQCT and to determine predictors of muscle density and type-2 diabetes risk.
Subjects: 82 women with varying BMI and physical activity levels.
Methods: Subjects had DXA and pQCT bone scans, a fasting blood draw, and completed a 4-day physical activity record.
Results: Fat and muscle distribution in the foreleg was highly correlated to total and central body adiposity. The pQCT device reliably measured muscle density (CV=0.8%), thus justifying use as surrogates for IMAT. Muscle density was positively related to physical activity ($r=0.29$; $p<0.05$) and negatively associated with markers of fat distribution and risk for type-2 diabetes [HOMA-IR ($r=-0.44$, $p<0.01$)].
Conclusions: Further research is necessary to determine whether specific fat or muscle depots can be targeted through exercise training to help with the prevention and treatment of obesity or type-2 diabetes.

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Chapter 1

Introduction

Clinical Problem and Rationale for Research

My dissertation stemmed from two separate research studies, first *Preliminary studies of bariatric surgery patients: comparison of Roux-en-Y gastric bypass vs. laparoscopic adjustable gastric banding procedures for effects on bone geometry, mass, and density at 6 months post-surgery* and secondly, *Repeatability of pQCT and evaluating effects of physical activity on muscle and fat parameters in premenopausal females*. The first, a prospective preliminary study, is part of an ongoing clinical investigation funded by a Research Acceleration Project grant from the Carilion Clinic Research Office (Roanoke, VA). The second project derived from a database generated by a 2+ year bone health study of women, conducted with Dr. William Herbert and my graduate student colleagues working in the Bone, Health, Nutrition and Exercise (BONE) Laboratory at Wallace Hall. My doctoral program consisted of two major components, Clinical Exercise Physiology and Human Nutrition. Therefore, I included issues in my dissertation work which incorporate both of these domains. My dissertation topics stemmed from interest two common chronic conditions, osteoporosis and related research with bariatric surgery patients and type-2 diabetes.

The first topic involved evaluation of bone mineral density (BMD) and content (BMC) changes and blood biomarkers prior to bariatric surgery compared to measurements at three and six months following surgery. Excess body weight provides additional skeletal loading and conventional thought has suggested the effect is osteogenic and associated with reduced risk for osteoporosis[1]. Rapid weight loss following bariatric surgery can reduce skeletal loading, and

also appears to negatively affect bone turnover, with several researchers noting bone loss following bariatric surgery[2]. The extent of weight loss is often correlated with bone loss and appears to be more prominent at the hip, an important weight bearing site[3]. Osteocalcin (OC) and the carboxy (C)-terminal peptide of type I collagen (CTx), both bone turnover markers are among the most sensitive to measure these changes[4]. Both markers have been documented to increase following bariatric surgery[3,5]. In addition, two adipokines, adiponectin and leptin have been proposed to have a potential impact on bone physiology[2]. However, limited research has been done with either in relation to potential influences on bone status and bone turnover markers following bariatric surgery. Nutritional factors may also contribute to bone loss, as more than 60% of bariatric surgery patients are deficient in vitamin D and between 25 to 48% show elevated parathyroid hormone (PTH) levels prior to surgery[6]. Bariatric surgery often exacerbates these issues; decreased absorption of nutrients in the gut, along with reduced food intake and rapid weight loss puts bariatric surgery patients at risk for bone loss[6]. As bariatric surgery becomes a more common procedure, especially in younger females, more research is necessary to evaluate the effects of two commonly performed procedures, Roux-en-Y gastric bypass (RYGB) and laparoscopic adjustable gastric banding (LAGB) surgery to determine effects of each on bone status in the short-term and suggest what might be the longer-term implications for late-life osteoporosis.

The subset of patients (n=9) included in my dissertation is preliminary pilot study data and does not represent sufficient sample size for publication. Therefore, measurements for this group were used to evaluate trends and forecast changes for further analysis within the larger sample group. The larger subset will include two groups of approximately ten patients each which will allow for additional statistical analysis to detect changes following bariatric surgery.

The second topic evaluated fat and muscle indices in the non-dominant foreleg and forearm in relation to total body adiposity measures, physical activity, and type-2 diabetes risk. The mass and distribution of adipose tissue affects cardiovascular and metabolic disease risk, especially when located centrally[7]. One repository of recent health interest is intermuscular adipose tissue (IMAT), the adipose tissue between muscle bundles[8]. This locus can be measured using magnetic resonance imaging (MRI), computed tomography (CT), and more recently, with peripheral quantitative computed tomography (pQCT). MRI and CT analysis have shown associations between higher concentrations of IMAT and insulin resistance, type-2 diabetes, and reduced muscular strength[9-12], yet little research has been done using the pQCT. Muscle density has been validated as an indicator of adipose tissue deposition in the muscle[13,14] and has been used as a surrogate for IMAT[15]. Physical activity appears to moderate the relationship of IMAT with type-2 diabetes risk, yet effects on fat depots in the limbs where pQCT is able to quantify hard and soft tissue are limited and indecisive[16,17]. Therefore, this study was necessary to evaluate the interrelations of fat and muscle indices in the lower limb assessed by pQCT, physical activity status, and potential risk of type-2 diabetes.

Study 1: An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D, and Calcium

Research Aims

The primary aim of this research was to comparatively evaluate, over 6 months, effects of Roux-en-Y gastric bypass (RYGB) vs. laparoscopic adjustable gastric banding (LABG) bariatric surgery on body weight loss, bone density and selected blood biomarkers related to bone status [osteocalcin, carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx), adiponectin, leptin, vitamin D, calcium and parathyroid hormone (PTH)]. This data represents a

small sample size, therefore, the primary purpose of this study was to examine potential trends and evaluate patterns for further analysis within a larger sample size.

Assumptions

1. Patients reported to fasting measurements without consumption of food at least 8-12 hours prior to the blood draw.

2. All laboratory equipment (DXA, stadiometer, scale) had been properly calibrated and maintained.

Delimitations

- The patients included in the study were all premenopausal, middle-aged Caucasian females.
- This study population consisted of a small sample of patients accepted for elective RYGB or LAGB bariatric surgery. There were 4-5 patients participating in each surgical group, each with similar age, body mass, and health status prior to surgery.
- The blood biomarkers measured included bone biomarkers: osteocalcin and CTx, adipokines: adiponectin and leptin and biomarkers related to nutrient and bone status: vitamin D, calcium and PTH.

Limitations

- The weight limit on the DXA machine is <136 kg (300 pounds), therefore all patients weighed less than 136 kg. This limited investigation of bone changes within morbidly obese patients.
- Only premenopausal females were studied to limit confounding effects of increased osteoporosis risk after menopause. The focus was on premenopausal females to evaluate potential later age-related risk of osteoporosis, thereby reducing potential effects of menopause and estrogen levels on bone status.

- Both leptin and adiponectin are adipokines believed to be involved with bone turnover, yet published data is limited with each in relation to bone changes following bariatric surgery. Adiponectin has only been measured in relation to bone density in two studies[18,19] and leptin has not been measured in bariatric surgery patients relative to BMD and BMC changes and blood bone biomarkers.
- Patients were recommended by a Registered Dietitian to consume calcium and vitamin D supplements after surgery; however there was not stringent reporting of all supplement use over the three and six month follow-up. Therefore, conclusions did not address the specific effects of supplement use on bone status.

Study 2: Fat and Muscle Indices Assessed by pQCT: Relationships with Physical Activity and Type-2 Diabetes Risk

Research Aims

The aim of this study was first to evaluate the distribution of muscle and fat indices evaluated by pQCT in the foreleg and to determine repeatability for intermuscular adipose tissue (IMAT) and muscle density. Two prediction models were evaluated to determine influences on muscle density and type-2 diabetes risk using physical activity and health-related measures.

Assumptions

1. Subjects reported to fasting measurements without consumption of food at least 8-12 hours prior to the blood draw.

2. All laboratory equipment (DXA, pQCT, stadiometer, and scale) had been properly calibrated and maintained.

3. Subjects wore the pedometer for all waking hours over a four day period and recorded any activities when the pedometer was not worn.

Delimitations

- Subjects included in the study were all young adult to middle aged premenopausal Caucasian females; therefore the results cannot be generalized to other gender or racial groups. Previous research has shown adipose tissue deposition to vary with race[20] and genders, with females generally having less IMAT than males[16]; thus, it was necessary to limit the inclusion criteria to control for potential variability within the group.
- IMAT was measured with the pQCT device. This measurement of IMAT included the adipose tissue between muscle bundles (intermuscular fat) and adipocytes within muscle fibers (intramuscular fat)[21]. The pQCT is not specific enough to discriminate between intermuscular and intramuscular adipose tissue, nor is it as precise as an MRI or CT scan.

- Physical activity was assessed through pedometer step count and self reported physical activity over a four day period.

Limitations

- The weight limit on DXA machine is <136 kg, which limited inclusion to subjects weighing less than 300 pounds.
- Measurement of IMAT was obtained with a pQCT instead of MRI or CT analysis which is more commonly reported in the literature. Most research with MRI or CT has measured the upper thigh, which has a greater concentration of IMAT than the calf, where measurements for this study were taken. However, Ruan et al.[22] demonstrated that calf IMAT assessed through MRI could be used to estimate whole body IMAT. Little research has been done with calf IMAT measured with pQCT, so there was not extensive data for comparison of results obtained in this study.
- Inferences on physical activity levels were based on pedometer step count and self report to assess overall habitual physical activity status, rather than exercise capacity as was done in Boettcher et al[16].

Definitions

Adipokine – a group of polypeptide hormones produced primarily by adipose tissue[23].
Adiponectin – an adipokine with three isoforms which targets skeletal muscle and the liver and has insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties[24].
Adjustable gastric banding (AGB) – a form of bariatric surgery in which an inflatable tube is placed around the stomach just below the gastroesophageal junction, thereby restricting entry of food into the stomach[25].
Bariatric surgery – a weight loss surgery for patients with a BMI > 40 kg/m^2 or those with a BMI > 35 kg/m^2 who have comorbidities[25].
Calcium – a water soluble mineral which is absorbed primarily by the duodenum and plays a role in bone mineralization[1].
Carboxy (C)-terminal cross-linked telopeptide of type I collagen (CTx) – a bone turnover marker which is generated from osteoclasts as a degradation product of type I collagen[26].
Computed tomography (CT) – a device which captures an image by measuring the X-ray absorption coefficients of the tissue. It is measured in Hounsfield Units (HU)[27].
Dual-Energy X-ray Absorptiometry (DXA) – the gold standard for measuring bone density[1].
Fat free soft tissue mass – also referred to as lean body mass or area in the body which is not fat. It includes bone, cartilage, ligaments, tendons and muscle and can be accurately measured by a DXA machine using a full body scan.
Glucose – a monosaccharide found in the blood and is an important fuel source. Impaired fasting glucose >126 mg/dL would classify someone as diabetic[28].
Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) – a calculation involving fasting insulin and glucose used to access insulin resistance and β cell function; calculation: fasting insulin (uU/L) x fasting glucose (mmol/L)/22.5[29].
Intermuscular adipose tissue (IMAT) – the adipose tissue between muscle bundles[21].
Insulin – a hormone released from the β cells of the pancreas which directs the body to metabolize and store glucose[28].
Insulin resistance – impairment of β cell production of insulin from the pancreas or reduced sensitivity to insulin production which is involved with development of type 2 diabetes[28].
Interleukin-6 – a circulating pro-inflammatory cytokine secreted by a variety of cells including immune cells, skeletal muscle, adipose tissue[24].
Magnetic resonance imaging (MRI) – a device which uses a magnetic field and pulses of radio waves to capture images of organs and structures in the body[30].
Laparoscopic – a minimally invasive surgical technique also referred to as "closed" which is often used to perform various forms of bariatric surgery[31].
Laparoscopic adjustable gastric banding (AGB) – a minimally invasive form of bariatric in which an inflatable tube is placed around the stomach just below the gastroesophageal junction, thereby restricting entry of food into the stomach[25].
Leptin – a protein hormone which affects food intake and energy expenditure[32].
Low bone mass – defined by the World Health Organization (WHO) as a T-score from a DXA scan between -1 and -2.5[1].
Amino (N)-terminal peptide of type I collagen (nTX) – a bone turnover marker which is generated from osteoclasts as a degradation product of type I collagen[26].

Osteocalcin – a bone turnover marker which is secreted by osteoblasts and is a protein marker of bone formation[33].
Osteoporosis – defined by the World Health Organization (WHO) as a T-score from a DXA scan $\leq$-2.5[1].
Osteoprotegerin (OPG) – a cytokine which binds to RANKL to inhibit osteoclast differentiation[34].
Parathyroid hormone (PTH) – a hormone secreted by the parathyroid which acts to maintain serum calcium level by stimulating exchange of calcium from bone to blood and can increase absorption of calcium from the kidneys[28].
peripheral Quantitative Computed Tomography (pQCT) – a peripheral computed tomography device smaller and less expensive than a computed tomography (CT) machine which is used to measure a peripheral site such as a the radius or tibia[27].
Receptor Activator for Nuclear Factor κ B Ligand (RANKL) – part of the tumor-necrosis factor family which, regulates osteoclast activity along with RANK and OPG[4]. Binding of OPG inhibits RANKL's osteoclast activity[34].
Roux-en-Y gastric bypass surgery (RYGB) – a form of bariatric surgery where a small pouch is created from the stomach, restricting food intake and then attached to the jejunum which bypasses the distal stomach, duodenum, and proximal jejunum[25].
Subcutaneous fat – a layer of fat found underneath the skin which is less metabolically active than visceral fat. Adipokines are produced here, but to a less extent than visceral fat with the exception of circulating adiponectin which is largely secreted by subcutaneous fat[35].
T-score – bone density in a specific area expressed in standard deviations from the mean value of a reference database of young Caucasian females[1].
TIBIA4S Macro – a macro option on the Stratec XCT 3000 pQCT (*White Plains, NY*) which analyzes the foreleg scan after taking images at a locations 4%, 14%, 38% and 66% from the distal end of the tibia.
Tumor necrosis factor-alpha (TNF-α) – an inflammatory cytokine involved in systemic inflammation and is produced primarily by macrophages but is also produced by adipose tissue[23].
Type-2 diabetes - a metabolic disorder characterized by hyperglycemia which results from defects in insulin secretion and/or action[36].
Visceral fat – a layer of fat located in the abdominal cavity which is associated with insulin resistance and cardiovascular disease[35].
Vitamin D – a hormone essential for calcium absorption, bone mineralization and activation of osteoblast activity[1]. It can be consumed in the diet or from UVB exposure. It's active form is $1,25(OH)_2D_3$ which plays a role in maintain normal serum calcium levels[37].

List of Abbreviations

BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
CRP	C Reactive Protein
CT	Computed Tomography
CTx	C-Terminal Cross-Linked Telopeptide of Type I Collagen
DXA	Dual-Energy X-ray Absorptiometry
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IL-6	Interleukin-6
ISCD	International Society for Clinical Densitometry
IMAT	Intermuscular Adipose Tissue
LABG	Laparoscopic Parathyroid Hormone Adjustable Gastric Banding
MET	Metabolic Equivalent
MRI	Magnetic Resonance Imaging
NTx	N-Terminal Cross-Linked Telopeptide of Type I Collagen
OC	Osteocalcin
OPG	Osteoprotegerin
pQCT	peripheral Quantitative Computed Tomography
PTH	Parathyroid Hormone
RANKL	Receptor Activator for Nuclear Factor κ B Ligand
RIA	Radio Immuno Assay
RYGB	Roux-en-Y Gastric Bypass
SST	Serum Separator Tube
TNF-α	Tumor Necrosis Factor-Alpha
WHO	World Health Organization

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Chapter 2

Literature Review

Introduction

Weight loss following bariatric surgery reduces comorbidities associated with obesity. However, it may raise the risk for another often overlooked disease, osteoporosis[1]. Excess body weight provides additional skeletal loading and conventional thought has suggested that this effect is osteogenic and associated with reduced risk for osteoporosis[1]. Rapid weight loss following bariatric surgery can reduce skeletal loading and also appears to increase bone resorption, with notable bone loss being documented in the year or so following surgery[2]. The International Society for Clinical Densitometry (ISCD) currently recommends females to receive a bone scan at the age of 65 years, but only younger premenopausal women should receive a scan if they have risk factors[3]. Bariatric surgery is now an option for adolescents and young adults[4]; therefore the long term significance of bone loss is critical since many women may not be aware of bone loss until much later in life. Therefore, additional research is necessary to evaluate the effects of the most widely used forms of bariatric surgery, Roux-en-Y gastric bypass (RYGB) and laparoscopic adjustable gastric banding (LAGB), on bone mass and how each procedure impacts bone turnover, as well blood markers related to nutrient status.

Along with osteoporosis, another disease of increasing health risk is type-2 diabetes. With rising rates of obesity, the prevalence of type-2 diabetes continues to rise and is expected to affect over 300 million individuals by 2025; more than double the rate of 135 million noted in 1995[5]. The mass and distribution of adipose tissue affects cardiovascular and metabolic disease risk, especially when located centrally[6]. One repository of recent health interest is intermuscular adipose tissue (IMAT), the adipose tissue within the fascia surrounding muscle[7]. Recent

research suggests the fat accumulated in muscle (IMAT) can contribute to insulin resistance and type-2 diabetes. Magnetic resonance imaging (MRI) and computed tomography (CT) analysis has shown associations between higher concentrations of IMAT and insulin resistance, type-2 diabetes, and reduced muscular strength[8-11], yet little research has been done using the pQCT to assess these patterns and potential health significance. Data from the pQCT on this issue are scarce; however the pQCT device is a smaller instrument with less radiation exposure than CT, less costly, and may be more practical to use in a research setting. Additional research is warranted to evaluate the interrelations of fat and muscle depots in the lower limb assessed by pQCT, physical activity and the associated risk of type-2 diabetes.

Therefore, to address these two needs for research, this literature review will cover the key areas pertinent to two research topics entitled, 1) *An exploratory study of bone changes following RYGB and LAGB: impact on bone biomarkers, adiponectin, leptin, vitamin D, and calcium status* and 2) *Fat and muscle indices assessed by pQCT: Relationships with physical activity and type-2 diabetes risk*. Thus, the following topics will be addressed in this literature review: 1) bariatric surgery; 2) impact of bariatric surgery on bone; 3) nutrition and bone changes following bariatric surgery; 4) intermuscular adipose tissue (IMAT); 5) insulin resistance and IMAT; 6) IMAT and inflammatory markers; 7) role of physical activity with IMAT; and 8) technical performance considerations for the DXA, pQCT, physical activity assessment, and selected bioassays to relevant to conducting investigation in these two topic areas. This chapter will conclude with a summary of the related literature and how each section relates to the dissertation research.

Bariatric Surgery – Comparison of RYGB and LAGB

Bariatric surgery is an increasingly selected intervention for weight loss among adults with clinically severe obesity that is near impossible to manage by conventional means. According to the National Institutes of Health Consensus Development Conference Panel, bariatric surgery is indicated as a treatment for obesity for adults with a body mass index (BMI) $\geq$ 40 kg/m^2 (class 3 obesity) or those with a BMI $\geq$ 35 kg/m^2 with comorbidities such as obstructive sleep apnea or type-2 diabetes[12]. Approximately 10 million Americans have a BMI $\geq$ 40 kg/m^2 and it is estimated that more than 220,000 bariatric surgeries will be performed in 2010 alone[13]. The primary goal for bariatric surgery is to dramatically reduce excess body weight, thereby lowering high risks of comorbidities associated with obesity. Substantial reductions of 30-50% of body weight are often achieved six months following surgery[14]. Reduction of excess body weight can significantly ameliorate or alleviate components of metabolic syndrome and conditions including obstructive sleep apnea, hypertension, type-2 diabetes, asthma, infertility, and arthritis[15].

Currently, Roux-en-Y gastric bypass (RYGB) is considered the favored approach in the United States (US)[16], with 85% of patients choosing this surgery[17]. Laparoscopic adjustable gastric banding (LAGB), a restrictive procedure, is more common in Europe and Australia but has gained increasing acceptance in the US[16]. Both lead to rapid weight loss, but involve different adaptive responses[16]. With LAGB a band is placed around the upper stomach that decreases food entry, thereby restricting intake. Initial weight loss after restrictive procedures is often less substantial initially than RYGB, but weight loss averages 50-60% of initial body weight and a 25-30% decrease in BMI two years post surgery[18]. Both restrictive and malabsorptive features are involved with RYGB which includes the reduction of the stomach to a small pouch which is attached to the middle of the small intestine, the jejunum. This procedure

bypasses the distal stomach and upper portion of the small intestine and may lower absorption of nutrients in the gut[16]. Weight loss is often quite significant after RYGB, with patients noting decreases of 65-70% of starting body weight and ~35% decrease in BMI[18].

Mortality rates are relatively low for bariatric surgery; a meta-analysis by Buchwald et al.[19] reported operative mortality (less than 30 days post-surgery) of 0.1% for purely restrictive procedures and 0.5% for RYGB procedures. However, short and long term complications are relatively common; infection, leaking, or hemorrhage occurs in 5-10% of patients and many experience long term complications including internal hernias, emotional disorders, and nutrient deficiencies[15]. Deficiencies of both macro and micro nutrients such as protein, iron, folate, calcium, vitamin D, and others are commonly reported after bariatric surgery[20]. Operating room time and length of hospitalization were lower with LAGB surgery, yet re-operative rates were higher compared to RYGB procedures[16]. According to review by Tice et al.[16], weight loss outcomes and patient satisfaction were higher in RYGB patients; however, LAGB remains an attractive option for many patients since it is less invasive and potentially reversible. Since both procedures are commonly performed in the US, additional research is necessary to compare each, especially regarding bone density and blood biomarker changes following surgery.

Impact of bariatric surgery on bone

Osteoporosis has become a significant health risk, as an estimated 10 million Americans over the age of 50 have the disease, with another 34 million at risk[21]. The 2004 Surgeon General's Report[21] projected that by 2020, one in two adults over age 50 will be at risk for a fracture from osteoporosis or low bone mass. Currently, an estimated 1.5 million people each year experience a fragility fracture which can lead to both short- and long-term morbidity and medical expenses[21]. Increased skeletal loading associated with excess body weight may protect

against osteoporosis, therefore it is often overlooked as a potential health risk in overweight or obese adults. Yet evidence on the relation between weight loss and bone loss in the overweight and obese population indicates loss of as little as 10% of body weight can result in 1-2% loss of bone[1]. Bariatric surgery may more dramatically increase one's risk for osteoporosis, not only through rapid weight loss, but also through changes in absorption of nutrients that could detrimentally affect calcium metabolism and thus the capacity to maintain bone mass.

The gold standard for assessing bone mass and density is dual energy X-ray absorptiometry (DXA). The DXA test quantifies bone density and risk for current or future fragility fracture by establishing threshold values (T-score or a Z-score) associated with such risks. Technical considerations and clinical applications of DXA are described in greater detail under the DXA measurement section of this chapter. Currently, DXA scans are not part of the routine pre- or post-operative care of bariatric surgery patients. However, Williams et al.[1] recommends a pre-operative DXA scan for bariatric patients to assess primary and secondary risk of bone disease, with a DXA scan repeated every 1-2 years, as indicated.

While RYGB and LAGB result in loss of excess body weight, the surgical impact on bone appears to differ between the two procedures[2]. Previous data show RYGB results in increased bone turnover and decreased bone mass[2], especially in the hip[22-25]. Similar results have been seen after biliopancreatic diversion (BPD), a primarily malabsorptive procedure[26]. Malabsorption of key nutrients such as calcium and vitamin D and others related to bone metabolism appears to play a critical role in bone changes noted after each of these surgeries[1]. Research is more limited following restrictive bariatric surgery, as it was previously assumed these procedures did not negatively impact bone turnover[1], since there is not a malabsorptive component. Results are more inconsistent following restrictive surgery; some bone loss appears

in the hip and minor changes were reported at other locations, including total body and the spine[27-31]. Guney et al.[28] noted bone loss following vertical banded gastroplasty (VBG, a restrictive procedure) and a medically supervised weight loss program and concluded that bone loss was independent of the weight loss method, but was rather dictated by the extent of weight loss. Fleischer et al.[24] also noted a correlation between the decline in hip bone mineral density (BMD) and weight loss after RYGB. Little published research is available to clarify the comparative effects of weight loss on skeletal health following RYGB and LAGB. Most research in this area has focused on either combination (RYGB) or restrictive surgery (adjustable gastric banding, AGB or VBG), but not both. Only one study has compared the two surgical procedures and this involved very small samples, i.e. n=9 and n=4[32]. In von Mach et al.[32], bariatric surgery patients lost significant weight after 24 months, yet BMD and bone mineral content (BMC) were both reduced after RYGB, but not after AGB. It is unclear whether the surgical procedure was the main contributor to bone loss, since RYGB patients lost significantly ($p<0.05$) more body weight (29% vs. 16% after AGB)[32].

The timing of measurements after surgery is often inconsistent in the few controlled studies that have been reported, with both retrospective and prospective follow up measurements ranging from three months to several years after surgery[2]. Johnson et al.[25,33] reported BMD changes are more prominent during the first year. Especially following RYGB, rapid weight loss is common leading to pronounced decreases in BMI and fat mass after just three months following surgery[34]. There is a need to compare both RYGB and LAGB surgical procedures at similar time points and to make adjustments in the comparison for weight loss to better understand the influence each procedure may have on bone. Measurements of biomarkers

related to bone and assessment of blood makers related to nutrient status are also critical to fully evaluate possible bone changes following surgery.

Blood biomarkers

A recent review[2] highlighted the effects of bone loss and bariatric surgery and noted that data on markers of bone formation were scarce among reviewed studies in this area. Along with BMD and BMC measurements, markers of bone turnover (both resorption and formation) are essential to understanding quantitative changes in skeletal turnover. For bone formation and resorption, respectively, osteocalcin (OC) and the carboxy (C)-terminal peptide of type I collagen (CTx) are among the most sensitive to measure these changes[35] and both markers have been documented to increase following RYGB bariatric surgery[22,24]. The second portion of this section discusses two adipokines, adiponectin and leptin, which were suggested in the same review on bone changes following bariatric surgery to have a potential impact on bone physiology[2]. However, limited research has been done with either in relation to potential influences on bone status following bariatric surgery. The following sections discuss these four markers which may influence bone, including OC, CTx, adiponectin, and leptin. CTx has similar properties to amino (N)-terminal cross-linked telopeptides of type I collagen (NTx) and therefore both markers will be discussed in this section. However, only measurement of CTx was performed in this research study.

Osteocalcin (OC)

Osteocalcin (OC), also referred to as bone γ-carboxyglutamic acid-containing protein (BGP), is the most abundant noncollagenous protein in bone and plays a role in bone mineralization, maturation and remodeling[36]. It is secreted by osteoblasts and is considered a protein marker of bone formation[37] which contributes to calcium homeostasis[38]. Osteocalcin is modified through carboxylation of three glutamate residue to γ-carboxyglutamate residues which

allows for calcium binding and increases osteocalcin's affinity for hydroxyapatite, the mineral complex of bone[39,40]. While carboxylated OC is intimately involved with bone, uncarboxylated OC appears to play a role in role in adiposity and insulin secretion. Research by Lee et al.[41] in 2007 concluded that uncarboxylated OC can contribute to the proliferation of pancreatic β cells and increase expression of insulin and adiponectin in β cells and adipocytes, thus providing evidence that the skeleton can impact energy metabolism. Osteocalcin can be measured as carboxylated, uncarboxylated, and as total osteocalcin and exhibits periodicity in circulation throughout the day[42]. Since carboxylation of OC is dependent on vitamin K, the percentage of carboxylated OC can be used as sensitive measure of vitamin K status[43]. Osteocalcin can be measured in urine or more commonly in serum and both measurement techniques have shown an association with bone loss. In a large sample of 75 year old women followed for five years, declines in both serum and urinary OC were associated with BMD losses in the spine and hip[44].

Osteocalcin is typically reduced in starvation, malnutrition, and anorexia nervosa[37]. Following bariatric surgery, large and significant increases in OC have been noted following both RYGB[24,32,45,46] and VBG[28], indicating increased bone turnover. When comparing RYGB patients ~one year after surgery to obese controls awaiting surgery, OC levels were 53% higher in post-surgical patients[22]. Calorie restriction and subsequent weight loss have also resulted in increased OC, although the increase was smaller than seen in VBG patients[28]. Changes in OC appear to occur relatively quickly, as Fleischer et al.[24] noted a 18% increase in serum OC only three months after RYGB and a 39% increase after the first year.

Carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx)

Type I collagen is the major structural component of the extracellular matrix of bone and contains peptides at either end of the collagen molecule, designated as carboxy (C) or amino (N) terminal telopeptides. Both carboxy (C)-terminal cross-linked telopeptides of type I collagen

(CTx) and amino (N)-terminal cross-linked telopeptides of type I collagen (NTx) are generated from osteoclasts as a degradation product of type I collagen[47]. During collagen breakdown of bone, both the N and C terminal peptides break off and are released into circulation before being cleared by the kidneys. Both CTx and NTx have considerable diurnal variation, with the highest levels reported in the morning and lowest in the afternoon and evenings[48]. CTx and NTx can be measured in either serum or in urine; yet serum measurements have less day to day variation[49]. Serum and urine measurements were found to be highly correlated in postmenopausal women, $r=0.76$, $p<0.01$[50]. Strong relationships have been observed between CTx and NTx, with correlations of 0.80 in serum and 0.87 in urine[50]. Both NTx and CTx appear to be good predictors of future bone loss, with stronger predictive accuracy seen in elderly adults compared to young women[51]. Several prospective, population based studies in older adults have shown that urinary CTx was associated with a two-fold increased risk for fractures in the hip and vertebrate two to five years later[52-54]. Measurement of NTx was also used to discriminate among men and women with normal bone mass, osteopenia, and osteoporosis, leading authors to conclude that NTx could be used to predict osteoporosis in men and women older than 50 years[55].

CTx and NTx are sensitive markers of bone changes and have therefore been measured pre- and post-bariatric surgery to assess bone turnover. Valderas et al.[56] showed that CTx measures were 65% higher in patients one to five years following RYGB compared to non-operated overweight controls. In Coates et al.[22], NTx was significantly elevated (288% higher) in post-RYGB patients compared to obese controls. However, no differences in post-surgical BMD were observed between the two groups (pre-surgical measurements were not taken). Fleischer et al.[24] noted significant increases in urinary NTx (57%) after only three months

following RYGB and a continual increase to 86% at six months and then to levels 106% over pre-surgical measurements at twelve months. These measurements mirrored the post-surgical response with OC, which also rose progressively after RYGB and were accompanied by significant decreases in femoral neck and total neck BMD[24]. Similar results were observed in Olmos et al.[57] which noted a two-fold increase in CTx along with a significant increase in OC three months following VBG. In Bruno et al.[46], RYGB patients had significantly elevated NTx at six months which remained elevated at 18 months following surgery, when most patients had achieved normal to overweight BMI status and weight loss had slowed. BMD measurements were not taken in this study; however, bone specific alkaline phosphate (BAP), another indicator of bone turnover, also increased following RYGB surgery[46].

Adiponectin

Adiponectin is an adipokine with three main configurations – a trimer (low molecular weight, LMW), a hexamer (medium molecular weight, MMW), and a high molecular weight (HMW) oligomer, with receptors found primarily in muscle and liver[58]. The HMW oligomer appears to be the most active form and plays a significant role in insulin sensitivity[58,59]. Total adiponectin can be measured in both serum and plasma and recently ELISAs have been developed which can detect the various isoforms of adiponectin (LMW, MMW, and HMW). Adiponectin circulation remains relatively constant throughout the day[60] and exhibits circadian rhythmicity[61].

Adiponectin may influence bone resorption and formation through alterations in osteoblast activity. Adiponectin is decreased in obesity and inversely related to BMD[62], even after adjustment for fat mass[62,63]. In one study, adiponectin increased post-RYGB and associated reductions in BMD were observed[23]. Other research has shown that adiponectin can inhibit osteoprotegerin (OPG), thus promoting nuclear factor κB ligand (RANKL) stimulation of

osteoclasts[64,65]. Conversely, adiponectin and its receptors have been located on bone forming cells and can stimulate osteoblasts[66-68]. In mice, adiponectin also appears to be regulated by osteocalcin, a protein made only by osteoblasts[41]. Vitamin D may also interfere in the relationship between adiponectin and OC; an association between vitamin D and adiponectin has been deomonstrated[69,70], yet mechanisms are not completely understood. In addition, 1,25-dihydroxyvitamin D_3 [$25(OH)D_3$] has been documented to enhance osteocalcin gene transcription[71], thus acting as a stimulus to osteoblast activity. In a recent article Nimitphong et al.[69] reported low adiponectin levels were associated with serum vitamin D deficiency and postulated this relationship was the result of vitamin D's influence on OC.

Unfortunately, current data on bone markers in relation to weight lost following bariatric surgery are scarce and only two articles have been published which measured adiponectin and BMD following bariatric surgery[23,72]. In Gomez et al.[72], data from different subjects were analyzed pre and post, allowing for no comparison within the group. While both $25(OH)D_3$ and adiponectin were measured, there was no discussion of any relationship between the two[72]. Carrasco et al.[23] concluded adiponectin may have an independent influence on BMD; however no other bone markers or cytokines were measured which would give further understanding of the relationship between adiponectin and bone. A recent article by Bruno et al.[46] reported increased adiponectin six months after RYGB along with increased BAP, NTx, and OC which indicated bone turnover was occurring. However, DXA measurements were not done to confirm changes in BMD following surgery. Several authors have concluded that adiponectin acts on bone metabolism[73,74], however current evidence is inconclusive and effects of adiponectin in the context of bone changes following significant weight loss are very limited.

Leptin

Leptin is hormone produced by adipocytes which plays a role in hunger and satiety regulation[60]. It also influences bone mass, however its role is complicated and appears to be regulated by two antagonistic pathways. Leptin is regulated by hormonal and nutrient status, with expression increased by insulin, glucose, glucocorticoids, and tumor necrosis factor-alpha (TNF-α) and decreased by β-adrenoceptor agonists and thiazolidinediones[60]. Leptin can be measured in serum and plasma and exhibits a circadian rhythmicity, reaching the highest levels during the night[60]. Leptin acts through the hypothalamus and sympathetic nervous system to inhibit osteoblast proliferation and increase expression of RANKL, thus favoring bone resorption[75]. Conversely, leptin can up-regulate cocaine-amphetamine-regulated transcript (CART) expression which decreases RANKL activity[76]. Leptin can also influence bone by increasing expression of OC, stimulating collagen synthesis and proliferation of osteoblasts[77]. Leptin is positively correlated with bone mass[78] and was found to be a significant predictor of BMD in postmenopausal women[79]. However, when controlled for fat mass, the relationship between leptin and BMD has been shown to be both positive[80] and negative[79]. Leptin appears to play a role as a mediator between fat mass and bone tissue, and studies have shown that leptin stimulates osteogenesis[81]. Ducy et al.[82]'s work with mice concluded that leptin inhibits bone formation, and proposed that leptin resistance may be the mechanism involved with obesity and increased BMD.

Leptin often decreases following bariatric surgery as a result of weight loss and reduced caloric intake[83]. Only a few studies have measured leptin following bariatric surgery in relation to bone turnover markers[46,57,84]; however DXA bone scans have not been done to confirm changes in BMD. Olmos et al.[57] reported decreased leptin three months after VBG along with increased bone resorption markers [OC, serum cross linked C telopeptide of type I collagen

(ICTP), and urinary CTx], but there was no correlation between leptin and related biomarkers. Reidl et al.[84] showed similar results with decreased leptin and increased OC one year after both RYGB and LAGB. In a recent article by Bruno et al.[85], the decrease in leptin was a significant predictor of increased NTx six months after RYGB; decreased leptin also correlated with increased OC, but was not significant. The author's postulated that the obese subjects may have leptin resistance which reduced leptin's central effects promoting bone resorption[46]. Subsequently when leptin levels decreased after surgery, leptin's role in bone turnover was resumed[46]. Overall, leptin's role in bone metabolism is incompletely understood[37], especially in the context of rapid weight loss following bariatric surgery.

Nutrition and bone changes following bariatric surgery

The next several sections are focused on vitamin D, calcium, and parathyroid hormone (PTH) and discuss the biologic origin, physiological role, and measurement techniques for each. Next is a discussion of the alterations of each following bariatric surgery and the interplay between vitamin D, calcium, and parathyroid hormone. The last section highlights research on the changes with each nutrient component following bariatric surgery and the impact on bone density.

Vitamin D, calcium, and parathyroid hormone: relationships with bone

Vitamin D

Vitamin D is a fat soluble vitamin with two forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D can be obtained from food sources, such as dairy products and can also be produced in the skin by UVB sun exposure. UVB rays are absorbed by 7-dehydrocholesterol (7-DHC) which is then transformed to previtamin D_3. After a heat dependent isomerization, previtamin D_3 then becomes $25(OH)D_3$ (cholecalciferol) and is then

transferred into circulation to be bound to the vitamin D receptor[86]. Ergocalciferol is found in plant sources and is used to fortify foods such as milk or cereal[87]. Receptors for vitamin D are located in bone, intestine, kidney, and parathyroid glands. Both cholecalciferol and ergocalciferol are hydroxylated at carbon 25 in the liver then again at carbon 1 in the kidney to produce the active form of vitamin D, 1,25 dihyroxycholecalciferol [$1,25(OH)_2D_3$] or calcitriol[86]. The current adequate intake recommendations for vitamin D is 400 IU per day[88].

Vitamin D functions in the body to maintain serum calcium and phosphorus levels within the normal ranges by enhancing absorption of both nutrients in the small intestine. Absorption of calcium is primarily in the duodenum and jejunum, while absorption of phosphorus is primarily in the jejunum and ileum[89]. Vitamin D also contributes to osteoclastogenesis by stimulating RANKL production and inhibiting OPG production, thus allowing the differentiation and maturation of osteclasts to occur[86]. Vitamin D can be most accurately measured as $25(OH)D_3$ by serum ELISA, although RIA can also be used[90]. It should be noted that vitamin D is subject to seasonal variations based on location relative to the equator and daily sun exposure[91]. A recent review by Higasi et al.[87] suggested that liquid chromatography LC coupled with mass spectrometry may be the next most utilized procedure for assessment of vitamin D due to its sensitivity, specificity, and versatility.

Calcium

Calcium is a water soluble mineral found predominantly in the bone (99%), but is highly regulated in the blood. Adequate calcium levels are necessary for cell signaling, neural, and muscular function as well as bone metabolism[92]. Within the bone, calcium forms calcium-phosphate complexes as hydroxyapatite which provides skeletal strength and serves as storage for intra- and extra-cellular calcium[93]. Calcium is typically measured in serum, but can also be measured in urine with normal healthy serum values ranging from 8.8-10.4 mg/dL[93]. Calcium

needs vary by age and gender, ranging from approximately 1,000-1,500 mg/day[88]. Supplemental calcium is typically through calcium carbonate or calcium citrate; the latter has greater bioavailability[94]. Calcium is absorbed in the body primarily in the duodenum, with active transport by $1,25(OH)_2D_3$ playing an important role when calcium intake is low[93]. Regulation in the body is tightly controlled by the parathyroid glands through parathyroid hormone and its receptor along with vitamin D and its receptor[93]. Calcium balance is affected by several other nutrients, including caffeine, oxalate, phosphorous, protein, and sodium. Vitamin D increases absorption, while caffeine, oxalates, and phosphorus decreases absorption; protein and sodium increase excretion of calcium[95].

