

**UTILIZING pQCT & BIOMARKERS OF BONE TURNOVER TO STUDY INFLUENCES OF PHYSICAL
ACTIVITY OR BARIATRIC SURGERY ON STRUCTURAL & METABOLIC STATUS OF BONE**

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UTILIZING pQCT & BIOMARKERS OF BONE TURNOVER TO STUDY INFLUENCES OF PHYSICAL ACTIVITY OR BARIATRIC SURGERY ON STRUCTURAL & METABOLIC STATUS OF BONE

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Abstract

Bone health in the context of two common maladies, osteoporosis and obesity, has spurred research in the area of physical activity (PA) and bariatric surgery (BarS).

Objectives: To examine: 1) relationships between PA and the skeleton utilizing the peripheral Quantitative Computed Tomography (pQCT) and Dual-energy X-ray Absorptiometry (DXA) in pre-menopausal women; 2) effects of adjustable gastric banding (AGB) vs. Roux-en-Y gastric bypass (RYGB) surgeries on pQCT and DXA measures; 3) 6-month time course changes on serum biomarkers of bone turnover and associated adipokines induced by AGB vs. RYGB.

Methods: Standard DXA and pQCT measurements were taken for all subjects. PA tertiles (PA-L, PA-M, PA-H) were based on a calculated average MET-min/day determined from 4-d self-reported PA and pedometer step counts. For BarS subjects, bone measurements were taken pre-surgery, 3- and 6-months post-surgery along with serum (or plasma) from fasting blood draws, with ELISA assays for total OC, undercarboxylated OC, CTx, adiponectin, and leptin.

Results: Minimal DXA differences between the highest and lowest PA tertiles were seen, while pQCT tibial measures and polar strength-strain index (SSI_p) indicated differences along the tibial shaft. Comparing the two instruments and adjusting for BMI, the DXA leg and hip BMD and BMC showed differences ($p<0.05$) between PA-M and PA-L as well as PA-H and PA-L. Similarly, the pQCT tibial cortical area, BMC, and SSI_p were progressively greater for the different levels of PA ($p<0.05$).

3- and 6-months post-BarS weight, fat-free mass, fat mass, central body fat, tibial and radial subcutaneous fat, and radial MCSA decreased ($p<0.05$). Comparing the AGB and RYGB and adjusting for weight, DXA BMC showed decreases ($p<0.01$) at both time points for RYGB. RYGB demonstrated differences ($p<0.05$) in bone measures at 3- and 6-months post-surgery along the tibial shaft that are indicative of increases in bone strength, and at 6-months, total OC, undercarboxylated OC, and HMW adiponectin increased, while leptin decreased.

Conclusions: PA is associated with increases in bone, but pQCT data are more discriminatory and sensitive. 6-months post-RYGB, pQCT measures indicate increases in bone strength parameters, and greater bone adaptation was evidenced by biomarkers of increased osteoblastic activity.

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Chapter 1

Introduction

Clinical Problem and Rationale for Research

This dissertation will incorporate data derived from parts of two separate research studies, first *Preliminary Studies of Obstructive Sleep Apnea Syndrome (OSAS): Searches for a Gene Expression Profile and Relationship to Type 2 Diabetes and Skeletal Fragility* and secondly, *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*. The skeletal fragility portion of the first study was conducted in the Bone, Osteoporosis, Nutrition and Exercise (BONE) Laboratory at Virginia Tech in Blacksburg, Virginia, and the protocol was approved by the Virginia Tech Institutional Review Board (IRB). It was originally constructed to determine the repeatability and coefficient of variance for the BONE lab for the peripheral quantitative computed tomography (pQCT) instrument. It was later expanded to include a larger number of subjects and allowed for the development of a cross-sectional database of healthy premenopausal women. Part 2 of the second research study, was a prospective clinical investigation funded by a Research Acceleration Project (RAP) grant from the Carilion Clinic Research Office in Roanoke, Virginia, and the protocol was approved by the Carilion Clinic IRB. These subjects were premenopausal women undergoing either Roux-en-Y Gastric Bypass (RYGB) or Adjustable Gastric Banding (AGB) elective surgery and thus experiencing accelerated weight loss. This doctoral program in

human nutrition has been centered on two common maladies, osteoporosis and obesity. The axis of my research, bone health, has focused on physical activity and bariatric surgery.

The first area of focus involved evaluation of physical activity in a group of healthy, premenopausal Caucasian women utilizing dual-energy x-ray absorptiometry (DXA) and pQCT measurements. The loss of bone in younger populations, especially younger women, increases the potential for osteoporosis related complications later in life. In the United States, osteoporotic fractures are a significant issue for the elderly with an estimated 2 million new incidences each year and an estimated annual cost in excess of \$19 billion [1]. It was estimated in 2006 that 44 million people in the United States and 200 million people worldwide had or are at risk of osteoporosis [2]. Low bone mass in the elderly has been attributable to either failure to accrue sufficient peak bone mass during development or an excessive rate of the inevitable loss associated with age [3]. Development of osteoporosis and a subsequent increased risk of fracture are not only influenced by the bone mineral content, but also the structural properties and additional material properties of bone [4]. Although adequate nutrition and mineral intake are important in the developing skeleton, physical activity alone has been shown to increase periosteal and endosteal circumferences in the tibia in developing children [5]. Turner states that the size and shape of bones are the major determinants of their resistance to fracture and further states that these characteristics are largely determined by the mechanical stresses placed on them [6]. Adaptive bone strength is driven by the strains imposed on the bones, and muscle forces produce the largest strains [7]. In a recent study comparing muscle cross-sectional area (MCSA) derived from peripheral quantitative computed tomography (pQCT) to isokinetic dynamometric peak grip torque, Frank et al [8], found that MCSA accounted for 68-76% of variance in peak torque and that MCSA as well as the isokinetic, concentric and eccentric grip strength measures

all accounted for 89-90% of variance in radial strength strain index (SSI). Although muscle size or cross-sectional area cannot account for all of the variation in muscle strength, they have been used as a surrogate for muscle strength [9]. Because physical activity is a modifiable behavior that has been shown to affect both bone and muscle, it is possible to construct research to examine the relationships between physical activity and bone, muscle or fat.

The majority of research related to the effects of exercise on bone has utilized DXA measurements as endpoints. Although the parameters of hard and soft tissues can be measured with magnetic resonance imaging (MRI) or computed tomography (CT), the peripheral quantitative computed tomography (pQCT) device may be a more efficient tool for researchers. It is a less expensive, smaller instrument and produces less radiation exposure than its larger counterparts. It has no weight limitations and is only limited by the size of the gantry, which will accommodate the forearm or foreleg of children and adults. Additionally, the pQCT allows researchers to evaluate geometric parameters and true volumetric bone mineral density (BMD) as well as the soft tissues of muscle and the various compartments of adipose tissue, which cannot be isolated with a DXA instrument.

The second area of focus involved evaluation of DXA and pQCT measurements as well as blood biomarkers in a group of premenopausal Caucasian women who were accepted for elective bariatric surgery (BarS). Bariatric surgery has become an ever increasing means of managing the obesity epidemic and is often utilized by young premenopausal women and men of similar ages. RYGB is one of the most common procedures and has been shown to resolve type 2 diabetes mellitus in as many as 84% of cases and often within the first couple of weeks after surgery, before any significant weight loss has occurred [10]. Additionally, hyperlipidemia is relieved in nearly 71% of cases, hypertension in 68% of cases, and sleep apnea in 85% of cases, also often

before significant loss of excess body weight [11]. Because of these results, BarS is becoming a common treatment option for these comorbidities related to obesity. One of the primary desired results of BarS, weight loss, is attributable to the restrictive nature of the surgeries, the malabsorption created by certain procedures and alterations in hormones. In a recent study of BarS patients, Bradley et al. [12] found that after a 20% reduction in excess body weight subjects had improved insulin sensitivity and β -cell function regardless of which surgery, AGB or RYGB, that they underwent. Weight loss associated with BarS patients produces increased bone loss and turnover which in turn may have an indirect effect on energy metabolism as well as the decrease in bone mass [13].

In recent years, the skeleton has become a focus of investigation for hormonal alterations that affect energy metabolism at the level of the pancreas and adipose tissue [14]. Until recently a relationship had not yet been identified, until leptin, an adipocyte-derived hormone, was shown to be a major regulator of bone turnover [15]. Working on the assumption that there must be a feedback mechanism and utilizing a mouse model, Lee et al. [16] identified osteocalcin (OC) as the bone related hormone involved in sugar metabolism. Osteocalcin, a key marker of bone turnover, is a γ -carboxyglutamate protein expressed predominantly by osteoblasts. In rodents, undercarboxylated osteocalcin (unOC) has been shown to play a role in the regulation of glucose homeostasis, and it is likely that a similar relationship exists in humans [17]. In a review by Zhang et al. [18], OC was shown to improve insulin sensitivity through actions on the pancreas and increased production of adiponectin mRNA. Hwang et al. [19] found similar results with plasma OC associated with improved glucose tolerance, increased β -cell function and insulin sensitivity; however, they found no association with plasma adiponectin levels. Few studies to date have examined the relationships of weight loss and OC. Reinehr and Roth [20] studied

obese children in a cross-sectional analysis as well as undergoing a 1-year diet and exercise induced weight loss program and found that OC levels were lower in obese children and were related to insulin resistance and leptin. Schafer et al. [21] demonstrated that changes in unOC induced by the osteoporosis medications parathyroid hormone and alendronate are associated with changes in metabolism in a group of postmenopausal women, and greater increases in unOC correlated with greater decreases in body weight and fat mass and greater increases in adiponectin.

Study 1: Influences of physical activity in healthy premenopausal women on appendicular skeleton

Research Aims:

The aim of this study was to examine the relationships between habitual physical activity and the hard and soft tissues of the appendicular skeleton utilizing the pQCT and DXA instruments. Via the pQCT, BMD, bone mineral content (BMC), bone geometry and bone strength measures were examined as well as the soft tissue measures of muscle density and fat (subcutaneous, marrow and inter-muscular), while the regional (lumbar spine, proximal hip, and distal radius) and whole body DXA measures of BMD and BMC were also evaluated. In addition, a comparison of the two technologies, relative to the status of bone, fat mass and fat-free mass was also conducted.

Assumptions:

1. All laboratory equipment (DXA, pQCT, stadiometer, and scale) had been properly calibrated and maintained.
2. Subjects wore the pedometer for all waking hours over a four day period and accurately recorded any activities when the pedometer was not worn.

Delimitations:

1. Subjects included in the study were restricted to young adult to middle aged premenopausal Caucasian females; therefore the results cannot be generalized to other gender or racial groups.
2. To assess overall habitual physical activity status, pedometer step counts and self-reported physical activity over a four day period were translated into a numerical value, a metabolic equivalent (MET), utilizing a compendium of physical activity

Limitations:

1. This study design is cross-sectional and therefore will not allow for causal determinations.
2. The weight limit on the DXA instrument is 136 kg, which limited inclusion to subjects weighing less than 300 pounds.
3. The subjects all had relatively high levels of physical activity, which did not allow for analysis across levels based on the 2008 Physical Activity Guidelines for Americans of low, moderate and high physical activity.

Study 2: Comparative influences of two forms of bariatric surgery in premenopausal women on changes in DXA and pQCT measures of bone, muscle, and fat

Research Aims:

The aim of this study was to examine the effects of AGB and RYGB surgeries on whole body fat mass, fat-free mass, as well as whole body and localized areal BMC, BMD and T-scores for osteoporotic risk (measured by DXA) and volumetric BMC, BMD, bone geometry and bone strength measures, muscle cross-sectional areas, and fat cross-sectional areas (measured by pQCT) and to determine if changes could be detected at early time points than have previously been reported. In addition, a comparison of the two technologies, relative to the status of bone, fat mass and fat-free mass was also conducted.