Parathyroid hormone (PTH)

The parathyroid glands are located in the neck and produce parathyroid hormone (PTH), which plays a crucial role in maintaining adequate calcium and phosphate in the blood. PTH has a relatively short half life in circulation, ranging from three to eight minutes and is primarily degraded by the liver[96]. Circulating PTH exhibits periodicity over 24 hours[42] with diurnal variations[97]. The active form is PTH 1-84 (the bio-intact form), indicating a peptide with 84 amino acids. Commercial assays which measure the bio-intact PTH are the preferred method, although previous assays which measured the PTH 7-84 fragment have shown similar measurements[98]. Since PTH controls calcium and phosphorus levels, it is recommended to evaluate serum PTH in the context of serum calcium and phosphorus as well[96].

Parathyroid hormone secretion is modulated by the calcium-sensing receptor located on the surface of chief cells[99]. Parathyroid hormone acts by three different mechanisms to restore calcium to normal levels: 1) in the kidneys, PTH increases receptor-mediate tubular reabsorption of calcium; 2) in bone, PTH stimulates osteoclast resorption to release skeletal calcium from the bone; and 3) in the bowel, PTH increases the activity of renal 1 hydroxylase which produces

$1,25(OH)_2D_3$, thus increasing calcium absorption[92]. Hyperparathyroidism or elevated PTH secretion can exist both as a primary and secondary condition. Increased PTH secretion promotes resorption of calcium, which is especially concerning to long-term bone health when calcium is removed from the bone[100]. Primary hyperparathyroidism occurs when one of the four parathyroid glands secretes excess PTH, leading to hypercalcemia. This is often due to an adenoma which can be surgically removed[92]. Secondary hyperparathyroidism occurs when one or more components involving calcium homeostasis fails[92]. The cause of secondary hyperparathyroidism can be varied and may be associated with gastrointestinal, vitamin D, kidney, or genetic issues[92]. In a large study of a healthy population, secondary hyperparathyroidism was noted in only 1.2% of subjects and the most common cause was low calcium intake or low $25(OH)D_3$ levels[101]. Secondary hyperparathyroidism is a significant concern following bariatric surgery, as calcium and vitamin D intake and absorption are often reduced, especially following RYGB or malabsorptive procedures[102].

Nutrient malabsorption/deficiencies following bariatric surgery

Changes in bone turnover may be related to nutrient deficiencies secondary to malabsorption and/or decreases in food consumption after surgery. Vitamin deficiencies of both water soluble and fat soluble vitamins have been noted following bariatric surgery and it is suggested that bariatric surgery patients are at risk for deficiencies in vitamins B_1, B_{12}, C, folate, calcium, A, D, and K along with trace minerals iron, selenium, zinc, and copper[20,103]. For the purposes of this literature review, the focus will be on nutrients most affecting bone (i.e. vitamin D and calcium). Specifically, alterations in vitamin D, calcium and/or PTH have been noted following bariatric surgery[24,25,46,57]. Morbid obesity is often associated with vitamin D deficiency[1], which may be exacerbated post-surgery[104]. Several studies have documented low

vitamin D levels in up to 60-80% of preoperative patients[105-107]. A prospective review showed that 25% of bariatric surgery patients had secondary hyperparathyroidism prior to surgery and 21% had low 25(OH)D_3 levels; low calcium was observed in only 3.5% of patients[108]. While vitamin D is primarily absorbed in the jejunum and ileum[20], RYGB bypasses the duodenum and proximal jejunum, the main sites of calcium absorption,[20] further complicating this issue and often resulting in calcium malabsorption after combination surgery[109,110]. Calcium deficiencies are not commonly reported prior to surgery, however malabsorption[109] and hypocalcemia[111] following RYGB have been reported due to 1) bypassing of the duodenum during RYGB surgery, 2) reduced intake of calcium or vitamin D (associated with reduced caloric intake), or 3) decreased intake of dairy products[95]. Decreased vitamin D can further contribute to calcium malabsorption. Low vitamin D prompts PTH levels to rise in a compensatory manner which induces calcium resorption from the bone[102]. This cycle of reduced vitamin D and calcium and elevated PTH often continues post-surgery, with vitamin D deficiency and secondary hyperparathyroidism seen after malabsorptive surgery[24,112], but not primarily restrictive surgery[27,29].

Changes in vitamin D, calcium, and PTH and their effects on bone after bariatric surgery

Ott et al.[45] was one of the early researchers reporting increased risk of metabolic bone disease 10 years following RYGB. Authors observed decreased calcium and 25(OH)D_3 and increased OC and alkaline phosphatase in RYGB patients compared to a control group who had lost weight through dietary restriction[45]. Although BMD measurements were similar, they concluded that metabolic bone disease may be present in the RYGB patients[45]. A more recent study by Duran et al.[113] evaluating RYGB patients longer than eight years following surgery also showed patients had low urinary calcium and most patients were vitamin D deficient, with 67%

having osteopenia in the spine and 40% showing osteopenia in the hip. Short term changes in vitamin D, calcium, and PTH have also been noted along with decreased BMD[24,25] in RYGB patients, but not necessarily restrictive procedures[2,114]. Fleischer et al.[24] noted decreased urinary calcium and increased PTH 3 months after RYGB; although serum $25(OH)D_3$ did not change, BMD significantly decreased in the femoral neck and total hip. Johnson et al.[25] demonstrated decreased serum calcium, increased PTH and no change in $25(OH)D_3$, yet BMD at the total hip and spine decreased significantly 1 year after RYGB. Bruno et al.[46] noted no changes in calcium or PTH, but increased $25(OH)D_3$ six months after RYGB surgery. Pugnale et al.[27] failed to show changes in serum calcium or $25(OH)D_3$, yet PTH decreased significantly along with the BMD of the femoral neck one year after VBG. Serum measurements of bone turnover markers NTx, OC, and bone alkaline phosphate increased, suggesting increased bone turnover[46]. Olmos et al.[57] observed increased serum calcium and $25(OH)D_3$ and decreased PTH after VBG. While BMD was not measured, OC and CTx increased, indicating increased bone turnover[57]. Taken together, these studies[46,57] suggest bone turnover may persist after rapid weight loss even in the presence of normal vitamin D and PTH levels[114]. Supplement use was not tightly controlled with these studies; therefore, it can be challenging to evaluate bone changes related to nutrient intake without measurement of pre-operative nutrient levels in conjunction with supplemental use following surgery.

It is well known that vitamin deficiencies exist following bariatric surgery[20]. Dietary supplement recommendations in this setting often focus on effects of malabsorptive procedures, however restricted nutrient intake or food intolerances may also contribute to deficiencies after restrictive procedures[20]. Unfortunately, vitamin supplements do not necessarily prevent deficiencies, as deficiencies have been reported both with and without supplementation[57,115]. In

a two year retrospective study, researchers found that 34% of patients required at least one supplement in addition to a standard multi-vitamin immediately following RYGB; this number increased to 59% at six months and then to 98% after two years[115]. In addition, it is difficult to predict preoperatively which patients will have vitamin deficiencies[116]. Follow-up with bariatric surgery patients depends on weight loss over time, clinical symptoms or complications and the type of procedure; restrictive procedures such as AGB require more clinical follow-up to adjust the volume of saline in the band, while RYGB requires more nutritional follow-up[117]. The American Society for Metabolic and Bariatric Surgery recommends monitoring vitamins B_1, B_6, B_{12}, A, E, D, K, as well as folate, iron, zinc, and protein. They also suggest a multi-vitamin along with supplemental vitamins and minerals including: calcium, iron, fat soluble vitamins, and B complex vitamins[118], however recommendations for monitoring and supplement use vary among institutions. Unfortunately, no controlled trials exist to give recommendations on the type and dosage of vitamin supplements for bariatric surgery patients[116]. Until such research exists, the bariatric surgery team must rely on patient education regarding multi-vitamin supplementation along regular blood tests to monitor nutrient status in order to help prevent deficiencies[116].

As this section has described, the nutritional factors including vitamin D, calcium, and PTH may play a significant role in bone changes which may occur following surgery. To further understand the nutrient impact on bone turnover following bariatric surgery, additional prospective research on nutrient intake and blood levels of these parameters, along with changes in bone density following RYGB and LAGB is necessary.

Intermuscular Adipose Tissue (IMAT)

Intermuscular adipose tissue (IMAT) has gained recent attention as a marker related to type-2 diabetes and inflammatory status. The next several sections discuss quantification of IMAT, the physiological role of insulin, how insulin resistance develops, and then IMAT's relationship with insulin resistance and inflammatory markers.

The mass and distribution of adipose tissue affects cardiovascular and metabolic disease risk, especially when located centrally[6]. Visceral fat tends to impair normal lipid and glucose metabolism, while subcutaneous fat does not seem to have the same effects[119]. One issue of special interest relates to possible health effects of variations in intermuscular adipose tissue (IMAT), the adipose tissue within the fascia surrounding muscle cells[7]. This locus can be measured using magnetic resonance imaging (MRI), computed tomography (CT), and more recently, with peripheral quantitative computed tomography (pQCT). Other techniques including percutaneous or whole muscle biopsies and quantitative histochemistry[120,121] have been used to measure intramuscular adipose tissue, the lipids contained within the muscle fibers. Intermuscular adipose tissue refers to adipose tissue that is between muscle bundles (intermuscular fat) and adipocytes within muscle fibers (intramuscular fat)[122]. While pQCT is able to quantify IMAT, it is not specific enough to discriminate between intramuscular adipose tissue and intermuscular adipose tissue. Therefore, it is important to note IMAT measurement with pQCT includes both intermuscular and intramuscular adipose tissue. This literature review will focus on measurement of intermuscular adipose tissue which can be measured through non-invasive means with the pQCT, MRI, and CT.

Intermuscular adipose tissue is strongly correlated to total adiposity in both men and women and also appears to be greater in patients with type-2 diabetes than in controls[123]. MRI analysis of IMAT in the calf was positively related to body weight, BMI, and total body fat

percentage in a group of males and females at risk for type-2 diabetes[124]. Similarly, MRI measurement of IMAT in the spine of older men showed associations with BMI, total, and regional body fat distribution[125].

One article by Miljkovic-Gacic et al.[7] has quantified IMAT using the pQCT to evaluate associations between IMAT and type-2 diabetes in African American adults. A second article[126] by the same group used pQCT measured muscle density as a surrogate for IMAT. Muscle density has been validated by others as an indicator of adipose tissue deposition in the muscle[127,128], with greater fat infiltration indicating reduced muscle density. The researchers concluded that ectopic fat accumulation in the calf muscle was associated with type-2 diabetes and that this relation was independent of total body fat and central obesity[126]. Results were similar to reports using MRI or CT scans[9,10,129,130]; higher IMAT in males of normal weight was associated with type-2 diabetes, independent of age, BMI, and total fat and muscle areas[7]. It is important to note that Miljkovic-Gacic et al.[7,126]'s work was done with African Americans, who may have greater[8] or similar[131] levels of IMAT compared to Caucasians.

Measurement of IMAT is of relatively recent interest since it appears to negatively contribute to the pathogenesis of metabolic disease and aging. Its biological origin is still being investigated, but evidence suggests muscle satellite stem cells may undergo adipocyte differentiation, thus forming intermuscular fat in the adipose tissue of muscle fibers[132]. Adipocyte differentiation appears to increase with the addition of peroxisome proliferator-activated reception (PPAR)γ2, leptin, and adiponectin[132]. In addition, inflammatory cytokines may also contribute to IMAT infiltration, which is discussed in greater detail in a later section of this chapter. A review by Hulver and Dohm[133] examining the influence on intramyocellular lipids on insulin resistance concluded that intramyocellular lipids and intermediates such as fatty

acid CoA, diacylglycerols and ceremides are correlated with insulin resistance and may affect insulin signaling by activating protein kinase C. Goodpaster et al.[134] confer with this relationship and research by Griffin et al.[135] suggesting that excess triglycerides in the muscle can increase diacylglycerols which then leads to activation of protein kinase C. Activation of protein kinase C can deactivate insulin receptors through phosphorylatation, leading many to conclude that protein kinase C causes insulin resistance in skeletal muscle[133]. Therefore, it appears that lipid intermediates, but not necessarily triglycerides themselves can alter insulin action[134].

Precision of measurement and variability of IMAT

Repeatability estimates of IMAT are somewhat limited and varied in the method of precision, with many published reports not including the precision data for IMAT measurement[11,124,136,137]. Gallagher et al.[122] reported a coefficient of variation (CV) of 5.9% for the IMAT in four repeated readings of the same four MRI whole body scans. Song et al.[138] reported strong precision measures with a CV of 0.59% for IMAT measured with the MRI after one technician conducted three repeated readings of the same scan. Using a cadaver to compare measurements from both an MRI and CT device, Mitsiopoulos et al.[139] reported strong relationships (r=0.92) among IMAT measured with MRI and the cadaver, but standard error of estimate for repeated measures of IMAT was quite high at 30%. Intramuscular adipose tissue measurement has a lower CV values typically less than 10%[140] due to greater measurement precision with magnetic resonance spectroscopy[141] or CT[127].

IMAT appears to be a dynamic tissue[142]; interventions have shown alterations in IMAT with obesity[11], insulin resistance[11], type 2 diabetes[7], ageing[136], weight loss,[11] and exercise[134,143]. In addition, researchers have noted variation with IMAT across race, gender, and age. IMAT is positively associated with age[136,144]; a five year prospective study in older adults documented

increases in IMAT in both males and females regardless of changes in body weight or skeletal muscle[144]. Yim et al.[145] showed that IMAT measured with MRI was greater in Asian and African-American men compared with Caucasian men and African-American women and was lowest in Caucasian and Asian women. After adjusting for age, gender, and total adipose tissue, IMAT in the femoral-gluteal region remained highest in African-American and Asian men[145]. Miljkovic-Gacic et al.[126] suggested that up to 35% of the variability in fat infiltration may be attributable to polygenic influences after controlling for environmental factors. In a large sample of African American, Asian, and Caucasian men and women, IMAT measured by MRI was higher in men by ~0.19 kg compared to women[122]. In the same population, Gallagher et al.[122] also observed a nine gram per year increase in IMAT after adjusting for total adipose tissue. These disparities highlight the need to control for race and ethnicity and suggest caution when generalizing data to other racial sub-groups.

Muscle density and IMAT

Muscle density has been examined as a possible surrogate for IMAT. Muscle attenuation measured in Hounsfield units from the CT scanner is a measure of muscle density; lower attenuation values indicate increased lipid accumulation since lipid has a negative attenuation value with the CT device[10]. Muscle attenuation has relatively good repeatability; a convenience sample from a large study by Visser et al.[146] showed a CV of less than 5% for muscle area and muscle density. Two separate groups[127,128] have validated muscle density assessed from CT scans or percutaneous biopsy as a measure of adipose tissue infiltration in the psoas, thigh and calf. Goodpaster et al.[127] measured muscle attenuation using CT and found higher muscle attenuation in lean subjects than subjects that were obese without diabetes and subjects who were obese with type-2 diabetes. Negative associations between muscle lipid content and muscle

attenuation measured by CT (r=-0.58, p<0.01) and muscle biopsy of the vastus lateralis (r=-0.43, p=0.019) were observed[127]. Similar relationships were observed by Larson-Meyer et al.[128], with strong negative correlations observed between muscle attenuation measured by CT in the soleus and tibialis anterior and intramuscular adipose tissue measured by magnetic resonance spectroscopy.

Similar to IMAT, muscle density is affected by physical activity and appears to be related to insulin resistance. A training effect study in older adults by Taaffe et al.[147] found that 24 weeks of detraining led to reduced muscle attenuation in the quadriceps, but no significant increases in IMAT. Retraining for 12 weeks increased muscle attenuation and trended toward significant decreases in IMAT[147]. The authors cited high variability in IMAT measures and small sample size (n=13) for insignificant changes in IMAT[147]. Muscle density measured by CT was positively related to insulin sensitivity (r=0.41, p<0.01) in both males and females and was negatively associated with subcutaneous fat and IMAT[10]. The same researcher concluded that muscle attenuation indicating the presence of adipose tissue infiltration was a strong independent predictor of insulin resistance, but only in obese subjects[148]. Similar results were seen in another study with HIV-infected patients. Researchers found CT measured muscle attenuation to be a strong independent predictor of hyperinsulinemia after controlling for BMI, visceral and subcutaneous fat[149].

Muscle fiber type and intramyocellular lipids

One disadvantage of measurement of IMAT with pQCT, MRI, or CT is the inability to discern between intramyocellular lipids and extracellular lipids and therefore examine intramyocellular lipids within specific muscle fibers[150]. Muscle is composed of two different fiber types; type I, generally considered slow-twitch and type II, considered fast-twitch. Type I

muscle fibers are more oxidative, more insulin sensitive, and also have a greater proportion of intramyocellular lipids[150]. Type I fibers are found predominately in elite athletes specializing in long distance or endurance sports. Type II muscle fibers consist of type IIB and type IIA. They both have a high glycolytic capacity, with type IIB having a low oxidative capacity and type IIA has a medium-high oxidative capacity[151]. Type II muscle fibers are more common in athletes who excel at sprinting sports or weight lifting and is associated with a lower VO_{2max}[151]. Type I fiber distribution is negatively associated with adiposity[152]. In trained individuals, increased intramyocellular lipids found predominantly in type I muscle fibers has been hypothesized to be a fuel source for aerobic training to maximize fat oxidation. However, intramyocellular lipids are also increased in sedentary subjects, possibly due to elevated fatty acid concentrations or a high fat diet, thus suggesting a paradox with adipose accumulation in the muscle. Gray et al.[152] noted higher intramyocellular lipids measured by histology in type I muscle fibers prior to gastric bypass surgery. Following surgery, significant decreases in intramyocellular lipids were observed in both type I and type II muscle fibers, with both fiber types showing a 44% decrease in the intramyocellular lipids/muscle fiber area ratio[152]. Also, the homeostasis model assessment of insulin resistance (HOMA-IR) decreased by 93%, consistent with other literature suggesting decreases in intramyocellular lipids may contribute to increased insulin sensitivity[152].

Insulin

Measurements of insulin and glucose are necessary to understand key components of metabolism as well as assessing risk or diagnosing type-2 diabetes. Insulin is a hormone produced by the β cells of the pancreas. Its main function is to regulate blood sugar in the body which occurs after food consumption; insulin stimulates cells in the liver, muscle, and fat tissue to uptake glucose to be stored as glycogen in the liver and muscle. Insulin exerts specific effects

in adipose tissue, muscle, and liver, but also targets the brain, pancreatic β cells, heart, and vascular endothelium. In adipose tissue, insulin decreases lipolysis to reduce free fatty acids circulation; in muscle, insulin increases glucose uptake by stimulating GLUT4 glucose transport; and in the liver, insulin inhibits gluconeogenesis by depressing the activity of enzymes necessary for gluconeogenesis[153].

The most sensitive measurement for insulin resistance is through the hyperinsulinemic-euglycemic clamp technique[154]. This method is highly reproducible, but is time consuming and very expensive. Therefore, other techniques such as an oral glucose tolerance test are often used in clinical practice, or more simply, calculations such as quantitative insulin sensitivity check index (QUICKI) or HOMA-IR are used. QUICKI gives an index of insulin sensitivity, has high predictive power and is calculated as 1/[log fasting insulin (U/mL) + log fasting glucose (mg/dl)][154]. HOMA-IR is used to assess the influence of insulin resistance and β cell function on fasting hyperglycemia levels[155]. HOMA-IR is calculated from fasting insulin and glucose measurements; [fasting insulin (U/mL) – fasting glucose (mmol/L)]/22.5. Significant correlations have been shown between HOMA-IR and measurements from the euglycemic clamp[156].

Insulin resistance and type-2 diabetes risk

Research suggests the fat accumulated in muscle (IMAT) can contribute to insulin resistance and type-2 diabetes[9,10]. Insulin resistance can occur when the target cell or organism has a decreased sensitivity or responsiveness to insulin levels. Insulin resistance leads to an increase in circulating free fatty acids and ectopic fat accumulation and is independently associated with increased risk for type-2 diabetes, cardiovascular disease, and hypertension[157]. Insulin resistance is also a contributing factor to metabolic syndrome, a clustering of symptoms

which can increase cardiovascular or metabolic disease risk when in combination with other components including central obesity, dyslipidemia, and hypertension. Over time, insulin resistance and β cell failure can contribute to development of type-2 diabetes. Type-2 diabetes is a metabolic disorder characterized by hyperglycemia which results from defects in insulin secretion and/or action[158]. Type-2 diabetes occurs in 90-95% of diabetics, but a small proportion have type-1 diabetes, which occurs when the β cells of the pancreas fail to produce sufficient insulin[159]. According to the World Health Organization (WHO)[160] and the American Diabetes Association[161], type-2 diabetes is diagnosed by a fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) or a glucose level ≥11.1 mmol/L (200 mg/dL) two hours after ingestion of a 75 gram oral glucose load. Hemoglobin A1C may also be measured to provide an indirect estimate of average plasma glucose over the previous few months, and can also be used to diagnose type-2 diabetes if values exceed 6.5%[162]. The treatment cost of diabetes is high; it is estimated treatment of diabetes and related complications in the US costs over 100 billion dollars each year[163]. Therefore, it is important to evaluate IMAT as a potential marker for insulin resistance to help determine therapies to reduce IMAT accumulation.

IMAT and insulin resistance

Specific patterns of fat distribution may be a more important marker for risk of type-2 diabetes and metabolic syndrome than overall fat mass[164]. Goodpaster et al.[8] suggested even normal weight individuals may be at risk for metabolic disorders if they exhibit IMAT and abdominal visceral fat accumulation. Several researchers have demonstrated a relationship between IMAT and insulin resistance[9,10,129,130]. Underlying mechanisms of this association are incompletely understood, but may include increased concentration of fatty acids and impaired blood flow to the muscle caused by muscle lipid accumlation[124]. In addition, IMAT proximity to

skeletal muscular could affect skeletal muscle glucose uptake or metabolism[145]. It has also been suggested that increased IMAT in the muscle is related to defects in fatty acid oxidation, causing more storage and therefore accumulation of lipids in the muscle. In a study measuring fatty acid metabolism using muscle biopsy in both lean and obese subjects, Kelley at el.[165] concluded that accumulation of triglycerides in the muscle is more related to an inability to manage fatty acid oxidation, rather than an increase in fatty acid uptake. Furthermore, insulin sensitivity may be mediated through the actions of cytokines and adipokines, rather than directly through IMAT[166]. Goodpaster's group has done extensive research regarding adipose depots, most with IMAT measurements of the mid-thigh using the CT scanner. Their data collected in older adults characterized IMAT as a marker of obesity and insulin resistance[8,10]. However, a few studies found no significant relationship between IMAT and insulin resistance after adjusting for body fat[10,11,167]. This suggests adipose accumulation between muscle fibers may be a physiologically important means of fat storage in the body[10,167]; yet unidentified factors may determine how this tissue modulates health risk, especially in relation to insulin resistance and type-2 diabetes[11].

IMAT and inflammatory markers

Chronic inflammation has been linked with obesity, insulin resistance, and type-2 diabetes. However, Greenfield et al.[168] have questioned whether inflammation is directly involved with the development of insulin resistance and type-2 diabetes. One proposed mechanism is that increased adiposity elevates circulating inflammatory markers which then act to mediate inflammation and insulin resistance[168]. One inflammatory cytokine of key interest is tumor necrosis factor-alpha (TNF-α) which tends to be associated with insulin resistance[169]. Also critical are the adipokines adiponectin and leptin, which may play a pivotal insulin-sensitizing role through increasing fatty acid oxidation and decreasing muscle lipid

accumulation[170]. Inflammatory markers have been evaluated in relation to visceral fat, and correlations documented with C reactive protein (CRP), interleukin-6 (IL-6), and TNF-α[171,172], among others. Visceral adipose tissue plays a significant role in insulin resistance[11] and is related to metabolic risk, even after weight loss[122]. Intermuscular adipose tissue appears to have depot sizes similar to visceral adipose tissue[130], suggesting accumulations may confer similar negative metabolic consequences. While inflammatory markers may show greater associations with specific fat depots than overall fat mass[171]; prior research to evaluate the role(s) of these circulating inflammatory markers in the context of IMAT has been limited and the finding inconsistent[11,137,173]. Beasley et al.[137] reported that thigh IMAT measured with CT was positively associated with serum IL-6 and TNF-α in older black and white males and females. Mazzali et al.[11] documented that there were no significant associations between mid-thigh CT measurements of IMAT and serum levels of leptin or adiponectin in middle age females who were obese. Zoico et al.[173] reported that elderly males showed an association between IMAT measured in the spine with MRI and serum leptin, but no significant relationships were reported with serum adiponectin or IL-6. Overall, current research suggests IMAT is a novel fat depot associated with insulin resistance and inflammatory markers. However, there is a need for additional research with subjects of the same race and gender to further understand these relations and a need to evaluate IMAT in the context of physical activity status.

Role of physical activity with IMAT

The potential role of physical activity in modifying the relationship between IMAT and health risks such as type-2 diabetes has often been overlooked and deserves additional attention. Exercise is often recommended for prevention or treatment of type-2 diabetes, as exercise increases GLUT4 transporters and activates hexokinase and glycogen synthase, resulting in

improved glucose and insulin action in the cell[166]. Physical activity presents a confounding variable to the relationship of IMAT and insulin resistance, as exercise trained subjects exhibit greater IMAT[134], or similar levels to sedentary subjects with obesity or those with type-2 diabetes[166]. Similar inconsistencies were noted with measurement of intramuscular adipose tissue; training studies showed increases[174] and decreases in intramuscular adipose tissue[175]. Goodpaster et al.[166] found that intramuscular adipose tissue was greater in a small group of trained subjects than sedentary subjects and they also had a 65% greater oxidative capacity. This paradox suggests that higher levels of IMAT in physically trained individuals may represent a heightened capacity for triglyceride fuel use during exercise[166]. High active premenopausal females were documented in a recent abstract to have greater muscle area and higher IMAT (after normalization for BMI) in the calf compared to low active subjects, as assessed with pQCT[176]. Conversely, in a group of middle-aged men and women at risk for type-2 diabetes, VO_{2peak} was negatively correlated with IMAT of the calf computed with MRI[124].

Some training studies have also measured IMAT following both following acute and chronic exercise. Most of the acute training research involves invasive measures of intramyocellular adipose tissue. Overall conclusions[150] based on electron microscopy or magnetic resonance spectroscopy measurements in highly trained subjects show that intramyocellular adipose tissue is depleted following distance running events[177,178], suggesting that intramyocellular adipose tissue serves as a fuel source to be oxidized.

Decreased activity also affects IMAT. In a group of healthy young adults, four weeks of unilateral limb suspension significantly increased IMAT measured by MRI in the calf (20%) and in the mid-thigh (14.5%) in a group of healthy young adults[179]. Janssen et al.[130] found that a ten percent weight loss (through diet only or through diet and exercise) in premenopausal obese

females led to reductions of 10-24% in IMAT. However, they could not conclude that changes in IMAT in this study population were indicative of changes in metabolic risk due to high CV measures reported in their lab[139]. Goodpaster et al.[136] randomized a group of older overweight males and females to regular physical activity which included a combination of aerobic, strength, flexibility, and balance exercises. After 12 months, IMAT did not change significantly in the intervention group, yet the control group showed an 18% increase in IMAT.[136] This suggests the possibility that physical activity may moderate risk of type-2 diabetes in part by countering age-related increases in IMAT, however additional research is necessary to understand IMAT's relationship with physical activity level and muscle mass accumulation.

Measurement techniques and instruments

The following section describes in detail the main modalities for accessing BMD, BMC, as well as body composition and muscle and fat deposition in specific depots of the lower limb. All subjects underwent dual-energy x-ray absorptiometry (DXA) and peripheral Quantitative Computed Tomography (pQCT) scans. In addition, physical activity was assessed using a pedometer and a four day physical activity record. The last portion of this section includes a detailed table of the assays which will be used in this study, including the location for measurement and CV values for each.

Dual energy X-ray absorptiometry (DXA)

The dual energy X-ray absorptiometry (DXA) is a machine used to measure bone density, assess fracture risk, diagnosis osteoporosis and monitor response to treatment; it has been widely used in clinical practice since 1987[180]. The DXA uses a small amount of radiation (5-7 μSv effective dose for the entire body) to differentiate between bone, fat and fat free soft tissue. The DXA can quantify bone mineral density (BMD) and bone mineral content (BMC) as well as T-

and Z-scores at specific sites, including the spine, hip and forearm, since these are areas most susceptible to fracture risk. DXA is also considered the most accurate and precise tool to assess body composition at total and regional locations[181]. The International Society of Clinical Densitometry (ISCD) recommends bone density testing for the following populations: women aged 65 and older, premenopausal women under age 65 with risk factors for fracture, women during the menopausal transition with clinical risk factors for fracture, such as low body weight, prior fracture, or high-risk medication use, men aged 70 and older, men under age 70 with clinical risk factors for fracture, adults with a fragility fracture, adults with a disease or condition associated with low bone mass or bone loss, adults taking medications associated with low bone mass or bone loss, anyone being considered for pharmacologic therapy, anyone being treated, to monitor treatment effect, and anyone not receiving therapy for whom evidence of bone loss would lead to treatment[182]. According to the World Health Organization (WHO), normal bone density is classified as a T-score ≥-1, low bone mass as a T-score between -1 and -2.5 and a T-score ≤-2.5 indicates osteoporosis[183]. A T-score is calculated as a standard deviation (SD): T-score = [Measured BMD – Young (adult mean BMD)/Young adult population SD][180]. T-scores are recommended for postmenopausal women and men above age 50[182]. A Z-score is calculated in a similar manner, however it is it matched for age, gender and ethnic group; Z-score = [(Measured BMD – Age matched mean BMD)/Age matched population SD]. Z-scores are recommended for females prior to menopause and men younger than age 50[182]. A Z-score of -2.0 or lower is defined as "below the expected range for age," and a Z-score above -2.0 is "within the expected range for age"[182]. The DXA is a very accurate and precise tool for quantifying osteoporosis risk. Long term precision measurements are strong, with CVs between 1-1.5% for the spine and total hip BMD and CVs of 2-2.5% for the femoral neck BMD[184].

Newer DXA machines can also accommodate a greater range of people, as these devices can now hold patients up to 450 pounds.

The BONE Laboratory in 229 Wallace Hall (Virginia Polytechnic Institute and State University, Blacksburg, VA) contains the Hologic QDR 4500-A Elite DXA (*Bedford, MA*). Quality control procedures were performed prior to each scan using an anthropomorphic phantom lumbar spine (Hologic) for quality control of bone density. The CV for the phantom spine is 0.39%. An external soft tissue bar (Hologic) was scanned weekly for quality control of soft tissue masses. The CVs for test–retest reliability in 15 young adult men and women tested on separate days were 0.73%, 1.75%, 1.07% and 1.79% for total body, fat mass, fat free soft tissue mass, and percentage body fat, respectively[185]. The CV measurements for BMD were done on 15 subjects with three separate scans; CVs for the spine BMD were 1.54% and 1.15% for the hip BMD[185].

All subjects in the research study had four scans performed: full body, lumbar spine, non-dominant hip and non-dominant forearm. These scans were analyzed by one trained and ISCD certified technician to determine BMD and BMC of the total body, lumbar spine, hip, and radius. T-scores for the lumbar spine, hip, and radius were also recorded. From the full body scan, the technician determined the percentage of total body fat, fat mass, and fat free soft tissue mass; a central abdominal fat analysis[186] was used to quantify the percentage of central body fat.

peripheral Quantitative Computed Tomography (pQCT)

Quantitative computed tomography was introduced in the 1970's as a way to measure BMD in 2-D slices. Computed tomography (CT) can be used to scan the full body, or smaller, less expensive machines can be used to measure peripheral sites such as the radius and tibia with a peripheral CT (pQCT). The radiation dose with the pQCT is very low; the approximate

effective dose per slice of the tibia is <0.003 mSv[187]. The pQCT device can quantify BMD, BMC, cortical width, and volume and cross sectional area of the radius or tibia. Three-dimensional measurement of both cortical and trabecular bone to quantify bone geometry and trabecular structure along with cross sectional area gives the pQCT machine some advantages over the DXA machine[187]. The pQCT device can also be used to quantify muscle and fat parameters, including subcutaneous fat and IMAT in the radius or tibia, similar to measurements obtained from a CT or MRI scanner. Measurements from the pQCT cannot be used to diagnosis osteoporosis, nor can the WHO T-score categories be applied to the pQCT; however, there is fair evidence that forearm pQCT at the ultra-distal radius can predict hip fragility fractures in postmenopausal women[187]. Although limited precision data exists for the pQCT, overall precision is high with BMD CVs of 0.3-1.5% and CVs of 1-3% for muscle cross sectional area[188]. A recent study showed precision was higher for bone (CV <1.0%) than muscle (CV <1.5%) or fat (<3.0%); while precision was strong between two technicians, precision decreased with length of time in between scans[188].

The BONE Laboratory in 229 Wallace Hall (Virginia Polytechnic Institute and State University, Blacksburg, VA) contains the Stratec XCT 3000 pQCT (*White Plains, NY*). Quality control procedures were performed prior to each scan using a phantom cone device (Stratec) using the methodology from the ISCD Precision Calculating Tool, accessed online at: http://www.iscd.org/visitors/resources/calc.cfm. Coefficients of variation were calculated after pQCT scans were done on 15 patients with three scans taken over a two week period. The CVs for area and density measures of cortical tissue ranged from 0.5 to 2.7% and for trabecular tissue from 1.8 to 8.0%[189]. For areal and density measures of soft tissues (marrow, muscle, fat) CVs ranged from 1.7 to 13.1%[189]. These measurements were similar to other data showing that

precision is greater in hard tissue compared to soft tissue; however the soft tissue measurements were slightly higher than others observed and may be because others did not use the ISCD precision tool for calculating CVs[188] or that measurements were taken on the same day with repositioning of subjects. A recent study by Swinford et al.[188] showed that subject weight, BMD DXA-derived lower extremity lean body mass explained 21-52% of the variance in the precision of BMC measured with pQCT. There was considerable variability in subject weight; lab data was used from subjects with BMI's ranging from 18.3-44.5 kg/m^2.

Subjects had their non-dominant tibia measured using the TIBIA4S mask and analyzed using software included with the Stratec XCT; analysis is explained in greater detail in the Appendices under the pQCT Scan Analysis section. The TIBIA4S takes images at sites 4%, 14%, 38% and 66% from the distal end of the tibia. One technician analyzed the results at a point 66% from the tibial distal end to quantify total area, muscle and fat cross sectional areas, including IMAT and subcutaneous fat.

Physical activity assessment

Pedometers are small, relatively inexpensive devices which are worn at the hip to assess step count throughout the day. They are being increasingly used to monitor and motivate subjects' physical activity. A recent meta-analysis found that pedometer use was associated with a significant increase in walking steps (approximately 2,000 steps/day, roughly equal to one mile) and reductions in body weight and blood pressure[190]. In contrast to accelerometers, pedometers cannot accurately provide an accurate estimate of energy expenditure; however, due to their simplicity and ease of wear, they can provide a useful and objective index of physical activity status[191]. Validity estimates between accelerometers and pedometers was shown to be concordant in a larger group of older adults with average step counts of 6,495 with

accelerometers and 6,712 with pedometers[192]. Accuracy has also been verified with pedometers, yet there is variation between brands[193]. The Accusplit was shown to be more accurate than L.L. Bean or Eddie Bauer models[194], yet another study suggested that the Accusplit underestimated step counts[193]. In the research studies conducted at Virginia Tech, all subjects were given the same brand of pedometer; therefore, conclusions were drawn based on comparisons between subjects.

Subjects were given an Accusplit Eagle pedometer (*Livermore, CA*) and were instructed to wear the pedometer through all waking activities over a four day period. The pedometer did not accurately assess volume (intensity x duration) of activity[191], therefore other physical activity when the pedometer was not worn (i.e. more strenuous or water related exercise) was recorded on a physical activity log to assess overall activity level. Four day self-reported physical activity and pedometer step count were translated into corresponding MET levels, multiplied by the length of time subjects were engaged in each activity and then averaged over four days to obtain MET·min/day to quantify overall physical activity status. By using a combination of measurement techniques for physical activity, this provided a measure of both unplanned (daily step counts) and planned activity (more intense activities when the pedometer was not worn) to better reflect overall daily exercise patterns.

Table 1. Assays and CV measurements

Measurement	Specimen	Vendor	Performing Lab	Intra-assay CV (%)	Inter-assay CV (%)
Insulin	Serum	Siemens (New York City, NY)	Frank Gwazdauskas, PhD Laboratory Blacksburg, VA	3.1-9.3	4.9-10
Adiponectin	Serum	Alpco Immunoassays (Salem, NH)	Carilion Community Basic Research Laboratory, Roanoke VA	5.3-5.4	5.0
Leptin	Serum	Alpco Immunoassays (Salem, NH)	Carilion Community Basic Research Laboratory, Roanoke VA	3.7-5.5	5.8-6.8
Osteocalcin	Serum	Alpco Immunoassays (Salem, NH)	Carilion Community Basic Research Laboratory, Roanoke VA	4.7-5.0	5.7-8.3

CTx	Serum	Immunodiagnostic Systems (Scottsdale, AZ)	Carilion Community Basic Research Laboratory, Roanoke VA	1.7-3.0	2.5-10.9
Parathyroid hormone	Serum	Seimens (New York, NY)	Carilion Labs, Roanoke VA	3.4-5.2	1.5-5.8
25(OH) Vitamin D	Serum	Immunodiagnostic Systems (Scottsdale, AZ)	Carilion Labs, Charlotte NC	2.3	5.0-6.0
Calcium	Serum	Beckman Coulter (Fullerton, CA)	Carilion Labs, Roanoke VA	0.3-0.4	0.5-0.6

Summary of Literature Review

Overall, this literature review highlights the need for additional research regarding bone health effects of two diseases which are increasing in prevalence in U.S. adults, specifically as related to risks of osteoporosis and type-2 diabetes in women. Bariatric surgery is becoming an increasingly common procedure, yet only one study has compared changes in bone density following RYGB and LAGB procedures[32]. Rapid weight loss following bariatric surgery can put patients at risk for bone loss[2], yet additional prospective research needs to be done to clarify the effects on bone loss and bone turnover on various surgery types. The first study addressed this by comparing the effects of RYGB vs. LABG on total bone mineral density and content and blood biomarkers related to bone at both three and six month follow-up. This research can help guide physicians in selecting an appropriate bariatric surgery procedure based on patient's current bone health and provide evidence supporting the use of DXA to monitor patient's pre- and post-bariatric surgery.

The pQCT is a novel device with a lower cost and less radiation dose than MRI or CT scanners. IMAT is now being measured with MRI and CT devices and appears to have an association with insulin resistance and therefore potentially contribute to the pathophysiology of type-2 diabetes[10]. However, other research suggests IMAT may be a physiological means for fuel storage in activity people[166]. The second study evaluated fat and muscle distribution of the non-dominant foreleg assessed by pQCT relative to physical activity status and overall body fat

and risk for type-2 diabetes. This research can provide greater understanding into the development of metabolic disease and also aid in defining physical activity interventions which may help to prevent or treat type-2 diabetes.

Overall, both research projects will provide valuable data which could be used by physicians and clinicians to help better educate patients prior to bariatric surgery on potential bone health changes and also provide insight into IMAT assessed by pQCT and its relationship with physical activity and type-2 diabetes risk.

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Chapter 3

Manuscript 1: An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D, and Calcium

Abstract

Purpose: To comparatively evaluate, over six months, effects of Roux-en-Y gastric bypass (RYGB) vs. laparoscopic adjustable gastric banding (LABG) bariatric surgery on body weight loss, bone density, and selected blood biomarkers related to bone status [osteocalcin (OC), carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx), adiponectin, leptin, vitamin D, calcium and parathyroid hormone (PTH)].

Methods: Nine premenopausal Caucasian women (Mean age ± SD 36.9 ± 8 yr) accepted for elective RYGB, n=5 or LAGB, n=4. Patients underwent dual energy X-ray absorptiometry **(**DXA) scans and blood measurements at baseline, three, and six months following surgery.