Assumptions:

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

Delimitations:

1. 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.

Limitations:

1. The weight limit on the DXA instrument is 136 kg, which limited inclusion to subjects weighing less than 300 pounds.

Study 3: Effects of weight loss on osteocalcin in premenopausal women undergoing bariatric surgery

Research Aims:

The primary objective of this study was to examine changes in serum biomarkers of bone turnover, OC and carboxy-terminal peptide of type I collagen (CTx), and associated adipokines, leptin and adiponectin, from before surgery to 3 months and 6 months post-surgery in the context of the accelerated weight loss associated with two different BarS types, AGB and RYGB. In addition, OC was further evaluated to determine the undercarboxylated fraction that has been identified as part of the connection between bone and energy metabolism.

Assumptions:

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

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

Delimitations:

1. 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.

Limitations:

1. The weight limit on the DXA instrument is 136 kg, which limited inclusion to subjects weighing less than 300 pounds.

Definitions

Adipokine – a group of polypeptide hormones produced primarily by adipose tissue.

Adiponectin – an adipokine with three isoforms which target skeletal muscle and the liver that have insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties [22].

Adjustable gastric banding (AGB) – a form of bariatric surgery in which an inflatable band is placed around the upper part of the stomach, creating a small pouch which limits the amount of food that can be consumed at one time and produces a quicker sense of satiety [23].

Bariatric surgery – a weight loss surgery for patients with a BMI $> 40 \text{ kg/m}^2$ or those with a BMI $> 35 \text{ kg/m}^2$ who have comorbidities [23].

Carboxy-terminal cross-linked telopeptide of type I collagen (CTx) – a peptide sequence cleaved by osteoclasts during bone resorption, and its serum levels are therefore proportional to osteoclast activity [24].

Computed tomography (CT) – a device that uses computer-processed x-rays to produce virtual slices of a scanned object, cross-sectional images that are used for diagnostic and therapeutic purposes in various medical disciplines [25].

Dual-Energy X-ray Absorptiometry (DXA) – a device that uses two x-ray beams with different energy levels to measure BMD and BMC and is the most commonly used means of measuring these data [25].

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 [25].

Leptin – an adipose derived protein that regulates energy intake and expenditure through appetite and metabolism [22].

Low bone mass – defined by the World Health Organization (WHO) as a T-score from a DXA scan between -1 and -2.5 [26].

Osteocalcin (OC) – a protein secreted exclusively by osteoblasts and utilized as a serum marker of bone formation [24].

Osteoporosis – defined by the World Health Organization (WHO) as a T-score from a DXA scan ≤ -2.5 [26].

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 the radius or tibia.

Roux-en-Y gastric bypass (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 [23].

T-score – bone density in a specific area expressed in standard deviations from the mean value of a reference database of young Caucasian females [26].

Table 1-1. List of Abbreviations

AGB	Adjustable Gastric Banding
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
CT	Computed Tomography
CTx	C-Terminal Cross-Linked Telopeptide of Type I Collagen
DXA	Dual-energy X-ray Absorptiometry
ELISA	Enzyme-Linked Immunosorbent Assay
ISCD	International Society for Clinical Densitometry
MET	Metabolic Equivalent
MRI	Magnetic Resonance Imaging
OC	Osteocalcin
pQCT	peripheral Quantitative Computed Tomography
RYGB	Roux-en-Y Gastric Bypass
SSI	Strength-Strain Index
WHO	World Health Organization

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Chapter 2

Background

Introduction

Bone health in the context of two common maladies in today's society, osteoporosis and obesity, has spurred ongoing research in the area of physical activity and more recently bariatric surgery (BarS), which is becoming an increasingly common means of weight loss. Modifying physical activity behavior as well as significant weight changes can have a direct impact on bone mass, strength, and geometry, all of which influence bone health. While increasing physical activity may improve bone health, the type, duration, and intensity of exercise to create these improvements has yet to be determined. In addition, the popularity of weight loss surgeries to aid in the reduction of obesity and several associated comorbidities has led to another potential risk of osteoporosis due to decreased loading of the skeleton. Because younger individuals, especially younger women, are choosing to utilize BarS to aid them in losing weight, there is a greater potential for osteoporosis related complications later in life due to the associated bone loss. To address the needs for bone related research in the areas of physical activity and BarS, this literature review will explore the following topics: 1) physical activity and bone, 2) bariatric surgery: adjustable gastric banding (AGB) and Roux-en-Y gastric bypass (RYGB), 3) bariatric surgery and bone, 4) bariatric surgery and biomarkers of bone turnover, and 5) measurement techniques and instruments, which includes the dual-energy x-ray absorptiometry (DXA) and peripheral Quantitative Computed Tomography (pQCT) instruments and physical activity assessment. This chapter will conclude with a summary of the related literature and how each section relates to the dissertation research.

Physical activity and bone

With an estimated 2 million new incidences in the United States and an annual cost in excess of \$19 billion, osteoporotic fractures have become a significant issue for the elderly [1]. Those that had or were at risk of osteoporosis in 2006 was estimated to be 44 million people in the United States, 68% of which are women, and 200 million people worldwide [2]. Failure to accrue sufficient peak bone mass during development or an excessive rate of the inevitable loss associated with age have been the cause of low bone mass in the elderly [3]. Hence, the loss of bone in younger individuals, especially younger women, increases the potential for osteoporosis-related complications later in life. Structural and material properties of the skeleton as well as bone mineral content influence the development of osteoporosis and the subsequent increased risk of fracture [4]. Physical activity (PA) alone has been shown to increase periosteal and endosteal circumferences in the tibia in developing children, factors known to reduce susceptibility to fracture, in spite of the importance of adequate nutrition and mineral intake for the developing skeleton [5]. The majority of research related to the effects of exercise on bone has utilized dual-energy X-ray absorptiometry (DXA) measurements of bone mineral content (BMC) and bone mineral Density (BMD) as endpoints. However, because the structural properties of bone play a significant role in bone strength and its ability to accommodate compressive and torsional loads, it is therefore logical that to effectively examine these issues researchers must not only evaluate bone density and content, but also bone geometry and microarchitecture. Turner [6] states that the size and shape of bones are the major determinants of their resistance to fracture and further states that these characteristics are largely determined by the mechanical stresses placed on them. Animal studies have demonstrated that significant

increases in bone strength and resistance to structural failure are produced where strains are highest despite only modest gains in bone mineral density (BMD) [7, 8].

Bariatric Surgery: Adjustable Gastric Banding and Roux-en-Y Gastric Bypass

For those individuals that are clinically obese and are unable to lose weight through exercise, diet, or other conventional means, BarS has become an increasingly popular means of accomplishing this objective and has become an effective means of dealing with several comorbidities associated with obesity. The primary goal for BarS is to dramatically reduce excess body weight, thereby lowering high risks of comorbidities associated with obesity.

According to the National Institutes of Health Consensus Development Conference Panel, BarS is indicated as a treatment for obesity for adults with a body mass index (BMI) $\geq 40 \text{ kg/m}^2$ (class 3 obesity) or those with a BMI $\geq 35 \text{ kg/m}^2$ in the presence of high-risk comorbidities [9].

According to the American Society for Metabolic and Bariatric Surgery, more than 15 million Americans are morbidly obese and patients lose an average of between 30 to 50 percent of their excess weight within 6 months and an average of 77 percent as early as one year after surgery [10]. Reductions in excess body weight can ameliorate a myriad of adverse health conditions associated with obesity, including type 2 diabetes mellitus, hypertension, gastroesophageal reflux disease, obstructive sleep apnea, and certain types of cancer [11].

Although adjustable gastric banding (AGB) is more common in Europe and Australia, it has gained increased acceptance in the United States, the most common weight loss surgery is Roux-en-Y gastric bypass (RYGB) [12]. Approximately 85% of patients opt for the RYGB surgical type [13]. AGB surgery is a restrictive procedure, while RYGB is both restrictive and malabsorptive; however, both produce significant and rapid weight loss. The restrictive portion of each is accomplished by reducing the size of the stomach and therefore the amount of food a

person can eat at any given time. In the AGB procedure, a band is placed around the upper stomach to create the smaller stomach pouch that has an adjustment port placed subcutaneously on the abdomen. This band functions similarly to the pyloric sphincter at the bottom of the stomach, allowing small amounts of food to pass through the normal gastrointestinal tract. Although weight loss after AGB surgery is often less than that after RYGB surgery, weight loss averages between 50 and 60% of initial body weight with a decrease of approximately 25% in BMI two years post-surgery [9]. RYGB surgery involves stapling off the stomach to create the small food pouch and attaching it directly to the small intestine, approximately 75 to 150 cm past the stomach and duodenum, in the jejunum. This rerouting or bypassing of part of the gastrointestinal tract produces the malabsorptive component of this procedure. Weight loss after RYGB surgery is generally greater, with patients noting two thirds of their pre-surgical body weight with approximately 35% decrease in BMI within two years [9]. Mortality rates are quite low for BarS with less than 0.5% for RYGB and 0.1% for AGB procedures [14]. Short-term complications like infection, leaking, or hemorrhage occur in 5-10% of patients and long-term complications, including internal hernias, emotional disorders, and nutrient deficiencies are also common [15]. Operating times and hospitalization were lower with AGB than RYGB surgeries; however both are now commonly performed laproscopically and these times are similar [12]. Because of the more favorable weight loss outcome, patient satisfaction is higher in RYGB than AGB, although it is less invasive and potentially reversible [12].

Bariatric surgery and bone

Osteoporosis has been estimated to affect 200 million women world-wide, with approximately one-tenth of women aged 60 at risk of the disease [16]. In 2004, the first ever Surgeon General's Report on bone health and osteoporosis projected that by 2020, one in two adults over age 50

will be at risk for a fracture from osteoporosis or low bone mass [17]. Obesity as well as osteoporosis is a worldwide health problem associated with significant morbidity and mortality. Although conventional thinking has suggested that increased body mass can provide additional skeletal loading that leads to a reduced risk for osteoporosis, excess body weight carries its own health risks [18]. In light of this, most doctors advocate weight loss for the obese; however, many people find it difficult to lose weight. The relationship 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 [19]. The difficulty in losing weight has led to an increasing number of bariatric surgeries being performed, with a conservative estimate that there are currently over 200,000 surgeries per year [20]. BarS may lead to increased risk for osteoporosis, due to the rapid weight loss produced by both common forms of the surgery and alterations in nutrient absorption produced by malabsorptive procedures like RYGB. Weight loss consequences with BarS include reductions in fat mass as well as fat-free mass, which is evidenced by decreases in bone mineral density (BMD) documented by areal measures from DXA [21-23]. This loss in bone can be a result of the reduction in mechanical loading due to weight loss and is also affected by reduced applied forces by the skeletal muscles. Therefore, the fat-free mass losses associated with BarS may also increase the osteoporotic fracture risk [24]. Bariatric surgical protocols currently do not routinely require DXA scans as part of the patient's pre- or post-operative care; however, it has been suggested that patients be assessed for primary and secondary risk of bone disease pre-operatively and then again post-operatively every 1-2 years [19]. The surgical impact of the differing types of BarS produce the loss of excess body weight, however, malabsorptive procedures like RYGB results in increased bone turnover and decreased bone mass [21, 22, 25]. Some studies have reported limited deleterious changes in bone

parameters following the simple restrictive procedure, AGB [26]. Thus, there are some inconsistent results between the two most common procedures performed.

Bariatric surgery and biomarkers of bone turnover

The concerns of bone loss and increased bone turnover from BarS induced weight loss have led researchers to examine BMD and BMC along with biomarkers of bone turnover. For bone formation and resorption, respectively, osteocalcin (OC) and the carboxy-terminal peptide of type I collagen (CTx) are among the most sensitive of the bone-derived biomarkers [27] and both have been documented to increase following BarS [28]. Additionally, adiponectin and leptin, which are produced by the adipocytes, have also demonstrated an impact on bone physiology [25]. Limited research has been done with either adipokine in relation to potential influences on bone status and bone turnover markers following BarS. This led researchers to explore the possible connection between bone and energy metabolism. Leptin has been identified as a major regulator of bone turnover [29], and the skeleton has become the focus of investigation for hormonal alterations that affect energy metabolism [30]. Lee et al. [31] has identified in animal models that osteocalcin (OC) is the bone related hormone involved in glucose metabolism. Osteocalcin, a γ -carboxyglutamate protein expressed predominantly by osteoblasts, is a key marker of bone turnover. Rodent models have shown undercarboxylated osteocalcin (unOC) to play a role in the regulation of glucose homeostasis, and it is likely that a similar relationship exists in humans [32]. In a review by Zhang et al. [33], OC was shown to improve insulin sensitivity through actions on the pancreas and increased production of adiponectin mRNA. Hwang et al. [34] found similar results with plasma OC associated with improved glucose tolerance, increased β -cell function and insulin sensitivity; however, they found no association with plasma adiponectin levels. Although few studies to date have examined the relationships of

weight loss and OC, Reinehr and Roth [35] found that OC levels were lower in obese children and were related to insulin resistance and leptin in a cross-sectional analysis and 1-year diet and exercise induced weight loss program. Greater increases in unOC have been correlated with greater decreases in body weight and fat mass in addition to greater increases in adiponectin when induced by the osteoporosis medications parathyroid hormone and alendronate in a group of postmenopausal women [36].

Measurement techniques and instruments

This section describes the modalities for accessing the bone, muscle, and fat in the subjects as well as the assessment of physical activity. Each subject underwent DXA and pQCT scans in the laboratory and completed a four day physical activity record while wearing a pedometer.

Dual-energy X-ray absorptiometry

The DXA is the primary instrument used to measure bone mineral density (BMD) to diagnosis osteoporosis, assess fracture risk, and monitor response to treatment [37]. Additionally, it is considered superior to many other methods used to determine body composition for the whole body as well as in specific regions of the body due to its simple and easy means of measurement [38]. For bone analysis, the DXA quantifies BMD and BMC and calculates T- and Z-scores for the specific regions of lumbar spine, proximal hip, and distal radius. T-scores (based on a reference database of 30 year-old Caucasian women) and Z-scores (based on age, gender, and ethnicity matched databases) are numbers that represent one's risk of osteoporosis and are calculated by dividing the difference of the measured BMD and the mean reference database BMD by the mean reference database standard deviation [37]. According to the World Health Organization (WHO) a T-score greater than or equal to -1.0 is considered normal, between -1.0 and -2.5 is low bone mass, and less than -2.5 is osteoporosis.

A Hologic QDR 4500-A Elite DXA (*Bedford, MA*) was located in the BONE Laboratory in 229 Wallace Hall (Virginia Polytechnic Institute and State University, Blacksburg, VA). The manufacturer's quality control procedures were followed at the beginning of each day of scanning using an anthropomorphic phantom lumbar spine (Hologic; CV = 0.39%) and an external soft tissue bar (Hologic) was scanned at least weekly. The CVs for this lab and personnel for test-retest reliability in 15 young adult men and women tested on separate days were less than 1.8% for total body, fat mass, fat free soft tissue mass, percentage body fat, lumbar spine and hip BMD.

All subjects in the each of the research studies had four scans performed – whole body, lumbar spine, non-dominant proximal hip and non-dominant distal radius. These scans were analyzed by a single ISCD certified clinical densitometrist 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 percentage of total body fat, fat mass, fat-free tissue mass, and central abdominal fat [39] were determined.

Peripheral Quantitative Computed Tomography

Although DXA is considered the gold standard for the diagnosis of osteoporosis, pQCT has displayed similar capability of predicting a combined index of mechanical strength at the hip, spine, and radius, is less prone to soft tissue errors, and allows separation of analysis for trabecular and cortical regions of bone [40]. The gantry of the pQCT is not large enough to accommodate an entire person but allows for scanning of the appendicular skeletal sites of the forearm and foreleg. The pQCT instrument quantifies BMD, BMC, cortical thickness, as well as trabecular and cortical cross-sectional areas. In addition, it can also be used to quantify muscle

cross-sectional area and density and areas of fat, including subcutaneous, intramuscular, and marrow fat depots.

A Stratec XCT 3000 pQCT (*White Plains, NY*) was located in the BONE Laboratory in 229 Wallace Hall (Virginia Polytechnic Institute and State University, Blacksburg, VA). The manufacturer's quality control procedures were followed at the beginning of each day of scanning using a phantom cone device (Stratec). The CVs for this lab and personnel for test-retest reliability in 15 young adult men and women tested on separate days ranged from 0.5 to 2.7% for cortical area and density measures, from 1.8 to 8.0% for trabecular tissue, and from 1.7 to 13.1% for soft tissue (marrow, muscle, fat) area and density measures [41].

For each subject in the each of the research studies, a scout scan to find the end of the bone for both the non-dominant tibia and non-dominant radius prior to the four tibial scans (TIBIA4S Mask; 4, 14, 38, and 66% from the distal end) or two radial scans (RADIUS2S Mask; 4 and 66% from the distal end). These scans were analyzed by a single ISCD certified clinical densitometrist utilizing the manufacturer's TIBIA4S and RADIUS2S Macros. In addition, the LOOP procedure was conducted on each scan and output to a database file for analysis.

Physical activity assessment

Pedometers are small, inexpensive, and easy to wear devices that have been used to monitor physical activity by counting steps taken while worn. Although they cannot accurately provide an estimate of energy expenditure, they can provide a useful and objective index of physical activity status [42]. Pedometer use has been shown to be associated with increasing daily steps walked and aiding in reductions in body weight and blood pressure [43].

All subjects completed a physical activity log and wore an Accusplit Eagle pedometer (Livermore, CA) through all waking activities over a four day period. The pedometer was

attached at the hip each morning and was removed each evening for sleep. Subjects removed the pedometer for running, swimming or any activity more strenuous than walking and recorded the activity with the duration and intensity on the physical activity log. The reported physical activity and pedometer step counts were translated into corresponding MET levels utilizing a compendium of physical activity, 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. Utilizing both the unplanned (daily step counts) and planned activity (more intense activities when the pedometer was not worn) better reflected the subject's overall daily exercise patterns.

Summary

Physical activity, a modifiable behavior, has direct impact on the hard and soft tissues of bone, muscle and fat. Although the parameters of hard and soft tissues can be measured with magnetic resonance imaging (MRI) or computed tomography (CT), the peripheral quantitative computed tomography (pQCT) device may be a more efficient tool for researchers. In addition to BMC and BMD measures, it produces regional and compartmental breakdown of the long bones most directly affected by PA and provides cortical and trabecular areas as well as calculated measures of bone strength. It also is a less expensive, smaller instrument and produces less radiation exposure than its larger counterparts. It has no body size/weight limitations and the capacity to accommodate measurements in the appendicular skeleton of a given subject's limbs is limited only by the size of the gantry. There are few studies that have examined bone parameters in the context of PA that have utilized pQCT data as endpoints.

The effects on bone quality (bone mineral density, bone mineral content, geometry and strength measures) and muscle quality may provide a better assessment of the risk of osteoporotic fracture

[44]. It is therefore important to evaluate bone status along with soft tissues in patients undergoing BarS in the short-term to determine the longer-term consequences of these procedures. The pQCT instrument is still not commonly used in the assessment of osteoporotic status or studies of bone health. Despite this, its demonstrated ability to discriminate those with recent fractures is best suited for examining the different hard and soft tissues of the peripheral musculoskeletal system [45, 46]. There is also a need to compare the most common surgical procedures, AGB and RYGB, at similar time points to better understand the impact each procedure may have on bone status. Measurement of biomarkers related to bone turnover are also critical to fully evaluate possible bone changes following surgery as well as exploring the possibilities of the interaction between bone and energy metabolisms.

This review has highlighted the need for additional research regarding bone health in the context of the common maladies, osteoporosis and obesity. Because PA is a modifiable behavior that has direct impact on both osteoporosis and obesity, examining its effects with novel instrumentation like the pQCT can improve the overall understanding. With the increasing use of BarS to combat the obesity epidemic and its possible effect on osteoporosis, examining bone health in those undergoing BarS is warranted. In addition, this research has provided valuable information that can be used by physicians and patients to help them make better choices related to issues of obesity and bone health.

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Chapter 3

Manuscript 1

Influences of physical activity in healthy premenopausal women on appendicular bone as measured with pQCT and DXA

Abstract

Purpose: Relationships between physical activity (PA) and parameters of bone density and strength were examined by peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA) in a group of healthy premenopausal women. Additionally, a comparison of these two technologies in the context of these measures was performed.

Methods: A total of 82 premenopausal women (age, mean $\pm$ SD; 38.6 $\pm$ 4.7 yr) underwent DXA and pQCT scans. DXA measurements were taken for whole body, lumbar spine, and non-dominant hip and radius, while pQCT measurements were taken at four locations along the non-dominant tibia and two locations for the non-dominant radius. Physical activity (PA) status was determined from 4-d self-reported PA and 4-d pedometer step counts along with a PA questionnaire. Subjects were grouped into tertiles, least to highest active (PA-L, PA-M, PA-H) based on an average MET-min/day calculation.

Results: Comparisons for DXA results, whole body bone mineral density (BMD) and lumbar spine bone mineral content (BMC), showed group differences ($p<0.05$) between the highest and lowest PA levels (PA-H greater than PA-L by 4.6% and 12.0%, respectively). Likewise the pQCT tibial cortical area at 14%, 38%, and 66% loci differed ($p<0.05$) between the highest and lowest PA tertiles (CoA; PA-H values higher than PA-L by 8.2%, 9.5%, and 12.0%, respectively; PA-H also higher than PA-M by 7.8% at the 14% locus). Further, total BMC (ToC;

PA-H was higher than PA-L by 9.2%, 9.3%, and 9.6%, respectively). Additionally the polar strength-strain index (SSI_p) at 4%, 14% and 66% loci were greater ($p<0.05$) in highest vs. lowest PA groups for the (PA-H was higher than PA-L by 9.5%, 14.2%, and 15.5%, respectively; The SSI_p for PA-H was also higher than PA-M by 11.9% at the 14% locus). Finally, the 66% locus was greater ($p<0.05$) in highest vs. lowest PA groups for the circular model of cortical thickness ($CoTh_c$; PA-H was higher than PA-L by 11.5%). The pQCT radial 4% locus differed ($p<0.05$) between the highest and lowest PA levels for the periosteal circumference ($Peri_c$; PA-H was higher than PA-L by 9.6%). To compare the two instruments, the four pQCT tibial loci were utilized as repeated measures and compared to the DXA leg and hip measures. The data were also adjusted for body mass index (BMI). The DXA leg BMD, leg BMC, hip BMD, and hip BMC all showed differences ($p<0.05$) between the moderate and lowest as well as the highest and lowest PA levels (PA-M was higher than PA-L by 5.7%, 9.4%, 9.2% and 14.7%, respectively; PA-H was higher than PA-L by 7.2%, 14.5%, 9.6%, and 15.2%, respectively). Likewise the pQCT tibial measures of CoA and ToC differed ($p<0.05$) between the moderate and lowest as well as the highest and lowest PA levels (PA-M was higher than PA-L by 8.2% and 8.6%, respectively; PA-H was higher than PA-L by 13.1% and 13.1%, respectively). Additionally the SSI_p differed ($p<0.05$) between all levels of PA (PA-M was higher than PA-L by 11.0%, PA-H was higher than PA-L by 19.3%, and PA-H was higher than PA-M by 9.4%). Similarly, the two pQCT radial loci were compared to the DXA arm and radius measures; however, none of these were significantly different.