Results: Following surgery, RYGB patients had greater reductions compared to LAGB in overall body weight at both three (mean weight loss: 19 vs. 9%) and six months (26 vs. 11%), $p<0.01$. Bone loss was most apparent at the hip, especially in RYGB patients who had an average 7% loss in hip bone mineral density (BMD) at six months. For all patients, hip BMD loss at six months was correlated ($p<0.05$) to the decrease in leptin ($r=0.88$) and increase in adiponectin ($r=-0.82$). Serum OC and CTx increased dramatically six months following both surgeries by an average of 130% and 456%, respectively, suggesting increases in bone turnover; these changes were more remarkable following RYGB than LAGB. Vitamin D was low in four patients prior to surgery and decreased in six patients six months after surgery. Small decreases in calcium and increases in PTH were observed, but were not significant.

Conclusions: This preliminary analysis showed bariatric surgery leads to reductions in body weight, fat mass, and fat free soft tissue mass which was more substantial after RYGB surgery compared to LAGB procedures. Changes in BMD were most evident in the hip which may be related to increased bone turnover markers, low vitamin D, or potential relationships with increased adiponectin and decreased leptin. Additional research with larger sample sizes is warranted to better understand changes in bone and related biomarkers and the potential implications for late-life osteoporosis risk following RYGB and LAGB.

Introduction

Excess body weight provides additional skeletal loading and conventional thought has suggested this body mass effect is osteogenic and associated with reduced risk for osteoporosis[1]. Rapid weight loss can reduce skeletal loading and also appears to detrimentally affect bone turnover, with several studies noting with bone loss following bariatric surgery[2]. The extent of post-surgical weight loss is often correlated with bone loss and appears to be more prominent at the hip, an important weight-bearing site[3]. Nutritional factors may also contribute to bone loss, as more than 60% of bariatric surgery patients are deficient in vitamin D and between 25 to 48% show elevated levels of parathyroid hormone (PTH) prior to surgery[4]. Bariatric surgery often exacerbates these issues; decreased absorption of nutrients in the gut, along with reduced food intake and rapid weight loss puts bariatric surgery patients at risk for bone loss[4].

Roux-en-Y gastric bypass (RYGB) is currently considered the favored bariatric surgery approach in the US[5] due to rapid weight loss attributable to both restriction of food intake (from decreased stomach size) and malabsorption of nutrients (from decreased length of the small intestine used); RYGB is therefore considered a combination bariatric surgery procedure. Laparoscopic adjustable gastric banding (LAGB), a primarily restrictive procedure, is gaining popularity in the US and results in weight loss through restriction of food entry into the stomach by the placement of a band at the top of the stomach. Little published research is available to clarify the comparative effects of RYGB- and LAGB-induced weight loss on skeletal health. Most research has focused on either combination (i.e. RYGB) or restrictive surgery (LAGB or vertical banded gastroplasty, VBG), but not both. Only one study has compared the two surgical procedures regarding bone mineral density (BMD) and content (BMC) changes following surgery and this involved very small samples, i.e. n=9 and n=4[6]. Research shows RYGB leads to increased bone turnover and decreased bone mass[2], especially in the hip[3,7-9]. Results are

inconsistent following restrictive surgery; some bone loss appears in the hip and minor changes have been reported at other locations, including total body and the spine[10-14].

Along with BMD and BMC, markers of bone turnover (both resorption and formation) are essential to understanding quantitative changes in skeletal turnover. For bone formation and resorption, respectively, osteocalcin (OC) and carboxy (C)-terminal peptide of type I collagen (CTx) are among the most sensitive to measure these changes[15] and both markers have been documented to increase following RYGB[3,7]. In addition, two adipokines, adiponectin and leptin have been proposed to have a potential impact on bone physiology[2]. However, limited research has been done with either adipokine in relation to potential influences on bone status and bone turnover markers following bariatric surgery.

Rates of bariatric surgery are increasing; it is projected that an estimated 220,000 bariatric surgeries will be performed in 2010 alone[16]. Bariatric surgery is commonly performed in premenopausal females; therefore it is important to evaluate these two surgical procedures to determine effects of each on bone status in the short-term and suggest what might be the longer-term implications for late-life osteoporosis. This was a non-randomized prospective study to comparatively evaluate, over six months, effects of RYGB vs. LABG bariatric surgery on body weight loss, bone density, and bone mass. Changes in BMD and BMC were evaluated in relation to serum levels of selected blood bone biomarkers (OC and CTx), adipokines which may impact bone status (adiponectin and leptin), and blood markers related to nutrient and bone status (vitamin D, calcium, and PTH). This data represents a small sample size; therefore, the primary purpose of this study was to examine potential trends and evaluate patterns for further analysis within a larger sample size.

Methods

Patients

Patients were nine Caucasian premenopausal women (Mean age ± SD 36.9 yr ± 8) who were accepted for elective bariatric surgery at the Carilion Clinic in Roanoke, VA. Women with an Epworth Sleepiness Score greater than ten indicating a possibility of obstructive sleep apnea were excluded, as well as patients taking any medications known to affect bone. Patients underwent either RYGB or LAGB procedures which were performed by one of three surgeons at the Carilion Clinic. The RYGB surgery resulted in a limb length ranging from 100-150 cm and a pouch which could hold approximately ¼ cup of food. The LAGB procedure was performed with the Lap-Band (Allergan, *Irvine, CA*) or the Realize band (Ethicon, *Cincinnati, OH*). Measurements for this study were taken prior to surgery and repeated at three and six months following surgery.

Blood draw/Assay measurement

Patients underwent a blood draw following an overnight fast for at least 8 hours and then serum was separated, centrifuged, and stored in a -80^{o}C freezer prior to biomarker assay. Serum calcium and 25 hydroxy [25$(OH)_2$] vitamin D were assayed by Carilion Laboratories using kits purchased from Beckman Coulter (*Fullerton, CA*), while serum PTH was assessed from a kit from Seimens (*New York, NY*). Intra- and inter-assay coefficients of variation (CV) were as follows: calcium: 0.3-.4% and 0.5-0.6%; 25(OH) vitamin D: 2.3% and 5-6%; PTH: 3.4-5.2% and 1.5-5.8%. Serum adiponectin, OC, and leptin were purchased from Alpco Diagnostics (*Salem, NH*) and CTx was purchased from Immunodiagnostic Systems (*Scottsdale, AZ*). Intra- and inter-assay CVs were as follows: adiponectin: 5.3-5.4% and 5%; OC: 4.7-5% and 5.7-8.3%; leptin: 3.7-5.5% and 5.8-6.8%; CTx: 1.7-3% and 2.5-10.9%.

DXA measurement/anthropometrics

Body weight and height were measured with a calibrated electronic scale (ScaleTronix, *Wheaton, IL*) to the nearest 0.1 kg and a calibrated, wall-mounted digital stadiometer (Heightronic, Measurement Concepts, *North Bend, WA*) to the nearest 0.1 centimeter (cm). Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Waist circumference was measured at the mid-distance between the last rib margin and the top of the iliac crest. Hip circumference was measured at the maximal circumference of the buttocks. Waist and hip circumferences were both measured to the nearest cm; circumferences were measured twice and averaged if the difference between the two measurements was <5 mm.

A full body dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500-A Elite DXA *Bedford, MA*) scan was completed and then the scans analyzed by one technician to determine the percentage of total body fat, fat mass, and fat-free soft tissue mass; a central abdominal fat analysis was used to quantify the percentage of central body fat[17]. Women greater than 136 kg were excluded from the study due to limitations of the DXA instrument. Using the whole body scan and subsequent scans of the lumbar spine, non-dominant hip, and non-dominant radius, both BMD and BMC were measured. A T-score was established for the spine, hip, and radius. Test–retest reliability for the DXA measurements were established in a separate study of 15 young adult men and women; CVs were 0.73%, 1.75%, 1.07% and 1.79% for total body BMD, fat mass, fat free soft tissue mass, and percentage total body fat, respectively[18].

IRB approval

The study protocol was authorized by the Institutional Review Board for the Carilion Clinic in Roanoke, VA and patients provided informed consent.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences, version 18.0 (SPSS, *Chicago, IL*); a p-value <0.05 was considered statistically significant. Means and standard deviation (SD) were calculated for age, anthropometrics, DXA measurements, and biochemical blood parameters. Due to the small sample size in each surgical group, one-way analysis of variance (ANOVA) was used to assess the impact of bariatric surgery across all patients and Student's t-tests were used to compare group differences between RYGB and LAGB surgery. Pearson correlation analyses were used to evaluate relationships among changes in anthropometric, bone, and blood biomarkers following surgery.

Results

Table 1 displays baseline patient characteristics for both RYGB and LAGB patients and Table 2 displays baseline BMD and BMC measurements. There were no significant differences between surgical patients for these baseline measurements.

Both RYGB and LAGB patients had similar body weight and BMI prior to surgery and experienced significant reductions in body weight (Figure 1), fat mass, and fat free soft tissue mass at both three and six months following surgery. These changes were more pronounced in RYGB patients as compared to LAGB at both three and six months following surgery, $p<0.05$. Comparison of measures following RYGB vs. LAGB at six months was as follows: %weight loss: 26 vs. 11%; %fat mass loss: 39 vs. 18%; %fat free soft tissue mass loss: 13 vs. 4%.

With the exception of one patient with a baseline lumbar spine T-score of -2.8, all other patients began with T-scores indicating no osteoporotic risk prior to surgery (T-score greater than -1.25). While many patients experienced some losses in BMD and/or BMC, T-scores remained within normal reference ranges (greater than -1.25) for all other subjects. For most patients, changes in BMD tended to be more pronounced at six months as compared to three

months (Figure 2-3); interestingly, many patients had an increased radial BMD (Figure 4) at six months as compared to the previous follow-up. When evaluating all bariatric surgery patients, the change in BMD and BMC measures for total body, lumbar spine, and radius were not significantly different from baseline measurements. The greatest bone changes were observed in the hip; all RYGB patients had hip BMD losses at both follow-up periods compared to only one LAGB patient at three months and two patients at six months (Figure 5). The difference in percent hip BMD loss between RYGB and LAGB patients trended toward significance at both three month ($p=0.055$) and six month ($p=0.059$) periods. Despite high intra-group variability, among all subjects there was a significant decrease in hip BMD from baseline to six months (Figure 6). In addition, hip BMD was the only bone measure significantly related to weight loss at three months (Figure 7).

With the exception of one patient who had a low baseline calcium level of 8 mg/dL, all patients had calcium and PTH values within the clinical normal ranges throughout the study. Parathyroid hormone was not available at baseline and four subjects did not have PTH measured at any time point throughout the study. At three months, calcium tended to decrease slightly or increase; six months after surgery most patients had increased calcium and PTH from baseline measures. Four patients (two RYGB and two LAGB) began with low 25(OH) vitamin D levels (less than 32 ng/mL), which remained low at the both three and six month follow-up in three of the four patients. Decreased vitamin D at three months was correlated with the change in lumbar spine BMD ($r=0.81$, $p<0.01$) and BMC ($r=0.79$, $p<0.05$), but was not related to other DXA bone measures. Among all patients, there were no significant differences in follow-up measures of vitamin D, calcium, or PTH as compared to baseline.

All patients began with similar levels of leptin, adiponectin, OC, and CTx. Following surgery, leptin decreased while adiponectin, OC, and CTx tended to increase in both patients groups. Changes in CTx, OC, and leptin at both three and six months were significantly different from baseline for all patients ($p<0.05$), although changes were more pronounced for RYGB patients (Figures 8-11). The percentage decrease in leptin was highly correlated with the percentage increase in CTx, $r=-0.85$, $p<0.05$ at three months (Figure 12). At both three and six months, CTx and OC values were highly correlated, $r=0.76$ and $r=0.78$, respectively, $p<0.05$. The ratio of CTx/OC decreased with time in both surgical groups; OC continued to increase until the six month follow-up, while CTx decreased for most patients between three and six months.

A few significant relationships were observed between the change in blood biomarkers and BMD. The change in hip BMD at six months was highly correlated ($p<0.05$) to the change in both leptin ($r=0.88$) and adiponectin ($r=-0.82$), Figure 13. There was also a significant relationship of hip BMD change with leptin at three months, $r=0.86$, $p<0.05$. The change in OC was negatively correlated ($p<0.05$) to total body BMD and BMC changes at three and six months (BMD: $r=-0.92$ and $r=-0.87$; BMC: $r=-0.83$ and $r=-0.91$). The percent change in radial BMD at six months was highly correlated to the change in CTx, $r=-0.86$, $p<0.05$. After controlling for the percent weight loss at each time point, these relationships were diminished with the exception of OC. The relationship remained significant at three months for BMD and at six months for BMD and BMC, after controlling for weight loss.

Discussion

Patients undergoing both surgical procedures began with similar anthropometric, bone, and blood measurements. Similar to other research[5], RYGB patients experienced greater body weight and fat loss at three and six months following surgery compared to LAGB patients.

Other literature suggests substantial reductions of 30-50% of body weight are often achieved six months following surgery[19]; however, patients' weight loss in this study was considerably lower, averaging 25% (range: 18.1-37.7%) six months following RYGB surgery and only 11% (range: 8.4-13.2%) after LAGB surgery. Therefore, it was expected that changes in bone status may not be as substantial as other literature documenting higher weight loss.

With the exception of one patient who began with a lumbar T-score in the osteoporotic range, bariatric surgery did not dramatically change patient's osteoporosis risk; additionally, all patients had hip BMD measurements throughout the study within the normal ranges reported from the NHANES III data[20]. This suggests BMD and BMC changes at three and six months may not be clinically significant in this small subset of patients. All patients undergoing RYGB had reduced hip BMD and BMC after surgery, similar to other research[3,7-9]. Some decreases in BMD and BMC were also observed with LAGB patients, mainly at the hip and spine, but were less substantial than changes noted following RYGB surgery. Guney et al.[11] noted bone loss following VBG, a restrictive form of bariatric surgery and a medically supervised weight loss program and concluded that bone loss was independent of the weight loss method, but was rather dictated by the extent of weight loss. Research suggests loss of as little as 10% of body weight can result in 1-2% loss of bone[1]. In this study, the decrease in hip BMD at three months was the only bone measure which correlated with percent change in body weight at three months, suggesting other factors beside weight loss and decreased skeletal loading may have contributed to bone changes, including cellular changes such as those indicated by bone turnover markers. Bruno et al.[21] reported elevated bone turnover markers 18 months after RYGB when body weight was in the normal-overweight BMI range and suggested that bone turnover is influenced by other factors than skeletal unloading following rapid weight loss.

Previous research suggests changes in bone turnover may be related to nutrient deficiencies secondary to malabsorption and/or decreases in food consumption after surgery[22]. RYGB tends to be associated more with nutrient deficiencies due to the nature of the surgery, which bypasses the duodenum and proximal jejunum, the main sites of calcium absorption[22]. Several articles have documented nutritional issues such as vitamin D deficiency[23], hypocalcemia[24], and secondary hyperparathyroidism[3,25] following malabsorptive bariatric surgery. In our patient group, 44% of patients had clinically low 25(OH) vitamin D levels prior to surgery which remained low throughout the follow-up period in 33% of those patients. Overall, 25(OH) vitamin D decreased in 66% of patients throughout the follow-up periods. The change in vitamin D was significantly related to changes in lumbar spine BMD and BMC measures. However, there was not a concomitant decreased in calcium or increase in PTH levels. Other research has found similar inconsistent results with nutrient status following surgery and concluded that that bone turnover may still be occurring if bone turnover markers are elevated[21,26].

Baseline measurements of OC, CTx, adiponectin, and leptin were within ranges reported for other patients prior to bariatric surgery, with a few patients slightly above other reported values at six months[21,27]. Increased OC and CTx in all patients at three months suggested increased bone turnover following surgery; elevations were more remarkable in RYGB compared to LAGB patients. The rate of CTx increase appeared to slow from three to six months, while OC levels continued to rise to an average of 456% (range: 6-2154%) above baseline at six months. Therefore, the ratio of CTx to OC was lower at six months than at three months, suggesting relatively greater bone accretion than absorption. These results vary some from other literature; Fleisher et al.[3] observed progressive increases in both amino (N)-terminal

peptide of type I collagen (NTx, a similar marker of bone resorption) and OC accompanied by significant decreases in femoral and total neck BMD up to twelve months after RYGB. Coates et al.[7] noted a progressive increase in NTx at three, six, and nine months after RYGB, but increased OC tended to level off after three months. In addition, significant losses in BMD of the spine, femoral neck, hip, and total body were observed[7]. There was a strong negative correlation between the change in leptin and CTx at three months ($r=-0.85$, $p<0.05$), similar to data by Bruno et al.[21] with leptin and NTx ($r=-0.58$, $p<0.05$) six months after RYGB. Researchers noted that the change in leptin was a significant predictor of the change of NTx and hypothesized that decreased leptin following surgery may have signaled the body to increase bone turnover associated with decreased calorie consumption[21]. Unfortunately, BMD and BMC measurements were not completed in that study to make comparisons with related biomarkers.

Research has suggested adiponectin and leptin may influence bone density after surgery[8,21]. Additional research is warranted to clarify and better understand their role following bariatric surgery[26,28] since both adipokines are highly related to fat mass[29]. Leptin was positively correlated to measures of body fat and fat mass, primarily at three and six months after surgery. It has been suggested that loss of bone following bariatric surgery is related to decreased leptin resistance[2]; however previous literature has focused on leptin and bone turnover markers[21,26,28] and additional research is necessary to evaluate these changes in the context of BMD and BMC measurements. Adiponectin is decreased in states of obesity and inversely related to BMD[30], even after adjustment for fat mass[30,31]. Additionally, changes in adiponectin were inversely correlated with decreased total and central body fat percent and fat mass at six months. Only one other study has observed changes in adiponectin and BMD measures following bariatric surgery; Carrasco et al.[8] noted increased adiponectin post-RYGB which correlated with decreased total

body BMD. This data were similar; adiponectin increased following surgery for all but one patient at three months and one patient at six months, while relationships with BMD and BMC at sites other than the hip were not significant. In this study, the changes in both leptin and adiponectin were highly correlated to hip BMD loss at six months, but were not significant after controlling for weight loss. This suggests alterations in these adipokines may be more related to body weight changes than bone loss.

The study group was homogenous with respects to gender and race and can be used to evaluate trends in bone changes and blood biomarkers which are useful to determine appropriate measures for evaluation in a larger subset of patients. Several limitations must be addressed within this small sample. Adequate sample size is necessary for publishable results and was limited in this study by patient's interest and distance to the study site. To minimize missing data and improve patient flow into the study, improved communication and coordination with the bariatric surgical team is necessary to ensure routine clinical bariatric surgery standard procedures are being completed for all patients. Additional measurements regarding supplement intake and exercise habits would strengthen the study and provide further parameters to evaluate in the context of bone changes following bariatric surgery. Patients were recommended by a Registered Dietitian to consume supplements; however, supplement use was not closely monitored through the study. Challenges in patient reporting and follow-up led to a significant portion of missing exercise data which was not able to be evaluated in this study.

Conclusions

This is only the second article to compare bone changes and related biomarkers following two different commonly performed bariatric surgery procedures, RYGB and LAGB. While bone loss was more pronounced in RYGB patients, this research shows that some bone loss can also occur following LAGB and that bone turnover was occurring in all patients as indicated by

increased CTx and OC. There is a need for studies of larger sample sizes to compare both RYGB and LAGB surgical procedures at similar post-surgical time points and to normalize comparisons for weight loss to better understand the influence each procedure may have on bone. Measurements of bone biomarkers and additional research with the adipokines adiponectin and leptin along with BMD and BMC are also critical to fully quantify changes in bone status and possible health implications following surgery. There is a also a need for well-controlled trials to provide recommendations on the type and dosage of vitamin supplements for bariatric surgery patients to help minimize risk of bone loss and nutrient deficiencies.

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Tables

Table 1. Baseline descriptive data for RYGB and LAGB patients.

	RYGB (n=5)	**LAGB** (n=4)
Anthropometric data (n=9)		
Age (yrs)	40.2 ± 4.5	32.8 ± 10.0
Weight (kg)	112.8 ± 3.6	114.3 ± 8.9
BMI (kg/m^2)	43.1 ± 3.8	42.1 ± 4.4
Waist circumference (cm)	119.8 ± 10.4	110.6 ± 6.3
Hip circumference (cm)	138.6 ± 3.4	137.3 ± 6.7
DXA body composition data (n=9)		
Total body fat (%)	46.9 ± 5.8	49 ± 2.8
Central body fat (%)	50.3 ± 5.5	52.0 ± 4.6
Fat mass (kg)	53.1 ± 6.0	56.3 ± 6.2
Fat free soft tissue mass (kg)	58.0 ± 7.7	56.2 ± 4.1
Blood biomarkers related to nutrient status (n=8)[1]		
Calcium Clinical range: 8.5-10.7 mg/dL	9.0 ± 0.6	8.8 ± 0.6
$25(OH)_2$ vitamin D Clinical range: 32-100 ng/mL	39.5 ± 16.6	45.0 ± 23.9
Parathyroid hormone Clinical range: 9.2-79.5 pg/mL	N/A	N/A
Bone biomarkers and adipokines (n=6)[2]		
Adiponectin (µg/mL)	7.9 ± 6.2	5.4 ± 1.3
Leptin (ng/mL)	73.3 ± 19.6	88.0 ± 17.4
CTx (ng/mL)	0.29 ± 0.16	0.35 ± 0.15
Osteocalcin (ng/mL)	7.6 ± 8.9	9.2 ± 31
CTx/OC ratio	0.076 ± 0.05	0.041 ± 0.02

Mean ± SD. N/A, data not available. There were no differences between RYGB and LAGB surgical groups at baseline.

[1]Basline data not available for one LAGB patient for calcium and one RYGB patient for vitamin D, baseline data for PTH was not available. [2]Baseline data was unavailable for three patients, complete data is presented for 3 RYGB and 3 LAGB patients.

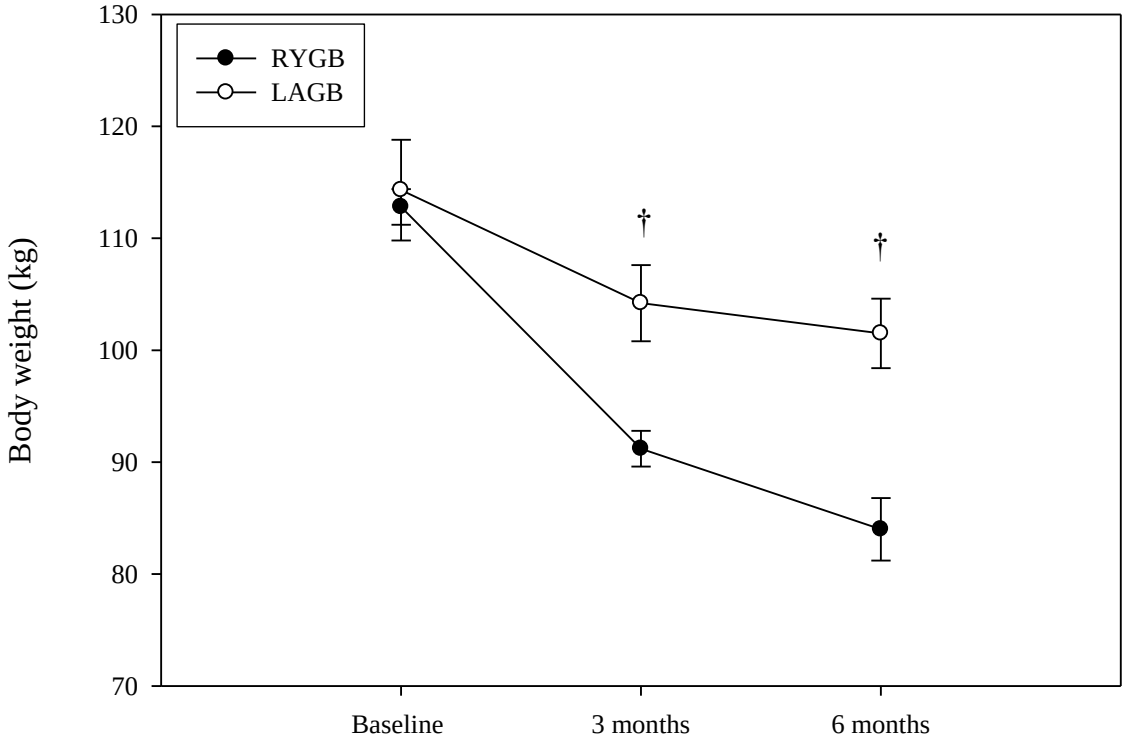
Table 2. Baseline DXA bone mineral density (BMD) and content (BMD) measures for RYGB and LAGB patients.

	RYGB (n=5)	**LAGB** (n=4)
DXA Bone Mineral Density (g/cm^2)		
Total BMD	1.170 ± 0.11	1.085 ± 0.062
Lumbar spine BMD	1.046 ± 0.15	1.052 ± 0.066
Non-dominant Hip BMD	1.006 ± 0.11	0.918 ± 0.13
Non-dominant Radius BMD	0.644 ± 0.033	0.632 ± 0.059
DXA Bone Mineral Content (g)		
Total BMC	2402.6 ± 337.9	2202.6 ± 205.2
Lumbar spine BMC	54.6 ± 11.4	56.1 ± 4.2
Non-dominant Hip BMC	32.7 ± 7.2	27.7 ± 3.8
Non-dominant Radius BMC	6.7 ± 1.4	7.9 ± 1.0

Mean ± SD. There were no differences between RYGB and LAGB surgical groups at baseline.

Figures

Figure 1. Mean body weight changes at baseline, three, and six months after bariatric surgery.



Values are means ± SEM. Body weight at three and six months was significantly different from baseline for all patients, †$p<0.001$

Figure 2. Percent change total body BMD at three and six months following bariatric surgery for RYGB (n=5) and LAGB (n=4) patients.

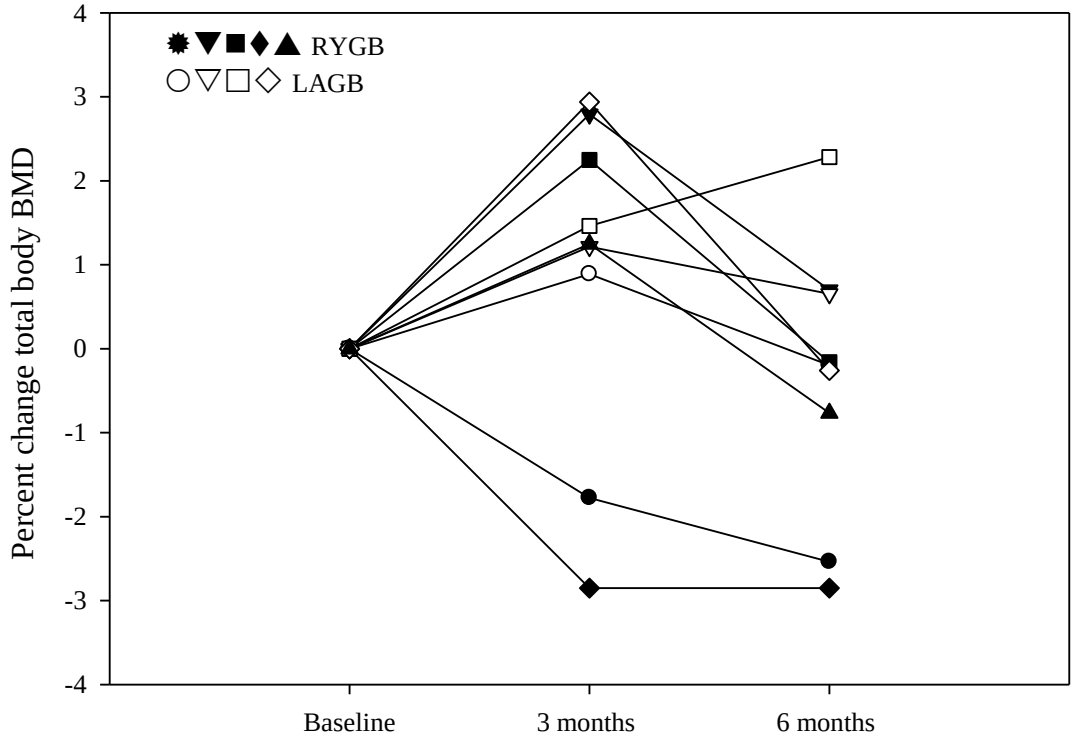


Figure 3. Percent change lumbar spine BMD at three and six months following bariatric surgery for RYGB (n=5) and LAGB (n=4) patients.

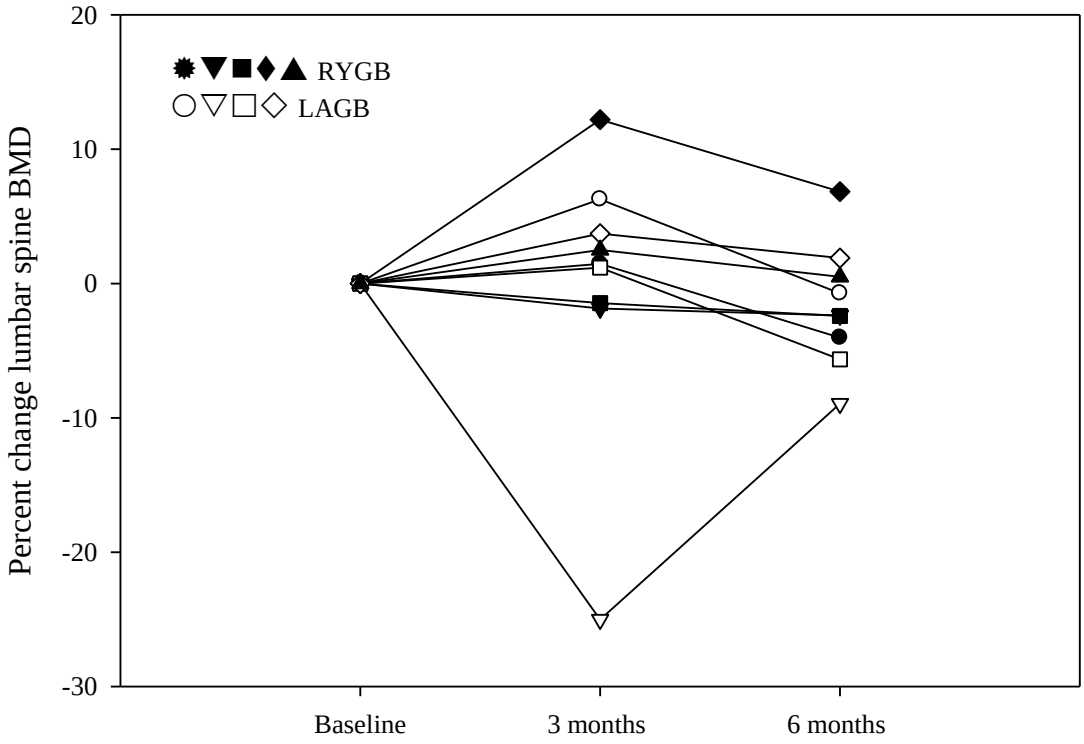


Figure 4. Percent change non-dominant radius BMD at three and six months following bariatric surgery for RYGB (n=5) and LAGB (n=4) patients.

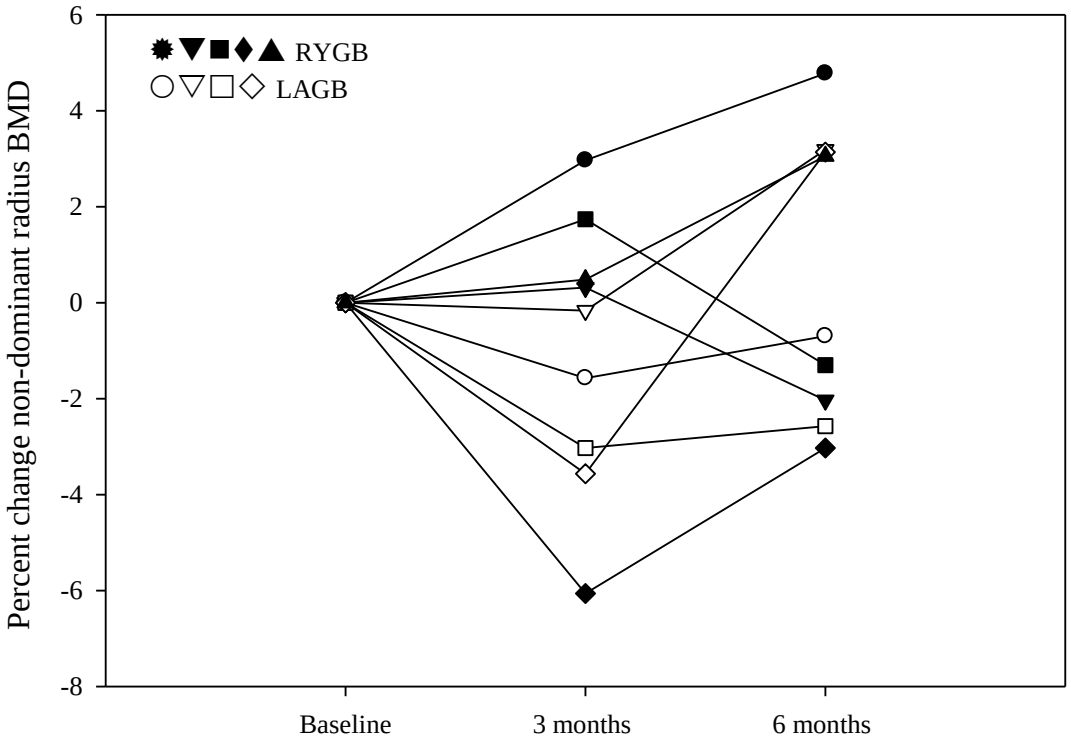


Figure 5. Percent change non-dominant hip BMD at three and six months following bariatric surgery for RYGB (n=5) and LAGB (n=4) patients.

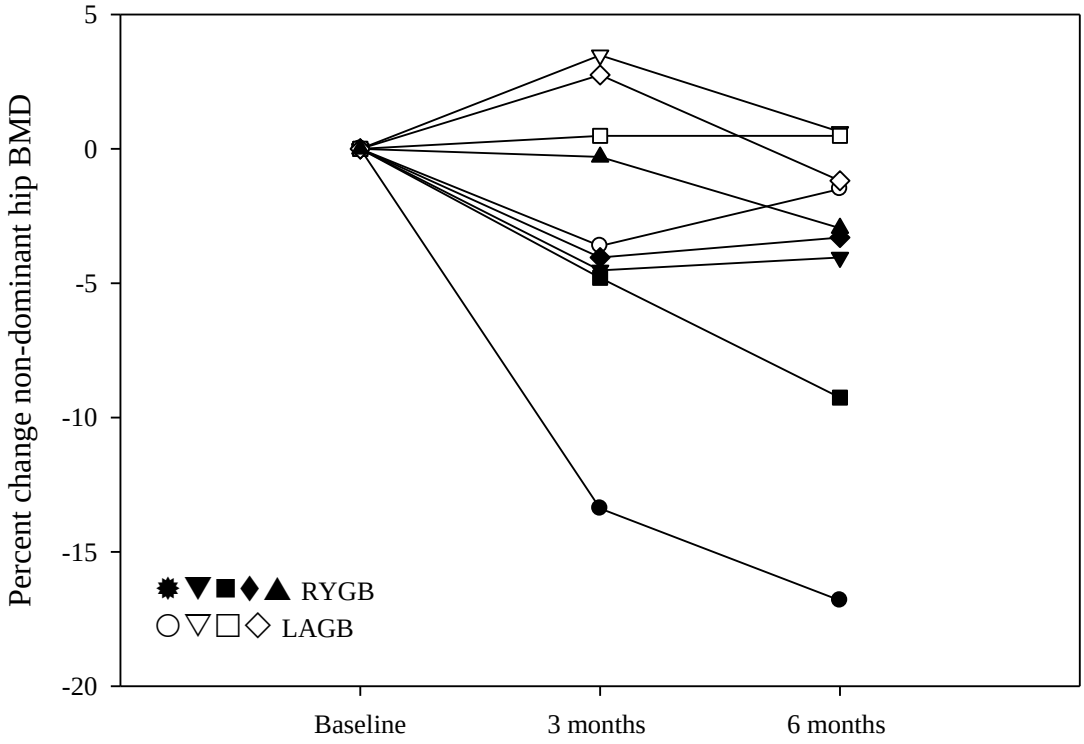
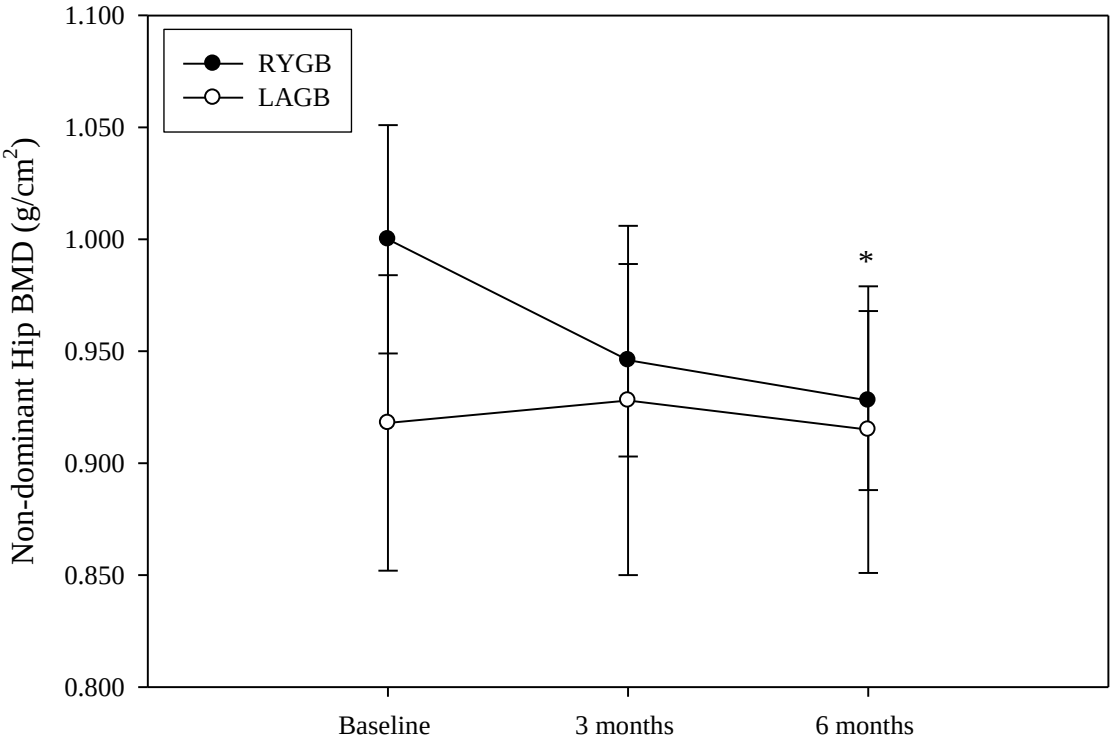


Figure 6. Non-dominant hip BMD at baseline, three, and six months following bariatric surgery.



Values are means ± SEM. Hip BMD at six month measurement was significantly different than baseline for all subjects, *$p<0.05$.

Figure 7. Relationship between changes in non-dominant hip BMD and weight loss at three months following bariatric surgery.