Conclusion: Both DXA and pQCT revealed differences in parameters of weight-bearing bones in groups where combined planned and unplanned PA averaged greater than ~300 MET-min/d. The areal images from DXA only provide evidence of increased BMC and BMD for the various

regions of clinical interest in response to higher levels of PA. In contrast, the pQCT can delineate smaller regions of BMC, BMD, and structural factors, and these parameters are more sensitive than those provided by DXA. Additional intervention studies are necessary to determine the types and magnitude of PA that can be discriminated by these instruments.

Introduction

In the United States, osteoporotic fractures are a significant issue for the elderly with 44 million people diagnosed or at risk of osteoporosis, 68% of which are women, and an estimated 2 million new incidences each year with an estimated annual cost in excess of \$19 billion [1,2]. Low bone mass in the elderly has been attributable to either failure to accrue sufficient peak bone mass during development or an excessive rate of the inevitable loss associated with age [3]. Therefore, the loss of bone in younger individuals, especially younger women, increases the potential for osteoporosis-related complications later in life.

Development of osteoporosis and a subsequent increased risk of fracture are influenced not only by the bone mineral content, but also the structural and material properties of bone [4]. Although adequate nutrition and mineral intake are important in the developing skeleton, physical activity (PA) alone has been shown to increase periosteal and endosteal circumferences in the tibia in developing children, factors known to reduce susceptibility to fracture [5]. Additionally, it has been shown that the increase in cortical bone size of the tibia and radius via periosteal expansion during youth persists in older men and that continued exercise at old age may decrease endosteal bone loss in weight-bearing bones [6]. Because osteoporosis is such a significant issue, understanding the relationship between PA and skeletal health is important. The majority of research related to the effects of exercise on bone has utilized dual-energy X-ray absorptiometry (DXA) measurements of bone mineral content (BMC) and bone mineral Density (BMD) as endpoints. However, because the structural properties of bone play a significant role in bone strength and its ability to accommodate compressive and torsional loads, it is therefore important to effectively examine not only bone density and content by planar imaging techniques, like those of DXA, but also bone geometry and microarchitecture via three-dimensional imaging like

that of the peripheral quantitative computed tomography (pQCT) or high-resolution instrument (HR-pQCT).

Turner [7] stated that the size and shape of bones are the major determinants of their resistance to fracture and, further, that these characteristics are largely determined by the mechanical stresses placed on them. Animal studies have demonstrated that significant increases in bone strength and resistance to structural failure are produced where strains are highest despite only modest gains in bone mineral density (BMD) [8, 9]. Although the parameters of hard and soft tissues can be measured with magnetic resonance imaging (MRI) or computed tomography (CT), the pQCT device may be a more efficient tool for researchers. In addition to BMC and BMD measures, it produces regional and compartmental breakdown of the long bones most directly affected by PA and provides cortical and trabecular areas as well as calculated measures of bone strength. It also is a less expensive, smaller instrument and produces less radiation exposure than its larger counterparts. It has no body size/weight limitations and the capacity to accommodate measurements in the appendicular skeleton of a given subject's limbs is limited only by the size of the gantry. There are few studies that have examined bone parameters in the context of PA that have utilized pQCT data as endpoints. The purpose of this research, therefore, was to demonstrate the effectiveness of utilizing measures from pQCT, as well as DXA, to assess the differences in healthy premenopausal women with markedly different patterns of habitual PA.

Methods

Subjects

Subjects were 82 healthy, premenopausal Caucasian women (mean age $\pm$ SD 38.6 $\pm$ 4.7 yr) with body mass index (BMI) ranging from 18.3 to 46.6 kg/m² (mean BMI $\pm$ SD 24.5 $\pm$ 5.1). Women who were receiving prescriptive medications known to affect bone health were excluded.

Subjects were recruited from the Virginia Tech campus and the surrounding communities in southwest Virginia. 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.

Anthropometrics

Body weight was measured with a calibrated electronic scale (ScaleTronix, *Wheaton, IL*) to the nearest 0.1 kg, and height by a calibrated, wall-mounted digital stadiometer (Heightronic, Measurement Concepts, *North Bend, WA*) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The length of the non-dominant radius was measured from the highest point of the radial head to the highest point of the styloid process, to the nearest cm. 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 to the nearest cm.

DXA measurements

DXA (Hologic QDR 4500-A Elite DXA *Bedford, MA*) scans of the whole body, lumbar spine, non-dominant hip and non-dominant forearm were completed and analyzed by one technician to determine whole body fat mass, fat-free mass, central body fat [10], areal bone mineral density (aBMD) and bone mineral content (BMC), as well as site specific aBMD, BMC and T-score for osteoporotic risk. The coefficient of variance (CV) for test–retest reliability in 15 young adult men and women tested on separate days were < 1.8% for hard and soft tissue parameters measured by DXA [11].

pQCT measurements

Four scans were performed at loci of 4, 14, 38, and 66% from the distal end of the non-dominant tibia and two more at 4 and 66% from the distal end of the non-dominant radius, utilizing the

Stratec XCT 3000 pQCT (*White Plains, NY*). For each of these scan locations, one technician determined volumetric bone mineral density (vBMD), BMC, polar and axial strength strain index (SSI), cortical and trabecular bone cross-sectional areas, muscle cross-sectional area (MCSA), and cross-sectional areas for subcutaneous, marrow, and inter-muscular fat. Repeatability of hard and soft tissue parameters was established using the ISCD Advanced Precision Tool [12], which included three scans on non-consecutive days in the non-dominant forelegs and forearms of an independent group of 15 premenopausal females (mean age $\pm$ SD 39.8 $\pm$ 4.6 yr, mean BMI 25.4 $\pm$ 6.6 kg/m²).

Physical activity

Both subjective and objective measures were used to quantify PA status. Each subject completed the VA Palo Alto Physical Activity Questionnaire (PAQ) developed by Myers et al [13], a subjective measure that quantifies weekly activity and the previous year and lifetime recreational and occupational PA and has been shown to be a useful clinical screening tool for patients at risk of cardiovascular disease [14]. In addition, each subject wore a pedometer to record activity over a 4-day period. Subjects also recorded other physical activities when the pedometer was not worn and detailed the type, length of time, and intensity of activity. Using this data and a compendium of PA [15], these activities along with the pedometer step counts were translated into corresponding metabolic equivalents (MET), and values multiplied by duration of each activity. Resulting values were averaged and PA expressed as MET·min/day to quantify overall PA status. The integration of these two data sources, both unplanned (daily step counts) and planned activity (more intense activities when the pedometer was not worn), produced a more comprehensive index of overall daily exercise patterns, especially when compared to the recall of the PAQ.

Statistical analyses

Data were analyzed using the NCSS 8 Statistical Software (NCSS, LLC, Kaysville, Utah) and $p < 0.05$ was considered statistically significant throughout. Subjects were grouped by level of PA (least, PA-L; moderate, PA-M; highest, PA-H) based on tertiles of the MET-min/day calculation. Descriptive statistics for all relevant measures were computed using means and standard deviations. The effects of habitual PA were independently evaluated for each of the DXA and pQCT variables of interest by analysis of variance (ANOVA). Finally, 2-way repeated measures analysis of covariance (ANCOVA) was also used to evaluate effects of PA level on pQCT parameters in a three-by-four model, with PA level (3 groups) and tibial loci (4 specific sites) representing the independent and repeated factors, while controlling for the effects of body size using BMI (PASW Statistics for Windows, Version 18.0; SPSS Inc. Chicago, Ill). ANCOVA was similarly used to analyze for effects PA in the pQCT variables measured at the radius in a three-by-two model. If significant F ratios were found through ANOVA or ANCOVA, post-hoc assessment was performed using the Tukey-Kramer test to isolate the source of these differences.

Results

Four of the 82 subjects failed to return their PA record, resulting only in data from 78 healthy females available for analysis. Weight, BMI, percent total and central body fat (by DXA) differed ($p < 0.05$) between two or more (PA-L, PA-M, and PA-H) of the PA levels (Table 3-1). Of the DXA bone regions of interest, only whole body BMD and lumbar spine BMC were significantly greater for the high (PA-H) group relative to the low (PA-L) group (Table 3-2). For the pQCT tibial measures, the SSI_p differed ($p < 0.05$) between the PA-L and PA-H groups at the 4, 14, and 66% loci, and also between the PA-M and PA-H groups at the 14% locus. The parameters that primarily contributed to these differences, ToC and CoA, were also different

(Table 3-2). Of the pQCT radial measures, only the Peri_c at the 4% location was different between the PA-L and PA-H levels ($p < 0.05$; not included in Table 3-2).

To further evaluate differences in bone parameters with the DXA and pQCT technologies, as a function of PA tertiles, the four slices along the shaft at 4, 14, 38 and 66% from the distal end of the tibia were treated as repeated measures for each subject; similar analyses also were performed with scans collected for the radius at the 4% and 66% loci. Table 3-3 displays the ANCOVA results for the DXA measures and the pQCT tibial repeated measures adjusted for body size. DXA BMD and BMC measures for the whole body, leg region, lumbar spine, and hip all differed between the PA-L and PA-H tertiles (range for F-ratio=2.99-6.93; range for p =0.057-0.002). The leg region and hip also differed between the PA-L and PA-M groups. For the pQCT tibial measures, SSI_p , CoA, and ToC, the PA-M and PA-H groups were each greater than the PA-L group and for the SSI_p the PA-H group was also greater than the PA-M group (F-ratio=11.55-15.21; prob level<0.001). None of the DXA or pQCT measures for the arm and radius differed between the PA groups, nor did the other tibial measures.

Discussion

In the current study, groups of healthy premenopausal women who differed by PA tertiles were compared on measures of appendicular bone status. These subjects were all relatively active, and despite the lack of a truly sedentary group, significant differences were still observed in bone measures with both the DXA and pQCT instruments, using simple delineation of tertiles based on PA. In addition, this was the first study to compare the two technologies utilizing the statistical technique of repeated measures for the pQCT loci. Although all the women in this study exceeded the 2008 Physical Activity Guidelines for Americans [16] recommendations for health, this study demonstrates that there can be notable differences in bone geometry and

strength for differences in PA. Initial observations indicated that the DXA discriminated between PA tertiles for measures of body composition, particularly with indicators of fat mass; however, with a few exceptions, DXA measures of bone status showed fewer differences according to PA tertiles. In contrast, several of the pQCT measures of bone were significant. This is consistent with the findings in a recent review by Gómez-Cabello, et al. [17], in which they noted changes in bone mass that were evident by pQCT, even when unremarkable by DXA and they indicated that pQCT may provide additional information about the effects of exercise on bone that cannot be elucidated by DXA. Additionally in a group of post-menopausal women, Adami, et al. [18] found increases in cortical area, density, and BMC of the ultra-distal radius ($2.8 \pm 15.0\%$, $2.2 \pm 15.8\%$, and $3.1 \pm 10.7\%$, respectively) and decreases in trabecular BMD and BMC ($-2.6 \pm 10.7\%$ and $-3.4 \pm 14.2\%$, respectively) despite finding no significant changes with DXA measurements. PA tertiles in the present study differed in BMI and fat related measures, prompting a need to control the analyses for this influence (BMI, as covariate). Similarly, in a study of obese postmenopausal women, Sornay-Rendu, et al. [19] concluded higher bone densities, areas of cortical and trabecular architecture, and strength indices as measured by high resolution pQCT were not in proportion to the excess of BMI and fat mass. This adjustment of measures produced a significant difference in the analyses, and the majority of bone measures obtained from both the DXA and pQCT were significant. In addition to the bone parameters evaluated in the current study, muscle cross-sectional areas for the forearm (F-ratio=1.97, $p=0.15$) and foreleg (F-ratio=1.78, $p=0.18$) was found not to differ across PA tertiles. This is in contrast to a findings in a study of elderly men and women by Edwards, et al. [20], in which muscle size and grip strength were positively associated with bone size and strength.