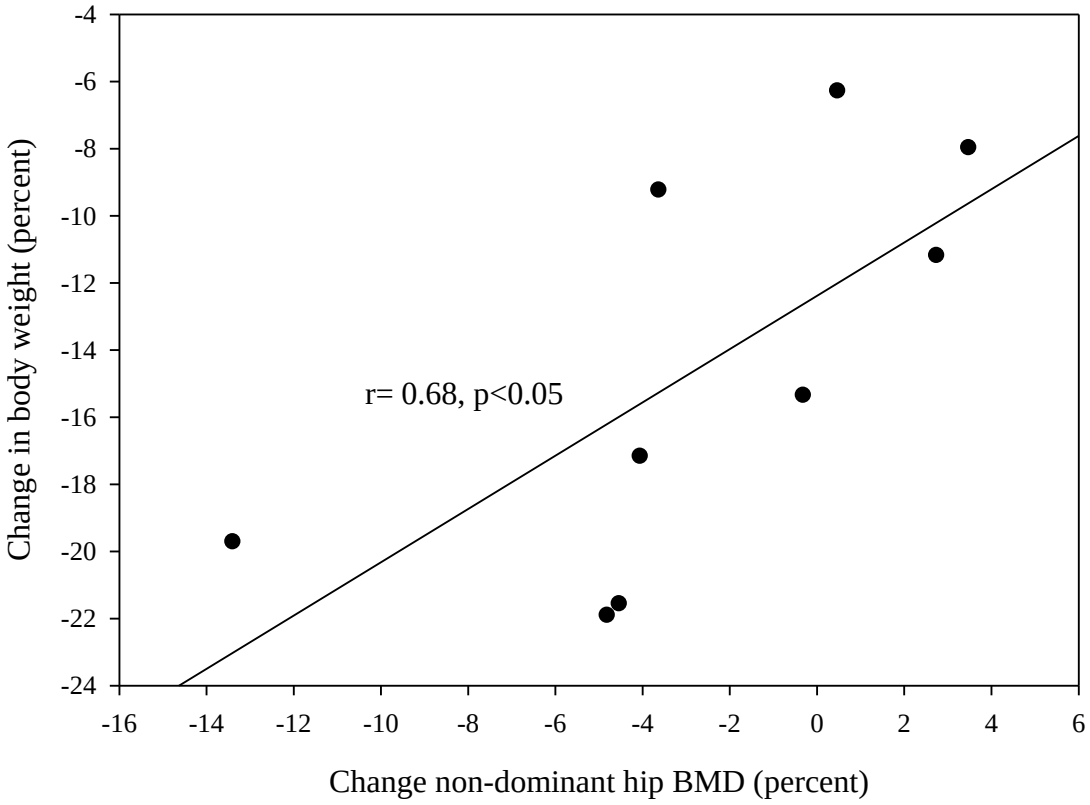
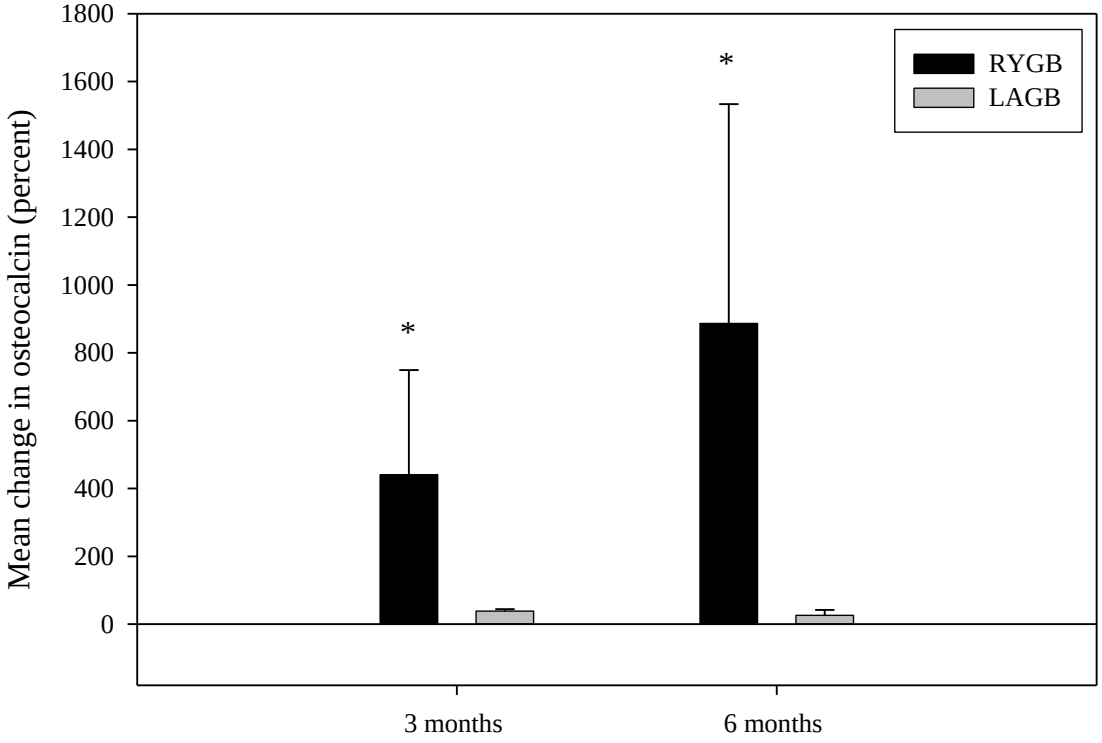
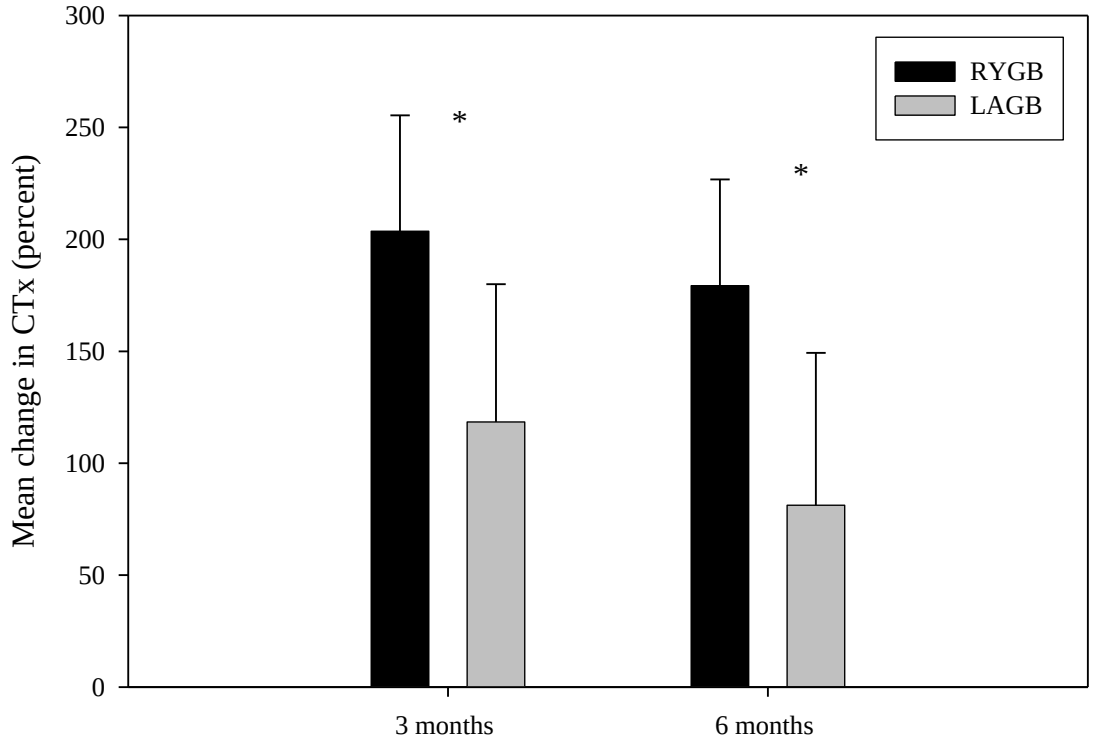


Figure 8. Mean change in osteocalcin at three and six months following bariatric surgery.



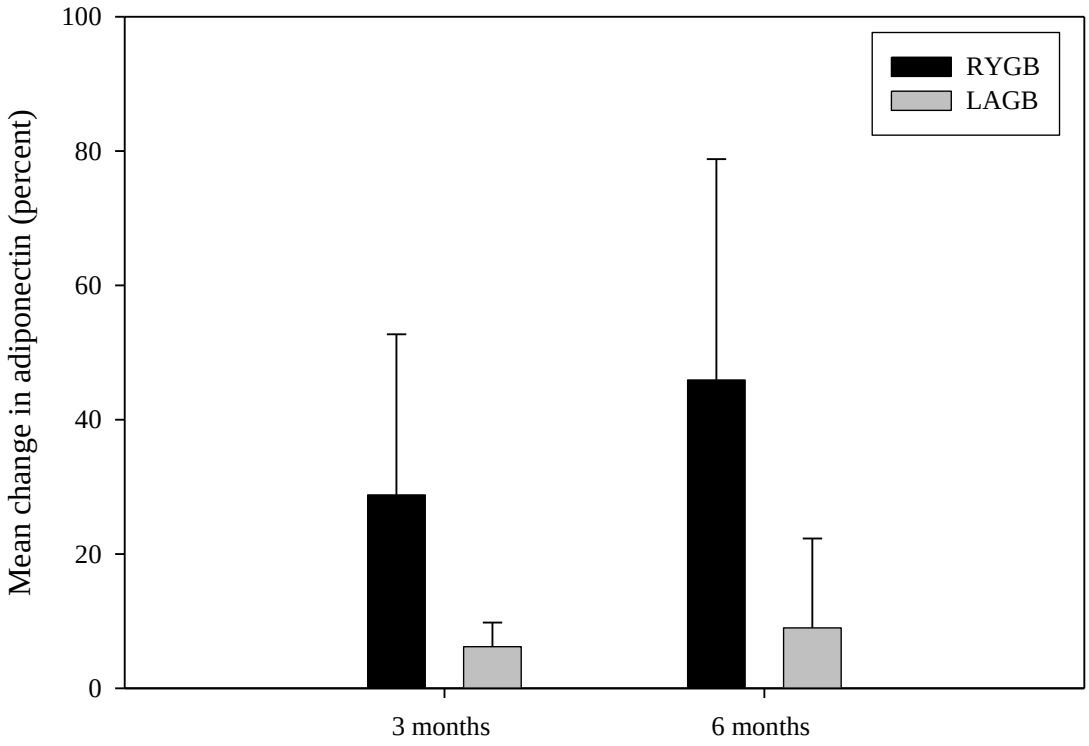
Osteocalcin at both three and six months was significantly greater than baseline measures for all patients, *$p<0.05$.

Figure 9. Mean change in CTx at three and six months following bariatric surgery.



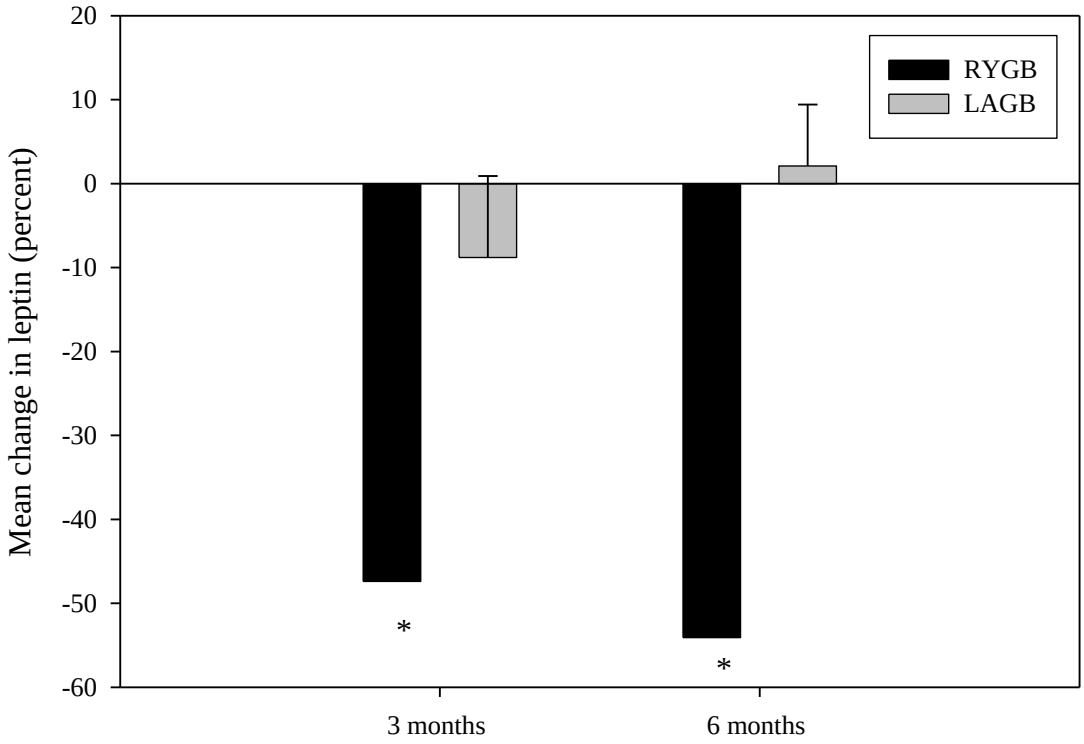
CTx at both three and six months was significantly greater than baseline measures for all patients,*$p<0.05$.

Figure 10. Mean change in adiponectin at three and six months following bariatric surgery.



Adiponectin at both three and six months was not significantly different than baseline measures for all patients.

Figure 11. Mean change in leptin at three and six months following bariatric surgery.



Leptin at both three and six months was significantly less than baseline measures for all patients, *$p<0.05$.

Figure 12. Relationship between change in CTx and leptin at three months following bariatric surgery.

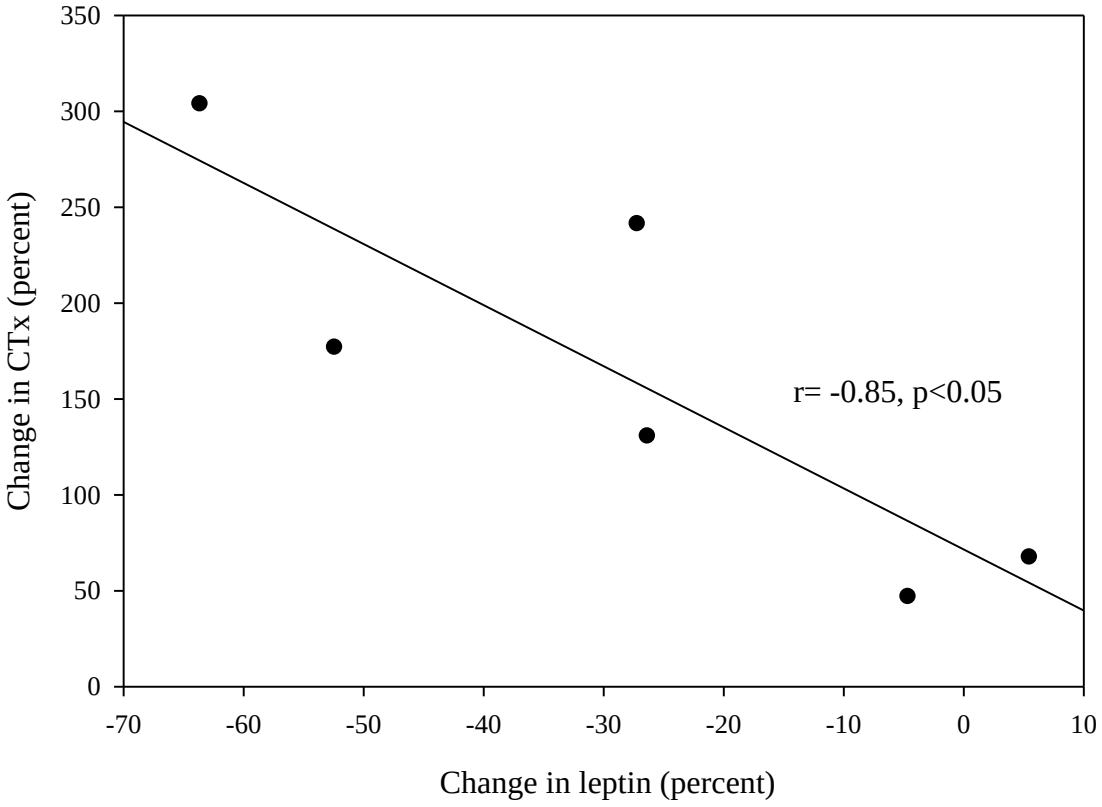
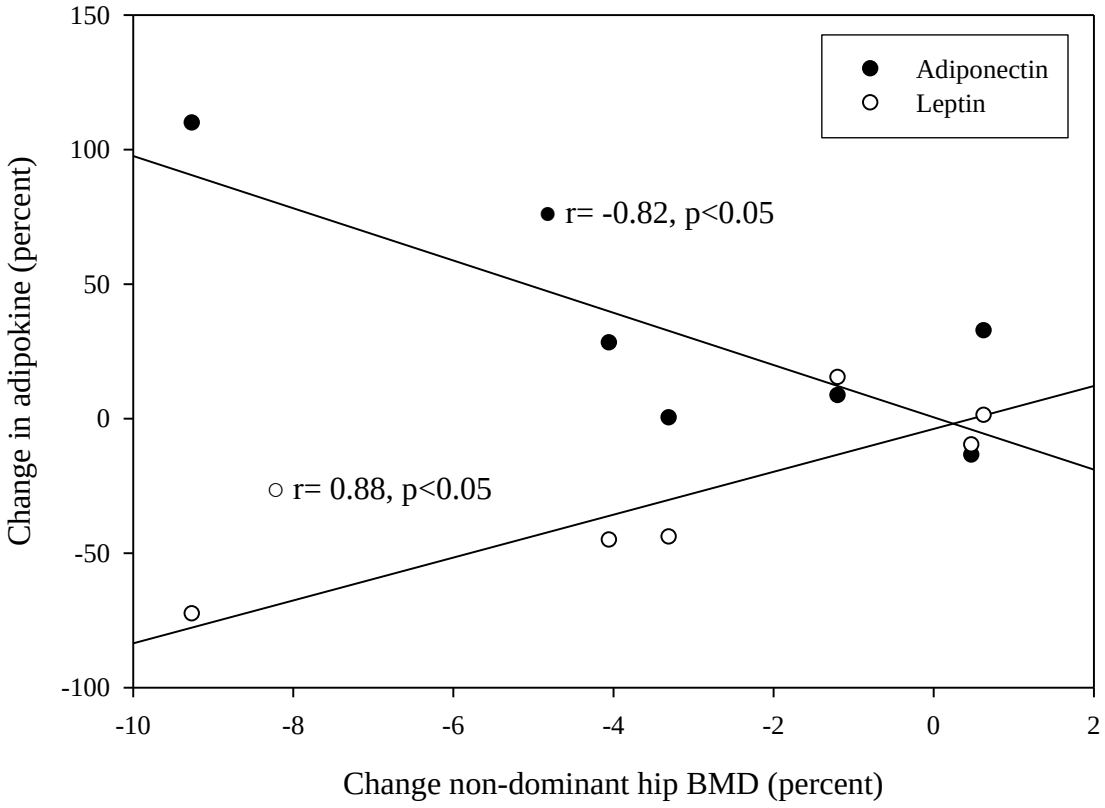


Figure 13. Relationship between change in non-dominant hip BMD and change in adiponectin and leptin six months after bariatric surgery.



Chapter 4

Manuscript 2: Fat and Muscle Indices Assessed by pQCT: Relationships with Physical Activity and Type-2 Diabetes Risk

Abstract

Purpose: To compare muscle and fat indices evaluated by the peripheral Quantitative Computed Tomography (pQCT) in the foreleg and to determine repeatability for intermuscular adipose tissue (IMAT) and muscle density. Two prediction models were evaluated to determine influences on muscle density and type-2 diabetes risk using physical activity status and health-related measures.

Methods: 82 premenopausal women (Mean age ± SD 38.6 ± 4.7 yr) underwent dual energy X-ray absorptiometry **(**DXA) scans to assess total fat, fat mass, fat free soft tissue mass, and central body fat. Muscle and fat parameters were quantified for the foreleg using the pQCT at a point 66% from the distal end of the tibia. Physical activity status was determined based on four-day self-reported physical activity and pedometer step counts.

Results: Fat and muscle distribution in the foreleg highly was correlated to total body adiposity. The pQCT device reliably measured muscle density in the foreleg; coefficient of variation (CV) was 0.8%, which was therefore used as a surrogate for IMAT. Precision was lower for IMAT in the foreleg with a CV of 15.1%. Muscle density was positively related to physical activity and negatively associated with markers of fat distribution and risk for type-2 diabetes. Foreleg subcutaneous fat and HDL-C were significant predictors of the homeostasis model assessment of insulin resistance (HOMA-IR) (R^2=0.48, $p<0.001$), while total body fat percentage was a significant negative determinant of foreleg muscle density (R^2=0.27, $p<0.001$).

Conclusions: The pQCT is a novel, noninvasive tool to assess IMAT and muscle density in the foreleg. Muscle density has stronger relationships to health-related markers than IMAT in this healthy population and can be used as a surrogate for IMAT to better understand the relationships with muscle and fat deposition and health risk. Additional research is necessary to understand the biology of IMAT and its relations with physical activity and potentially, with risks for cardiometabolic disease.

Introduction

The mass and distribution of adipose tissue affects cardiovascular and metabolic disease risk, especially when centrally located[1]. Visceral fat is associated with alterations in lipid and glucose metabolism, while subcutaneous fat does not seem to present the same increased risk[2]. One related index of recent health interest is intermuscular adipose tissue (IMAT), the adipose tissue between muscle bundles[3]. This locus can be measured using magnetic resonance imaging (MRI), computed tomography (CT), and more recently, with peripheral quantitative computed tomography (pQCT). The pQCT device is a smaller instrument with less radiation exposure, less costly, and may be more practical to use in a research setting compared to MRI or CT. Measurements with MRI and CT have shown associations between higher concentrations of IMAT, insulin resistance, type-2 diabetes, and reduced muscular strength[4-7]. Research by Miljkovic-Gacic et al.[8] showed similar relationships with IMAT measured by pQCT and type-2 diabetes risk. Similar conclusions were found in a separate article by the same researchers where muscle density was used as a surrogate for IMAT with the rationale that greater fat infiltration is an indicator of reduced muscle density[9]. Muscle density has been validated by others as an indicator of adipose tissue deposition in the muscle[10,11].

Specific patterns of fat infiltration may be a more important marker for risk of type-2 diabetes and metabolic syndrome than overall fat mass[12]. Goodpaster et al.[4] suggested even normal weight individuals may be at risk for metabolic disorders if they exhibit higher levels of IMAT and abdominal visceral fat accumulation. Physical activity appears to moderate this relationship, yet effects on fat depots in the limbs where pQCT is able to quantify hard and soft tissue are limited and indecisive[13,14]. Limited research shows physical activity may decrease[15,16]

or increase[17,18] IMAT, the latter possibly being beneficial to increase metabolic flexibility during exercise[19].

Measurement of IMAT is typically done in the leg, a weight-bearing site, with a majority of research focused on either the upper thigh or calf region. Therefore, the purpose of this study was first to evaluate the distribution of muscle and fat indices evaluated by pQCT in the foreleg and to determine repeatability for IMAT and muscle density. Two prediction models were evaluated to determine influences on muscle density and type-2 diabetes risk using physical activity status and health-related measures.

Methods

Subjects

Subjects were 82 healthy, premenopausal Caucasian women (Mean age ± SD 38.6 ± 4.7 yrs) with body mass index (BMI) ranging from 18.3 to 46.6 kg/m^2 (Mean BMI ± SD 24.5 ± 5.1). Women who were taking any prescriptive medications known to affect bone health were excluded. Subjects were recruited from the Virginia Tech campus and the surrounding communities in the New River Valley, VA.

Blood draw/assay measurement

Subjects underwent a blood draw following an overnight fast for at least 8 hours; serum was separated, centrifuged, and stored in a -80^{o}C freezer prior to assay analysis. Glucose and lipids were measured on a whole blood sample using a Cholestech Lipid Profile and GLU cassette (Cholestech, *Hayward, CA*). Serum insulin was measured using Coat-a-Count insulin radioimmunoassay (RIA, Siemens, *New York, NY*) with an inter-assay coefficient of variation (CV) of 4.9-10% and an intra-assay CV of 3.1-9.3%. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting insulin and glucose measurements, [fasting insulin (U/ml) x fasting glucose (mmol/l)]/22.5[20].

Anthropometrics

Body weight and height, respectively, were measured with a calibrated electronic scale (ScaleTronix, *Wheaton, IL*) to the nearest 0.1 kg and a calibrated, wall-mounted digital stadiometer (Heightronic, Measurement Concepts, *North Bend, WA*) to the nearest 0.1 cm; BMI was calculated as weight divided by height in meters squared. The length of the non-dominant tibia was measured from the highest point of the medial condyle of the tibia to the highest point of the medial malleolus, and the length was recorded to the nearest cm.

DXA measurement

A full body dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500-A Elite DXA *Bedford, MA*) scan was completed and analyzed by one technician to determine percentage of total body fat, fat mass, and fat free soft tissue mass; a separate analysis was used to determine the percentage of central body fat[21]. Women greater than 136 kg were excluded from the study due to capacity limitations of the DXA instrument. The CVs for test–retest reliability in 15 young adult men and women tested on separate days were 1.75%, 1.07%, and 1.79% for fat mass, fat free soft tissue mass, and total body fat percentage, respectively[22].

pQCT measurement

The non-dominant tibia was scanned using the Stratec XCT 3000 pQCT (*White Plains, NY*) TIBIA4S mask, and then analyzed using the corresponding macro software. One technician analyzed the results at a point 66% from the tibial distal end to quantify total, muscle, and fat cross-sectional areas and muscle density. A percentage for each index was calculated based on the total cross-sectional area of the foreleg. A secondary analysis using the Stratec software was necessary to determine IMAT. Total area was established using contour mode 3 (iterative contour search), peel mode 1 (concentric peel), and filters F03:F05:F05 (muscle smooth 3). The analysis was run a second time with an additional filter (F07) which excluded fat. These two

total areas were subtracted to calculate IMAT. Repeatability of muscle density and IMAT was established using the Advanced Precision Tool by the ISCD[23] which included three scans on non-consecutive days in the non-dominant foreleg of 15 premenopausal females (Mean age ± SD 39.8 ± 4.6 yr, BMI 25.4 ± 6.6 kg/m^2).

Physical activity

Both subjective and objective measures were used to quantify physical activity status. Each subject wore a pedometer to record all activities over a four day period. Subjects recorded other physical activities when the pedometer was not worn and detailed the type, length of time, and intensity of activity. These activities were translated into corresponding metabolic equivalent (MET) levels, multiplied by the length of time in each activity and then averaged to obtain MET·min/day to quantify overall physical activity status. By using a combination of measurement techniques for physical activity, this provided a measure of both unplanned (daily step counts) and planned activity (more intense activities when the pedometer was not worn) to better reflect overall daily exercise patterns.

IRB approval

The study protocol was authorized by the Institutional Review Board at Virginia Polytechnic Institute and State University, Blacksburg, VA and each subject gave informed consent.

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences, version 18.0 (SPSS, *Chicago, IL*); a p-value <0.05 was considered statistically significant. Pearson correlation analyses were used to evaluate relationships among variables of interest and multiple regression analyses were used to determine predictors of muscle density and type-2 diabetes risk.

Results

Subject characteristics are displayed in Table 1. Subjects were relatively healthy; on average BMI was within the normal range and fasting blood glucose values indicated subjects were not at risk for type-2 diabetes. Overall, strong correlations of both fat and muscle parameters of the foreleg measured by pQCT were noted with total and central body fat percentage and fat mass measured by DXA (Table 2).

Coefficient of variation of IMAT measured with pQCT was 15.1% for the foreleg. IMAT was first examined as raw data and then normalized separately for height and muscle cross-sectional area; no significant relationships were observed with health-related measures. Repeatability with muscle density was robust; the CV was 0.8% for the foreleg.

Inverse relationships were noted between both HOMA-IR and markers of health risk and muscle density and markers of health risk, Table 3. This indicated higher muscle density was associated with increased physical activity and lower body fat measures. Fifty percent of the variance in HOMA-IR was explained by foreleg muscle density and percentage of subcutaneous fat, physical activity, HDL-cholesterol (HDL-C), and total body fat percentage (R^2=0.50, $p<0.001$), Table 4. Following stepwise multiple regression, foreleg subcutaneous fat and HDL-C remained significant predictors with an R^2 of 0.48 ($p<0.001$). The same independent variables along with HOMA-IR explained 33.1% of the variance in foreleg muscle density, Table 5 ($p<0.001$). After stepwise linear regression, only total body fat percentage remained a negative predictor with an R^2 value of 0.27, $p<0.001$.

To compare influences of habitual physical activity on muscle density and IMAT, subjects were divided into tertiles based on physical activity status. Through subject interview, the four-day log of both pedometer steps and planned activity was representative of their typical habitual exercise patterns. Based on previous literature, we expected to see differences with

IMAT when comparing the high and low physically active subjects. High active subjects had lower BMI, total and central body fat percentage, and HOMA-IR levels, $p<0.01$. No significant differences were observed with IMAT, but muscle density of the foreleg tended ($p=0.054$) to be greater in high active subjects. Overall, physical activity level was positively correlated with foreleg muscle density ($r=0.29$, $p<0.05$), Figure 1 and was negatively correlated with HOMA-IR ($r=-0.44$, $p<0.01$), Figure 2.

Discussion

We believe this is the first article to use the rigorous standards prescribed by the ISCD[23] to determine repeatability of both IMAT and muscle density of the foreleg measured by pQCT. The pQCT measurement of IMAT includes the adipose tissue that is between muscle bundles (intermuscular fat) and adipocytes within muscle fibers (intramuscular fat)[24]. The pQCT is not specific enough to discriminate between intermuscular and intramuscular adipose tissue, nor is it as precise as an MRI or CT scan. Coefficient of variation for the foreleg IMAT (15.1%),was higher than those obtained in other studies, using MRI measurements with repetitive readings taken of the same scan[24,25]. However, foreleg IMAT CV was significantly better than Mitsiopoulos et al.[26] which reported a standard error of estimate of 30% for repeated measures of IMAT measured with MRI and CT compared to a cadaver. Length of time between scans and subject anthropometrics can contribute significantly to the variance in pQCT measurements with precision error increased in measurements of fat compared to muscle[27]. Variation between measurements was considerably lower with muscle density with a CV of 0.8%and was therefore used as a surrogate for IMAT.

Our results were similar to Ducher et al.[28] which demonstrated adiposity indices in the foreleg and forearm measured by pQCT were similar to DXA total body fat percentages in pre-

pubertal children. Fat distribution appears to be similarly dispersed among the total body and specific depots in the foreleg in this population.

Intermuscular adipose tissue (IMAT) appears to have depot sizes similar to visceral adipose tissue[24], suggesting accumulations may confer similar negative metabolic consequences. In addition, IMAT is strongly correlated to total adiposity in both men and women[13,29] and appears to be greater in subjects with type-2 diabetes than in controls[30]. This subject population did not appear to be at risk for type-2 diabetes, as fasting glucose values were below thresholds (≤126 mg/dL) defined by the World Health Organization (WHO)[31] and the American Diabetes Association[32]. While several researchers have demonstrated a relationship between IMAT and insulin resistance[5,6,33,34], key characteristics of the subjects examined in those studies were dramatically different from our healthy Caucasian females and included the elderly[4], those with diabetes[5], or predominantly overweight or obese women[33,34]. Underlying mechanisms of the association with IMAT and insulin resistance are incompletely understood, but may include increased concentrations of fatty acids, impaired blood flow to the muscle caused by muscle lipid accumlation[13], or alterations in skeletal muscle glucose uptake or metabolism[35].

An article by Miljkovic-Gacic et al.[8] quantified IMAT with pQCT to evaluate associations with type-2 diabetes in African American adults and a second article by the same group used pQCT to measure muscle density as a surrogate for IMAT[9]. Findings from Miljkovic-Gacic et al.[8,9] were similar to those reported by other investigators using MRI or CT scans[5,6,33,34] and showed higher IMAT in males of normal weight was associated with type-2 diabetes independent of age, BMI, and total fat and muscle areas. In this study with healthy females, IMAT did not show associations with comparable health parameters, even after normalization for height or muscle cross-sectional area.

Two separate groups[10,11] have validated muscle density assessed from CT scans or percutaneous biopsy as a measure of adipose tissue infiltration. Muscle density was therefore used to evaluate relationships with type-2 diabetes risk in our study population and showed negative associations with several health parameters, including HOMA-IR. There were no relationships between muscle density and HOMA-IR after controlling for body fat, which is consistent with other literature[6,7,36]. After stepwise multiple regression, only total body fat percentage remained a significant negative predictor of muscle density. Overall, these results suggest adipose accumulation between muscle bundles may be a physiologically important means of fat storage in the body[6,36]; yet unidentified factors may determine how this tissue modulates health risk, especially in relation to insulin resistance and type-2 diabetes[7].

Physical activity presents a moderating risk factor to the relationship of IMAT and insulin resistance, as exercise trained subjects exhibit greater IMAT[18], or similar levels to those with type-2 diabetes[14]. Goodpaster et al.[14] reported that intramuscular adipose tissue was greater in a small group of trained subjects than in sedentary subjects and they also had a 65% greater oxidative capacity, indicating a heightened capacity for triglyceride fuel use during exercise. Limited research has evaluated exercise training effects on IMAT, resulting in both increased[37] and decreased[38] IMAT. Pruchnic et al.[17] noted increases in intramyocellular lipids assessed by muscle biopsies and increased oxidative capacity after a 12 week aerobic exercise program in older adults. Goodpaster et al.[39] randomized a group of older males and females to regular physical activity which included a combination of aerobic, strength, flexibility, and balance exercises. After 12 months, IMAT measured by CT did not change significantly in the intervention group, yet increased by 18% in control subjects[39]. This suggests the possibility that

physical activity may moderate risk of type-2 diabetes in part by countering age-related increases in IMAT.

Conclusions

This study is the first to assess and establish repeatability for IMAT and muscle density of the foreleg and to compare pQCT measurements to total body measurements by DXA in adults. This cross-sectional analysis was strengthened by a subject sample of Caucasian women with varying BMI, body fat distribution, and physical activity levels. Measurement of IMAT by pQCT does not correlate with variations in biomarkers of risk for type-2 diabetes and atherosclerosis in apparently healthy women. However, muscle density had low CV values and was negatively related to markers of health and metabolic disease risk. Other researchers noted variation with IMAT across gender and race[24,35,40,41]; therefore research must be done with homogenous sub-populations. The pQCT device is smaller than a MRI or CT machine with less radiation dose and could be used as a prognostic marker to assess disease risk or in training studies to determine changes in IMAT or muscle density associated with exercise adaptations. More research is necessary to understand the biology of IMAT[3] and to help determine whether IMAT can be targeted through exercise training to help with the prevention and treatment of obesity or type-2 diabetes.

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Tables

Table 1. Subject characteristics (n=82).

	Mean ± SD	Range
Age (yrs)	38.6 ± 4.7	29-46
BMI (kg/m^2)	24.5 ± 5.1	18.3-46.6
Total body fat (%)	29.2 ± 7.8	10.6-51.6
Central body fat (%)	25.0 ± 10.0	8.9-48.7
Fasting blood glucose (mmol/L)	86.5 ± 7.5	72-108
HDL-cholesterol (mg/dL)	58.8 ± 16.0	32-100
Physical activity (MET·min/day)	534 ± 272	99-1702

Table 2. Relationships between total body DXA measurements and foreleg pQCT measurements (n=82).

pQCT Foreleg Measures	DXA Measures			
	Total body fat (%)	**Central body fat** (%)	**Fat mass** (g)	**Lean body mass** (g)
Total Foreleg Cross-Sectional Area (mm^2)	0.67†	0.61†	0.77†	0.53†
Subcutaneous Fat (%)	0.83†	0.67†	0.76†	0.10
Muscle Area (%)	-0.81†	-0.65†	-0.75†	-0.09
IMAT (%)	-0.34†	-0.27*	-0.29†	-0.16
Muscle Density (mg/cm^3)	-0.53†	-0.48†	-0.55†	-0.34†

Percentage of subcutaneous fat, muscle area, and IMAT refer to the percentage based on the total cross-sectional area of the foreleg. †$p<0.01$,*$p<0.05$

Table 3. Correlations between foreleg muscle density, HOMA-IR and related health indices (n=82).

	Foreleg Muscle Density (mg/cm^3)	**HOMA-IR**
Anthropometrics and Physical Activity		
BMI (kg/m^2)	-0.45†	0.68†
Physical activity (MET·min/day)	0.29*	-0.44†
DXA Body Composition Measures		
Total body fat (%)	-0.53†	0.59†
Central body fat (%)	-0.48†	0.56†
Fat mass (g)	-0.55†	0.60†
Fat free soft tissue mass (g)	-0.34†	0.22
Blood Measures		
HOMA-IR	-0.31†	N/A
Insulin (U/ml)	-0.33†	0.99†
Glucose (mmol/l)	0.13	0.03
LDL-C (mg/dl)	-0.11	0.32†
HDL-C (mg/dl)	0.34†	-0.42†

N/A, not applicable, †$p<0.01$,*$p<0.05$

Table 4. Multiple regression analysis to determine predictors of HOMA-IR.

Independent Variable	β	p
Foreleg muscle density (mg/cm^3)	0.08	0.46
Physical activity (MET·min/day)	-0.18	0.16
Total body fat (%)	-0.11	0.54
HDL-C (mg/dl)	-0.18	0.11
Foreleg subcutaneous fat (%)	0.65	<0.001†

$R^2 = 0.50$, $p<0.01$†

Table 5. Multiple regression analysis to determine predictors of foreleg muscle density.

Independent Variable	β	p
HOMA-IR	0.11	0.46
Physical activity (MET·min/day)	-0.15	0.29
Total body fat (%)	-0.38	0.07
HDL-C (mg/dl)	0.29	0.03*
Foreleg subcutaneous fat (%)	-0.17	0.37

R^2=0.33, $p<0.05$

Figures

Figure 1. Relationship with foreleg muscle density and physical activity (n=78).

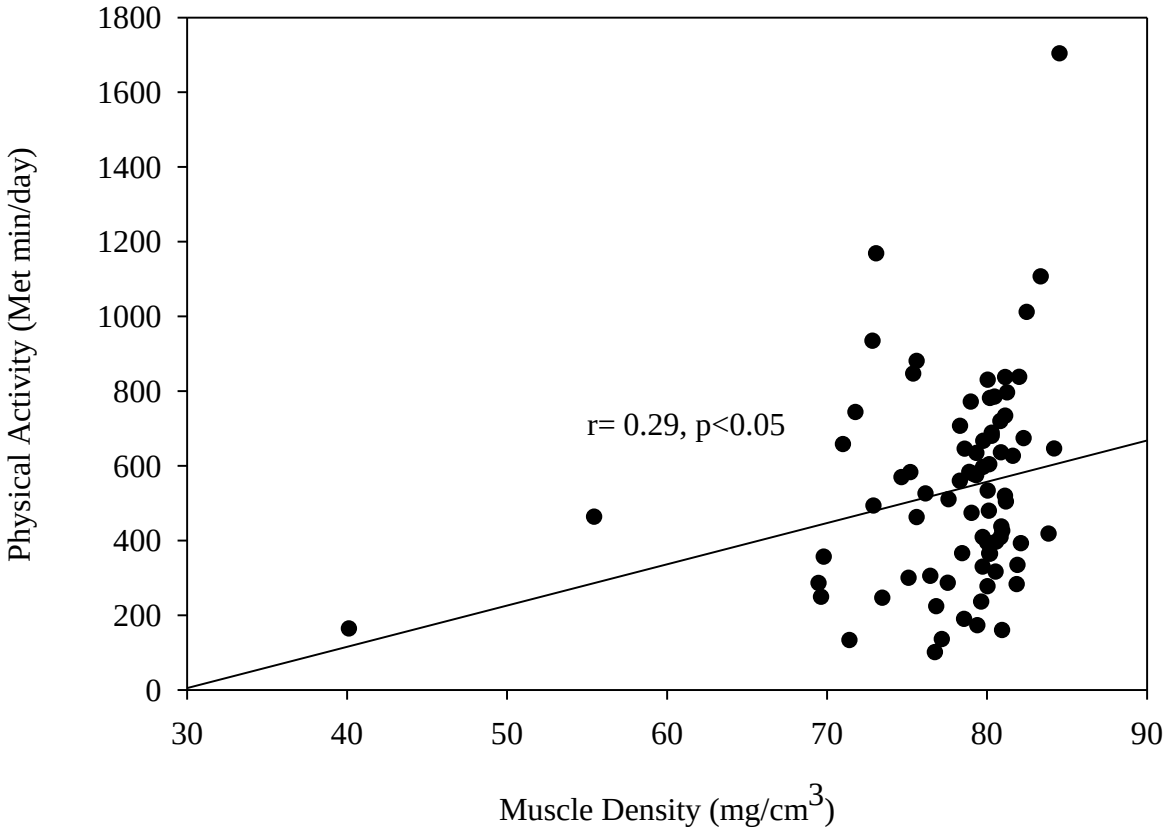
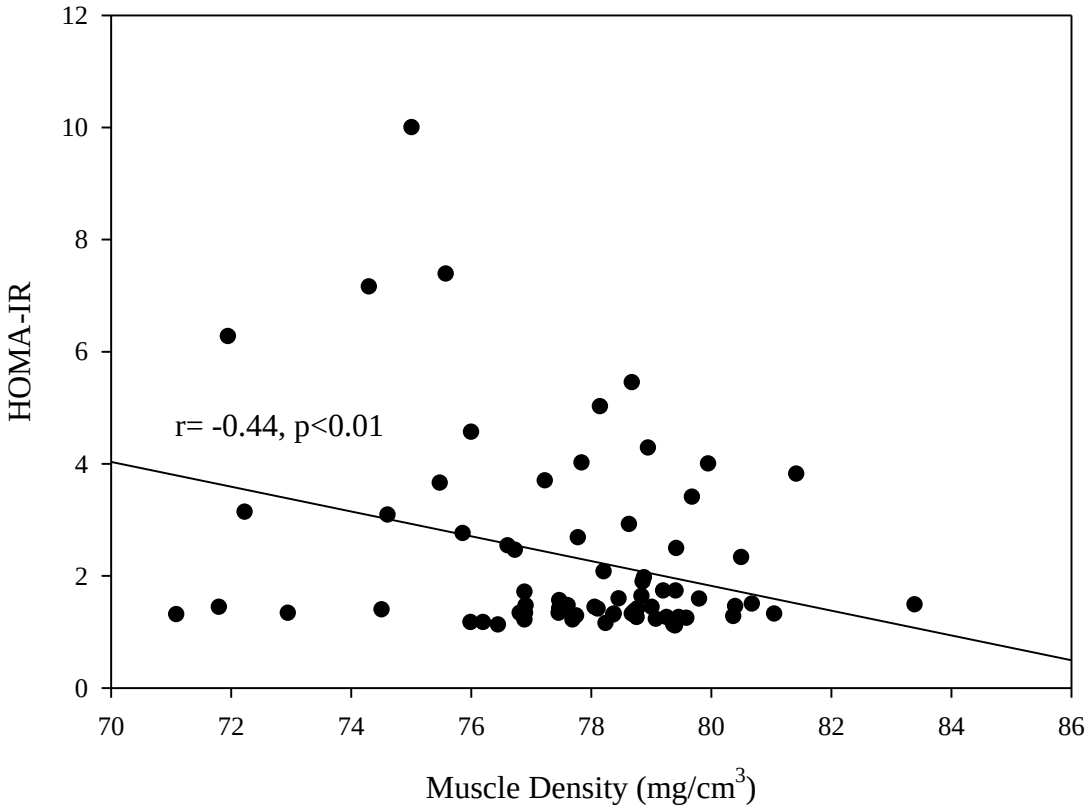


Figure 2. Relationship between foreleg muscle density and HOMA-IR (n=73).



Chapter 5

Overall summary and conclusions: applications and recommendations for future clinical research

Summary and Conclusions

An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D, and Calcium

This research showed bariatric surgery leads to significant reductions in body weight, fat mass, and fat free soft tissue mass at both three and six month periods following surgery. These changes were more pronounced after Roux-en-Y gastric bypass (RYGB) compared to laparoscopic adjustable gastric banding (LABG) surgery. Weight loss ranged from 17-25 kg three months after RYGB and 7-14 kg after LAGB. Six months after surgery, weight loss was 21-35 kg after RYGB and 9-18 kg after LAGB, which was lower than other research documenting weight loss of up to 30-50% of body weight six months following surgery[1]. Many patients experienced some reductions in bone mineral density (BMD) and content (BMC) following bariatric surgery. There were significant reductions in hip BMD from baseline measures assessed at six months; these changes were more apparent after RYGB procedures. Due to high variability between patients, the changes at six months in BMD and BMC for total body, lumbar spine, and radius were not significantly different than baseline measurements. Three months following surgery, hip BMD changes were significantly related to weight loss ($r=0.68$, $p<0.05$). At the six month follow-up, hip BMD was correlated ($p<0.05$) to decreased leptin ($r=0.88$) and increased adiponectin ($r=-0.82$). These adipokines were related to measurements of adiposity after surgery. Osteocalcin (OC) and carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx) significantly ($p<0.05$) increased in all patients three

months after surgery which confirmed the occurrence of bone turnover. Leptin was inversely correlated to CTx, $r=-0.85$, $p<0.05$; this relationship has been observed by other researchers and may be related to a decrease in leptin resistance associated with rapid weight loss following surgery[2]. Vitamin D was clinically low in four patients prior to surgery and had decreased in six patients six months after surgery. Decreased vitamin D at three months was correlated with the change in lumbar spine BMD ($r=0.81$, $p<0.01$) and BMC ($r=0.79$, $p<0.05$), but was not related to other DXA bone measures or other blood biomarkers. Small decreases in calcium and increased parathyroid hormone were observed, but were not significant and remained within clinically normal ranges.

Despite some bone loss following both surgeries and increased bone turnover markers, overall hip BMD measures remained within the normal NHANES III reference range[3]. Changes in bone within this small subset may not be clinically significant and it appears osteoporosis risk did not increase significantly for the patients evaluated in this study. Additional research is necessary with a larger subset of patients and with longer follow-up time periods to better compare changes in bone density and related biomarkers after RYGB and LAGB and to evaluate implications for late-life osteoporosis risk.

Fat and Muscle Indices Assessed by pQCT: Relationships with Physical Activity and Type-2 Diabetes Risk

This is the first research to use the rigorous standards prescribed by the International Society for Clinical Densitometry (ISCD)[4] to determine repeatability of both intermuscular adipose tissue (IMAT) and muscle density of the foreleg measured by peripheral Quantitative Computed Tomography (pQCT). The pQCT device reliably measured muscle density in the foreleg; coefficient of variation (CV) was 0.8%. Precision was lower for IMAT of the foreleg with a CV of 15.1%. Muscle density has been validated as an indicator of adipose tissue

deposition in the muscle[5,6] was therefore used as a surrogate for IMAT. Fat and muscle distribution of the foreleg measured by pQCT were highly correlated to total body adiposity assessed by dual energy X-ray absorptiometry (DXA), similar to research in other populations[7]. Previous literature has shown that IMAT is strongly correlated to total adiposity in both men and women[8,9] and appears to be greater in subjects with type-2 diabetes than in controls[10]. In this group of apparently healthy women not at risk for type-2 diabetes, IMAT was not significantly related to markers of health risk. Muscle density was related to several parameters of health status; positive relationships were noted with physical activity, while inverse correlations were observed with markers of fat distribution and risk of type-2 diabetes. Using multiple linear regression, 50% percent of the variance in HOMA-IR was explained by foreleg muscle density and the percentage of foreleg subcutaneous fat, physical activity, HDL-cholesterol (HDL-C), and total body fat percentage, $p<0.001$. The same independent variables along with HOMA-IR explained 33% of the variance in foreleg muscle density, $p<0.001$. After stepwise multiple linear regression, foreleg subcutaneous fat and HDL-C remained significant predictors of HOMA-IR ($R^2=0.48$, $p<0.001$), while total body fat percentage was a significant negative determinant of foreleg muscle density ($R^2=0.27$, $p<0.001$).

Physical activity may present a confounding variable to the relationship of IMAT and type-2 diabetes risk, but data are inconsistent. Some researchers have shown increased IMAT in trained subjects, possibly utilized as a fuel source during exercise[11], while other researchers observed a negative relationship with exercise capacity and IMAT[9]. In this population, IMAT did not differ between high and low physically active subjects and foreleg muscle density tended ($p=0.054$) to be greater in highly active subjects. Overall, physical activity level was positively

correlated ($p<0.05$) with muscle density in the foreleg ($r=0.29$)) and was negatively correlated with HOMA-IR ($r=-0.44$, $p<0.01$).

This research establishes the repeatability of foreleg muscle density measured with pQCT and confirms the use of this measure as a surrogate for IMAT to evaluate relationships with health status. More research necessary to understand the biology of IMAT[12] and to help determine whether IMAT or muscle density can be targeted through exercise training to help with the prevention and treatment of obesity or type-2 diabetes.

Practical and Clinical Applications

An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D, and Calcium

Osteoporosis has become a significant health risk, as an estimated 10 million Americans over the age of 50 have the disease, with another 34 million at risk[13]. The 2004 Surgeon General's Report[13] projected that by 2020, one in two adults over age 50 will be at risk for a fracture from osteoporosis or low bone mass. Increased skeletal loading associated with excess body weight may protect against osteoporosis, therefore osteoporosis is often overlooked as a potential health risk in overweight or obese adults. Yet evidence on the relation between weight loss and bone loss in the overweight and obese population indicates that loss of as little as 10% of body weight can result in 1-2% loss of bone[14]. It is estimated more than 220,000 bariatric surgeries will be performed in 2010 alone[15]. Bariatric surgery may dramatically increase one's risk for osteoporosis, not only through rapid weight loss, but also through changes in absorption of nutrients that could detrimentally affect calcium metabolism and thus the capacity to maintain bone mass. Currently, DXA scans are not part of the routine care of bariatric surgery patients. Williams et al.[14] recommends a pre-operative DXA scan for bariatric patients to assess primary and secondary risk of bone disease, with a DXA scan repeated every 1-2 years, as indicated.

Additional research of bone changes in bariatric surgery patients can provide evidence supporting the use of DXA to monitor patient's bone status post-bariatric surgery and perhaps help guide physicians in selecting an appropriate surgical procedure based on patient's current bone health.

Fat and Muscle Indices Assessed by pQCT: Relationships with Physical Activity and Type-2 Diabetes Risk

With rising rates of obesity, the prevalence of type-2 diabetes continues to increase and is expected to affect over 300 million individuals by 2025, more than double the rate of 135 million noted in 1995[16]. Recent research suggests the fat accumulated in muscle (IMAT) appears to have an association with insulin resistance and therefore potentially contribute to the pathophysiology of type-2 diabetes[17]. IMAT may also have an association with inflammatory markers[18]. Other research suggests IMAT may be a physiological means for fuel storage in active people[11]. Magnetic resonance imaging (MRI) and computed tomography (CT) analysis has shown associations between higher concentrations of IMAT and insulin resistance, type-2 diabetes, and reduced muscular strength[17,19-21], yet little research has been done using pQCT to assess these patterns and potential health significance. Data derived from the pQCT on this issue are scarce; however the pQCT device is a smaller instrument with less radiation exposure than CT, less costly and may be more practical to use in a research setting. This research suggests measurement of IMAT or muscle density using the pQCT device could be used as a prognostic marker to assess disease risk or in training studies to evaluate changes in IMAT or muscle density associated with exercise adaptations.

Recommendations for Future Research

An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D, and Calcium

The purpose of this dissertation study was to examine potential trends and evaluate patterns for further analysis within a larger sample size. The study protocol should continue to be followed rigorously with an emphasis on communication and coordination with the bariatric surgical team to ensure routine clinical bariatric surgery standard procedures are being completed for all patients. It is anticipated that continuation of the current protocol to increase sample size will allow for additional statistical analysis to better evaluate the changes in bone density and related biomarkers in the context of rapid weight loss after bariatric surgery.

Bariatric surgery is now an option for adolescents and young adults[22]; therefore the long term significance of bone loss is important since many women may not be aware of bone loss until much later in life. Bariatric surgery is becoming an increasingly common procedure, yet this research is only the second[23] to compare changes in bone density following both RYGB and LAGB procedures in relation to biomarkers of bone turnover, adipokines, and blood measures related to bone and nutrient status (vitamin D, calcium, parathyroid hormone). Deficiencies of both macro and micro nutrients are commonly reported after bariatric surgery[24] and supplementation may not prevent deficiencies[25,26]. Additional clinical trials are necessary to provide recommendations on the type and dosage of vitamin supplements to help minimize risk of bone loss and nutrient deficiencies. It is critical to continue well-controlled research studies to evaluate bone changes in the context of bone biomarkers, adipokines, and nutrient related blood measures following various forms of bariatric surgery. This research can help provide more detailed guidelines for the bariatric surgery team to identify patients at potential risk for nutrient deficiencies and to avoid potential bone loss later in life.

Fat and Muscle Indices Assessed by pQCT: Relationships with Physical Activity and Type-2 Diabetes Risk

This research is the first to measure IMAT and muscle density using the pQCT in relation to physical activity status. Additional research is warranted to better understand relationships between IMAT, insulin resistance, and physical activity in other populations, including those at risk for type-2 diabetes. Other researchers noted variation with IMAT across gender and race[18,27-29]; therefore research must be done with homogenous sub-populations. Physical activity presents a moderating risk factor to the relationship of IMAT and insulin resistance, as exercise trained subjects exhibit greater IMAT[30], or similar levels to those with type-2 diabetes[11]. Better controlled clinical trials are necessary to evaluate exercise training effects on IMAT. Intermuscular adipose tissue appears to have depot sizes similar to visceral adipose tissue[28], suggesting accumulations may confer similar negative metabolic consequences. While inflammatory markers may show greater associations with specific fat depots than overall fat mass[31], prior research to evaluate the role(s) of circulating inflammatory markers in the context of IMAT has been limited and the findings inconsistent[18,21,32]. Additional research with IMAT and inflammatory markers can provide greater understanding into the development of metabolic disease and also aid in defining physical activity interventions which may help to prevent or treat type-2 diabetes.

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Appendix A: Detailed Methodology Carilion Bariatric Surgery Study

- **Recruitment**
- **DXA and pQCT bone scan procedures**
- **DXA and pQCT scan analysis**
- **Diet and physical activity records**
- **Blood draw and processing**

Recruitment

Subjects were recruited from the bariatric surgical population at Carilion Health System. No private records were used by the researchers as a means of identifying candidates. Open recruitment enabled bariatric surgical physicians to refer their patients, who may qualify. Study personnel assessed eligibility for the study, based on pre-determined inclusion and exclusion criteria.

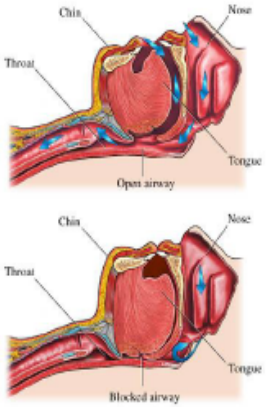
Brochure

Brochures were distributed by bariatric personnel and physicians and at monthly Bariatric Surgery interest group meetings. An example brochure is included below:

Preliminary Studies of Bariatric Surgery Patients:

Researchers at Carilion and Virginia Tech want to look at two different problems that happen to patients having bariatric surgery.

One study involves patients who suffer from OSAS, a nighttime breathing disorder. OSAS disrupts sleep patterns, causes excessive daytime sleepiness and can cause complications related to surgery.



- Undiagnosed OSAS increases risks of cardiovascular complications during and after surgery.

OSAS Research

- 70% of bariatric surgery patients may have OSAS. Early identification and treatment should reduce surgical risks.

- OSAS is not easily diagnosed. Testing blood for a special protein may help to identify patients who are at risk for OSAS.

- Treatment is nightly use of an at home sleep device. This may eliminate symptoms and complications from surgery.

- We hope to be able to find a connection between certain protein markers in the blood with the presence or absence of OSAS in bariatric surgery patients.

BONE Research

- The second study looks at why bariatric surgery may result in loss of bone density. This may cause risk of fracture, especially in women.

- For women under the age of 30, preventing post-operative bone loss may be even more critical, as they have not reached peak bone mass.

- We will use two different bone scanning techniques to evaluate bone density and bone quality related to body mass and activity.

- We hope to evaluate the impact of rapid weight loss on bone health following bariatric surgery.

Study Eligibility:

You may qualify to take part in one or both parts of the study.

You will be given questionnaires to determine your sleep habits, your health history, as well as your ability to perform certain tasks. Also, measurements of your neck, waist, and hip will be taken.

Researchers will use this information to determine what parts of the study best suit you.

If you are interested
or
For more information
please contact:

Study Coordinator:
Laura Newsome, MS
231B War Memorial Hall, Virginia Tech,
Blacksburg, VA, 24061
Phone: (540) 231-6473
E-mail: lnewsome@vt.edu

CARILION CLINIC

Virginia Tech
Invent the Future

Research Study

Bone Health and Gene Expression of Obstructive Sleep Apnea Syndrome (OSAS)

Preliminary study to isolate gene markers for OSAS in bariatric surgery patients and to assess bone health before and after bariatric surgery

Principal Investigator:
T.A. Lucktong, MD

Co-Principal Investigator:
William G. Herbert, PhD

Co-Principal Investigator:
Thomas W. Chittenden, MSc, PhD

Study Coordinators:
Laura Newsome

DXA and pQCT Bone Scan Procedures

Subject Arrival

Subject arrives at 229 Wallace Hall at scheduled time.

Researchers fill out parking pass and instruct subject to write in license plate number on pass and place in car.

Informed Consent

Researchers sit down with subject and explain informed consent with subject. Give time to read, review and ask questions before subject signs form. Remind subject of components of study: bone scans, food and physical activity record and blood draw prior to surgery and then to be repeated at three and six months after surgery. Make two copies of the signed consent – one for the subject, one for us and the original will be taken to Carilion.

Pregnancy Test

Send subject to rest room for urine sample for pregnancy test.

Complete pregnancy test. If test is negative, continue with protocol. Check off on Screening and Measurement Data Sheet that pregnancy test has been completed.

DXA/pQCT Measurement Data Sheet

Ask subject which surgical procedure she will be having.

If Roux-en Y, subject code begins with BR followed by the number previously assigned. If subject is having laparoscopic adjustable gastric banding, subject code begins with BL followed by the number previously assigned.

Fill in subject code, date, and check off which visit this is for subject (baseline, 3 mo, 6 mo) in the Appendix E: DXA AND pQCT MEASUREMENT DATA SHEET.

Measurements: body weight, height, radius and tibia length

Body Weight

Instruct the subject to remove shoes, any extra jackets or layers and anything from her pockets. Ask subject to step onto the scale in room 229A. Record her weight to the nearest kilogram and to the nearest pound. Subjects exceeding 136 kilograms of body weight will be excluded from the study.

Ask subject to carefully step off of scale.

Height

At the wall-mounted stadiometer, ask subject to stand with back against the wall (without shoes) facing toward you. Position arm so it meets the top of the subject's head. Record her height to the nearest centimeter and to nearest inches.

Take subject's body weight (measure in kg and lbs) and height (measure in cm and inches) and record on Appendix E: DXA AND pQCT MEASUREMENT DATA SHEET.

Radius Length

If subject is wearing a long sleeve shirt, instruct subject to pull up sleeve arm on non-dominant arm. Have the subject bend the elbow at a 90 degree angle and raise their forearm in front of their chest parallel to the floor. Measure from the highest point of the radial head to the highest point of the styloid process of the radius, and record the length to the nearest millimeter using a measuring tape and record on the DXA/pQCT Measurement Data Sheet.

Tibia Length

If subject is wearing pants, ask the subject to pull up his/her pant leg so that the lower leg is exposed. Instruct the subject to sit in a chair and cross their non-dominant leg over the other leg so that the ankle of the non-dominant leg rests upon the top of the knee of the other leg. Measure from the highest point of the medial condyle of the tibia to the highest point of the medial malleolus, and record the length to the nearest millimeter using a measuring tape and record on the DXA/pQCT Measurement Data Sheet.

DXA Scans

Before beginning the bone scans, ask subject if they are wearing any items that contain metal, buttons, snaps, zippers, or hard plastic (including underwire bras). If she is, offer clothing to change into before the DXA scans.

The subjects' identifying information is entered into the DXA computer. The subject is led to the DXA table and given brief information about the four scans to be performed. The following scans will be conducted:

a. whole body
b. lumbar spine
c. non-dominant hip
d. non-dominant forearm.

After the subject is finished both scans, on technician completes analysis on the four scans completed.

DXA Scan Analysis

Four separate scans were performed and then analyzed using the DXA: whole body, lumbar spine, non-dominant hip and non-dominant forearm according to the instructions in the QDR 4500 Instruction Manual. Central body fat analysis was measured between the first and fourth lumbar intervertebral disks by adjusting the lines of the rib box as outlined in Svendsen et al. Measurement of abdominal and intra-abdominal fat in postmenopausal women by dual energy X-ray absorptiometry and anthropometry: comparison with computerized tomography. (*Int J Obes Relat Metab Disord* 1993;17(1):45-51).

After analyses, the following output scans were printed and placed in the subject data file, with the listed data being entered into the database:

a. Central abdominal fat analysis (screen 2 / 1 page)
    i. Trunk % body fat
b. whole body (screens 1 & 3 / 1 page)
    i. Total BMC
    ii. Total BMD
    iii. Total % body fat
    iv. Total fat mass
    v. Total fat free soft tissue mass
c. lumbar spine (screens 1 & 2 / 1 page)
    i. L1-L4 BMD
    ii. L1-L4 BMC
    iii. T-score
d. total hip (screen 1, plot screen 1 / 1 page)
    i. Total BMC
    ii. Total BMD
    iii. Total T-score
e. radius (radius screen 1, plot radius screen 1 / 1 page)
    i. Radius BMC
    ii. Radius BMD
    iii. Total T-score

pQCT Scans

The subjects' identifying information is entered into the pQCT computer. The subject is led to the pQCT and given brief information about the scans to be performed. The TIBIA4S and RADIUS2S scans will be performed.

The TIBIA4S will be completed first on the subject. Set up the pQCT with the leg stabilizer and foot stirrup.
Ask the subject to sit in the pQCT chair, and adjust the seat for the appropriate non-dominant limb. Place the bottom of the foot of the non-dominant limb in the stirrup through the gantry of the device with the stabilizing arm placed just above the knee so that the knee is slightly bent. The leg should be very nearly parallel to the ground and in the center of the gantry. Subject is instructed to remain still and keep lower limb steady in the device.
Conduct the scan.

pQCT Scan Analysis

Scans were analyzed using the TIBIA4S Macro according to the instructions in the Stratec XCT 3000 Instruction Manual.

After analyses, the following output scans will be printed and placed in the subject data file, with the listed data being entered into the database:

a. TIBIA4S Macro (2 screens)
    i. 66% Total area
    ii. 66% Subcutaneous Fat
    iii. 66% Muscle Area

iv. 66% Muscle Density

IMAT Analysis

The IMAT was calculated using the scan located at 66% of the non-dominant tibia and the CALCBD analysis using the Stratec software. Two separate analyses were done using the Stratec software and then subtracted from one another to determine IMAT. The first scan used the following parameters: threshold 41 mg/ccm, trabecular area 100%, contour mode 3 (iterative contour search), peel mode 1 (concentric peel), and filters F03F05F05 (muscle smooth 3) to determine total area at the 66% location. The analysis was run a second time with an additional filter (F07) which excluded fat. These two total areas were subtracted to calculate IMAT

Diet and Physical Activity Record

Diet Record Instructions

Record subject code at the top of the Food and Physical Activity Record and record visit (baseline, 3 mo, 6 mo). Give subject Food and Activity Record and thoroughly explain top instruction sheet, noting contact information at the top of the page if any questions arise later. The food record contains the time, location where food was consumed, detailed description of food including the method of preparation, amounts and special notes. During the session, subjects will be given instructions on how to measure portion sizes and instructed to give details about the food consumed including the brand name of the food if applicable. Subjects will be shown a sample food record and how to complete the diet record. Subjects will be instructed to keep the food record with them throughout the day and to record each meal after it is eaten, rather than waiting until the evening to record all food consumed for the day.

Instruct subject to record all food and beverages consumed over a four day period. The Food and Activity Record needs to be completed over 4 days, 3 weekdays and 1 weekend day. Subjects are asked to complete the record from Wednesday-Saturday or Sunday-Wednesday.

It is important that subject's keep the record with them throughout the day so they can make a recording after each meal/snack, rather than relying on memory to record their meals at the end of the day.

Subjects will include detailed record of each food item consumed, including the time of day, the location, the food, and the portion size. Ask subjects to include recipes and any less common ingredient labels from foods/drinks they have consumed.

Arrange a time for subject to return Food and Activity Record. If subject will not be bringing record to next visit, give subject an addressed, stamped envelope to record the record to the researchers. Inform subjects they will be notified upon receipt of the Food and Activity Record.

Pedometer Instructions

During the same 4 day period of maintaining a diet and physical activity log, subjects wore wear a pedometer for all waking hours for 4 continuous days. The pedometer will be attached at the hip on the right side of the body. The pedometer will be removed each evening for sleep. After removing the pedometer, subjects will open the pedometer face and record the number of steps taken that day. After recording, they will hit the reset button and wait for the pedometer to zero.

Subjects will then attach the pedometer to their right hip and go about their daily activities. Subjects will remove the pedometer for running, swimming or any activity more strenuous that walking and then record the activity including the duration and the intensity at the top of each day's diet/physical activity record. Researcher will explain to subject how to rate their intensity level using a BORG scale (6-20). A level 6 is comparable to sitting down watching TV, while a level 20 would refer to extremely strenuous activity. Subjects will also note whether the pedometer was removed for any activities. Additionally, subjects will also remove the pedometer when bathing or showering, as water will damage the instrument. Remind subjects they will be using this pedometer again at the three month and six month follow up and that they will be allowed to keep the pedometer following completion of this study.

The following pages include the instructions for the subjects and a copy of the dietary and physical activity record.

Instructions for Completing the 4-Day Diet and Activity Record

It is extremely important that you take this part of the study seriously. We need for you to be as complete and specific as possible. If you have any questions while completing this, please email me at **kbutner@vt.edu.**

1. Please write down **everything** you eat and drink (that has calories) as soon as possible after you consume it. Include **anything** you eat including hard candy, gum, etc. There is no need to include water or drinks without calories, but if you put cream and sugar in your coffee, please make sure to list those.
2. Please fill out each column for each food item: time, place, food description, portion size.
3. Please be especially detailed on the food description. We need this to be able to enter your food intake correctly into the computer. If you are eating at a restaurant/take out, please list the restaurant name and portion size; for most restaurants we can look up the nutrition information online.

Use your best judgment, and here are some tips:

   a. Try to use amounts such as cups, tablespoons, ounces, etc. if possible, but just describe the amount if you are not sure
      i. 1 cup is about the size of a baseball
      ii. ½ cup is about the size of a tennis ball (usually 1 scoop of side dish at a cafeteria)
      iii. 1 teaspoon is about the size of the end of your thumb – 3 teaspoons is 1 tablespoon
      iv. ¼ cup is about the size of a golf ball
      v. 3 ounces of meat is about the size of a deck of cards
   b. Please include drinks with calories, the SIZE and tell us whether it was regular or diet, sweet or unsweetened, and anything you added (i.e. cream, sugar, etc.)
   c. For any foods that you can, please tell us the **BRAND NAME and PRODUCT NAME** and whether it was fat free, low fat, etc.

4. **Please don't forget to write down your physical activity** for the day on the front of the diet/activity record. Please make sure to note the type of activity, the total time spent, the intensity and whether or not you removed your pedometer.

5. **Please rate the intensity of your activity on a BORG scale 6-20.**

BORG Rating of Perceived Exertion (RPE) Scale

6	No exertion at all	14	
7	Very, very light	15	Hard
8		16	
9	Very light	17	Very hard
10		18	
11	Fairly light	19	Very, very hard
12		20	Maximal exertion
13	Somewhat hard		

Subject ID: _____ Diet Record: Baseline 3 mo 6 mo **Pedometer Step Count: _____**

Date: _____ Day of the week: _____ Day (circle): 1 2 3 4

Physical Activity Record

Please list any physical activity you did today. Please remove your pedometer for any activity more strenuous than walking or any water activity. Please rate your intensity on a 6-20 scale (see instruction page for further details).

Type of Activity	**Time**	**Intensity** (6-20 from mild-heavy)	**Pedometer on/off**
Example: Elliptical Machine, level 6 hill interval	*20 minutes*	*10*	*Off*

Food Intake Record

Remember: please do not alter your normal diet while keeping this record. Keep the record for 4 consecutive days. Use additional pages for each day if necessary. For foods eaten out, indicate where foods were purchased. For mixed foods, include recipe on a separate page.

Time	**Place** (Restaurant, home, etc.)	**Food Description** (Please specify, if known: brand names, cooking method, type of product, and include labels when possible)	**Portion Size** (How much? Serving size)	**Additional Notes**
7:00 am	*Home*	*Cheerios and skim milk*	*1 cup Cheerios* *½ cup skim milk*	

Time	**Place** (Restaurant, home, etc.)	**Food Description** (Please specify, if known: brand names, cooking method, type of product, and include labels when possible)	**Portion Size** (How much? Serving size)	**Additional Notes**

At the end of the visit

Thank subject for their time and participation in study.

Inform subject they will receive the results of their measurements after the completion of the study.

If bone scans are completed before blood draw appointment: Let subject know the researchers will be there at the blood draw visit to take additional vials of blood and that the subject will need to be fasting prior to this visit. Ask subject whether they would like a phone or email reminder to be fasted.

If bone scans are completed after the blood draw appointment: Let the subject know a researcher will be in contact with them three months after surgery to arrange their second visit.

Blood Draw and Processing

Researchers will contact subjects with the following instructions the day before they are scheduled for their blood draw.

1. Remember that the fast is for at least 8 hours, but no more than 16 hours prior to your scheduled appointment.
2. During the fasting period, drink only water. You may take any prescribed medications, as long as they do not require that you take them with food.
3. Contact your physician with any medical concerns regarding fasting.
4. Avoid excessive exercise prior to your blood draw appointment.

Blood is drawn as it will be for current clinic patients and drawn by same certified personnel who currently does blood draws for these patients, e.g. Carilion Labs, Inc. Subjects will have 75mL drawn and analyzed for clinical purposes. Carilion Labs Inc. is provided with the following instructions and one of the researchers will be present during the blood draws and will complete the blood processing.

Blood Collection

1. Pull 4 SST – (red/grey speckled stopper), 3 EDTA – (lavender stopper), tube for blood collection.
2. All tubes will be labeled with subjects ID #
3. Take blood in the standard protocol sequence adding the additional tubes for research. (SST, EDTA)
4. Inoculate each tube 5 times before putting in the rack.
5. Label date and time of draw on the tubes.
6. Place EDTA and SST tubes in holding rack inside biohazard bag and into the cooler.

Blood Processing and Storage

After blood is drawn by Carilion Lab personnel, the tubes containing the research samples will be labeled with subject numbers and then immediately transported to the Carilion Community Research Lab by individuals authorized by Carilion Clinic to do such. Upon arrival at the lab the

blood samples will be processed to separate plasma and serum and stored at -80°C for later cytokine analyses. Serum will be used for analysis of the following biochemical markers: osteocalcin, carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx), adiponectin, and leptin. Serum vitamin D [25(OH)D_3], calcium and parathyroid hormone (PTH) will be measured by Carilion Laboratories in accordance with the standard bariatric surgery clinical pathway.

Important Pre-procedure Notes:

*All procedures using blood should be conducted in the Biosafety hood and blood should be treated as infectious (i.e., standard precautions in effect).

*All tubes should be sprayed with ethanol prior to entry into the hood. Tubes should be opened only inside the hood using multiple, folded kimwipes to absorb any blood from the top of the tube as the stopper is removed as this is a splatter hazard.

*Ensure LABELS have three identifiers: 1) patient ID code, 2) date, and 3) visit designation)

*Consider the volumes you will need for additional testing when aliquotting serum and plasma to cryovials. Base volumes per tube on amounts required for these later assays; a maximum of 3 freeze/thaw cycles is generally acceptable for general downstream applications such as ELISAs.

* If processing must be delayed once blood is brought to the lab, place tubes on tube roller (back bench) at RT for up to 2 hours.

*Use the pipet-aid on SLOW or MED speed for processing until you are comfortable with the control.

*Note: This procedure is to be done *individually* for each study participant although multiple samples may be processed simultaneously. Special care must be taken to record specimen ID, date and visit designation on each vessel used. Work carefully and label well to ensure that samples from two patients are not mixed at any point during the procedure.

Procedure for SST (tiger top tube)*:* [see "Tech Talk" Vol. 4, No. 2; Nov 2005 for additional info]

*Note: this tube should be inverted ~5 times immediately after drawn before being placed upright to clot!

1. Allow the tube to clot for up to 30 minutes undisturbed, vertically at room temperature (RT) (in a rack on the bench).
2. Once clot has formed, spin tubes for 10 minutes at RT at 1200 x g in the (tissue culture) Legend RT+ benchtop centrifuge. [Program #1 on Legend cfg but ADD 5 more minutes]
3. Label appropriate number of cryovials with the specimen/patient code ID and other identifiers and the letter "S" for serum. Repeat labeling for each unique patient ID. (note volume of serum on the vial where warranted for convenience when thawing for subsequent use).
4. Carefully remove the tubes from the centrifuge, so as not to disturb the gel barrier, and place them in the hood.

5. Aliquot 500-1000 μl of serum (top layer above gel) into pre-labled cryovials (from step3) using 1 ml micropipettor and sterile tips.
6. Place the serum vials in the designated box in the -80°C freezer. Record the location of the vials on a box map, with complete identifiers, so that they may be entered into Cryotrack software by the lab staff.

Procedure for K_2EDTA (purple top tube):

*Note: Tubes should be inverted ~8-10 times immediately after drawn before being transported!

1. Balance tubes (opposite buckets) and spin tubes for 10 minutes at RT at 1200 x g in the (tissue culture) Legend RT+ benchtop centrifuge. [Program #1 on Legend cfg but ADD 5 more minutes]
2. Label appropriate number of cryovials with the specimen/patient code ID and other identifiers and "EDTA-P" for plasma. Repeat labeling for <u>each</u> unique patient ID. (note volume of plasma on the vial where warranted for convenience when thawing for subsequent use).
3. Carefully remove the tubes from the centrifuge, so as not to disturb the gel barrier, and place them in the hood.
4. A. If more than one EDTA tube has been drawn per patient, pool the plasma (yellow-ish, top layer above gel in tubes) from all tubes into a sterile 15 ml (or 50 ml) concial tube and mix gently, by pipetting up and down, to ensure homogeneity of plasma before aliquotting to freeze.

   B. Aliquot 500-1000 μl of EDTA plasma (top layer above gel) into pre-labled cryovials (from step2) using 1 ml micropipettor and sterile tips. [As long as the tip remains sterile, you may use the same tip for all aliquots of one patient. Change tips if it touches anything besides the plasma/tubes OR when you begin a new patient sample.]
5. Place the serum vials in the designated box in the -80°C freezer. Record the location of the vials on a box map, with complete identifiers, so that they may be entered into Cryotrack software by the lab staff.

Appendix B: Detailed Methodology Virginia Tech Bone Study

- **Recruitment**
- **DXA and pQCT bone scan procedures**
- **DXA and pQCT scan analysis**
- **Diet and physical activity records**
- **Blood draw and processing**

Recruitment

Subjects were recruited from the resident and student population at Virginia Tech and in the communities surrounding the campus and through flyers posted at a local gym. No private records were used by the researchers as a means of identifying candidates. Study personnel assessed eligibility for the study, based on pre-determined inclusion and exclusion criteria.

DXA and pQCT Bone Scan Procedures

DXA and pQCT bone scan procedures were identical to those described in Appendix A: DXA and pQCT Bone Scan Procedures. However, subjects were coded starting with the letter B_0#. The first subset of 15 subjects returned to the lab for a total of three pQCT scans to establish repeatability. All other subjects had one DXA and one pQCT scan completed.

DXA and pQCT Scan Analysis

DXA and pQCT bone scan analyses were identical to those described in Appendix A: DXA Scan Analysis and pQCT Scan Analysis.

Diet and Physical Activity Record

Diet and physical activity records were identical to those described in Appendix A: Diet and Physical Activity Record.

Blood Chemistry Fasting Instructions

Blood Draw and Processing Researchers will contact subjects with the following instructions the day before they are scheduled for their blood draw.

1. Remember that you should fast for at least 8 hours, but no more than 16 hours prior to your scheduled appointment.
2. During the fasting period, drink only water and take all prescribed medications.
3. Avoid excessive exercise prior to your blood draw appointment.
4. Contact your physician with any medical concerns regarding fasting.
5. After your blood draw, complimentary snacks will be provided for you.

For this study, blood was drawn by a phlebotomist and laboratory technician at Virginia Tech, Janet Rinehart in Wallace 229.

Blood Draw and Processing

Venous Collection

Before subject arrives:

1. Create new subject file.
2. Put a copy of "Blood Draw" in subject's chart.
3. Take out turnicate, yellow needle shield, vacutainer needle or butterfly, alcohol swabs, gauze, bandages, and gloves.
4. Pull 3 serum (red/grey speckled stopper), 3 plasma (lavender stopper), and 1 whole blood (green stopper) tube for blood collection.
5. Fill plastic container with crushed ice.

After subject arrives:

1. Have subject sit in black exam chair and rest for about 5 minutes

2. Have phlebotomist go through "Blood Draw" questions.
    a. If subject did not get sufficient sleep, or ate, had caffeine, or alcohol in the previous 8 hours, reschedule blood draw
3. Take subjects seated resting blood pressure
    a. If value is considerably higher/lower than previous testing, attempt to determine reason and potentially reschedule.
4. Proceed with venipuncture
5. Inoculate each plasma tube (lavender) and the whole blood tube (green) 10-12 times before putting on ice.
6. Place plasma blood tubes in crushed ice (at least 75% covered) and serum tubes in holding rack

After venipuncture:

1. Place all needles in red sharp disposal container.
2. Take subject's blood pressure and compare to pre-draw measurement.
3. Give subject snack of juice and crackers.
4. Allow subject to leave after 5 minutes supervision and no signs/symptoms of abnormal response.

Discard used gloves, gauze, band aids, alcohol wipes, and any other disposable material contaminated with blood in the red biohazard disposal box.

Cholestech Procedures

Cholestech Summary

The Cholestech Lipid Profile and GLU cassette measures total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TRG) and glucose (GLU). The Lipid Profile and GLU cassette also calculates the TC/HDL ratio, non-HDL cholesterol, and estimates the level of LDL cholesterol. The test takes about 5 minutes and 55 µL of whole blood or serum is needed as the sample amount.

Cholestech Instructions

1. Let the Cholestech cassette sit at room temperature for 10 minutes.
2. Put on latex gloves provided by the laboratory.
3. Remove the cassette from its pouch. Do not touch the black bar or the brown stripe. Put the cassette on a clean, flat surface.
4. Press RUN on the Cholestech Analyzer.
5. Using whole blood from the 2mL tube collected via venipuncture, use the MiniPet Pipette to place blood into the cassette within 5 minutes of collection.
6. Keep the cassette flat after the blood sample has been applied. Place the cassette into the drawer of the Analyzer at once. The black bar must face the Analyzer. The brown stripe must be on the right.
7. Press RUN on the Analyzer. The drawer will close.
8. Put everything that touched the blood sample in a biohazard waste container.
9. When the test is complete, the Analyzer will beep and print an output of lipid and glucose measurements.