Because the regions of interest for the DXA and pQCT are not the same, it is somewhat problematic to compare their utility and discriminative value with regard to assessing PA related differences. The DXA produces areal images of the whole body or select anatomical regions of bones, while the pQCT provides a 3-dimensional slice at a given location on an individual limb. The data available from the DXA are BMC and BMD from the three clinical regions of interest (lumbar spine, hip and radius), as well as the whole body and select regions (arms, legs and torso). Due to the size of the gantry, the pQCT is limited to the foreleg and forearm. To compensate for these differences in the two technologies, not only the individual pQCT site locations on the appendicular skeleton were examined, but the separate slice data along the tibial and radial shafts were also utilized as repeated measures. This allowed us to analyze the effects of PA on the entire tibia and radius to make better comparisons between the DXA and pQCT. Figure 3-1 demonstrates the extent to which geometric and strength parameters in the tibia systematically differ at representative points along the tibia, as a function of PA tertile classification. Comparisons of BMC of the tibia (pQCT) and leg (DXA) are seen in Figure 3-2 and indicate that the pQCT measures are more sensitive to PA differences despite the similar finding with DXA. Figure 3-3 demonstrates that the index of BMD as measured by pQCT lacks the potential to separate individuals according to habitual PA even after accounting for effects of body size, therefore, it may not be a clear indicator of bone status. Finally, the hip region may dominate the DXA leg measures of BMC (Figure 3-2) and BMD (Figure 3-3).

There are several limitations in the current study. The activities that these subjects undertook over the 4-day reporting period were those that primarily affect the lower extremities, such as walking, running, and cycling. This may explain the lack of similar patterns of difference in the arm and radius regions of interest. Because PA was assessed with pedometer step count and self-

reported PA from a 4-day record, some inferences were made in the conversion of this data to the MET-min/day. This group of subjects was relatively active and the majority of subjects were meeting or exceeding the 2008 Physical Activity Guidelines for Americans [16] recommended PA for health. That guideline calls for 500-1000 MET-minutes/week and the average for these subjects was over 3700 MET-minutes/wk. Additionally, the cross-sectional nature of the study precludes interpretation of any causal relationships to PA and the studied population of young (20 – 45 yr) healthy Caucasian women precludes the generalization of the data to the overall population.

Understanding the relationship between PA and skeletal health is important because of the significant impact of osteoporosis on society. Recent studies have shown that PA has a positive impact on increasing or maintaining bone including in pre- to early-pubertal children [21], young adults [22] and post-menopausal women [23]. Bone geometry and strength as well as bone mineral content and density are each important factors in evaluating risk for osteoporotic fracture. In a study of adolescent males and females, McKay, et al. [24] found that impact PA is positively associated with bone architecture and strength. Additionally in a review in post-menopausal women by Polidoulis, et al. [25] reported that exercise decreases bone loss by maintaining cortical and trabecular volumetric BMD. Although the DXA provides information for the whole body, specific regions, and the clinically relevant regions of interest used for diagnosis of osteoporosis, it cannot provide information about bone geometry and strength. The pQCT not only provides total BMC and BMD information for each given slice, but also cortical and trabecular BMC and BMD components as well as the areas of each of the bone compartments, muscle, and fat.

Conclusions

Although BMD measures are often used to evaluate bone status in the context of assessing possible relations to PA or specific modalities of exercise training, it is evident from the current study that BMD yields information of limited utility to understanding such outcomes. On the other hand, BMC, cortical area, and the polar strength-strain index all showed discriminative advantage between PA tertiles. Additionally, the regional information on the DXA and the localized scans on the pQCT provided specific information for those regions of bones that may relate to the type of PA performed. PA influences bone strength measures as measured with pQCT and DXA in healthy premenopausal women. Additionally, the pQCT is more sensitive than the DXA in detecting differences between activity levels in the appendicular skeleton.

Table 3-1. Descriptive characteristics

	PA-L (n=27)	PA-M (n=26)	PA-H (n=25)
MET-min/day	270.4 ± 88.2	521.0 ± 72.4	831.8 ± 227.9
Age (years)	37.9 ± 4.1	38.6 ± 5.1	39.7 ± 4.7
Height (cm)	164.4 ± 7.4	164.5 ± 7.5	166.8 ± 7.7
Weight (kg)	74.1 ± 17.8	61.9 ± 10.3 ^a	62.8 ± 9.0 ^b
BMI (kg/m ²)	27.4 ± 6.0	22.8 ± 3.0 ^a	22.5 ± 2.0 ^b
BF (%)	34.6 ± 7.2	28.0 ± 5.5 ^a	23.9 ± 5.5 ^{bc}
Central BF (%)	31.8 ± 10.0	23.1 ± 8.1 ^a	19.0 ± 6.8 ^b

Data are displayed as mean ± SD. BMI: body mass index, BF: body fat.

^a – difference between PA-L and PA-M, p<0.05

^b – difference between PA-L and PA-H p<0.05

^c – difference between PA-M and PA-H, p<0.05

Table 3-2. ANOVA results from selected DXA and tibial pQCT measures

	PA-L	PA-M	PA-H	F-ratio	P
DXA					
T_BMD (g/cm ²)	1.1 (0.01)	1.2 (0.01)	1.2 (0.02) ^b	3.70	0.029
L_BMC (g/cm)	59.5 (2.0)	61.1 (2.0)	67.7 (2.1) ^b	4.38	0.016
pQCT (tibia)					
ToD (mg/mm³)					
4%	296.0 (7.5)	301.6 (7.7)	294.3 (7.8)	0.25	0.781
14%	547.3 (13.8)	557.8 (14.1)	561.8 (14.3)	0.28	0.754
38%	934.2 (11.3)	955.2 (11.5)	961.0 (11.7)	1.52	0.225
66%	712.7 (17.6)	754.3 (17.9)	746.7 (18.3)	1.56	0.216
ToC (mg/mm)					
4%	276.2 (7.2)	284.6 (7.4)	291.4 (7.5)	1.07	0.349
14%	219.6 (4.9)	226.7 (5.0)	241.7 (5.1) ^b	5.08	0.009
38%	322.2 (6.9)	337.5 (7.1)	355.3 (7.2) ^b	5.50	0.006
66%	352.2 (7.8)	368.6 (8.0)	389.6 (8.1) ^b	5.48	0.006
CoA (mm²)					
4%	182.8 (4.4)	188.4 (4.5)	197.5 (4.5)	2.77	0.069
14%	154.5 (3.2)	155.2 (3.3)	168.3 (3.3) ^{bc}	5.53	0.006
38%	251.9 (6.9)	271.5 (7.0)	278.4 (7.1) ^b	3.90	0.025
66%	270.9 (6.9)	287.9 (7.0)	307.8 (7.2) ^b	6.92	0.002
CoTh_c (mm)					
4%	1.8 (0.03)	1.8 (0.04)	1.9 (0.04)	1.31	0.276
14%	2.5 (0.06)	2.4 (0.07)	2.6 (0.07)	1.29	0.282
38%	5.2 (0.21)	5.7 (0.23)	5.5 (0.23)	1.53	0.224
66%	4.1 (0.12)	4.5 (0.12)	4.6 (0.12) ^b	5.57	0.006
SSI_p (mm³)					
4%	1157.3 (31.9)	1173.1 (32.5)	1278.6 (33.1) ^b	4.05	0.021
14%	1172.3 (35.3)	1203.8 (36.0)	1366.4 (36.7) ^{bc}	8.23	0.001
38%	1365.0 (45.2)	1431.3 (46.0)	1505.6 (47.0)	2.33	0.104
66%	1928.3 (74.7)	2036.1 (76.1)	2282.5 (77.6) ^b	5.63	0.005

Data are displayed as mean (SEM). DXA: T_BMD: whole body bone mineral density, L_BMC: lumbar spine bone mineral content; pQCT: ToD: total bone mineral density, ToC: total bone mineral content, CoA: total cortical area, CoTh_c: cortical thickness in the circular ring model, SSI_p: polar strength-strain index.

^a – difference between PA-L and PA-M, p<0.05

^b – difference between PA-L and PA-H p<0.05

^c – difference between PA-M and PA-H, p<0.05

Table 3-3. ANCOVA results for DXA and repeated measures pQCT with BMI as a covariate

	PA-L	PA-M	PA-H	F-ratio	P
DXA					
Leg_BMD (g/cm ²)	1.155 (0.0172)	1.225 (0.0175) ^a	1.245 (0.0178) ^b	5.59	0.005
Leg_BMC (g)	392.0 (11.1)	432.9 (11.3) ^a	458.5 (11.5) ^b	6.93	0.002
T_BMD (g/cm ²)	1.136 (0.0147)	1.184 (0.0150)	1.198 (0.0152) ^b	3.65	0.031
T_BMC (g)	2202.9 (49.8)	2340.3 (50.8)	2459.0 (51.8) ^b	5.10	0.008
L_BMD (g/cm ²)	1.020 (0.021)	1.060 (0.022)	1.103 (0.022) ^b	2.99	0.057
L_BMC (g)	58.3 (2.0)	61.7 (2.0)	68.4 (2.1) ^b	5.36	0.007
H_BMD (g/cm ²)	0.890 (0.0210)	0.980 (0.0214) ^a	0.984 (0.0218) ^b	4.80	0.011
H_BMC (g)	27.8 (1.2)	32.6 (1.2) ^a	32.8 (1.3) ^b	3.95	0.023
pQCT (tibia)					
ToD (mg/mm ³)	626.2 (11.3)	640.5 (10.7)	638.8 (11.0)	0.434	0.650
ToC (mg/mm)	282.6 (6.1)	309.2 (5.8) ^a	325.3 (5.9) ^b	11.548	<0.001
SSI _p (mm ³)	1332.0 (39.5)	1496.5 (37.5) ^a	1651.1 (38.6) ^{bc}	15.212	<0.001
CoA (mm ²)	209.6 (4.0)	228.4 (3.8) ^a	241.2 (3.9) ^b	14.545	<0.001
CoTh _c (mm)	3.4 (0.1)	3.6 (0.1)	3.6 (0.1)	2.398	0.098
Endo _c (mm)	58.6 (1.1)	59.4 (1.1)	61.8 (1.1)	2.116	0.128
Peri _c (mm)	79.8 (0.9)	82.1 (0.8)	84.6 (0.9) ^b	7.162	0.001

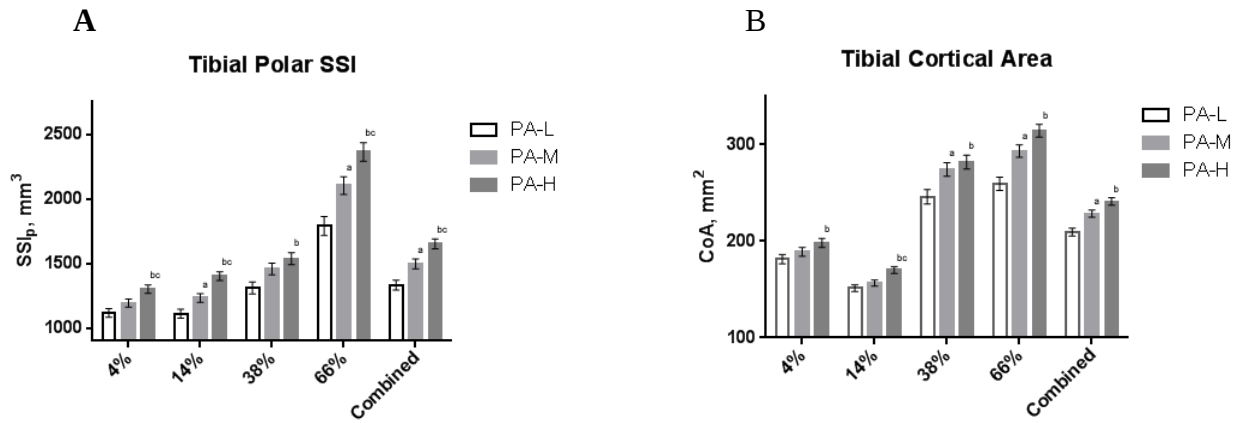
Data are displayed as mean (SEM). The covariate of BMI was evaluated at 24.287 in the model. PA: physical activity. DXA: Leg_BMD: non-dominant leg bone mineral density (BMD), Leg_BMC: non-dominant leg bone mineral content (BMC), T_BMD: whole body BMD, T_BMC: whole body BMC, L_BMD: lumbar spine BMD, L_BMC: lumbar spine BMC, H_BMD: proximal hip BMD, H_BMC: proximal hip BMC. The pQCT repeated measures were the four slices along the shaft at 4, 14, 38 and 66% from the distal end of the tibia. pQCT: ToD: total BMD, ToC: total BMC, SSI_p: polar strength-strain index, CoA: total cortical area, CoTh_c: cortical thickness in the circular ring model, Endo_c: endosteal circumference in the circular ring model, Peri_c: periosteal circumference in the circular ring model.