10. When the drawer opens, remove the cassette and discard in a biohazard waste container. Close the Analyzer drawer and leave empty when not in use.
11. Label printed output with subject's number and date and file with subject's existing data.

Blood Processing and Storage

1. Place 2 large-hole tube holders across from one another in the centrifuge.
2. Place small tube holders in the other 2 slots, so that small is straight across from small, and large is straight across from large.
3. Place 2 small plasma tubes in the center of the small tube holder.
4. Screw the clear plastic lid on the small tube holder and place in centrifuge
5. Place the third plasma tube and a plasma tube filled with water in the same slots on the second small tube holder.
6. Screw the clear plastic lid on the small tube holder and place in centrifuge
7. Close the centrifuge and press mode until 15:00 appears.
8. Press the green start key.
9. This will spin for 15 minutes at 2500RPM at 4 degrees C.
10. If the centrifuge stops suddenly after 10-15 seconds, it is not balanced. Make sure that all tubes are placed exactly as described earlier.
11. While blood is spinning, take out one small biohazard bag, 12 storage microcuvettes, and the box containing pipettes.
12. Label the biohazard bag with the subject's initials, code number, and date.
13. Label 6 microcuvettes with the subject's initials, code number, date, and a large P (plasma)
14. Label 6 microcuvettes with the subject's initials, code number, date, and a large S (serum)
15. When blood finishes spinning, remove tubes from centrifuge.
16. Tube should contain 2 layers: plasma (clear-yellowish) and red blood cells (red)
17. Carefully remove the lavender stoppers and the lids of the P labeled microcuvettes.
18. Compress pipette and dip into the middle of the top plasma layer.
19. Slowly release pressure on the pipette, watching plasma be removed from the tube and be sucked into the pipette.
20. Expel plasma into microcuvettes.
21. Carefully continue this process until roughly ¼ inch of plasma remains (leave this, as you do not want to disturb the layers).
22. Evenly distribute plasma into microcuvettes, ideally having 1.5-1.8 ml plasma in each microcuvette.
23. Firmly close each microcuvette and place in labeled biohazard bag.
24. Replace the stopper and place the tube and pipette in sharps disposal container.
25. After 30-45 minutes sitting at room temperature, place serum tubes in centrifuge
26. Place 2 large serum tubes at opposite sides of the large tube holder.
27. Screw the clear plastic lid on the large tube holder and place in centrifuge
28. Place the third serum tube and a serum tube filled with water in the same slots on the second large tube holder.
29. Screw the clear plastic lid on the large tube holder and place in centrifuge

30. Close the centrifuge and press mode until 15:00 appears.
31. Press the green start key.
32. This will spin for 15 minutes at 2500RPM at 4 degrees C.
33. If the centrifuge stops suddenly after 10-15 seconds, it is not balanced. Make sure that all tubes are placed exactly as described earlier.
34. When blood finishes spinning, remove tubes from centrifuge.
35. Tube should contain 3 layers: serum (yellowish-clear), fibrous clot (yellow-white-clear), and red blood cells)
36. If serum clots, remove stopper and dip wooden twist stick or pipette into the middle of the top yellow/clear layer.
37. Slowly spin the wooden stick or pipette as the clot begins to attach.
38. When the clot has wrapped around the stick or pipette completely, slowly pull from the tube and discard.
39. After completing this process on all 3 serum tubes, replace in the centrifuge, making sure the tubes are balanced as before.
40. Spin on the same setting as before, but tubes can be removed after 5 minutes of spinning to extract serum.
41. Carefully remove the red/grey stoppers and the lids of the S labeled microcuvettes.
42. Compress a new pipette and dip into the middle of the top serum layer (clear/yellow).
43. Slowly release pressure on the pipette, watching serum be removed from the tube and be sucked into the pipette.
44. Expel serum into microcuvettes.
45. Carefully continue this process until roughly ¼ inch of serum remains (leave this, as you do not want to disturb the layers).
46. Evenly distribute serum into microcuvettes, ideally having 1.0-1.8 mL serum in each microcuvette.
47. Firmly close each microcuvette and place in labeled biohazard bag.
48. Replace the stopper and place the tube and pipette in sharps disposal container.
49. Close seal on biohazard bag and place in bottom drawer of -80^{o}C freezer with other samples.

Appendix C: Institutional Review Board Protection of Human Subjects

- **Carilion Bariatric Surgery Study**
- **Virginia Tech Bone Study**

CARILION CLINIC

January 16, 2009

Tananchai Lucktong, MD
213 McClanahan Street, #404
Roanoke, VA 24014

RE: Preliminary Studies of Bariatric Surgery Patients: 1) Monocyte Transcription Profiling Risk Markers of Obstructive Sleep Apnea Syndrome (OSAS); and 2) Comparison of Roux-en-Y vs. Laparoscopic Adjustable Gastric Banding Procedures for Effects on Bone Geometry, Mass, and Density at 6 Months Post-Surgery

Dear Dr. Lucktong:

I am pleased to inform you that the Institutional Review Board (IRB) of Carilion Clinic has reviewed the Protocol Amendment #2, dated 12/16/08, for the above-mentioned protocol at our regularly scheduled meeting on 1/14/09. The protocol amendment also included attachments consisting of the Final Report and the Home Sleep Study Questionnaire. Additional research team members have been added. The Board required changes to the Protocol, Final Report, and the Questionnaire. All revisions have been received. Protocol Amendment #2, protocol version dated 1/15/09, and all attachments have been approved.

I would like to remind you that the principal investigator must provide the Institutional Review Board with a report summarizing the status of the project every year. The principal investigator should submit a continuing review application thirty (30) days prior to the expiration date, providing a summary of the project to date and requesting permission for continuation of the original project. It is also your responsibility to report to the IRB serious adverse events, as outlined in the IRB Guidelines, which can be attributed to this study within seven (7) business days of notification. In addition, copies of reports from Data Monitoring Committees or auditing/monitoring reports from a sponsor must also be sent to the IRB Research Compliance Officer within seven (7) business days. Any changes to the research study must receive IRB approval before those changes can be implemented unless subject safety is directly affected. The IRB must be notified immediately about subject safety issues. The IRB must be notified within seven (7) business days if and when a project is discontinued.

The Institutional Review Board of Carilion Clinic would like to thank you for allowing us the opportunity to review this protocol. We look forward to learning of your results.

Sincerely,

Charles Hite, MA, CIP
Human Protections Administrator, Carilion Clinic Institutional Review Board

cc: file

CARILION CLINIC

July 14, 2010

T. A. Lucktong, MD
3 Riverside Circle
Roanoke, VA 24016

RE: Preliminary Studies of Bariatric Surgery Patients: 1) Monocyte Transcription Profiling Risk Markers of Obstructive Sleep Apnea Syndrome (OSAS); and 2) Comparison of Roux-en-Y vs. Laparoscopic Adjustable Gastric Banding Procedures for Effects on Bone Geometry, Mass, and Density at 6 Months Post-surgery

Dear Dr. Lucktong:

I am pleased to inform you that the Institutional Review Board (IRB) of Carilion Clinic has reviewed the Protocol Revision, dated 6/11/10, for the above-mentioned protocol in an expedited manner. The research staff changes do not affect the scientific validity of the study or adversely alter the assessment of the risks and benefits of the study. The revisions have been approved.

I would like to remind you that the principal investigator must provide the Institutional Review Board with a report summarizing the status of the project every year. The principal investigator should submit a continuing review application thirty (30) days prior to the expiration date, providing a summary of the project to date and requesting permission for continuation of the original project. It is also your responsibility to report to the IRB serious adverse events or unanticipated problems, as outlined in the IRB Guidelines, which can be attributed to this study within seven (7) business days of notification. In addition, copies of reports from Data Monitoring Committees or auditing/monitoring reports from a sponsor must also be sent to the IRB Research Compliance Officer within seven (7) business days. Any changes to the research study must receive IRB approval before those changes can be implemented unless subject safety is directly affected. The IRB must be notified immediately about subject safety issues. The IRB must be notified within seven (7) business days if and when a project is discontinued.

The Institutional Review Board of Carilion Clinic would like to thank you for allowing us the opportunity to review this protocol. We look forward to learning of your results.

Sincerely,

Charles Hite, MA, CIP
Human Protections Administrator, Carilion Clinic IRB

cc: file

Institutional Review Board
2001 Crystal Spring Avenue, SW, Suite 202 Roanoke, VA 24014-2465 P.O. Box 13367 Roanoke, VA 24033-3367
540-853-0728 p 540-985-5323 f

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24061
540/231-4991 Fax 540/231-0959
e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572(expires 1/20/2010)
IRB # is IRB00000667

DATE: June 25, 2007

MEMORANDUM

TO: William G. Herbert
Donald Zedalis
John Gregg

Approval date: 6/11/2007
Continuing Review Due Date:5/26/2008
Expiration Date: 6/10/2008

FROM: David M. Moore

SUBJECT: **IRB Full IRB Approval:** "Preliminary Studies of Obstructive Sleep Apnea Syndrome (OSAS): Searches for a Gene Expression Profile and Relationships to Type 2 Diabetes and Skeletal Fragility" , IRB # 07-291

The above referenced protocol was submitted for full review and approval by the IRB at the June 11, 2007 meeting. The board had voted approval of this proposal contingent upon receipt of responses to questions raised during its deliberation. Following receipt and review of your responses, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective June 11, 2007.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3. Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must ubmit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtained re-approval from the IRB before the study's expiration date.
4. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

Important:
If you are conducting **federally funded non-exempt research**, this approval letter must state that the IRB has compared the OSP grant application and IRB application and found the documents to be consistent. Otherwise, this approval letter is invalid for OSP to release funds. Visit our website at http://www.irb.vt.edu/pages/newstudy.htm#OSP for further information.

cc: File
Department Reviewer:Kathy Hosig

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24060
540/231-4606 Fax 540/231-0959
e-mail irb@vt.edu
Website: www.irb.vt.edu

MEMORANDUM

DATE: May 11, 2010

TO: William G. Herbert, Donald Zedalis, John Gregg, Stephen Guill, Sharon M. Nickols-Richardson, Thomas Chittenden

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires June 13, 2011)

PROTOCOL TITLE: Preliminary Studies of Obstructive Sleep Apnea Syndrome (OSAS): Searches for a Gene Expression Profile and Relationships to Type 2 Diabetes and Skeletal Fragility

IRB NUMBER: 07-291

As of June 11, 2010, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the continuation request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at http://www.irb.vt.edu/pages/responsibilities.htm (please review before the commencement of your research).

PROTOCOL INFORMATION:
Approved as: **Full Board Review**
Protocol Approval Date: **6/11/2010**
Protocol Expiration Date: **6/10/2011**
Continuing Review Due Date*: **5/27/2011**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:
Per federally regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Appendix D: Informed Consent

- **Carilion Bariatric Surgery Study**
    - **Preliminary Screening Informed Consent**
    - **Bone Study Informed Consent**
- **Virginia Tech Bone Study**

T. A. Lucktong, MD, FACS
Principal Investigator
3 Riverside Circle
Roanoke, VA 24016
Phone: 540-224-5170

TITLE: Preliminary Studies of Bariatric Surgery Patients: 1) Monocyte transcription profiling risk markers of Obstructive Sleep Apnea Syndrome (OSAS); and 2) Comparison of Roux-en-Y vs. Laparoscopic Adjustable Gastric Banding procedures for effects on bone geometry, mass, and density at 6 months post-surgery.

Screening Consent

Co-INVESTIGATORS: William G. Herbert, PhD., Thomas W. Chittenden, MSc, PhD., Frank Biscardi, MD., Bruce Long, MD., Jonathan Dort, MD.

WHAT IS INFORMED CONSENT?

You are being asked to take part in a research study because you have been accepted for elective bariatric surgery. Carilion Clinic sponsors the research. The person running this study locally is Tananchai Lucktong, MD. Before you can decide whether to take part in the research, you should be told about the possible risks and benefits with this study. This process is known as informed consent. Please ask as many questions as you need to make sure that you know what will happen to you in this study and why you are being asked to be in it. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family.

Be aware that the role of the research doctor is different from the role of your personal doctor. Your personal doctor decides how to treat your specific problem in order to help you. The research doctor treats all subjects under a specific protocol to obtain general knowledge that may or may not benefit you. Be sure to ask your doctors questions to help you know more about these different roles.

WHY IS THIS RESEARCH BEING DONE?

This research will look at two different problems that happen to patients having bariatric surgery.

First, many patients having bariatric surgery may suffer from Obstructive Sleep Apnea Syndrome (OSAS), a nighttime breathing disorder. OSAS increases surgical risks but is difficult and costly to diagnose. Researchers want to examine genetic susceptibility to OSAS using studies of blood cells. Testing patients' blood could help identify patients that should be diagnosed and treated for sleep apnea to reduce risks of bariatric surgical complications.

Second, bariatric surgery may also result in loss of bone mass that may increase the risk of fracture, particularly in women. Researchers are not certain why this effect is occurring. One possibility is that the surgery may decrease calcium absorption. After bariatric surgery, we will look at bone scans to review bone density and bone quality. This will help us to understand how body weight and bone structure are related.

Part of the purpose of this study is to find out if basic questions about the two problems can be answered. If this study is successful, then researchers hope to do a larger study using many more patients.

Locally there will be 48 subjects taking part in this research. The length of time you can expect to be in this research is six months following your surgery.

WHAT WILL HAPPEN IN THIS RESEARCH STUDY?

The study that you are being asked to take part in is made up of two parts. One part is looking for markers for sleep apnea in your blood. A second part is looking at bone health after Roux-en Y gastric bypass or Laparoscopic adjustable gastric banding surgery. You may qualify to be in the sleep apnea part, the bone health part, or both parts of the study. This preliminary consent will allow the investigators to gather some information on you to determine what parts of the study best suit you. In this preliminary part of the study, you will be given questionnaires to determine your sleep habits, your health history, as well as your ability to perform certain tasks. Also, measurements of your neck, waist, and hip will be taken.

If you agree to take part in the screening part of the study, investigators will determine what other part(s) of the study are best suited for you. You then will be given an additional consent(s) that explain what will happen in the other part(s) of the study.

WHAT ARE THE RISKS OF BEING IN THIS RESEARCH STUDY?

There is minimal risk associated with the questionnaires and measurements in this study. Your name will not appear on the questionnaires. The questionnaires will be identified only by the patient identification number that is assigned to you at the beginning of the study.

WHAT ARE THE BENEFITS OF BEING IN THIS RESEARCH STUDY?

Taking part in this research study will not benefit your outcome with bariatric surgery. The questionnaires you complete for the screening part of the study will not provide you with any information concerning sleep apnea or bone density. They will provide you with information on your eating and activity habits.

ARE THERE ANY OPTIONS TO BEING IN THIS RESEARCH STUDY?

You do not have to be in this research to receive bariatric surgery. You may choose to have bariatric surgery alone without taking part in the research. Your doctor will discuss all of the choices with you.

WILL I RECEIVE NEW INFORMATION ABOUT THIS RESEARCH STUDY?

Sometimes new information comes out that may affect your health, welfare, or willingness to stay in a study. If that happens, the researchers will tell you about that information. They will also tell you about other options for your care. You may need to sign another form with your consent to continue in the study.

WHAT ABOUT CONFIDENTIALITY?

Individual subject data from this study will be kept strictly confidential. This means that all of your answers to questions that you are asked, measurement values, and blood test results will be kept confidential. Results will be kept in an electronic database which is password protected. A master list of subjects' code numbers will be kept in a locked filing cabinet separate from completed data, which will also be maintained in a locked filing cabinet. Paper files will be stored in a locked cabinet. All questionnaires, data collection sheets, data analysis sheets, blood collection and storage containers will be identified by code number and not by your name.

The investigators of this study and the investigators' students will be allowed access to data. In addition, data for the study may be examined by federal or state regulatory agencies that oversee human subjects research or by auditors from the committee at Carilion Clinic charged with protecting human subjects in research. During analyses and written reports of this research, the information provided will have names removed and only a three-digit code number (excluding social security numbers) will identify subjects. The Principal Investigator will keep a list of subject's identifications, with corresponding coding to properly identify individual subject data.

HIPAA AUTHORIZATION

There is a federal law that protects the privacy of health information. This law is known as HIPAA. HIPAA stands for the "Health Insurance Portability and Accountability Act." Because of this law, your records cannot be looked at without your permission. The researchers for this study may need to look at and use information from your records. You need to give your permission for them to be able to do this. If you are giving permission for someone other than yourself, this permission applies to that person's health information.

Researchers may get new information about you from procedures, tests, surveys or interviews. In some cases, researchers may talk to your treating doctors about you.

The researchers will use the information they get about you. They may share it with others, including:

- researchers at other places who are taking part in the research
- other international, federal, state and local agencies that have authority over research
- Carilion Clinic Institutional Review Board (CC IRB), a committee that checks for quality and safety of research
- other organizations that also check for quality and safety

Some of the groups that receive information about you while you are in the research study could share it with others. If this happens, HIPAA may not apply. Other state and federal privacy regulations may protect your personal health information. However, absolute confidentiality cannot be guaranteed.

You must give your permission to access and use your health information in this study. Your permission will not expire unless you cancel it. Otherwise, your information will be used as long as it is needed for the study. You have the right to access your records about this research study but you may have to wait until the study is completed.

You may refuse to give permission to use your health information. This will not change your ability to get health care outside of the research study. If you do not give your permission to use and share your health information, you cannot be in the research study.

You may change your mind at any time and cancel your permission to use your personal health information. To cancel your permission, you must put it in writing and give it to your research doctor. However, any information the researchers got before you cancelled your permission may still be used. Also, any of your information that is being used for safety monitoring or certain other uses may still be used even after you withdraw.

IRB SURVEY:

The IRB is a group of people that review research to protect the rights of research subjects. One job of the IRB is to make sure the research is done in a way that is respectful to subjects. To help it do this, the CC IRB would like to send you a survey about your experiences while taking part in research. While your name and address will be given to the IRB in order to mail the survey, the IRB will destroy the list of names and addresses as soon as the survey is mailed. The survey itself will be anonymous. You do not have to allow the IRB to send you this survey. Please check below if you would like the IRB to send you the survey or not:

_____ Yes, I agree to CC IRB sending me a survey about my experiences while taking part in research.

_____ No, I do not want CC IRB to send me such a survey.

WHAT WILL TAKING PART IN THIS RESEARCH STUDY COST OR PAY?

There is no cost associated with taking part in this preliminary study. If you are eligible and decide to take part in either or both of the follow-up studies, you will be given information about any costs or payments associated with those in the future.

WHAT WILL HAPPEN IF I HAVE COMPLICATIONS OR IF I AM INJURED BY THIS RESEARCH STUDY?

In this preliminary study, there is no chance for injury. If you are eligible and decide to take part in either or both of the follow-up studies, possible injuries and compensation for those injuries will be discussed in the future.

WHAT IF I WANT TO STOP BEING IN THE STUDY BEFORE IT IS FINISHED?

Being in this research is voluntary. You may refuse to take part or you may withdraw at any time. Your decision not to take part or your decision to withdraw will not affect your ability to get care from your doctors. The researchers may take you out of the research study for any reason, without your consent, if they feel it is in your best interest. The reason for any exclusion will be explained to you.

ARE RESEARCHERS BEING PAID TO DO THIS STUDY?

This study is funded through Carilion Clinic. None of the investigators or research staff will receive money or other types of payment from this study.

WHO ARE THE CONTACT PERSONS?

If you encounter medical problems, complications or have any questions about the study, you may call the researcher, Tananchai Lucktong, MD, during the day at 540 342-6346. If you have questions about your rights as a research subject, you may contact staff of the Carilion Clinic IRB at (540) 853-0728.

CONSENT SIGNATURES:

RESEARCH SUBJECT: The research study described in this consent form, including the risks and benefits, has been explained to me and all of my questions have been answered to my satisfaction. I consent to participate in this research study. My consent is given willingly and voluntarily. I may withdraw my consent at any time. I will receive a signed copy of this consent form.

_____
Printed Name of Research Subject

_____ _____
Subject's Signature Date

PERSON OBTAINING CONSENT: I certify I was present for the informed consent discussion and that the subject or legally authorized representative had an opportunity to ask questions about and appeared to understand the information presented and agreed to participate voluntarily in the research.

_____
Printed Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

T. A. Lucktong, MD, FACS
Principal Investigator
3 Riverside Circle
Roanoke, VA 24016
Phone: 540-224-5170

TITLE: Preliminary Studies of Bariatric Surgery Patients: 1) Monocyte transcription profiling risk markers of Obstructive Sleep Apnea Syndrome (OSAS); and 2) Comparison of Roux-en-Y vs. Laparoscopic Adjustable Gastric Banding procedures for effects on bone geometry, mass, and density at 6 months post-surgery

Bone Density Study Consent

Co-INVESTIGATORS: William G. Herbert, PhD., Thomas W. Chittenden, MSc, PhD., Frank Biscardi, MD., Bruce Long, MD. Jonathan Dort, MD.

WHAT IS INFORMED CONSENT?

You are being asked to take part in a research study because you have been accepted for bariatric surgery. Carilion Clinic sponsors the research. The person running this study locally is Tananchai Lucktong, MD. Before you can decide whether to take part in the research, you should be told about the possible risks and benefits with this study. This process is known as informed consent. This consent form will give you information about this study and your rights as a research subject. Please ask as many questions as you need to make sure that you know what will happen to you in this study and why you are being asked to be in it. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family.

Be aware that the role of the research doctor is different from the role of your personal doctor. Your personal doctor decides how to treat your specific problem in order to help you. The research doctor treats all subjects under a specific protocol to obtain general knowledge that may or may not benefit you. Be sure to ask your doctors questions to help you know more about these different roles.

WHY IS THIS RESEARCH BEING DONE?

Bariatric surgery may result in loss of bone mass that may increase the risk of fracture, particularly in women. Researchers are not certain why this effect is occurring. One possibility is that the surgery may decrease calcium absorption. We will look at your bone scans to review your bone density and bone quality before and after your surgery. This will help us to understand how body weight and bone structure are related.

During this study, you will have blood drawn for research purposes. The blood will be analyzed to find out the differences between Roux-en Y gastric bypass (RYGBS) and

Laparoscopic adjustable gastric banding (LAGB) and their effects on your bones. The tubes of blood will be labeled with only a subject number. The blood samples will be processed at the Carilion Community Research Lab.

Your blood drawn for this research study will not be used for any other purpose or research other than this one. Any results from the genetic portion of the study will not be shown to anyone else outside the research group. All the blood samples obtained will be disposed of according to regulations of biohazards, and will not be held after the study is completed.

Locally there will be 30 subjects taking part. The length of time you can expect to be in this research is six months following your surgery.

WHAT WILL HAPPEN IN THIS RESEARCH STUDY?

You are being asked to take part in all sessions of the study described below. Once you agree to take part, you will be asked to attend one testing session before your surgery and two testing sessions after your surgery. All three sessions will require you to travel to the Virginia Tech Bone, Osteoporosis and Nutrition Examination (BONE) Lab on West Campus Drive in addition to your already scheduled visits at Carilion Clinic.

SESSION ONE:

During this testing session, you will receive a pedometer to help the researchers assess your physical activity. You will also be asked to take a pregnancy test to ensure that you are not pregnant before receiving the bone mineral density (BMD) test. During the routine blood draw that occurs at this time, you will have an extra 5 tablespoons of blood drawn for this study. The session will take approximately 45 minutes.

The bone mineral density (BMD) test measures the amount of minerals (such as calcium) in your bones using a special X-ray called a DXA scan. It is not safe to have this test if you are pregnant. You will also have measurements taken of your non-dominant forearm and non-dominant leg below the knee by peripheral quantitative computed tomography called a pQCT. Peripheral quantitative computed tomography is another bone mineral density test used for making measurements of the bone strength. You will be given the results of this testing.

SESSIONS TWO AND THREE:

You will be asked to repeat the bone scans at three and six months after your surgery. You also will be asked to complete questionnaires about physicial activity history, diet and general health. Both of these sessions will take approximately 45 minutes as well. During the routine blood draw that occurs at the 3 and 6 month follow up at your regularly scheduled doctors visit, an additional amount of blood will be drawn for this study (about 5 tablespoons). If the additional study blood is not able to be drawn at the

same time as the 3 or 6 month follow up, we will ask that you come in another morning to the 4th floor CCL at Roanoke Community Hospital for the fasting study blood draw. This will take approximately 10-15 minutes.

WHAT ARE THE RISKS OF BEING IN THIS RESEARCH STUDY?

There is minimal risk involved in blood draws. A bruise may result from blood collection procedures. You may rest for as long as needed after your blood is drawn.

This research study requires a small amount of radiation from the DXA and pQCT scans. This radiation exposure is for research purposes only. The total amount of radiation that you will receive from this study is from three (3) DXA sessions (comprised of 4 different scans) and three (3) pQCT sessions (comprised of a tibia and radius scan). This amount of radiation for all the scans is equal to the amount the average person in the United States receives every day from natural background sources, such as the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. There will be three sessions (pre-surgical, and 3 and 6 months post surgery). The amount of radiation you will receive is less than the amount permitted by the Food and Drug Administration (FDA) per year. The total amount you will receive is equal to 1/20 of a chest x-ray.

The more radiation you receive over the course of a lifetime, the more likely the risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however the exact increase in such risk is not known. You should not be pregnant due to the risk from the DXA scan radiation to the embryo or fetus. You will first undergo a pregnancy test prior to having each DXA scan. You will not be eligible to participate in this study if you are pregnant.

It is not possible to identify all potential risks in an experimental study; however the study doctors and study staff will take all possible safeguards to minimize any known potential risks to patient well-being. We believe that overall risks of participation are minimal. All of the procedures are well established and used routinely. Side effects are possible in any research study despite high standards of care, and could occur through no fault of the subject or the study staff.

WHAT ARE THE BENEFITS OF BEING IN THIS RESEARCH STUDY?

This study will not provide any direct benefits to you. You will be provided with your bone density results as well as patient education should your results indicate a dietary supplement is needed. Although you may not personally benefit from taking part in this study, the knowledge gained may benefit others.

ARE THERE ANY OPTIONS TO BEING IN THIS RESEARCH STUDY?

You do not have to be in this research to receive bariatric surgery. You may choose to have bariatric surgery alone without taking part in the research. Your doctor will discuss all of the choices with you.

WILL I RECEIVE NEW INFORMATION ABOUT THIS RESEARCH STUDY?

Sometimes new information comes out that may affect your health, welfare, or willingness to stay in a study. If that happens, the researchers will tell you about that information. They will also tell you about other options for your care. You may need to sign another form with your consent to continue in the study.

WHAT ABOUT CONFIDENTIALITY?

Individual subject data from this study will be kept strictly confidential. This means that all of your answers to questions that you are asked, measurement values, and blood test results will be kept confidential and shared only with you. Results will be kept in an electronic database which is password protected. A master list of subjects' code numbers will be kept in a locked filing cabinet separate from completed data, which will also be maintained in a locked filing cabinet. Paper files will be stored in a locked cabinet. All questionnaires, data collection sheets, data analysis sheets, blood collection and storage containers will be identified by code number and not by your name.

The investigators of this study and the investigators' students will be allowed access to data. During analyses and written reports of this research, the information provided will have names removed and only a three-digit code number. The Principal Investigator will keep a list of subject's identifications, with corresponding coding to properly identify individual subject data.

HIPAA AUTHORIZATION

There is a federal law that protects the privacy of health information. This law is known as HIPAA. HIPAA stands for the "Health Insurance Portability and Accountability Act." Because of this law, your records cannot be looked at without your permission. The researchers for this study may need to look at and use information from your records. You need to give your permission for them to be able to do this. If you are giving permission for someone other than yourself, this permission applies to that person's health information.

Researchers may get new information about you from procedures, tests, surveys or interviews. In some cases, researchers may talk to your treating doctors about you.

The researchers will use the information they get about you. They may share it with others, including:

- the sponsor of the research
- groups working with the sponsor
- researchers at other places who are taking part in the research

- the Food and Drug Administration (FDA)
- other international, federal, state and local agencies that have authority over research
- Carilion Clinic Institutional Review Board (CC IRB), a committee that checks for quality and safety of research
- other organizations that also check for quality and safety
- your health insurer for treatment not paid for through the research

Some of the groups that receive information about you while you are in the research study could share it with others. If this happens, HIPAA may not apply. Other state and federal privacy regulations may protect your personal health information. However, absolute confidentiality cannot be guaranteed.

You must give your permission to access and use your health information in this study. Your permission will not expire unless you cancel it. Otherwise, your information will be used as long as it is needed for the study. You have the right to access your records about this research study but you may have to wait until the study is completed.

You may refuse to give permission to use your health information. This will not change your ability to get health care outside of the research study. If you do not give your permission to use and share your health information, you cannot be in the research study.

You may change your mind at any time and cancel your permission to use your personal health information. To cancel your permission, you must put it in writing and give it to your research doctor. However, any information the researchers got before you cancelled your permission may still be used. Also, any of your information that is being used for safety monitoring or certain other uses may still be used even after you withdraw.

WHAT WILL TAKING PART IN THIS RESEARCH STUDY COST OR PAY?

Taking part in this research study will not add any costs to your bariatric surgery. No procedures done just for research will be billed to you or your insurance company. You will not be paid to be in this research study.

You will receive a $20 gift card for each of your three trips that you make to Virginia Tech. You have the option to drive yourself or to take the SmartWay shuttle. The SmartWay shuttle provides service to residents between the Roanoke Valley and the Virginia Tech campus. The fare is $4 each way.

WHAT WILL HAPPEN IF I HAVE COMPLICATIONS OR IF I AM INJURED BY THIS RESEARCH STUDY?

If you have a medical problem that happens because you are in this study, you will be able to get treatment. The treatment will be billed to you or your insurer at the usual

charge. The study does not make any provisions for the payment of these costs. You will not receive any other financial compensation. However, you do not give up any legal rights to seek compensation for injury by signing this consent form.

WHAT IF I WANT TO STOP BEING IN THE STUDY BEFORE IT IS FINISHED?

Being in this research is voluntary. You may refuse to take part or you may withdraw at any time. Your decision not to take part or your decision to withdraw will not affect your ability to get care from your doctors or from Carilion Clinic. The researchers may take you out of the research study for any reason, without your consent, if they feel it is in your best interest. The reason for any exclusion will be explained to you.

ARE RESEARCHERS BEING PAID TO DO THIS STUDY?

This study is funded by a grant from Carilion Clinic to help cover basic costs involved in research. None of the investigators or research staff will receive money or other types of payment from this study.

WHO ARE THE CONTACT PERSONS?

If you encounter medical problems, complications or have any questions about the study, you may call the researcher, Tananchai Lucktong, MD, during the day at 540 342-6346. If you have questions about your rights as a research subject, you may contact staff of the Carilion Clinic IRB at (540) 853-0728.

CONSENT SIGNATURES:

RESEARCH SUBJECT: The research study described in this consent form, including the risks and benefits, has been explained to me and all of my questions have been answered to my satisfaction. I consent to participate in this research study. My consent is given willingly and voluntarily. I may withdraw my consent at any time. I will receive a signed copy of this consent form.

_____
Printed Name of Research Subject

_____ _____
Subject's Signature Date

PERSON OBTAINING CONSENT: I certify I was present for the informed consent discussion and that the subject or legally authorized representative had an opportunity to ask questions about and appeared to understand the information presented and agreed to participate voluntarily in the research.

_____
Printed Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

Virginia Polytechnic Institute and State University
Informed Consent for Participants of Investigative Projects
(Proposed Revision of February 18, 2008)

Title of Project: Preliminary studies of Obstructive Sleep Apnea Syndrome (OSAS): Searches for a gene expression profile and relationships to Type 2 diabetes and skeletal fragility.

Location of Study: 231 War Memorial Hall and 229 Wallace Hall, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Investigators: William G. Herbert, Ph.D., Don Zedalis, MD, John M. Gregg, DDS, PhD, Sharon Nickols-Richardson, RD, PhD, Stephen Gulli, PhD

I. Purpose: One purpose is to determine if adults with obstructive sleep apnea syndrome (OSAS) have a unique set of proteins in their blood that suggest a clear genetic basis of this disorder. Other purposes are to determine if those with OSAS are at increased risk for developing either cardiovascular disease or Type 2 diabetes, or might be at increased long-term risk for osteoporosis. OSAS is a disorder that occurs when a person stops breathing many times during the night while asleep. This study is designed to help better understand the relationships between OSAS and risks for these important chronic diseases. If there are clear associations found through this study, the results might lead to better prevention and treatment of those with OSAS. Another purpose, related only to women participating in the repeat bone scans with the pQCT device (described later), is to establish measurement reliability for numerous detailed measurements of the lower leg and forearm, which are assessed by this device.

II. Procedures: Upon giving Informed Consent, you will be asked to complete a screening process to determine your eligibility. Before you may be selected, you will be asked to answer questions about your health history, complete health and sleep questionnaires. You will not be eligible to take part in the study if you currently have or have a history of any of the following:

- Heart problems, including past heart attack, chest pain that may be related to heart problems (this is called angina pectoris), surgery for your heart or its blood vessels, or heart failure;
- Blood pressure higher than 160/100 mmHg;
- Chronic lung diseases (including asthma);
- Diabetes mellitus;
- Use of medications known to affect bone (e.g., steroid or thyroid hormones, bisphosphonates, anticonvulsants, glucocorticoids);
- Use of blood pressure medications or antihistamines (cold or allergy medicine);
- Use of any tobacco or nicotine products (only non-smokers can participate);
- Infectious illnesses, such as cold, sinus infection, etc. during the previous 6 weeks;
- Engaging in a regular physical fitness program during the last 6 months (> 30 minutes of moderate to vigorous activity per day for 3 or more days per week OR – see next paragraph for women who may be eligible for one aspect of the study, regardless of physical activity habits;
- For women, if you are pregnant or might be pregnant.

Women ages 30-45 who may not qualify for the sleep portion of the study may still be eligible to complete the radiologic measures portion of the study (bone scans and body composition procedures). All but one of the exclusion criteria described above apply. The only difference is that physically active women are not excluded.

If researchers are concerned by any part of your health history that may affect your eligibility and you have a personal physician, you may still qualify if you can secure documentation from your physician that clarifies the issues of concern. Only you can ask your physician to do this and he/she must

secure your written permission to release this information to us. We will provide you with a fax phone number (maintained in a secured area) or mailing address for your physician to use in sending information to us, should this be needed.

Once it is determined that you are eligible, you will be asked to complete the following procedures:

Preliminary Visit - Explanation of Study, Informed Consent, and Screening

This will take about 60 minutes, but you may take as much time as you need to fill-out the questionnaires and forms. Before session 1, you will be provided a copy of this informed consent form, either through standard mailing, email, or the study website. You will also receive a simple health history form, sleep questionnaires, and a health-related quality of life questionnaire. Please read all of these documents carefully and write down any questions you may have for the research team before you report to our lab for the first meeting.

Session 1 – Anthropometrics, Home Sleep and Heart Monitor Tests

If you are a woman eligible only for the radiologic part of the study, you will report only to the Bone, Osteoporosis and Nutrition Evaluation (BONE) Lab in 229 Wallace, where the researchers will measure your height, body weight, and use a tape to measure the size of your waist, hips, and length of forearm and lower leg. You will then follow the steps outlined in Session 3 of this document. You will also be asked in your first session to complete the 4-day diet record and record of physical activity history, and take home a pedometer to wear for counting your daily steps (see second paragraph under description for Session 2 for these steps on the next page). You also will be asked to return to the BONE lab in Wallace on two follow-up test sessions, to repeat the bone scans of your leg and arm, with the pQCT device (described later in this document).

You will report to the Laboratory for Health and Exercise Science in 231 War Memorial Hall on the Virginia Tech campus. We will provide a map for you to show this location and information about parking, should you be driving to campus. Once there, a researcher will review all of these forms with you and answer any questions or concerns that you may have. The researcher may ask you additional questions after reviewing your completed forms to clarify any aspects of your health or exercise habits that may affect your eligibility. If you have consented and wish to proceed as a subject in the study at this point, a researcher will measure your height, body weight, and use a tape to measure the size of your neck, waist, and hips. If any of these values do not meet the study requirements, you will not be eligible to continue in this study.

Next, you will be taught how to set-up and use the heart monitor (this is called a Polar R-R device) and the home sleep test device (this is called an Embletta® device). Both are small pocket-sized recorders. You will be asked to wear the heart monitor on one normal (typical) day while you are awake (at least 10 hours). For the home sleep test, you will be asked to wear the Embletta® for one full night. Should one of the recorders fail to provide the information required, you will be asked to repeat these measurements on different days. If either fails again, you will not be asked to wear the devices a third time and you may not be eligible for further participation in the study.

The heart monitor requires 2 small electrodes to be placed on your sternum and left chest wall and wires that lead to the small recorder, which will be affixed to your belt or clothing waistband. The electrodes are disposable and gelled on the inside; the gel very rarely may cause slight skin irritation, but if so this should last no more than one day. You will be instructed on how to properly set-up and use the heart monitor and given an instruction sheet with pictures showing how to wear and activate the device. Throughout the day you wear the heart monitor, you will also be asked to record major changes in your physical activities and changes in your feelings about your stress level (on a scale of 1-4). Before going to bed that evening, you will remove the device and electrodes, turn off the

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recorder. You will be asked to return this monitor to the investigators the next day along with the Embletta® (see next paragraph for explanation about when and how to do the home sleep test with the Embletta®).

The Embletta® sleep test device is equipped with straps, wires, and small sensors. You will wear the Embletta® for one entire night at home, while you sleep. The sensors detect airflow through your nose, movement of your chest wall during breathing, your pulse, and the level of oxygen in your blood. The Embletta® is a harmless, non-invasive (no needles) monitor that is sometimes used by sleep doctors to screen people who may need more medical tests for possible nighttime breathing disorders. The researchers will make plans for you to take the Embletta® home, assist you by phone if needed to properly set it up for one night and arrange for you to return the device the next day. For this sleep test, you will first attach a flexible strap to your abdomen and chest to measure its motion while you breathe during sleep. You will also wear a nasal cannula (tiny tube that fits around your ears) and attaches to your nostrils to measure airflow while you breathe. You will also attach a small sensor to your finger, which will measure any changes in your blood oxygen level while you sleep. The length of time for the home sleep study will depend on how long you sleep that night, which should at least 6-7 hours for an adequate test. During the night and in the first few hours of the morning after your home sleep test, you will be asked to refrain from eating or drinking anything but water (12 hours). This fasting period will be from about 8-10 PM until about 8-10 AM the next morning, at which time you will be asked to report to the researchers' lab in 231 War Memorial Hall. When you come to the lab the next morning, you will be asked to return the Embletta® and perform the activities of Session 2 (see next section).

Session 2 – Blood Vessel Test, Instructions for Diet/Physical Activity Recording

On the morning that you return the heart monitor and Embletta®, you will be asked to do a blood vessel health test. This session will also take place in 231 War Memorial Hall on the Virginia Tech campus and will take about 55 minutes. This test is called plethysmography and measures the ability of your blood vessels to expand and contract. The test requires that you lie quietly for 10-20 minutes on a comfortable padded table with a pillow. You will have your blood pressure measured while lying quietly. If your blood pressure should be above 160/100 mmHg (after three successive checks), you will not be allowed to complete this blood vessel health test and you will be encouraged to see your personal physician for medical evaluation of your blood pressure; this finding may prevent you from further participation in the study. If your resting blood pressure is found to be acceptable, you will have inflatable cuffs placed around your upper arm and wrist. Next a delicate elastic band about the thickness of a string will be placed around your forearm. Then, the two cuffs will be inflated, increasing the outside pressure on your arm and you will lie quietly with the pressure elevated on your arm for 10 minutes. You will feel slight discomfort and numbness in your fingers; these sensations will quickly disappear after the cuff pressure is released at the end of 10 minutes. We have done this test in previous research studies, without any subjects reporting any lasting discomfort or problems. Other health researchers also have performed this test for at least 30 years, without any known adverse effects. After the test you will be provided breakfast snacks and juice.

Before you leave the lab, we will show you how to complete 4-day dietary and physical activity records and how to use a pedometer (a step counter, to be worn on your waistband or belt). You will also schedule a time to return the dietary and physical activity records and come in for the third testing session. The evening prior to session 3 you will receive a courtesy call or email to remind you to fast for 12 hours before the blood draw, as described in the next section. This means that you will not eat or drink any foods or fluids other than water after about 8 PM and also not to eat or drink anything but water in the morning before donating the blood sample.

Session 3 – Blood Sample and Body Fat and Bone Health Tests

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This session will be held in a different building than sessions 1 and 2 and you will be given directions or a map, with parking information if needed. When you arrive on the scheduled day at this facility, the BONE Lab, Room 229 Wallace Hall, you will do following:

(1) undergo a pregnancy test, if you are female (urinary test, 5 minutes);
(2) have a blood sample taken by a certified technician; the amount of blood that will be drawn through a needle inserted into your arm will be 75 mL (~5 Tbsp) or, ***if you volunteer and qualify for the genetic portion of the study, this total amount will be 120 mL (~8 Tbsp)*** (10 – 15 minutes);
(3) consume provided breakfast snacks and juice (10 minutes);
(4) lie on or sit next to the dual-energy X-ray absorptiometry machine (DXA) as directed by a Licensed Limited Radiologic Technologist, who will conduct scans of your whole body, lumbar spine, hip, and forearm for bone mineral density (BMD) and body fat testing (15 minutes);
(5) sit at the peripheral quantitative computed tomography (pQCT) machine as directed by a Licensed Limited Radiologic Technologist, who will conduct scans of your lower leg (tibia) and forearm (radius) for BMD and bone geometry testing (30 minutes);

Your total time required for this session will be approximately 75 minutes. If you are participating only in the radiologic portions of the study, the total time for this session will be approximately 45 minutes.

For women participating only in the radiologic portion of the study, you will be asked to return for two (2) more sessions on non-consecutive days, over a period of 1-2 weeks, to repeat only step 5 above with the pQCT procedure. Each of these two visits will require approximately 30 minutes.

There will be three separate lab visits with associated activities over the course of approximately one to two weeks. Your participation for these three testing sessions plus the time at home filing out forms and setting up the at-home testing equipment will require approximately 5.5 hours (this time commitment will be 3 hours, if you are participating only in the radiologic portion of the study) of your time (see attached appendix). You may require more or less time than this estimate to complete any of the procedures, and you will be given ample opportunity to complete all procedures in an unhurried manner.

> ***Ten Caucasian men who have higher home sleep test scores (suggesting at least moderate OSAS) and 10 who have very low home sleep test scores (very similar controls, but without scores suggesting OSAS) will be invited to participate in the genetic portion of this study. If you are in one of these two categories, are invited to participate and volunteer to participate, you will be asked to donate an additional blood sample that will be analyzed for possible genetic markers (proteins) that may predict who is and is not likely to have OSAS. The ability to identify individuals in this manner, through a specific blood protein pattern, could eventually lead to better diagnosis and treatment of OSAS. Essentially, the only difference for these 20 men will be a donation of an extra 3 Tablespoons of blood. The genetic analysis will not include anything other than specific proteins possibly involved in the development of OSAS. If you participate in this portion of the study, the researchers will keep confidential all genetic information about you. These genetic results will never be disclosed to anyone, other than in research reports that cannot be personally associated with you.***

III. Risks: The investigators are not aware of any specific risks associated with the at-home sleep test or the heart rate monitor. In fact, many people have this type of sleep test done to screen for sleep conditions without any problems. Potential risks while participating in this study exist during blood draws and DXA and pQCT scans.

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Inflatable cuffs pumped up for 10 minutes are used when the blood vessel test is done. This may produce slight discomfort and numbness in the fingers, which will subside quickly after the cuffs are removed.

There is minimal risk involved in blood draws. A bruise may result from blood collection procedures or slight risk of infection at the point of needle insertion; these risks are extremely small. To avoid or minimize bruising and reduce risk of infection, a Certified Medical Technologist will draw your blood and the skin on your arm will be cleansed beforehand with alcohol pad and a bandage placed over the puncture site afterwards. In addition, you will be allowed to sit or recline in the most comfortable position for you during your blood draw. You may rest for as long as needed after your blood is drawn and will be provided with breakfast snacks and juice after your blood is collected. Two attempts to draw your blood (or two needle sticks) will be allowed. If a second attempt is unsuccessful, no further tries for collection of your blood will be performed. All personnel involved in drawing and handling blood have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech. Precautions will be taken by research personnel during handling of your blood. In the event that blood exposure occurs to one of the researchers or the Technologist, hepatitis testing of your blood will be done as required by Virginia state law. If a technician or other person who handles your blood sample is accidentally exposed to your blood, you will also be required to have your blood tested for HIV/AIDS. This testing will be confidential and will be done at the Montgomery County Health Department. It is required that you provide the Montgomery County Health Department with your social security number and your name; if you have a positive test for HIV/AIDS, and only then, this result must be reported to the State Health Department (this is a legal requirement). The names of persons with HIV/AIDS positive tests that are reported to the state remain confidential; however, this information will be placed in your permanent medical records. The test facility requires pre-test and post-test counseling. They will contact you within 2 weeks to notify you that you must return there to receive your test results. No results will be given by phone.

This research study requires a small amount of radiation from the DXA and pQCT scans. It must be noted that this radiation exposure is for research purposes only. The total amount of radiation you will receive from this study is from four (4) DXA scans and six (6) pQCT scans (18 pQCT scans for the women participating only in the radiologic portion of the study (3 total pQCT sessions).

Using the standard way of describing radiation exposure, from one DXA or pQCT scan you will receive an effective dose of less than five microSievert (μSv). By comparison the average person in the United States receives this much radiation every day from natural background sources, such as the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. In this scan, the only part of the body exposed is the skin, which is less vulnerable to radiation than most other parts of the body. The combined total effective dose you will receive is ~13.25 μSv [made up of scans for the Total Body (2.1 μSv), Lumbar Spine (2.2 μSv), Total Proximal Femur (4.6 μSv), and Total Forearm (0.03 μSv) by DXA and for the Tibia (2.9 μSv), Radius (1.4 μSv) by pQCT]. If you are only participating in the radiologic portion of the study, you will receive an additional 8.6 μSv of effective dose (total ~22 μSv for the 3 sessions). The chance anyone has of eventually dying of cancer in their lifetime is 1 in 4. After receiving radiation exposure from scans in this study, for all practical purposes, your chances of death from cancer will remain 1 in 4. This low risk is the same for those completing all aspects of the study or women participating only in the bone scans.

If you are pregnant or think you may be pregnant, you will not be permitted to participate in the study. It is best to avoid radiation to the human embryo.

Having been informed of the risks, you may choose to not complete any one, a combination, or all of these DXA and pQCT scans. If any of your scans are unreadable or unusable, replacement scans will not be conducted to avoid further exposure.

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IV. Benefits of this Project: The investigators do not guarantee any specific benefit to you as a result of being in the study. If you are participating in all aspects of the study, you will receive the following tests and results after your participation: (1) screening and scoring for OSAS (scored by a certified sleep technologist, reviewed by a physician who is a sleep medicine specialist); (2) results of blood test, including blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides; (3) nutritionist's evaluation of diet and an exercise specialist's evaluation of your physical activity and; (4) blood pressure evaluation; and (5) determination of body fat percentage, BMD and BMI. You will be encouraged to see a healthcare professional of your choice, should your sleep test results suggest this is advisable or if your bone scan or blood pressure results suggest health risks. In particular, if you score a 10 or higher on the at-home sleep test, you will be provided with a Contact Information List of sleep study physicians within the area. Any and all costs related to such referral, including further sleep evaluation, will be your responsibility and not the responsibility of the researchers or Virginia Tech.

If you are a woman participating only in the radiologic portion of the study, you will receive (1) a report of your body fat percentage, BMD and BMI and (2) a nutritionist's evaluation of your diet record and an exercise specialist's evaluation of your physical activity history record. You will be encouraged to see a healthcare professional of your choice, should your bone scan results suggest health risks. Any and all costs related to such referral will be your responsibility and not the responsibility of the researchers or Virginia Tech.

The general public may benefit from your participation in this research as new understandings between OSAS and cardiovascular, metabolic and bone function will be evaluated.

V. Extent of Anonymity and Confidentiality: Your participation in this research will not be anonymous, meaning that the researchers and the researchers' students will know your name and that you are participating in this study. In addition, other subjects who are in this study may be in the laboratory for testing at the same time as you. These subjects will know that you are participating in the study. It is possible that another subject may know your name or hear you called by your name by the investigators or the investigators' students.

Your information will be kept confidential. This means that all of your answers to questions that you are asked, measurement values, and DXA and pQCT scan results will be kept confidential and shared only with you. A three-digit code number will be assigned to you. All questionnaires, data collection sheets, data analysis sheets, blood collection and storage containers, and DXA and pQCT scan sheets will be identified by code number and not by your name. The genetic material obtained from you will not be used for any other purpose or research other than described in this protocol. Any results from the genetic portion of the study, should you participate in this, will not be disclosed to anyone else outside the research group. The blood samples obtained from you will be destroyed, and will not be held after the study is completed. A master list of subjects' code numbers will be kept in a locked filing cabinet in the lead researcher's office, separate from completed data which will also be maintained in a locked office on the Virginia Tech campus. Only the investigators and the investigators' students will be allowed access to any data.

VI. Compensation: Four prizes of $50 will be given to subjects who participate in the study. The drawing and naming of the four winners will be determined at the end of the human testing for the study, likely between January and March, 2008. All those who qualify for the study, upon completion of their first scheduled lab session will be eligible for the drawing – even those who do not complete any other aspect of the study. If you also participate in the genetic portion of the study, you also will receive $10 as compensation for donating this extra blood sample. All subjects can keep their pedometers after measurements are finished.

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VII. Freedom to Withdraw: You are free to withdraw from this study at any time, without penalty. You are free to not answer any questions or to not participate in any procedure in the study, without penalty. There may be circumstances under which the researcher may determine that you should not continue to participate in this project.

VIII. Emergency Procedure: If a minor emergency arises during your participation in any of the testing sessions of this study, you will discontinue your participation and be advised to seek care from your Primary Care Physician. If a major emergency arises during your participation in any of the testing sessions for this study, local emergency personnel will be called (911) and they will care for you. The researchers or Virginia Tech will not be responsible for any medical care or costs for same that arise directly or indirectly from of your participation in this study.

IX. Approval of Research: This research project has been reviewed, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods and Exercise.

X. Subject's Responsibilities: You voluntarily agree to participate in this study. If you are participating in all parts of the study, your responsibilities are summarized below. If you are a woman participating only in the radiologic portion of the study, your responsibilities are described as follows:

For Subjects Participating in All Aspects of the Study:

You have the following responsibilities on the first testing day:

(1) provide written consent to complete procedures for this study;
(2) arrive at Room 231 War Memorial Hall on the Virginia Tech campus on the scheduled day and time;
(3) honestly and to the best of your knowledge complete all questionnaires, including those about health history, sleep, and health-related quality of life;
(4) have your height, weight, neck, waist, and hip circumference measured by researchers;
(5) receive instruction on how to complete the home sleep test and heart monitor tests (You will be provided with the sleep test and heart monitor device.).

For the heart monitor test, you have the following responsibilities:

(1) wear the device as instructed;
(2) contact the researchers, if you have any questions or problems with the heart monitor device or how to set it up;
(3) return the heart monitor as directed along with the Embletta®.

For the at-home sleep test, you have the following responsibilities:

(1) wear the Embletta® device, as instructed;
(2) contact the researchers if you have any questions or problems with the Embletta® device or its set-up;
(3) fast (do not eat any foods or drink any fluids other than water) from about 8 PM until after the blood vessel test is completed the next morning;
(4) return the Embletta® as directed the morning after completion of the home sleep test.

For the blood vessel health test, you have the following responsibilities:

(1) refrain from any possible movement during the test;
(2) be as relaxed as you can the entire testing time.

For the second visit you have the following responsibilities:

(1) follow the instructions for completing the 4-day dietary and physical activity records;
(2) receive instruction on how to use a pedometer (You will be provided with a pedometer);

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(3) schedule your next testing session;
(4) return these records together with the pedometer at the scheduled time;

During the third testing session, you have the following responsibilities:
(1) consume no food, caffeine, or nicotine products during the 12-hour period before arriving for the blood draw ;
(2) arrive at Room 229 Wallace Hall on the Virginia Tech campus on the scheduled day after having fasted overnight;
(3) If you are female, undergo a pregnancy test;
(4) have 75 mL (~5 Tbsp) or, if you are involved in the genetic portion of the study, 120 mL (~8 Tbsp) of blood drawn from your arm by a Certified Medical Technologist trained in blood draws;
(5) consume breakfast snacks and juice;
(6) have your BMD of the whole body, lumbar spine, hip, and forearm measured by DXA;
(7) have your bone geometry and density of the lower leg (tibia) and forearm (radius) measured once by the pQCT;
(8) Follow the directions of the investigator, as related to the project.

For Subjects (Women) Participating Only in the Bone Scan Aspects of the Study:

You have the following responsibilities on the first testing day:
(1) provide written consent to complete procedures for this study;
(2) consume no food, caffeine, or nicotine products during the 12-hour period before arriving for the blood draw;
(3) arrive at Room 229 Wallace Hall on the Virginia Tech campus on the scheduled day and time;
(4) honestly and to the best of your knowledge complete all questionnaires, including those about health history, physical activity, and health-related quality of life;
(5) have your height, weight, neck, waist, and hip circumference by researchers;
(6) undergo a pregnancy test;
(7) have 75 mL (~5 Tbsp) of blood drawn from your arm by a Certified Medical Technologist trained in blood draws;
(8) consume breakfast snacks and juice;
(9) have your forearm and lower leg length measured by researchers;
(10) have your BMD of the whole body, lumbar spine, hip, and forearm measured by DXA;
(11) have your BMD and bone geometry of the lower leg (tibia) and forearm (radius) measured by pQCT;
(12) follow the instructions for completing the 4-day dietary and physical activity records;
(10) receive instruction on how to use a pedometer (You will be provided with a pedometer);
(11) schedule your next testing session;

For your second visit, you have the following responsibilities:
(1) follow the instructions for completing the 4-day dietary and physical activity records;
(2) receive instruction on how to use a pedometer (You will be provided with a pedometer);
(3) return these records together with the pedometer at the scheduled time;
(4) undergo a pregnancy test;
(5) have your bone geometry and density of the lower leg (tibia) and forearm (radius) measured by the pQCT.
(6) schedule your next testing session.

For your third visit, you have the following responsibilities:
(1) Undergo a pregnancy test;

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(2) have your BMD and bone geometry of the lower leg (tibia) and forearm (radius) measured by pQCT.
(3) Follow the directions of the investigator, as related to the project.

XI. Subject's Permission: I have read the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project. If I participate, I understand that I may withdraw at any time, without penalty. I agree to abide by the rules of this project.

Participant Signature	Date
Researcher (Witness) Signature	Date

For genetic sub-study: *If I qualify based on the screening criteria for the genetic analysis, I give my voluntary permission for an extra 45 mL of blood (3 tablespoons) to be drawn for the purpose of genetic screening which will occur in the third session of testing.*

Participant's Signature	Date
Researcher (Witness) Signature	Date

Should you have any questions about this research or its conduct, you may contact:

Dr. William G. Herbert, Principal Investigator
(540) 231-5104; wgherb@vt.edu

Dr. David M. Moore, IRB Chair
(540) 231-4991; moored@vt.edu

Adrian Aron, M.S., Coordinator
Laboratory for Health & Exercise Science
(540) 231-6376; aronady@vt.edu

Dr. Kathy Hosig, HNFE Reviewer
(540) 231-4900); khosig@vt.edu

Kyle Creamer, Coordinator
BONE Laboratory
(540) 231-7367; kcreamer@vt.edu

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Appendix E: DXA and pQCT Measurement Data Sheet

Similar recording forms for used for both the Carilion Bariatric Surgery Study and the HNFE Department Bone Study. For the Carilion study, an additional header was added to indicate whether subjects were coming in for the baseline, three or six month follow up.

Virginia Tech / Carilion Medical Center		
Subject ID ☐☐☐☐	Date ☐☐☐☐☐☐	DXA/pQCT ☐ Baseline ☐ 3 Mo. ☐ 6 Mo.

DXA/pQCT Measurement Data Sheet

D.O.B_____ **Pregnancy Test Performed** ☐

Height _____ cm/_____ in **Weight _____ lbs. / _____ kg**

BMI _____ kg/m^2

Lengths

Non-dominant Side _____

Non-dominant Tibia _____ mm Non-dominant Radius _____ mm

Appendix F: Assay procedures

- **Insulin Radioimmunoassay procedure**
- **Osteocalcin ELISA assay procedure**
- **Carboxy (C)-terminal cross-linked telopeptides of type I collagen (CTx) ELISA assay procedure**
- **Adiponectin ELISA assay procedure**
- **Leptin ELISA assay procedure**

Insulin Radioimmunoassay Procedure

A Coat-a-Count Insulin Radio Immuno Assay (RIA) was purchased from Siemens, *Los Angeles, C*A. The insulin RIA was analyzed in Dr. Frank Gwazdauskas's laboratory located in Litton Reaves, on Virginia Tech's campus in accordance with the instruction manual produced by Siemens and provided in the assay kit. The instructions are detailed below.

1. Bring serum samples to room temperature

2. Plain tubes: label four plain (uncoated) 12x75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

3. Coated tubes: label 14 Insulin Ab-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody anti-body coated tubes, as in duplicate for controls and patient samples

4. Pipet 200 uL of the zero calibrator A into the NSB and A tubes and 200 uL of each of the remaining calibrator, control and each patient sample into the tubes prepared. Pipet directly into the bottom.

5. Add 1 mL of ^{125}I Insulin to every tube. Vortex.

6. Incubate for 24 hours at room temperature.

7. Decant thoroughly.

8. Count for 1 minute in a gamma counter.

Osteocalcin ELISA Assay Procedure

An osteocalcin (1-43/49) enzyme-linked immunosorbent assay (ELISA) was purchased from Alpco, *Salem, NH*. The osteocalcin ELISA was analyzed using serum in Carilion Community Basic Research Laboratory, located in Roanoke VA with the assistance of laboratory technician Frances Philp and was performed in accordance with the instruction manual produced by Alpco and available online at http://www.alpco.com/pdfs/43/43-OSNHU-E01.pdf.

The following laboratory equipment was used in for this assay:

- Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
- Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
- BioRad Laboratories Model 680 Microplate Reader along with Microplate manager software Version 5.2.1 Build 106 (*Hercules, CA*) to read the specimen at 450 nm with 595 nm background.

Carboxy (C)-telopeptide of Type I Collagen (CTx) ELISA Assay Procedure

A human carboxy (C)-telopeptide of type I collagen (CTx) CrossLaps enzyme-linked immunosorbent assay (ELISA) was purchased from Immunodiagnostic Systems (*Scottsdale, AZ*). The CTx ELISA was analyzed using serum in Carilion Community Basic Research Laboratory, located in Roanoke VA with the assistance of laboratory technician Frances Philp and was performed in accordance with the instruction manual produced by Immunodiagnostic Systems found on their website at http://www.idsplc.com/en-us/products/product.php?id=8521.

The following laboratory equipment was used in for this assay:

- Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
- Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
- BioRad Laboratories Model 680 Microplate Reader along with Microplate manager software Version 5.2.1 Build 106 (*Hercules, CA*) to read the specimen at 450 nm with no background.

Adiponectin ELISA Assay Procedure

An adiponectin enzyme-linked immunosorbent assay (ELISA) was purchased from Alpco, *Salem, NH*. The adiponectin ELISA was analyzed using serum in Carilion Community Basic Research Laboratory, located in Roanoke VA with the assistance of laboratory technician Frances Philp and was performed in accordance with the instruction manual produced by Alpco found on their website at http://www.alpco.com/pdfs/47/47-ADPHUT-E01.pdf.

The following laboratory equipment was used in for this assay:

- Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
- Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
- BioRad Laboratories Model 680 Microplate Reader along with Microplate manager software Version 5.2.1 Build 106 (*Hercules, CA*) to read the specimen at 490 nm with no background.

Leptin ELISA Assay Procedure

A leptin enzyme-linked immunosorbent assay (ELISA) was purchased from Alpco, *Salem, NH*. The leptin ELISA was analyzed was analyzed using serum in Carilion Community Basic Research Laboratory, located in Roanoke VA with the assistance of laboratory technician Frances Philp and was performed in accordance with the instruction manual produced by Alpco found on their website, http://www.alpco.com/pdfs/11/11-LEPHU-E01.pdf.

The following laboratory equipment was used in for this assay:

- Shaker, Model 2314FS, Fischer Scientific (*Dubuque, IO*), shaker at room temperature.
- Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
- BioRad Laboratories Model 680 Microplate Reader along with Microplate manager software Version 5.2.1 Build 106 (*Hercules, CA*) to read the specimen at 450 nm with no background.

Appendix G: Raw Data Chapter 3

Chapter 3: An Exploratory Study of Bone Changes Following RYGB and LAGB: Impact on Bone Biomarkers, Adiponectin, Leptin, Vitamin D and Calcium

- **Table 1. RYGB and LAGB baseline, three month, six month anthropometrics**
- **Table 2. RYGB and LAGB baseline, three month, six month DXA body composition**
- **Table 3. RYGB and LAGB baseline, three month, six month DXA bone measurements**
- **Table 4. RYGB and LAGB baseline, three month, six month blood nutrient measures**
- **Table 5. RYGB and LAGB baseline, three month, six month metabolic and bone markers**
- **Table 6. Baseline, three and six month measurements following RYGB and LAGB surgery.**
- **Table 7. Percent change from baseline measurements at three and six months following bariatric surgery.**

Table 1. RYGB and LAGB baseline, three month, six month anthropometrics

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement; N/A, data not available.

	Age (yr)	Height (cm)	Weight (kg)			Body Mass Index (mg/kg^2)			Waist Circumference (cm)			Hip Circumference (cm)		
Code			**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	42	169.8	108.1	98.1	99.2	37.5	34.0	34.4	107	108	102	132	132	123
BR_012	42	167.5	117.1	94.0	87.4	41.8	33.5	31.2	123.5	100.5	97.5	137	121	100
BR_013	45	155.2	114.5	89.8	79.4	47.5	37.3	33	115	94.5	87	142	122	117
BR_020	43	170.1	109.5	85.5	79.1	37.9	29.6	27.3	128	128	99	135	135	116
BL_021	40	165.3	107.7	99.1	94.1	39.4	36.3	34.4	120	107	102	135	125	124
BL_026	28	160.0	114.4	107.2	104.1	44.7	41.9	40.7	107	96	114	147	139	138
BR_033	34	157.4	114.1	94.5	93.4	46.0	38.1	37.7	128.5	108	N/A	142.5	128	N/A
BL_41	21	165.0	126.8	112.6	108.5	46.8	41.6	40.0	108	97	96	135	126	117
BR_042	37	161.0	108.7	92.0	80.8	42.2	35.7	31.4	104	94.5	90	136.5	124	113
Mean ± SD	36.9 ± 7.9	163.4 ± 5.4	113.4 ± 6.1	96.9 ± 8.5	91.8 ± 10.9	42.6 ± 3.8	36.4 ± 3.9	34.5 ± 4.4	116.7 ± 9.6	103.7 ± 10.7	98.4 ± 8.3	138 ± 4.8	127.9 ± 6.2	118.5 ± 10.8
RYGB	40.2 ± 4.5	162.1 ± 6.4	112.8 ± 3.6	91.1 ± 3.7	84 ± 6.2	43.1 ± 3.9	34.8 ± 3.4	32.1 ± 3.8	119.8 ± 10.4	105.1 ± 14	93.4 ± 5.8	138.6 ± 3.4	125.9 ± 5.8	111.5 ± 7.9
LAGB	32.8 ± 10	164.9 ± 4.0	114.3 ± 8.9	104.3 ± 7	101.5 ± 6.2	42.1 ± 4.4	38.5 ± 3.9	37.4 ± 10	110.6 ± 6.3	102 ± 6.4	103.5 ± 7.5	137.3 ± 6.7	130.5 ± 6.5	125.5 ± 8.9

Table 2. RYGB and LAGB baseline, three month, six month DXA body composition

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement.

	Total body fat (%)			Central body fat (%)			Total fat mass (g)			Fat free soft tissue mass (g)		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	46.0	45.0	42.6	46.1	48.0	41.5	50232.5	44564.3	42649.6	57049.9	52450.1	55566.8
BR_012	40.8	33.5	26.9	46.7	40.9	32.6	48609.0	32078.7	23780.2	67893.9	61184.5	62147.2
BR_013	53.8	41.8	43.6	50.8	38.9	42.0	61427.2	37684.3	35243.1	50216.5	49824.2	42961.9
BR_020	43.1	36.5	31.8	48.2	41.6	32.3	47770.4	31243.1	25460.5	60252.1	51728.7	52006.9
BL_021	48.0	45.9	42.2	51.7	54.9	45.0	51738	45413	40083.5	54031.2	51333.3	52717.6
BL_026	52.6	50.0	49.1	57.2	52.6	54.7	60364.9	53829.9	51339.8	52126.7	51498.4	51038.4
BR_033	44.2	41.2	40.7	46.2	43.2	41.9	50389.9	39089.3	39063.8	61560.0	53768.8	54799.4
BL_41	49.5	47.5	46.1	52.9	49.7	48.8	62774.6	53632.4	50677.4	61591.8	56732.6	56672.8
BR_042	52.4	52.4	47.3	59.7	56.6	46.5	57149.8	48782	38922.4	50051.5	42306.1	41373.4
Mean ± SD	47.8 ± 4.6	43.8 ± 6.1	41.1 ± 7.3	51.1 ± 4.9	43.4 ± 6.5	42.8 ± 7.2	54495.1 ± 5922.4	42924.1 ± 8463.4	38580 ± 9556.6	57197.1 ± 6099.6	52314.1 ± 5107.2	52152.7 ± 6531
RYGB	46.9 ± 5.8	41.1 ± 7.2	38.1 ± 8.5	50.3 ± 5.5	44.2 ± 7.1	39.1 ± 6.3	53069.3 ± 5951.0	37775.5 ± 7035.7	32494 ± 7373.0	57994.8 ± 7736.3	51762.5 ± 6818.7	50657.8 ± 8608.2
LAGB	49 ± 2.8	47.1 ± 2.2	45 ± 3.2	52.0 ± 4.6	51.3 ± 3.1	47.5 ± 5.6	56277.5 ± 6220.1	49359.9 ± 5060.0	46187.6 ± 5617.0	56199.9 ± 4126.7	52002.6 ± 2534.3	53998.9 ± 2582.9

Table 3. RYGB and LAGB baseline, three month, six month DXA bone measurements

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement.

	Total Body Bone Mineral Density (g/cm^2)			Total Bone Mineral Content (g)			Lumbar Spine Bone Mineral Density (g/cm^2)			Lumbar Spine Bone Mineral Content (g)		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	1.014	1.023	1.012	1980.75	2046.43	2012.75	0.958	1.018	0.951	56.58	59.72	54.58
BR_012	1.182	1.161	1.152	2569.32	2561.88	2496.35	1.092	1.108	1.048	64.86	68.80	63.58
BR_013	1.289	1.325	1.298	2562.20	2587.36	2575.12	1.180	1.158	1.152	61.40	62.11	61.60
BR_020	1.245	1.273	1.243	2769.23	2705.48	2642.98	1.157	1.14	1.129	61.67	63.91	61.27
BL_021	1.071	1.084	1.078	2122.21	2215.69	2120.25	1.095	0.821	0.997	61.80	38.54	52.59
BL_026	1.095	1.111	1.120	2242.13	2276.53	2230.24	1.098	1.111	1.036	53.89	51.73	48.38
BR_033	1.087	1.056	1.056	2176.07	2087.98	2019.48	0.804	0.902	0.859	39.19	44.64	44.86
BL_41	1.157	1.191	1.154	2465.35	2520.94	2483.48	1.054	1.093	1.074	52.28	56.46	57.23
BR_042	1.043	1.056	1.035	1936.07	1987.40	1941.05	1.003	1.028	1.008	45.78	49.56	49.36
Mean ± SD	1.132 ± 0.09	1.142 ± 0.1	1.128 ± 1	2313.7 ± 289.8	2332.2 ± 266.9	2280.2 ± 271.4	1.048 ± 0.1	1.042 ± 0.1	1.028 ± 0.9	55.3 ± 8.5	55.1 ± 9.8	54.8 ± 6.6
RYGB	1.170 ± 0.1	1.176 ± 0.1	1.576 ± 0.1	2402.6 ± 337.9	2386.0 ± 324.5	2335.0 ± 329.1	1.046 ± 0.2	1.068 ± 0.1	1.039 ± 0.2	58.5 ± 11.4	57.8 ± 10.2	56.1 ± 8.4
LAGB	1.085 ± 0.06	1.100 ± 0.07	1.091 ± 0.06	2202.6 ± 205.2	2264.9 ± 196.5	2211.7 ± 201.8	1.053 ± 0.07	1.010 ± 0.1	1.015 ± 0.05	56.1 ± 4.2	51.6 9.3	53.2 3.7

Table 3. RYGB and LAGB baseline, three month, six month DXA bone measurements continued

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement.

	Lumbar T-Score			Hip Bone Mineral Density (g/cm^2)			Hip Bone Mineral Content (g)			Hip T-Score		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	-1.17	-0.85	-1.15	0.801	0.772	0.789	23.64	23.03	25.23	-1.15	-1.39	-1.25
BR_012	0.4	0.69	0.11	1.053	0.912	0.876	42.19	34.56	33.37	0.91	-0.24	-0.54
BR_013	1.02	0.94	1.03	1.038	0.991	0.996	28.57	27.38	29.76	0.79	0.40	0.44
BR_020	0.84	0.86	0.84	1.145	1.090	1.039	38.44	37.25	35.40	1.67	1.21	0.80
BL_021	0.36	-2.14	-0.66	0.947	0.980	0.953	29.72	31.39	32.22	0.04	0.31	0.09
BL_026	0.28	0.41	-0.39	0.834	0.838	0.838	25.65	25.22	26.52	-0.88	-0.85	-0.85
BR_033	-2.77	-1.68	-2.08	0.939	0.901	0.908	29.38	28.66	29.19	-0.02	-0.33	-0.28
BL_41	-0.21	0.25	0.09	1.091	1.121	1.078	31.93	34.41	32.29	1.22	1.47	1.11
BR_042	-0.48	-0.38	-0.67	0.847	0.844	0.822	25.05	24.56	23.78	-0.77	-0.8	-0.99
Mean ± SD	-0.2 ± 1.2	-0.2 ± 1.1	-0.3 ± 1.0	0.9767± 0.1	0.938 ± 0.1	0.922 ± 0.1	30.5 ± 6.2	29.6 ± 5.0	29.8 ± 3.9	0.2 ± 1.0	-0.02 ± 1.1	-0.2 ± 0.8
RYGB	-0.2 ± 1.6	0.09 ± 1.1	-0.2 ± 1.3	1.00 ± 0.1	0.946 ± 0.1	0.928 ± 0.09	32.7 ± 7.2	30.5 ± 5.3	30.3 ± 4.5	0.5 ± 0.9	0.05 ± 0.8	-0.1 ± 0.7
LAGB	-0.2 ± .7	-0.6 ± 1.2	-0.5 ± 0.5	0.918 ± 0.1	0.928 ± 0.2	0.915 ± 0.1	27.7 ± 3.8	28.5 ± 5.3	29.1 ± 3.7	-.19 ± 1.1	-.1 ± 1.3	-.2 ± 1.1

Table 3. RYGB and LAGB baseline, three month, six month DXA bone measurements continued

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement; N/A, data not available.

	Radius Bone Mineral Density (g/cm^2)			Radius Bone Mineral Content (g)			Radius T-Score		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	0.57	0.56	0.57	6.73	6.73	5.12	-0.14	-0.30	-0.20
BR_012	0.61	0.63	0.64	7.94	7.87	8.47	0.53	0.85	1.06
BR_013	0.64	0.64	0.63	6.52	6.54	6.44	1.12	1.16	0.87
BR_020	0.69	0.70	0.68	8.30	8.50	8.41	2.08	2.3	1.92
BL_021	0.60	0.60	0.62	N/A	7.55	7.17	0.63	0.30	0.67
BL_026	0.66	0.64	0.64	8.17	8.27	8.06	1.50	1.12	1.19
BR_033	0.66	0.62	0.64	5.25	6.75	6.79	1.50	0.76	1.13
BL_41	0.70	0.68	0.72	8.74	8.68	7.48	2.26	1.80	2.66
BR_042	0.62	0.63	0.64	5.57	5.57	5.60	0.80	0.86	1.14
Mean ± SD	0.639 ± 0.04	0.632 ± 0.04	0.642 ± 0.04	7.7 ± 2.1	7.4 ± 1	7.1 ± 1.1	1.1 ± 0.8	1 ± 0.8	1.2 ± 0.8
RYGB	0.644 ± 0.3	0.643 ± 0.03	0.645 ± 0.02	6.7 ± 1.4	7 ± 1.2	7.1 ± 1.3	1.2 ± 0.6	1.2 ± 0.6	1.2 ± 0.4
LAGB	0.632 ± 0.06	0.619 ± 0.05	0.637 ± 0.07	9.0 ± 2.4	7.8 ± 0.9	7 ± 1.3	1.1 ± 1	0.7 ± 0.9	1.1 ± 1.2

Table 4. RYGB and LAGB baseline, three month, six month blood measures

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement; N/A, data not available.

	25 Hydroxy Vitamin D Total EIA (ng/L)			Calcium (mg/dL)			Parathyroid Hormone (pg/mL)		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	47.0	40.0	40.0	9.4	8.8	9.0	N/A	N/A	N/A
BR_012	54.0	38.0	29.0	9.4	9.3	9.5	N/A	N/A	42.7
BR_013	30.0	28.0	28.0	9.3	9.7	9.9	N/A	66.3	86.5
BR_020	53.0	42.0	52.0	9.4	9.3	9.4	N/A	39.2	48.2
BL_021	78.0	31.0	30.0	8.3	9.2	9.2	N/A	N/A	N/A
BL_026	25.0	26.0	25.0	N/A	9.4	9.2	N/A	N/A	53.5
BR_033	21.0	24.0	17.0	9.0	9.4	9.6	N/A	37.6	51.8
BL_41	30.0	31.0	40.0	8.6	9.5	9.2	N/A	16.2	12.7
BR_042	N/A	32.0	30.0	8.0	9.8	9.2	N/A	37.9	31.8
Mean ± SD	42.3 ± 19.3	32.4 ± 6.3	32.3 ± 10.2	8.9 ± 0.6	9.4 ± 0.3	9.4 ± 0.3	N/A	35.2 ± 19	46.7 ± 22.6
RYGB	39.5 ± 16.6	32.8 ± 7.3	31.2 ± 12.8	9 ± 0.6	9.5 ± 0.2	9.5 ± 0.3	N/A	18.5 ± 8.3	20.6 ± 9.2
LAGB	45.0 ± 23.9	32 ± 5.8	33.8 ± 7.5	8.7 ± 0.6	9.2 ± 0.3	9.2 ± 0.1	N/A	N/A	28.8 ± 20.4

Table 5. RYGB and LAGB baseline, three month, six month metabolic and bone markers

Color coding: White = RYGB; gray = LAGB.
B, baseline; 3, three month measurement; 6, six month measurement; N/A, data not available.
Means ± SD for all subjects, RYGB and LAGB patients are at the end of each table.

	Osteocalcin (ng/mL)			CTx (ng/mL)			Adiponectin (µg/mL)			Leptin(ng/mL)		
Code	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**	**B**	**3**	**6**
BL_003	N/A	8.31	10.08	N/A	0.43	0.25	N/A	2.49	3.09	N/A	68.86	72.56
BR_012	N/A	19.39	32.37	N/A	1.22	1.18	N/A	3.24	4.67	N/A	31.59	16.24
BR_013	17.78	20.10	23.31	0.46	1.06	0.85	14.94	17.19	19.11	92.80	68.36	50.72
BR_020	3.79	13.94	21.78	0.26	1.06	0.84	3.19	5.6.0	6.70	73.41	26.72	19.99
BL_021	6.39	8.33	10.05	0.42	0.61	0.4	3.94	3.95	5.22	81.22	77.48	82.06
BL_026	8.89	13.20	10.24	0.18	0.61	0.56	6.44	7.25	5.55	107.76	78.47	97.04
BR_033	1.14	12.99	25.69	0.15	0.42	0.51	5.56	5.34	5.57	53.54	25.48	29.88
BL_41	12.58	17.36	13.35	0.46	0.78	0.62	5.69	6.02	6.17	74.88	78.99	86.20
BR_042	N/A	8.86	12.0	N/A	0.64	0.66	N/A	4.2.0	4.99	N/A	81.29	48.0
Mean ± SD	8.4 ± 6.1	13.6 ± 4.6	17.7 ± 8.3	0.32 ± 0.14	0.76 ± 0.29	0.65 ± 0.28	6.6 ± 4.2	6.1 ± 4.4	6.8 ± 4.7	80.6 ± 18.5	59.7 ± 24.3	55.9 ± 30.0
RYGB	7.6 ± 8.9	15.1 ± 4.7	23 ± 7.4	0.29 ± 0.16	0.88 ± 0.34	0.81 ± 0.25	7.9 ± 6.2	7.1 ± 5.7	8.2 ± 6.1	73.3 ± 19.6	46.7 ± 26.2	33 ± 15.8
LAGB	9.3 ± 3.1	11.8 ± 4.4	10.9 ± 1.6	0.35 ± 0.15	0.61 ± 0.14	0.46 ± 0.17	5.4 ± 1.3	4.9 ± 2.1	5 ± 1.3	88 ± 17.4	76 ± 4.8	84.5 ± 10.1

Table 6. Baseline, three, and six month measurements following RYGB and LAGB surgery.