^a – difference between PA-L and PA-M, p<0.05

^b – difference between PA-L and PA-H p<0.05

^c – difference between PA-M and PA-H, p<0.05

Figure 3-1. pQCT tibial slice and repeated measures



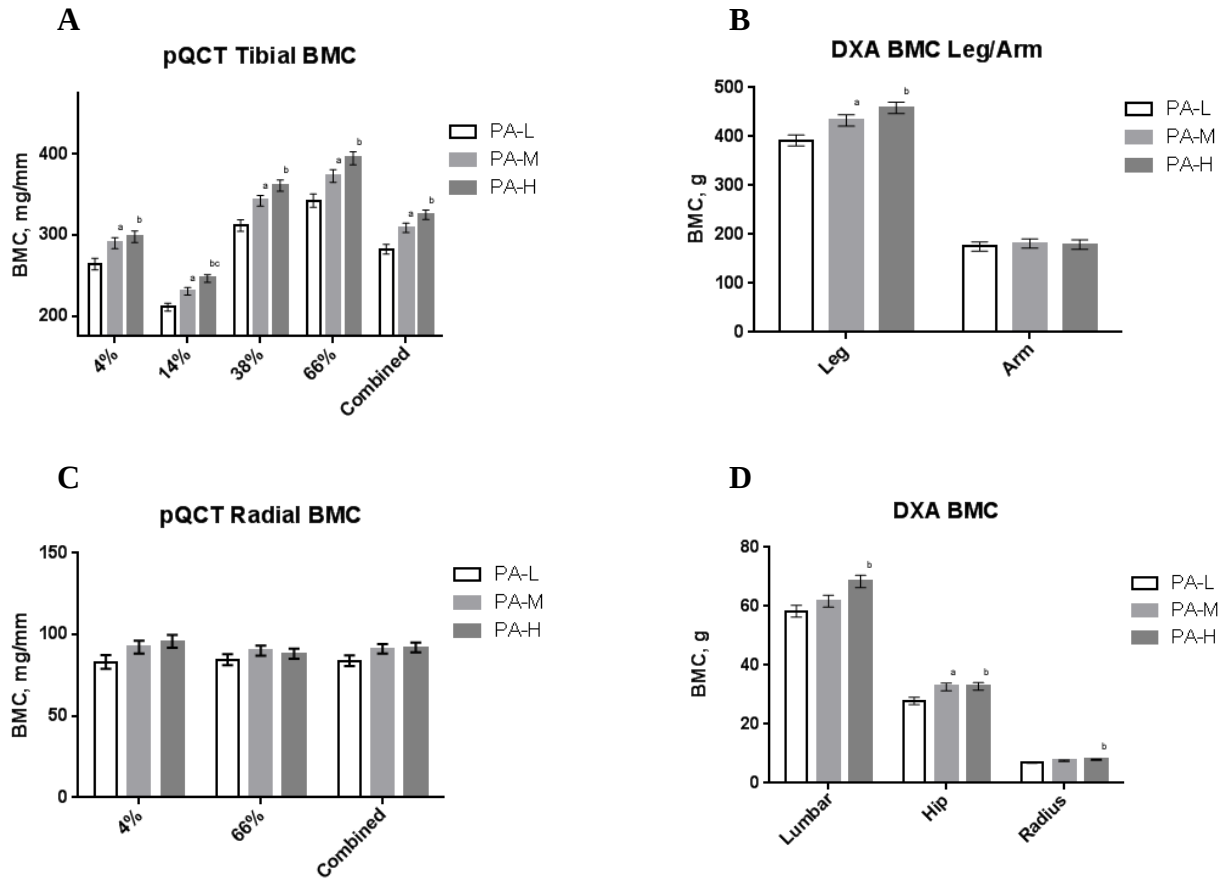
ANCOVA ($p < 0.05$; covariate was BMI). **(A)** pQCT tibial polar strength-strain index (SSI_p) by slice; **(B)** pQCT tibial cortical area (CoA) by slice.

^a – difference between PA-L and PA-M

^b – difference between PA-L and PA-H

^c – difference between PA-M and PA-H

Figure 3-2. pQCT slice and repeated measures and DXA measures



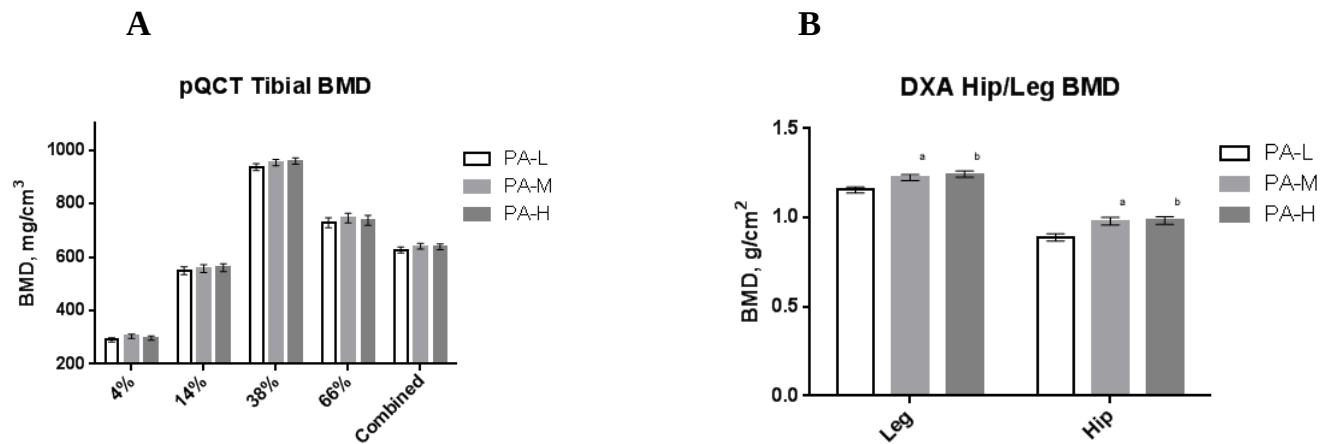
ANCOVA ($p < 0.05$; covariate was BMI). **(A)** pQCT tibial BMC by slice; **(B)** DXA Leg and Arm regions BMC; **(C)** pQCT radial BMC; **(D)** DXA clinical regions of interest BMC.

^a – difference between PA-L and PA-M

^b – difference between PA-L and PA-H

^c – difference between PA-M and PA-H

Figure 3-3. pQCT slice and repeated measures and DXA measures



ANCOVA ($p < 0.05$; covariate was BMI). **(A)** pQCT tibial BMD by slice; **(B)** DXA Hip and Leg BMD.

^a – difference between PA-L and PA-M

^b – difference between PA-L and PA-H

^c – difference between PA-M and PA-H

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Chapter 4

Manuscript 2

Comparative influences of bariatric surgery in premenopausal women on changes in DXA and pQCT measures of bone, muscle, and fat

Abstract

Purpose: This non-randomized 6-month prospective study compared the effects of adjustable gastric banding (AGB) and Roux-en-Y gastric bypass (RYGB) surgeries on bone, muscle and fat parameters measured with the dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT).

Methods: Subjects were 21 Caucasian premenopausal women (Mean age $\pm$ SD 35.0 $\pm$ 5.9 yr), volunteers who had been accepted for elective bariatric surgery (BarS) at the Carilion Clinic in Roanoke, VA. Measurements were taken before surgery and at 3 and 6-months post-surgery. DXA scans of the whole body, the lumbar spine, the non-dominant hip and the non-dominant forearm were completed to assess body fat mass, fat-free mass, central body fat, areal bone mineral density (BMD) and bone mineral content (BMC). The pQCT scans were performed at loci of 4, 14, 38, and 66% from the distal end of the non-dominant tibia and at loci of 4 and 66% from the distal end of the non-dominant radius. These pQCT measures of the forearm and foreleg enabled determination of volumetric BMD, BMC, polar and axial strength strain indices (SSI), cortical and trabecular bone cross-sectional areas, muscle cross-sectional area (MCSA), and cross-sectional areas for subcutaneous, marrow, and inter-muscular fat.

Results: Examining data for the entire sample, BarS was associated with decreases ($p<0.05$) in weight, fat-free mass, fat mass, and central body fat (DXA) and decreases in tibial and radial

subcutaneous fat and radial MCSA (pQCT) through 6 months post-surgery; however, bone measures of interest did not change over this period, either by DXA or pQCT. In comparing the two forms of BarS, analyses of change in all parameters were adjusted for weight loss. Among the DXA measures, only central body fat ($p<0.05$) and whole body BMC ($p<0.01$) showed decreases; these were observed at both post-surgical time points after AGB and RYGB surgeries. However, these temporal changes did not differ by type of surgery. For the pQCT measures at the 4% locus, the total BMD decreased ($p<0.01$) while the trabecular BMC ($p<0.01$), periosteal circumference (PERI) ($p<0.05$), and endosteal circumference (ENDO) increased ($p<0.05$) at both 3 months and 6 months post-surgery, but only for the RYGB surgery. Each of these were different ($p<0.05$) between surgeries at 6 months post-surgery, with the ENDO also different ($p<0.05$) at 3 months post-surgery. At the 38% locus, there were significant differences between surgeries at 3 months and 6 months post-surgery for the polar SSI, PERI and ENDO measures. The polar SSI increased ($p<0.05$) for AGB at 3 months while both polar SSI ($p<0.05$) and ENDO ($p<0.01$) increased for RYGB at 3 months as well as 6 months. At the 66% locus, the cortical thickness (COTH) was different ($p<0.05$) between surgeries at 3 months, muscle density (MD) was different ($p<0.01$) between surgeries at 6 months, and PERI and ENDO were different ($p<0.05$) between surgeries at both 3 months and 6 months post-surgery. Additionally, ENDO increased ($p<0.05$) for the RYGB surgical group at both time points and MD decreased ($p<0.05$) for the AGB group at 3 months and for the RYGB group at both 3 months and 6 months.

Conclusions: In this group of premenopausal women studied through 6 months after BarS, no loss of bone was observed, as measured either by DXA or pQCT, despite the finding of moderate reductions in body weight, fat-free mass, and fat mass. This finding is a positive indication that these women do not appear to be at increased risk of osteoporosis as a function of BarS, in

general, or by either of the two specific surgical procedures investigated. Differences in the two surgical types were evident with RYGB demonstrating comparatively greater increases in bone mechanical strength post-surgery. There were discordant results between the DXA and pQCT instruments, which demonstrate the value of utilizing both instruments in evaluating the effects of BarS on hard and soft tissues. In order to achieve a better understanding of these observations in the context of other studies, large scale randomized trials are needed that include considerably longer follow-up.

Introduction

Osteoporosis is a significant health risk for millions of Americans over the age of 50, two-thirds of which are women [1]. In 2004, the first ever Surgeon General's Report on bone health and osteoporosis projected that by 2020, one in two adults over age 50 will be at risk for a fracture from osteoporosis or low bone mass [2]. Although conventional thinking has suggested that increased body mass can provide additional skeletal loading that leads to a reduced risk for osteoporosis, excess body weight carries its own health risks [3]. Obesity is associated with a myriad of adverse health conditions, including type 2 diabetes mellitus, hypertension, gastroesophageal reflux disease, obstructive sleep apnea, and certain types of cancer [4]. In light of this, the great majority of physicians advocate weight loss for their obese patients; however, many people find it exceedingly difficult to lose weight. This difficulty has led to an increasing number of bariatric surgeries (BarS) being performed, with a conservative estimate that there are currently over 200,000 surgeries per year [5]. The two most common BarS procedures are adjustable gastric banding (AGB), which is a restrictive procedure that places a band at the top of the stomach to limit the amount of food an individual can eat at one time, and Roux-en-Y gastric bypass (RYGB), which is malabsorptive as well as restrictive and is accomplished by reducing the size of the stomach and bypassing a small length of the upper small intestine. Although adjustable gastric banding (AGB) is more common in Europe and Australia, it has gained increased acceptance in the United States, while the most common weight loss surgery performed is RYGB [6], with approximately 85% of patients opting for this surgical procedure [7].

In addition to overall weight loss and fat mass reductions, BarS has also shown evidence of fat-free mass losses evidenced by decreases in bone mineral density (BMD) documented by dual-

energy x-ray absorptiometry (DXA) [8-10]. This loss in bone may be a result of the reduction in mechanical loading during ambulation, due to diminished body mass after surgery and may also be further affected by reduced forces imposed on bone by the skeletal muscles [11]. Therefore, the fat-free soft-tissue mass losses associated with BarS also may increase the osteoporotic fracture risk [12]. The effects on bone quality (bone mineral density, mineral content, geometry and mechanical strength) and muscle quality may provide a much more comprehensive assessment of osteoporotic risk [13]. It is therefore important to evaluate bone status along with soft tissues in the short-term in patients undergoing BarS to determine the possibility for long-term implications. Losses observed in the short-term follow up, would point to the need for further investigation of long-term effects that one or both of these forms of BarS might lead to risk of early osteoporosis. The peripheral quantitative computed tomography (pQCT) instrument is still not commonly used in the assessment of osteoporotic status or studies of bone health. Despite this, its demonstrated ability to discriminate those with recent fractures is best suited for examining the different hard and soft tissues of the peripheral musculoskeletal system [14, 15]. This non-randomized 6-month prospective study compared the effects of AGB and RYGB surgeries on bone, muscle and fat comparing parameters obtained using DXA and pQCT imaging technologies.

Methods

Subjects

Subjects were recruited from patients applying for elective BarS at the Carilion Clinic in Roanoke, VA during their initial interview with the surgical staff between March of 2009 and October of 2010. The study protocol was authorized by the Institutional Review Board for the Carilion Clinic and patients provided informed consent. Of the eighty-seven Caucasian

premenopausal women that met the inclusion criteria of weighing less than 136 kg (the limitation of this DXA instrument), 35 agreed to participate. Additional exclusion criteria were an Epworth Sleepiness Score (ESS) greater than 10, indicating increased risk of obstructive sleep apnea, and subjects taking any medications known to affect bone. It has been shown that individuals with OSA are at 2.7 times the risk of osteoporosis than those without OSA, and females have a higher risk than younger and male counterparts [16]. Health insurance eligibility requirements and pre-surgical protocols delayed surgery for some subjects as much as 8 months. Subsequently, nine subjects either decided against surgery or failed to have surgery before the conclusion of data collection, two were denied surgery by their insurance carriers, and one had an ESS score of 18. One subject failed to return after the 3 month follow-up, and one dropped out before the 3 month follow-up. Therefore data from 21 subjects (Mean age $\pm$ SD 35.0 $\pm$ 5.9 yr) were available for the analyses (n=10 and n=11, for AGB and RYGB, respectively). Based on the initial interview, a required seminar, consultation with the surgeon and personal preferences, the subjects selected a desired surgical procedure, AGB or RYGB, and the desired surgical procedure was conducted by one of three surgeons at the Carilion Clinic. DXA and pQCT measurements for this study were taken prior to surgery and repeated at 3 and 6 months following surgery.

Anthropometrics

Body weight was measured without shoes in relaxed attire with a calibrated electronic scale (ScaleTronix, *Wheaton, IL*) to the nearest 0.1 kg, and height by a calibrated, wall-mounted digital stadiometer (Heightronic, Measurement Concepts, *North Bend, WA*) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The length of the non-dominant radius was measured from the highest point of the radial head to the highest point of the styloid process, to the nearest cm with a retractable tape measure. Likewise, 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 to the nearest cm.

DXA measurement

DXA (Hologic QDR 4500-A Elite DXA *Bedford, MA*) scans of the whole body, lumbar spine, non-dominant hip and non-dominant forearm were completed and analyzed by one technician to determine whole body fat mass, fat-free mass, central body fat [17], areal bone mineral density (BMD) and bone mineral content (BMC), as well as site specific areal BMD, BMC and T-score for osteoporotic risk. The coefficient of variance (CV) for test–retest reliability in 15 young adult men and women tested on separate days were < 1.8% for hard and soft tissue parameters measured by DXA [18].

pQCT measurement

Four scans were performed at loci of 4, 14, 38, and 66% from the distal end of the non-dominant tibia and two more at 4 and 66% from the distal end of the non-dominant radius, utilizing the Stratec XCT 3000 pQCT (*White Plains, NY*). For each of these scan locations, one technician determined volumetric BMD, BMC, polar and axial strength strain index (SSI), cortical and trabecular bone cross-sectional areas, muscle cross-sectional area (MCSA), and cross-sectional areas for subcutaneous, marrow, and inter-muscular fat utilizing the manufacturers included loop software with contour mode set to 31 and peel mode to 1 for both the CALCBD and CORTBD functions. Repeatability of hard and soft tissue parameters was established using the ISCD Advanced Precision Tool [19], which included three scans on non-consecutive days in the non-dominant forelegs and forearms of an independent group of 15 premenopausal females (mean age $\pm$ SD 39.8 $\pm$ 4.6 yr, mean BMI 25.4 $\pm$ 6.6 kg/m²).

Statistical analyses

Data were analyzed using the NCSS 8 Statistical Software (NCSS, LLC, Kaysville, Utah), a p -value <0.05 was considered statistically significant throughout. Descriptive statistics for all relevant measures were computed using means and standard deviations. One-way ANOVA was used to assess the impact of bariatric surgery across all patients at the three time points (baseline, 3-months, and 6-months post-surgery) and then two-way ANCOVA was used to analyze differences between RYGB and AGB surgery across the three time points with weight as the covariate. In both cases, if significant F ratios were found, post-hoc assessment was performed using the Tukey-Kramer test to isolate the source of these differences.

Results

The significant differences between the two surgical groups at baseline are displayed in Table 4-1, while changes in weight and body composition measures are displayed in Table 4-2.

Considering all study subjects, BarS resulted in successive reductions ($p<0.05$) in body weight, fat-free mass, fat mass, and central body fat (DXA), as well as reductions in tibial and radial subcutaneous fat and radial MCSA (pQCT) through 6 months post-surgery. However, no bone measures changed over this same 6 months of surgical follow-up.

Comparison of effects for AGB and RYGB on measures of weight, fat mass, and central body fat are presented in Figure 4-1, indicating greater weight and fat loss by those undergoing RYGB.

Subsequent statistical analyses employed in comparisons were adjusted for differences in weight between the two surgical types and accounting for differences in weight loss. Figure 4-2 displays the DXA measures of BMC, BMD, and fat-free mass. Although the DXA measure whole body BMC ($p<0.01$) showed decreases at both time points for RYGB surgery, there was no statistical difference between the two surgical types at either time point. No other differences were found over time or between types of surgery among the DXA measures investigated. With pQCT, several differences in bone measures were found at the various loci and these are displayed in

Figure 4-3. At the 4% locus, the total BMD decreased ($p<0.01$) while the trabecular BMC ($p<0.01$), periosteal circumference (PERI) ($p<0.05$), and endosteal circumference (ENDO) increased ($p<0.05$) at both 3 months and 6 months post-surgery for the RYGB surgery. Each of these were significantly different ($p<0.05$) between surgeries at 6 months post-surgery, with the ENDO also different ($p<0.05$) at 3 months post-surgery. At the 38% locus, there were significant differences between surgeries at 3 months and 6 months post-surgery for the polar strength-strain index (SSI_p), PERI and ENDO measures. The SSI_p increased ($p<0.05$) for AGB between baseline and 3 months while both SSI_p ($p<0.05$) and ENDO ($p<0.01$) increased for RYGB between baseline and 3 months as well as baseline and 6 months. At the 66% locus, the cortical thickness (CoTH) was significantly different ($p<0.05$) between surgeries at 3 months, muscle density (MD) was different ($p<0.01$) between surgeries at 6 months, and PERI and ENDO were different ($p<0.05$) between surgeries at both 3 months and 6 months post-surgery. Additionally, ENDO increased ($p<0.05$) for the RYGB surgical group at both time points and MD decreased ($p<0.05$) for the AGB group at 3 months and for the RYGB group at both 3 months and 6 months.

Discussion

This non-randomized 6-month prospective study is the first to examine bone and soft tissue mass changes with two widely used BarS procedures, AGB and RYGB, which facilitate weight loss by altering the digestive process in markedly different ways. Evidence of bone remodeling was seen by DXA and pQCT in RYGB surgery while not in AGB surgery, despite significant weight loss for both groups. In fact, the DXA and pQCT data were somewhat contradictory. After adjusting the analyses for differences in weight and differential weight loss related to the two types of surgery, the DXA results indicated a whole body loss in BMC for RYGB surgery only, while the pQCT results indicated an increase in endosteal and periosteal circumferences along the tibial

shaft, also for the RYGB surgery. The increase in periosteal circumference is indicative of increases in bone strength, although the SSI_p measures did not change significantly.

Bariatric surgery has become an ever increasing means of managing the obesity epidemic and is often utilized by young premenopausal women and men of similar ages. Of the two most common BarS procedures, RYGB has demonstrated the more dramatic effects on weight loss and reductions in BMD due to its malabsorptive component [20]. It has been shown to resolve type 2 diabetes mellitus in as many as 84% of cases and often within the first couple of weeks after surgery, before any significant weight loss has occurred [21]. Additionally, hyperlipidemia is relieved in nearly 71% of cases, hypertension in 68% of cases, and sleep apnea in 85% of cases, also often before significant loss of excess body weight [22]. Because of these results, BarS is becoming a common treatment option for these comorbidities related to obesity. In addition to these beneficial results, weight loss associated with BarS has also been shown to produce the deleterious effects of loss of bone [23]. Fleisher et al, found declines in hip BMD that were strongly and incrementally related to the amount of weight loss; yet, they found no significant declines in the lumbar spine and distal radius [9]. Carrasco et al, reported that BarS induced a significant total body BMD loss and that reductions in lumbar spine and hip BMD were directly correlated with changes in body composition, i.e. reductions in soft tissue mass. [8]. Yu et al, found that after one year post-surgery following BarS spine and hip BMD measured by DXA showed significant decreases while quantitative computed tomography (QCT) measures only demonstrated losses in the spine but none at the femur [10]. This is similar to the results of the study presented herein and implies that DXA findings of earlier studies may overstate the perceived negative effects of BarS on bone integrity.