	RYGB (n=5)			**LAGB** (n=4)		
	Baseline	**3 months**	**6 months**	**Baseline**	**3 months**	**6 months**
Anthropometric data (n=9)						
Age (yrs)	40.2 ± 4.5	--	--	32.8 ± 10.0	--	--
Weight (kg)	112.8 ± 3.6	91.2 ± 3.7	84.0 ± 6.2	114.3 ± 8.9	104.3 ± 6.9*	101.5 ± 6.2*
BMI (kg/m^2)	43.1 ± 3.8	34.8 ± 3.4	32.1 ± 3.8	42.1 ± 4.4	38.5 ± 3.9	37.4 ± 3.4
Waist circumference (cm)	119.8 ± 10.4	105.1 ± 14	93.4 ± 5.8	110.6 ± 6.3	102 ± 6.4	103.5 ± 7.5
Hip circumference (cm)	138.6 ± 3.4	125.9 ± 5.8	125.5 ± 8.9	137.3 ± 6.7	130.5 ± 6.5	125.5 ± 8.9
DXA body composition data (n=9)						
Total body fat (%)	46.9 ± 5.8	41.1 ± 7.2	38.1 ± 8.5	49 ± 2.8	47.1 ± 2.2	45 ± 3.2
Central body fat (%)	50.3 ± 5.5	44.2 ± 7.1	39.1 ± 6.3	52.0 ± 4.6	51.3 ± 3.1	47.5 ± 5.7
Fat mass (kg)	53.1 ± 6.0	37.8 ± 7.0	32.5 ± 7.3	56.3 ± 6.2	49.4 ± 5.1*	46.2 ± 5.7*
Fat free soft tissue mass (kg)	58.0 ± 7.7	51.8 ± 6.8	50.6 ± 8.6	56.2 ± 4.1	53.3 ± 2.5	54 ± 2.6
Blood markers related to nutrient status (n=9)						
Calcium Clinical range: 8.5-10.7 mg/dL	9.0 ± 0.6	9.5 ± 0.2	9.5 ± 0.3	8.8 ± 0.6	9.2 ± 0.3	9.2 ± 0.1*
Parathyroid hormone Clinical range: 9.2-79.5 pg/mL	N/A	39.5 ± 18.5	52.2 ± 20.6	N/A	16.2	33.1 ± 28.8
$25(OH)_2$ vitamin D Clinical range: 32-100 ng/mL	39.5 ± 16.6	32.8 ± 7.3	31.2 ± 12.8	45 ± 23.9	32 ± 5.8	33.8 ± 7.5
Bone and metabolic biomarkers[1] (n=6, 3 RYGB, 3 LAGB)						
Adiponectin (µg/mL)	7.9 ± 6.2	7.1 ± 5.7	8.2 ± 6.1	5.4 ± 1.3	4.9 ± 2.1	5 ± 1.3
Leptin (ng/mL)	73.3 ± 19.6	46.7 ± 26.2	33 ± 15.8	88 ± 17.4	76 ± 4.8	84.5 ± 10.1†
CTx (ng/mL)	0.29 ± 0.16	0.88 ± 0.34	0.81 ± 0.25	0.35 ± 0.15	0.61 ± 0.14	0.46 ± 0.17*
Osteocalcin (ng/mL)	7 ± 8.6	15.2 ± 8.2	24 ± 8.2	7.2 ± 1.7	9.6 ± 2.8	8.9 ± 1.5*
CTx/OC ratio	0.076 ± 0.05	0.059 ± 0.02	0.037 ± 0.01	0.041 ± 0.02	0.054 ± 0.01	0.041 ± 0.01

Mean ± SD, N/A, data not available. Differences between RYGB and LAGB surgical groups, †$p<0.01$,*$p<0.05$.

Table 7. Percent change from baseline measurements at three and six months following bariatric surgery.

	RYGB (n=5)		**LAGB** (n=4)	
	3 months (% change)	**6 months** (% change)	**3 months** (% change)	**6 months** (% change)
Anthropometric data				
Weight (kg)	-21.6 ± 1.5	-28.8 ± 2.3	-10 ± 1.5†	-12.8 ± 2.1†
DXA bone mineral density (g/cm^2)				
Total BMD	0.3 ± 1.1	-1.1 ± 0.7	1.6 ± 0.5	0.6 ± 0.6
Lumbar spine BMD	2.5 ± 2.5	-0.3 ± 1.9	-3.5 ± 7.3	-3.4 ± 2.4
Non-dominant Hip BMD	-5.4 ± 2.2	-7.3 ± 2.6	0.8 ± 1.6	-0.4 ± 0.6
Non-dominant Radius BMD	-0.1 ± 1.6	0.3 ± 1.5	-2.1 ± 0.8	0.8 ± 1.4
DXA bone mineral content (g)				
Total BMC	-0.6 ± 2.6	-2.8 ± 3.3	2.9 ± 1.3*	0.4 ± 0.9
Lumbar spine BMC	6.6 ± 4.9	4 ± 7	-7 ± 21	-4.8 ± 10.6
Non-dominant Hip BMC	-6 ± 6.8	-6.1 ± 9.5	2.3 ± 5.2	4.9 ± 3.3
Non-dominant Radius BMC	6.1 ± 12.6	7.3 ± 12.6	-9.5 ± 19.5	-20.4 ± 17
Bone and metabolic biomarkers				
Adiponectin (µg/ml)	28.8 ± 23.9	45.9 ± 32.9	6.2 ± 3.6	9.0 ± 13.3
Leptin (ng/ml)	-47.4 ± 11	-54.1 ± 9.3	-8.8 ± 9.7	2.1 ± 7.2*
CTx (ng/ml)	203.6 ± 51.8	179.3 ± 47.5	118.4 ± 61.6	81.2 ± 68.1
Osteocalcin (ng/ml)	440.4 ± 308.7	886.8 ± 646.4	38.9 ± 5.3	26.1 ± 15.7

Means ± SEM. Differences between RYGB and LAGB surgical groups, †$p<0.01$,*$p<0.05$.

Appendix H: Raw Data Chapter 4

Chapter 4: Fat and muscle distribution assessed by pQCT: Relationships with physical activity and type-2 diabetes risk

- **Table 1. Anthropometrics**
- **Table 2. Blood measurements**
- **Table 3. pQCT foreleg data**
- **Table 4. Physical activity data**
- **Figure 1. Foreleg muscle density CV**
- **Figure 2. Foreleg IMAT CV**

Table 1. Anthropometrics

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body fat (%)	Central body fat (%)	Fat mass (g)	Lean body mass (g)	SBP (mm HG)	DBP (mm HG)
B01	43	169.1	62.4	21.8	27.4	20.9	17315.9	43369.2	118	78
B02	46	166.6	75.3	27.1	37.2	37.8	28190.2	45650.2	122	86
B03	40	166.2	57.7	20.9	23.1	20.9	13508.2	42416.5	132	86
B04	31	162.6	73.0	27.6	29.7	33.1	21988.8	495552.6	N/A	N/A
B05	37	166.1	57.0	20.7	30.4	28.1	17623	38212.6	122	68
B06	36	159.3	54.7	21.6	23.8	16	13190.2	39981.6	98	58
B07	46	165.5	57.2	20.9	16.3	10.9	9452.7	45938.8	104	58
B08	43	157.9	58.7	23.6	32.2	20.2	19180.7	38462.1	118	70
B09	45	167.7	84.1	29.9	33.0	32.3	28043.3	54385.9	116	70
B10	33	161.0	62.2	24.0	25.9	18.1	16671.7	45152.3	122	80
B11	37	171.1	72.4	24.7	33.9	22.3	24892.7	46361.8	112	68
B12	40	160.1	81.2	31.7	39.9	41.0	32847.1	47250.2	138	78
B13	44	160.2	58.5	22.8	26.5	20.0	15685.7	41491.7	104	68
B14	37	165.3	80.7	29.5	34.6	36.8	28274	51290.1	180	100
B15	39	168.1	51.6	18.3	25.2	11.4	13167.4	36705.7	100	60
B16	42	164.8	57.9	21.3	21.3	11.6	13461.8	43606.8	N/A	N/A
B17	42	161.4	59.3	22.8	35.0	38.4	20934.8	37010.0	106	64
B18	44	165.4	54.9	20.1	19.7	16.6	10952.2	42326.7	112	70
B19	32	162.1	68.8	26.2	31.9	34.6	22064.5	44594.1	112	80
B20	38	173.8	134.3	44.5	47.2	48.1	63027.8	67625.4	138	80
B21	35	156.4	98.4	40.2	47.2	48.7	46997.9	50177.1	122	74
B22	45	162.8	50.1	18.9	20.5	9.3	10372.3	37989.1	N/A	N/A
B23	32	166.3	73.4	26.6	37.6	39.2	27823.6	44009.1	112	68
B24	41	166.3	56.0	20.3	22.7	14.9	12859.1	41546.8	104	70
B25	38	158.1	52.9	21.2	30.4	24.5	16362.1	35321.9	122	80
B26	37	160.1	74.2	28.9	39.7	41.8	29770.6	43252.9	118	78
B27	37	141.1	71.7	36.0	43.1	44.5	31294.1	39726.6	N/A	N/A
B28	32	177.3	93.2	29.6	39.3	32.4	36949.6	54657.2	124	72
B29	34	160.2	57.1	22.3	23.6	21.0	13668.1	42007.5	108	64
B30	32	163.4	74.0	27.7	41.2	32.0	30620.7	41448.2	116	74

Table 1. Anthropometrics continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m2)	Body fat (%)	Central body fat (%)	Fat mass (g)	Lean body mass (g)	SBP (mm HG)	DBP (mm HG)
B31	32	177.7	100.2	31.7	42.1	40.4	42605.7	55947	106	76
B32	44	159.2	68.0	26.8	33.9	34.4	32437.7	43612.8	134	90
B33	38	167	66.7	23.9	33.5	29.7	22749.8	42877.5	118	78
B34	41	178.7	87.8	27.5	37.3	31.2	33102.7	52750.0	138	80
B35	41	158.8	58.9	23.4	32.4	34.1	19432.1	38406.3	104	82
B36	44	172.4	71.0	23.9	31.9	22.3	23054.4	46940.3	102	74
B37	40	160.9	68.4	26.4	37.3	33.8	25513.4	41061.2	118	94
B38	36	171.3	77.9	26.5	28.9	28.4	22827.1	53670.1	118	76
B39	41	153.5	63.6	27.0	36.2	30.1	23346.2	38958.5	122	78
B40	34	159.3	63.4	25.0	24.4	20.6	15738.4	46524	106	68
B41	41	158.7	55.5	22.0	27.7	20.9	15680.1	38690.7	108	80
B42	35	169.1	66.1	23.1	31.1	25.5	20689.3	43596.1	122	82
B43	38	164.0	87.4	32.5	40.8	34.9	36186.1	50085.7	124	82
B44	31	155.9	113.2	46.6	51.6	47.2	59227.9	53094.9	128	76
B45	39	170.6	56.8	19.5	19.8	15.2	11551.6	44048.1	118	72
B46	34	163.1	64.2	24.1	23.6	17.3	15301.2	47074	120	74
B47	42	164.2	51.8	19.2	17.6	11.5	9268.6	41584.1	110	64
B48	45	151.0	45.0	19.7	20.1	14.4	9201.9	34515.2	118	70
B49	42	163.6	55.2	20.6	22.0	10.5	12409.9	41555.7	120	80
B50	38	164.4	64.2	23.7	26.5	25.5	17307.9	45604.7	114	76
B51	38	171.4	55.6	18.9	19.1	12.6	10769.6	43252.0	110	68
B52	40	163.3	59.9	22.5	23.7	20.6	14195.5	43466.4	118	80
B53	32	159.3	56.2	22.1	24.9	22.0	14023.1	40101.3	120	82
B54	40	169.3	63.6	22.2	24.3	20.9	15742.7	46552.9	112	70
B55	44	157.0	52.8	21.4	22.4	12.1	12070.5	39536.6	118	78
B56	38	160.5	61.3	23.8	35.7	38.0	22178.0	37585.1	104	70
B57	37	179.9	79.4	24.5	31.2	21.7	25228.5	53244	120	74
B58	31	178.1	75.9	23.9	29.8	28.5	23017.7	51730.4	122	76
B59	43	177.0	76.1	24.3	26.6	29.3	20472.7	54165.6	110	68
B60	40	160.2	60.6	23.6	25.3	17.7	15678.2	43670.0	110	72

Table 1. Anthropometrics continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects.

Code	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body fat (%)	Central body fat (%)	Fat mass (g)	Lean body mass (g)	SBP (mm HG)	DBP (mm HG)
B61	42	164.4	60.8	22.5	27.2	22.4	16691.6	42530.8	102	60
B62	29	185.5	85.7	24.9	30.8	26.9	26638.6	56854.6	102	60
B63	44	174.9	67.8	22.2	15.8	8.9	10856.7	55130.4	104	64
B64	44	160.0	57.8	22.6	25.6	27	15018.2	41538.5	120	72
B65	43	167.0	65.5	23.5	23.5	16.4	15683.5	48611.9	122	70
B66	43	164.5	65.3	24.1	27.6	23.3	18199.2	45507.1	120	78
B67	30	165.1	70.5	25.9	32.3	28.7	23068.2	45904.6	116	74
B68	42	166.6	57.1	20.6	20.8	12.3	12040.6	43482.3	122	70
B69	40	166.8	60.4	21.7	24.2	17.0	14919.6	44168.9	110	70
B70	45	172.2	82.6	27.9	40.9	40.5	34241.3	46799.8	112	70
B71	35	165.3	52.5	19.2	28.1	26.0	14999.2	36320.1	102	58
B72	41	180.1	78.2	24.1	29.0	22.9	22849.7	53235.8	118	78
B73	31	156.8	54.2	22.1	31.7	27.2	1736	35342	122	68
B74	31	169.1	69.4	24.3	27.3	27.4	19351.9	48964.8	112	80
B75	31	159.9	58.2	22.8	20.9	19.2	12320.8	44481.9	110	66
B76	44	170.9	55.2	18.9	25.7	18.2	14424.6	39834	102	60
B77	45	169.1	64.7	22.6	30	29.8	19630.7	43361.9	120	82
B78	41	171.0	63.2	21.6	25.6	21.5	16369.8	45175.1	120	70
B79	34	176.7	58.8	18.8	20.5	13.3	12304.5	44821	118	70
B80	42	162.1	52.7	20.0	24.5	14.4	13133.2	3312.4	114	64
B81	32	157.3	62.1	25.1	26.6	20.6	16799.8	43903.7	120	70
B82	42	152.3	52.2	22.5	10.6	8.9	5586.7	44925	122	78
Means ± SD	39 ± 5	165.2 ± 7.4	66.9 ± 14.7	24.5 ± 5.1	29.2 ± 7.8	25.1 ± 10.1	20218 ± 9220.9	44098.8 ± 7026.8	116 ± 12	73 ± 8
High Active	40 ± 5	166.2 ± 8.2	62.1 ± 9.5	22.4 ± 2	23.7 ± 5.4	18.8 ± 6.8	15290.8 ± 5253.1	45338.9 ± 5474.3	114 ± 8	72 ± 7
Mod. Active	38 ± 5	164.9 ± 7	62.8 ± 9.8	23.1 ± 3	28.7 ± 5.5	23.9 ± 8.2	18585.6 ± 5845.7	41443.2 ± 9311	114 ± 8	72 ± 7
Low Active	38 ± 4	164.5 ± 7.5	74.3 ± 7.5	27.4 ± 6.1	34.5 ± 7.4	31.8 ± 10.2	25879 ± 9552	45023.4 ± 5371.4	120 ± 17	76 ± 9

Table 2. Blood measurements

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	Blood Glucose (mg/dL)	Insulin (uUI/mL)	HOMA-IR
B01	260	>100	N/A	62	75	9.3	1.72
B02	237	50	141	227	95	19.7	4.62
B03	194	88	100	<45	79	7.2	1.40
B04	176	57	N/A	<45	96	N/A	N/A
B05	190	36	148	<45	81	27.2	5.44
B06	190	62	110	87	95	8.8	2.06
B07	188	69	107	59	83	12.1	2.48
B08	174	88	76	48	80	17.2	3.40
B09	100	38	55	<45	82	22.5	4.56
B10	188	68	111	48	76	6.8	1.28
B11	146	72	65	47	84	8.2	1.70
B12	138	54	59	125	86	23.6	5.01
B13	183	88	81	73	81	7.3	1.46
B14	184	40	122	110	90	16.6	3.69
B15	133	76	50	<45	79	13.7	2.67
B16	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B17	161	46	109	<45	96	16.9	4.01
B18	130	67	N/A	<45	85	6.2	1.30
B19	144	48	89	<45	72	8.9	1.58
B20	233	32	184	83	93	43.5	9.99
B21	162	33	114	75	78	37.1	7.15
B22	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B23	152	62	64	130	82	12.1	2.45
B24	186	56	109	106	87	19.9	4.27
B25	185	77	99	<45	87	10.8	2.32
B26	165	46	98	106	77	6.1	1.16
B27	214	90	111	65	97	N/A	N/A
B28	135	42	71	113	83	36.0	7.38
B29	175	74	83	87	85	19.0	3.99
B30	172	55	90	136	85	13.1	2.75

Table 2. Blood measurements continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	Blood Glucose (mg/dL)	Insulin (uUI/mL)	HOMA-IR
B31	164	34	108	111	84	30.2	6.26
B32	223	57	146	102	85	14.9	3.13
B33	137	49	79	46	86	11.9	2.53
B34	126	38	69	94	88	6.1	1.33
B35	252	72	159	106	84	8.3	1.72
B36	184	41	126	84	89	16.6	3.65
B37	170	50	103	83	82	15.2	3.08
B38	187	49	125	67	83	14.2	2.91
B39	170	53	108	47	84	6.9	1.43
B40	163	46	100	82	94	16.4	3.81
B41	198	70	121	<45	82	7.8	1.58
B42	134	58	62	66	77	10.3	1.96
B43	169	39	95	175	99	61.8	15.11
B44	178	62	99	89	84	23.3	4.83
B45	161	70	84	45	80	6.1	1.20
B46	145	46	81	91	102	6.1	1.54
B47	182	52	111	95	97	6.1	1.46
B48	191	74	90	134	73	6.1	1.10
B49	185	58	109	87	83	6.1	1.25
B50	219	40	148	153	93	8.2	1.88
B51	135	81	42	58	93	6.1	1.40
B52	150	40	95	75	74	6.1	1.11
B53	131	58	53	99	86	6.1	1.30
B54	147	76	64	45	90	6.1	1.36
B55	156	58	90	45	95	6.1	1.43
B56	175	51	117	45	76	6.1	1.14
B57	147	58	81	45	77	6.1	1.16
B58	193	59	117	89	81	6.1	1.22
B59	179	36	11	146	88	6.1	1.33
B60	136	57	69	52	93	6.1	1.40

Table 2. Blood measurements continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.

Code	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	Blood Glucose (mg/dlL	Insulin (uUI/mL)	HOMA-IR
B61	153	56	90	45	83	6.1	1.25
B62	145	60	76	45	92	6.1	1.39
B63	207	68	125	65	74	6.1	1.11
B64	173	71	90	62	96	6.1	1.45
B65	165	N/A	N/A	45	99	6.1	1.49
B66	146	40	89	81	80	6.1	1.20
B67	207	72	111	121	83	6.1	1.25
B68	173	81	83	45	84	6.1	1.27
B69	127	61	42	120	92	6.1	1.39
B70	181	48	106	135	103	6.1	1.55
B71	167	68	83	79	95	6.1	1.43
B72	153	55	79	153	88	6.1	1.33
B73	155	52	83	155	88	6.1	1.33
B74	153	40	102	53	90	6.1	1.36
B75	199	89	96	73	87	6.1	1.31
B76	143	63	62	90	87	6.1	1.31
B77	150	43	97	47	85	6.1	1.28
B78	134	58	70	45	86	6.1	1.30
B79	133	57	61	74	108	6.1	1.63
B80	159	66	79	72	98	6.1	1.48
B81	191	65	112	67	82	6.1	1.24
B82	192	96	88	45	87	6.1	1.31
Means ± SD	169 ± 30	59 ± 16	93 ± 29	80 ± 38	86 ± 8	11.0 ± 8.1	2.3 ± 1.7
High Active	177 ± 33	67 ± 17	91 ± 28	73 ± 34	84 ± 8	7.3 ± 2.7	1.5 ± 0.6
Mod. Active	160 ± 26	59 ± 15	86 ± 26	77 ± 3	87 ± 8	9.2 ± 4.9	2.0 ± 1.0
Low Active	173 ± 31	52 ± 13	103 ± 31	92 ± 45	87 ± 7	17.0 ± 11.1	3.6 ± 2.4

Table 3. pQCT foreleg data

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Total Area (mm^2)	Muscle Area (mm^2)	Muscle Density (mg/cm^3)	Subcutaneous Fat (mm^2)	IMAT (mm^2)
B01	10715.5	7039.5	79.2	2985.8	111.0
B02	10791.5	6433.8	75.6	3747.8	101.0
B03	8921.5	6111.3	77.5	2132.8	109.5
B04	9729.3	6396.3	81.3	2684.8	92.3
B05	9521.3	5541.5	78.7	3331.3	91.0
B06	8629	5666.5	78.2	2452.0	83.3
B07	10099.0	7197.3	79.4	2217.5	98.0
B08	12183.5	6540.0	79.7	5120.3	89.7
B09	13432	8904.5	76	3874.8	30.3
B10	10025.8	6798.8	77.8	2619.3	102.5
B11	12251.5	7244.0	76.9	4369.8	49.5
B12	12466.3	7518.5	78.2	4330.5	95.8
B13	10637.5	7556.8	76.9	2524.3	85.7
B14	10806.3	7252.5	77.2	3007.5	114
B15	9294.8	5356.0	77.8	3262.5	106.2
B16	9612.5	6222.0	79.8	2633.5	90.3
B17	9083.8	5708.5	77.9	2785.8	82.0
B18	9573.0	7050.8	71.1	1835.0	90.2
B19	10452.3	7037.3	78.5	2810.3	106.5
B20	19180.5	8665.8	75.0	9190.3	41.0
B21	15216.8	7317.0	74.3	7193.5	98.5
B22	9502.0	6059.3	78.8	2901.5	83.8
B23	11223.8	7195.8	76.7	3403.8	47.8
B24	8916.8	6083.0	79.0	2238.8	98.5
B25	8514.9	4917.0	80.5	3070.9	63.5
B26	10038.6	5807.2	76.0	3596.6	100.0
B27	14241.1	5535.7	73.6	7553.1	42.3
B28	10966.4	6533.0	75.6	3721.1	N/A
B29	9381.4	6074.6	80.0	2669.9	38.0
B30	12415.4	6319.8	75.9	5366.9	100.8

Table 3. pQCT foreleg data continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.
Means ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Total Area (mm^2)	Muscle Area (mm^2)	Muscle Density (mg/cm^3)	Subcutaneous Fat (mm^2)	IMAT (mm^2)
B31	12864.0	6700.0	72.0	4988.8	186.3
B32	10125.6	5961.4	72.2	3349.8	105.0
B33	11292.6	6994.6	76.6	3571.0	98.0
B34	13023.3	6300.0	73.0	5773.0	43.5
B35	9673.8	6628.6	79.4	2549.0	86.5
B36	12498.7	6789.4	75.5	4800.3	110.0
B37	9953.0	6130.2	74.6	3046.4	89.3
B38	9586.2	6522.1	78.6	2316.2	99.5
B39	10694.9	6683.7	71.8	3184.0	42.7
B40	9879.7	6709.6	81.4	2313.1	103.8
B41	8972.0	5887.2	79.8	2508.3	98.5
B42	9429.3	5589.9	78.9	3175.4	105
B43	11614.8	7444.0	77.3	3537.0	37.5
B44	18160.8	7316.0	76.4	9904.3	115.0
B45	8927.5	6380.5	77.7	1973.8	92.0
B46	10337	7321.0	79.5	2348.3	25.5
B47	7566.3	5273.3	77.6	1729.8	N/A
B48	6854.8	4644.3	79.4	1697.5	97.5
B49	9038.0	6357.0	79.3	2062.0	95.3
B50	9901.0	6886.3	78.9	2408.0	99.5
B51	9025.3	6542.5	78.1	1835.0	101.0
B52	9861.0	7198.8	76.5	1885.5	115.5
B53	8651.5	5866.5	78.7	2269.8	92.0
B54	9887.5	7154.0	80.2	2075.8	100.5
B55	10076.8	6939.0	79.0	2560.0	85.7
B56	9699.3	5928.0	78.3	3167.5	100.5
B57	10653.3	6790.5	76.2	3097.0	51.2
B58	10026.5	6504.5	79.1	2893.0	N/A
B59	12808.3	9328.0	76.9	2775.8	218.5
B60	9710.3	6657.3	78.8	2340.5	93.0

Table 3. pQCT foreleg data continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects.

Code	Total Area (mm^2)	Muscle Area (mm^2)	Muscle Density (mg/cm^3)	Subcutaneous Fat (mm^2)	IMAT (mm^2)
B61	8594.5	5890.5	78.8	2157.8	96.2
B62	11634.8	6476.5	74.5	4391.3	45.2
B63	9994.3	7469.3	79.4	1875.0	164.0
B64	9691.8	7050.3	80.4	2045.8	95.3
B65	10088.3	6290.5	80.7	3158.0	94.7
B66	10793.8	7504.0	76.9	2662.5	104.7
B67	11604	6674.8	79.5	4265.5	98.7
B68	9009.8	6053.3	80.4	2335.5	29.0
B69	9886.3	6800.5	77.6	2455.0	94.0
B70	11223.3	6466.5	77.5	4208.5	101.8
B71	7561.0	4862.0	78.1	2060.8	92.5
B72	11566.5	7473.0	76.8	3238.0	108.3
B73	8441.5	5050.5	77.5	2796.3	73.3
B74	12122.3	8367.3	78.7	3022.5	48.0
B75	9746.8	6711.5	78.7	2421.3	98.5
B76	7800	5266.8	78.4	1922.3	154.8
B77	10338	6521.8	78.9	3268.3	16.8
B78	8955.8	5734.8	78.4	2592.8	100
B79	11189.5	8018.8	78.9	2526.8	16.3
B80	7602.5	5131.5	83.4	1965.8	89.2
B81	9758.8	5910.0	79.6	3213.5	102.0
B82	10972.0	8574.5	81.1	1798.3	109.5
Means ± SD	10460.5 ± 2017.7	6576.0 ± 901.9	77.8 ± 2.3	3210.8 ± 1510.9	89.4 ± 33.9
High Active	9972.2 ± 1239.7	6756.3 ± 947.4	78.3 ± 2.2	2547.7 ± 711.5	100.5 ± 35.2
Mod. Active	10136.3 ± 1599.7	6466.6 ± 1029.3	77.9 ± 2.4	3023.0 ± 950.6	79.5 ± 31.2
Low Active	10986.4 ± 2431.8	6450.1 ± 769.1	77.0 ± 2.3	10986.4 ± 2431.8	92.2 ± 29.1

Table 4. Physical activity data

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects.
Mean ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

Code	Average 4 day Step Count	Average MET·min/day
B01	15511	1166
B02	4508	354
B03	10963	1009
B04	7897	328
B05	4482	280
B06	7417	275
B07	8274	828
B08	13984	576
B09	9897	491
B10	7870	1105
B11	5100	363
B12	3173	131
B13	7641	632
B14	6256	188
B15	15402	655
B16	14785	782
B17	6286	234
B18	18878	717
B19	11857	477
B20	5917	244
B21	5983	297
B22	9795	594
B23	5184	567
B24	9509	392
B25	11446	523
B26	2593	99
B27	4448	133
B28	9447	283
B29	7508	508
B30	7387	222

Table 4. Physical activity data continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.
Mean ± SD for all subjects, high, moderate and low physically active subjects are at the end of each table.

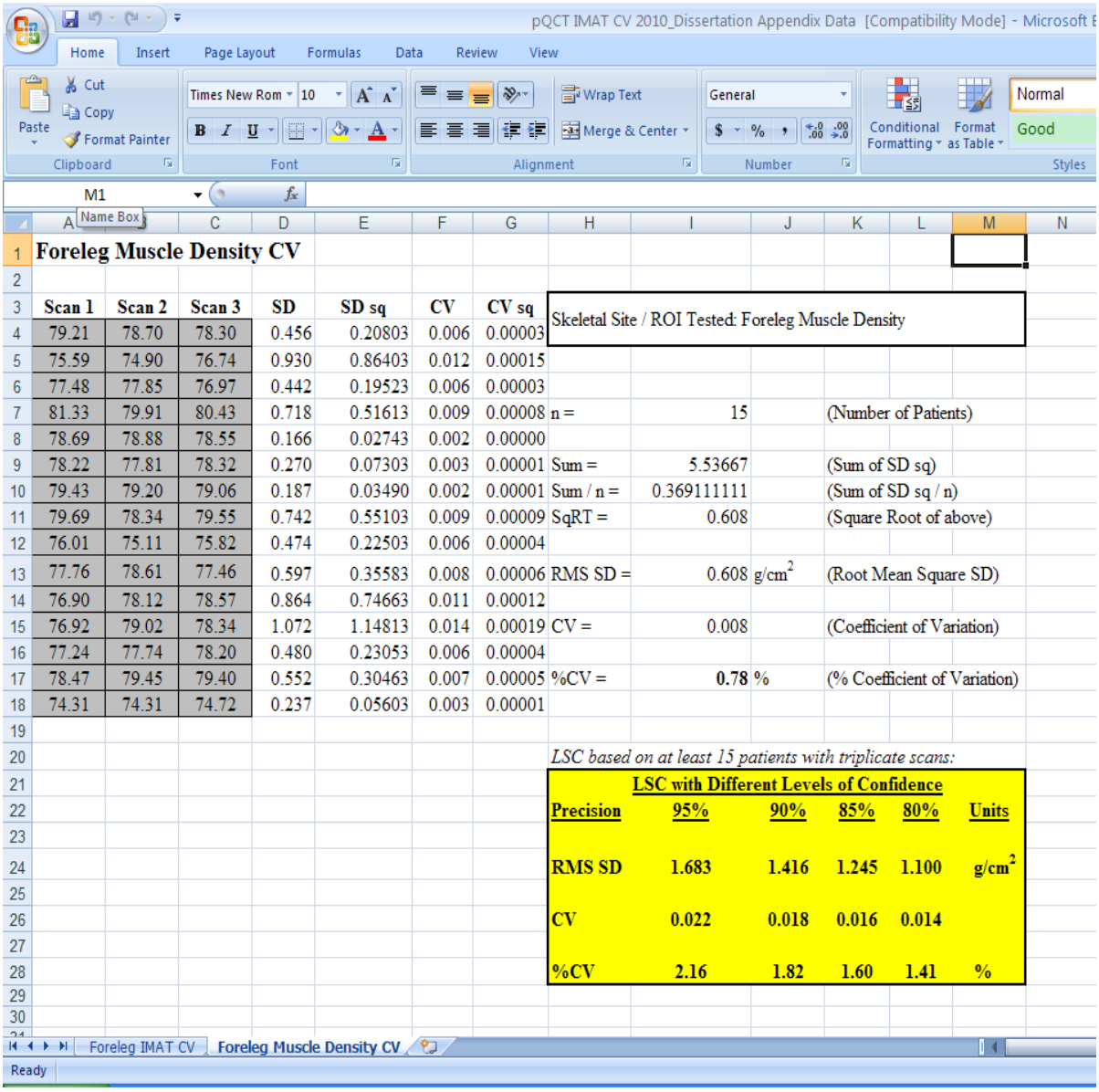
Code	Average 4 day Step Count	Average MET·min/day
B31	3214	246
B32	3923	162
B33	6891	284
B34	15268	580
B35	17827	677
B36	10694	406
B37	9560	394
B38	4482	170
B39	5794	471
B40	17477	664
B41	8737	332
B42	3815	157
B43	10094	303
B44	N/A	N/A
B45	12173	836
B46	N/A	N/A
B47	13470	779
B48	16012	633
B49	12069	362
B50	7595	424
B51	14660	557
B52	6321	769
B53	7124	390
B54	N/A	N/A
B55	10913	572
B56	7550	406
B57	8202	741
B58	6425	460
B59	8702	878
B60	6397	794

Table 4. Physical activity data continued

Color coding: Dark gray = high physically active subjects; light gray = moderate physically active subjects; white = low physically active subjects; N/A, data not available.

Code	Average 4 day Step Count	Average MET·min/day
B61	9547	363
B62	10984	643
B63	10064	932
B64	12841	686
B65	5368	671
B66	22222	844
B67	10658	644
B68	17738	732
B69	6526	501
B70	7614	314
B71	11442	435
B72	15505	705
B73	6211	624
B74	7970	517
B75	6829	835
B76	7105	461
B77	N/A	N/A
B78	12873	531
B79	7471	581
B80	8114	602
B81	6451	416
B82	9504	1702
Means ± SD	9292 ± 4121	533 ± 272
High Active	121890 ± 4576	824 ± 227
Mod. Active	9228 ± 2870	512 ± 73
Low Active	6458 ± 2460	266 ± 86

Figure 1. Foreleg muscle density CV



pQCT IMAT CV 2010_Dissertation Appendix Data [Compatibility Mode] - Microsoft E

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M1 | Name Box

Foreleg Muscle Density CV

Scan 1	Scan 2	Scan 3	SD	SD sq	CV	CV sq
79.21	78.70	78.30	0.456	0.20803	0.006	0.00003
75.59	74.90	76.74	0.930	0.86403	0.012	0.00015
77.48	77.85	76.97	0.442	0.19523	0.006	0.00003
81.33	79.91	80.43	0.718	0.51613	0.009	0.00008
78.69	78.88	78.55	0.166	0.02743	0.002	0.00000
78.22	77.81	78.32	0.270	0.07303	0.003	0.00001
79.43	79.20	79.06	0.187	0.03490	0.002	0.00001
79.69	78.34	79.55	0.742	0.55103	0.009	0.00009
76.01	75.11	75.82	0.474	0.22503	0.006	0.00004
77.76	78.61	77.46	0.597	0.35583	0.008	0.00006
76.90	78.12	78.57	0.864	0.74663	0.011	0.00012
76.92	79.02	78.34	1.072	1.14813	0.014	0.00019
77.24	77.74	78.20	0.480	0.23053	0.006	0.00004
78.47	79.45	79.40	0.552	0.30463	0.007	0.00005
74.31	74.31	74.72	0.237	0.05603	0.003	0.00001

Skeletal Site / ROI Tested: Foreleg Muscle Density

n =	15		(Number of Patients)
Sum =	5.53667		(Sum of SD sq)
Sum / n =	0.369111111		(Sum of SD sq / n)
SqRT =	0.608		(Square Root of above)
RMS SD =	0.608	g/cm^2	(Root Mean Square SD)
CV =	0.008		(Coefficient of Variation)
%CV =	**0.78**	%	(% Coefficient of Variation)

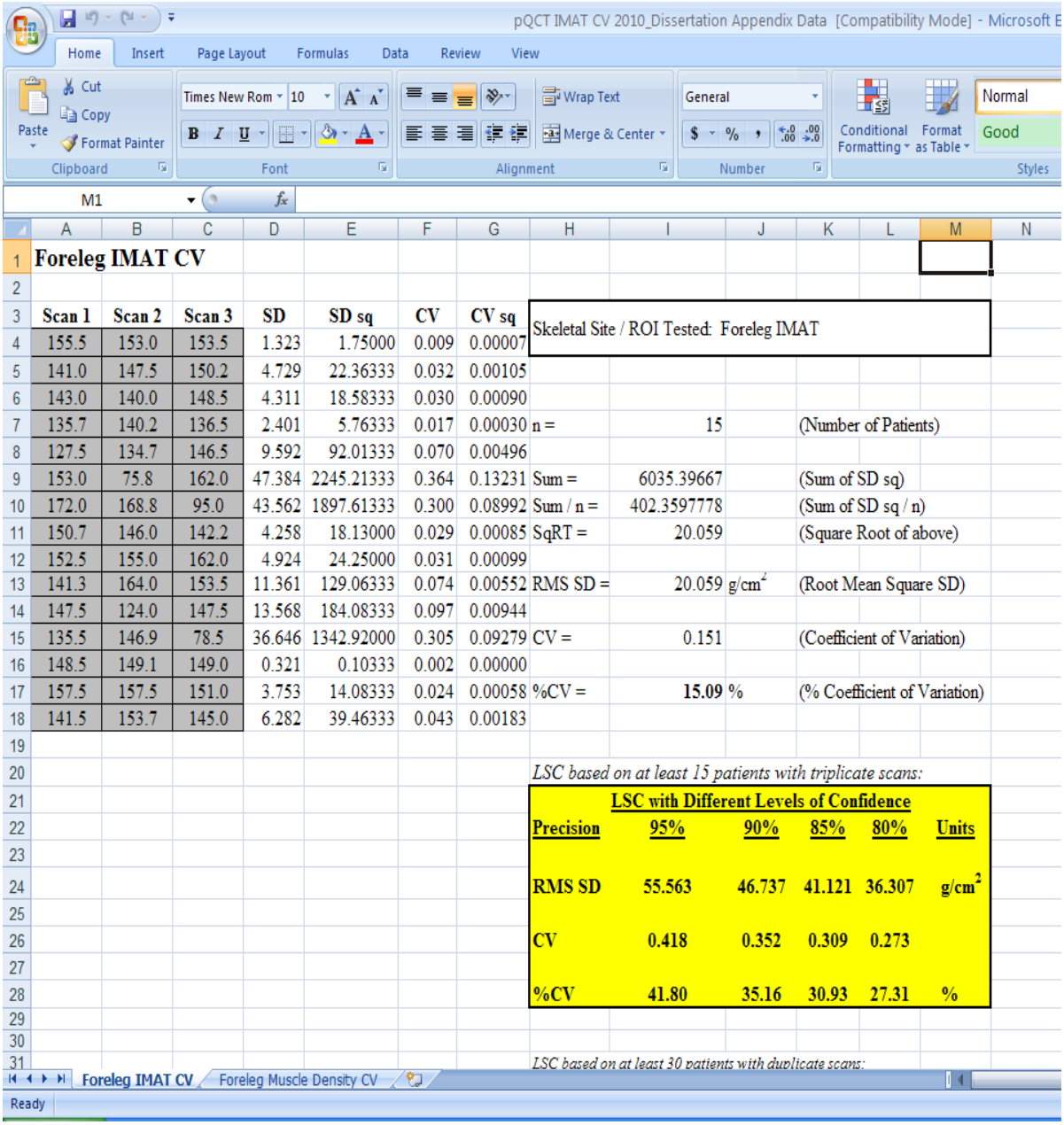
LSC based on at least 15 patients with triplicate scans:

LSC with Different Levels of Confidence					
Precision	95%	90%	85%	80%	Units
RMS SD	1.683	1.416	1.245	1.100	g/cm^2
CV	0.022	0.018	0.016	0.014	
%CV	2.16	1.82	1.60	1.41	%

Foreleg IMAT CV | Foreleg Muscle Density CV

Ready

Figure 2. Foreleg IMAT CV



pQCT IMAT CV 2010_Dissertation Appendix Data [Compatibility Mode] - Microsoft E

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M1

Foreleg IMAT CV

Scan 1	Scan 2	Scan 3	SD	SD sq	CV	CV sq
155.5	153.0	153.5	1.323	1.75000	0.009	0.00007
141.0	147.5	150.2	4.729	22.36333	0.032	0.00105
143.0	140.0	148.5	4.311	18.58333	0.030	0.00090
135.7	140.2	136.5	2.401	5.76333	0.017	0.00030
127.5	134.7	146.5	9.592	92.01333	0.070	0.00496
153.0	75.8	162.0	47.384	2245.21333	0.364	0.13231
172.0	168.8	95.0	43.562	1897.61333	0.300	0.08992
150.7	146.0	142.2	4.258	18.13000	0.029	0.00085
152.5	155.0	162.0	4.924	24.25000	0.031	0.00099
141.3	164.0	153.5	11.361	129.06333	0.074	0.00552
147.5	124.0	147.5	13.568	184.08333	0.097	0.00944
135.5	146.9	78.5	36.646	1342.92000	0.305	0.09279
148.5	149.1	149.0	0.321	0.10333	0.002	0.00000
157.5	157.5	151.0	3.753	14.08333	0.024	0.00058
141.5	153.7	145.0	6.282	39.46333	0.043	0.00183

Skeletal Site / ROI Tested: Foreleg IMAT

n =	15		(Number of Patients)
Sum =	6035.39667		(Sum of SD sq)
Sum / n =	402.3597778		(Sum of SD sq / n)
SqRT =	20.059		(Square Root of above)
RMS SD =	20.059	g/cm^2	(Root Mean Square SD)
CV =	0.151		(Coefficient of Variation)
%CV =	**15.09**	%	(% Coefficient of Variation)

LSC based on at least 15 patients with triplicate scans:

LSC with Different Levels of Confidence

Precision	95%	90%	85%	80%	Units
RMS SD	55.563	46.737	41.121	36.307	g/cm^2
CV	0.418	0.352	0.309	0.273	
%CV	41.80	35.16	30.93	27.31	%

LSC based on at least 30 patients with duplicate scans:

Foreleg IMAT CV | Foreleg Muscle Density CV

Ready