Although this study did not investigate physical activity behavior before or after surgery, anecdotal responses to inquiry during bone scanning indicate a possible reason for the increases seen in the pQCT data. The recovery protocol following BarS encourages patients to increase physical activity beginning approximately 3 months post-surgery. Physical activity (PA) alone has been shown to increase periosteal and endosteal circumferences in the tibia in developing children, factors known to reduce susceptibility to fracture, in spite of the importance of adequate nutrition and mineral intake for the developing skeleton [24]. This physical activity may become less burdensome for patients, as weight is reduced and body composition changes allow for greater mobility. Additionally, increased physical activity accompanying weight loss can create a greater lean mass to fat mass ratio. This change in body composition may increase loading on the musculoskeletal system that may counteract the reductions in loading due to weight loss, and lean mass has been shown to exert a greater effect on BMD than fat mass, with physical activity helping to prevent bone loss and potential osteoporosis [25].

Limitations of this study were the relatively small sample size (n=10 and n=11, for AGB and RYGB, respectively), short follow up period of only 6 months, and the small percentage losses in weight compared to other studies. Although, these subjects were class II or III obese at baseline, the absolute weight to be lost was smaller than that of other studies due to the mechanical limitation of the DXA instrument that allows for a maximum of 136 kg of body weight. There is a temporal delay in bone loss following weight loss due to bone adaptation following changes in mechanical loading; the short duration of 6 months for this study may have also limited the results. Increased physical activity accompanying weight loss can create a greater lean mass to fat mass ratio. Higher PA energy expenditure has been shown to correlate with higher appendicular lean mass, and moderate-to-vigorous intensity activity is associated with lean mass

[26]. Lean mass has been shown to exert a greater effect on BMD than fat mass, and physical activity is a proven means of helping to prevent bone loss and osteoporosis [25]. This change in body composition may increase loading on the musculoskeletal system that may counteract the reductions in loading due to weight loss.

Conclusions

This is the first study to examine bone and soft tissue mass changes with two widely used BarS procedures, AGB and RYGB, which facilitate weight loss by altering the digestive process in markedly different ways. In addition, innovative measurements of peripheral bone status as well as the traditional DXA measurements were utilized. In this group of premenopausal women studied through 6 months post-surgery, no significant loss of bone was observed, either with two- or three-dimensional imaging technologies, despite overall body weight losses averaging 26.9 %, overall. However after adjusting for weight loss, differences in the two surgical types were evident with RYGB demonstrating greater changes in bone adaptation. There were discordant results between the DXA and pQCT instruments, which may be indicative of the specific locations of the scans performed by the pQCT as well as the areas of initial bone turnover, but indicate the utility of including the latter newer technology for evaluating the effects of BarS on bone quality. Although other studies have indicated bone loss associated with weight loss after BarS, the finding of no significant loss at 6 months post-surgery is a positive indication. In fact, the increase in periosteal circumference indicates and adaptation toward increased strength. In order to achieve a better understanding of these observations in the context of other studies, additional studies that are longer in duration are needed.

Table 4-1. Subject baseline characteristics

	AGB (n=10)	RYGB (n=11)
Age (years)	33.7 ± 6.2	34.9 ± 6.1
Weight (kg)	118.9 ± 12.6	114.2 ± 9.6
BMI (kg/m ²)	43.2 ± 4.2	42.5 ± 2.7
FFM (kg)	58.0 ± 5.1	58.5 ± 6.2
BF (kg)	58.9 ± 8.8	53.8 ± 6.9
Whole body BMC (g/cm)	2391.4 ± 251.2	2461.3 ± 335.6
Whole body BMD (g/cm ²)	1.14 ± 0.08	1.18 ± 0.08
Lumbar BMC (g/cm)	57.7 ± 9.6	57.4 ± 11.0
Lumbar BMD (g/cm ²)	1.08 ± 0.12	1.07 ± 0.16
Hip BMC (g/cm)	31.5 ± 5.3	34.6 ± 6.7
Hip BMD (g/cm ²)	1.01 ± 0.12	1.06 ± 0.14
Radius BMC (g/cm)	8.1 ± 1.6	7.5 ± 1.7
Radius BMD (g/cm ²)	0.62 ± 0.06	0.63 ± 0.05

Data are displayed as mean ± SD. BMI: body mass index, FFM: fat free mass, BF: body fat, BMC: bone mineral content, BMD: bone mineral density.

Table 4-2. Changes in weight and body composition following bariatric surgery

	Baseline	3 months	6 months
Weight (kg)	116.5 ± 11.1	98.1 ± 13.1 ^a	91.8 ± 15.5 ^b
Weight Loss (kg)		18.3 ± 6.5	6.3 ± 5.0 ^c
BMI (kg/m ²)	42.8 ± 3.4	36.1 ± 4.4 ^a	33.8 ± 5.4 ^b
FFM (kg)	58.3 ± 5.6	52.8 ± 5.4 ^a	51.9 ± 5.9 ^b
BF (kg)	56.2 ± 8.1	43.7 ± 9.7 ^a	38.6 ± 11.8 ^b
Central BF(kg)	4.5 ± 1.5	3.0 ± 0.9 ^a	2.7 ± 1.0 ^b
Whole body BMC (g/cm)	2428.0 ± 293.3	2435.0 ± 279.6	2409.6 ± 300.2
Whole body BMD (g/cm ²)	1.16 ± 0.08	1.17 ± 0.09	1.16 ± 0.09

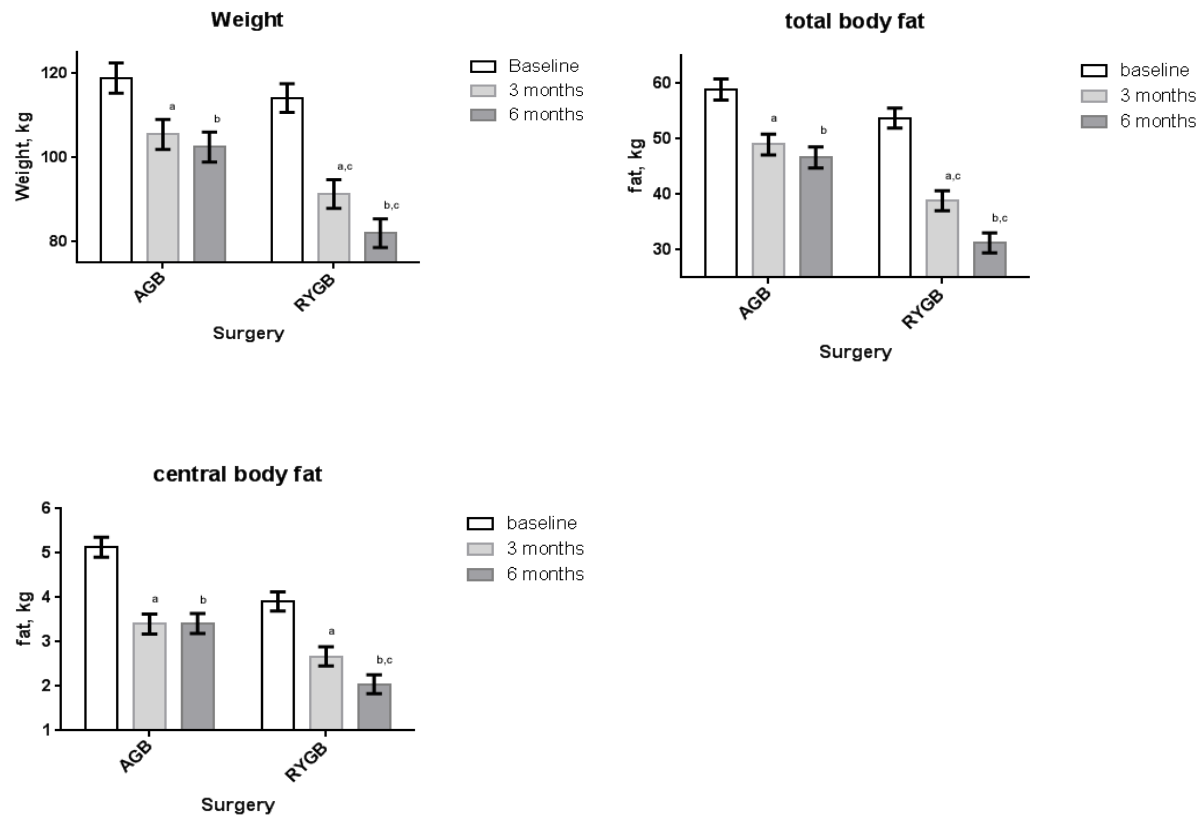
Data are displayed as mean ± SD. BMI: body mass index, FFM: fat free mass, BF: body fat, BMC: bone mineral content, BMD: bone mineral density

^a – difference between baseline and 3 months, p<0.01

^b – difference between baseline and 6 months, p<0.01

^c – difference between 3 months and 6 months, p<0.01

Figure 4-1. Weight and fat loss results by surgical type

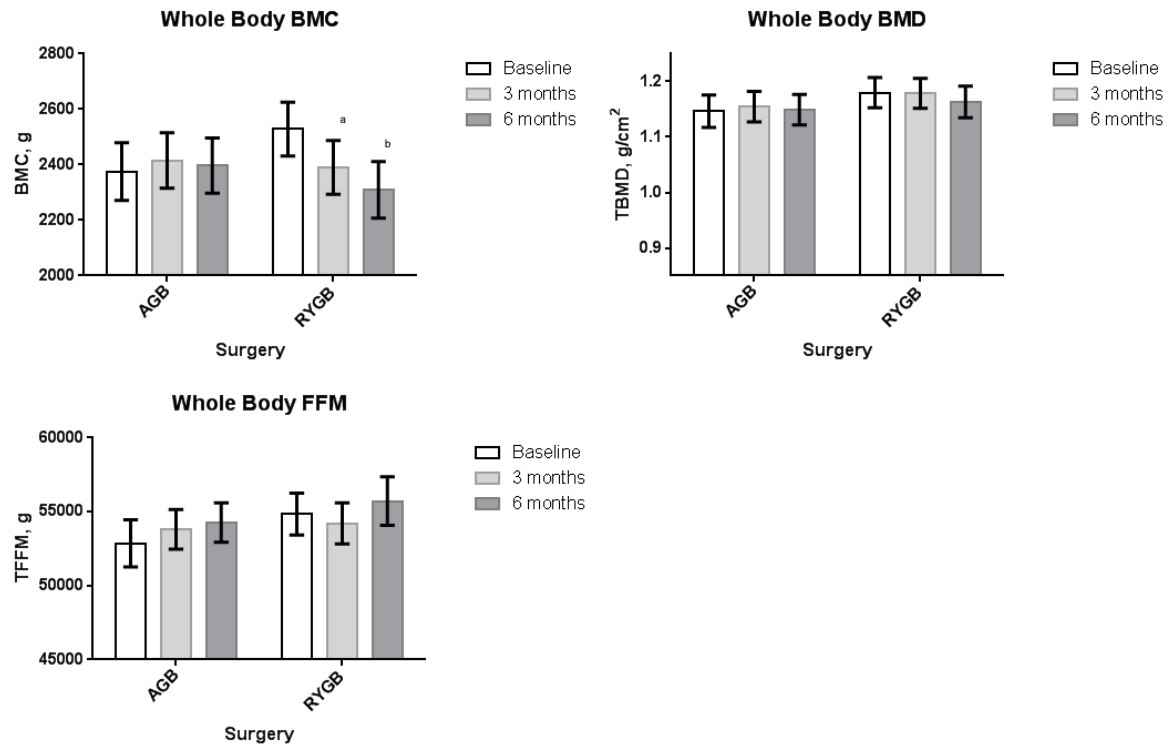


^a – difference between baseline and 3 months, $p < 0.05$

^b – difference between baseline and 6 months, $p < 0.05$

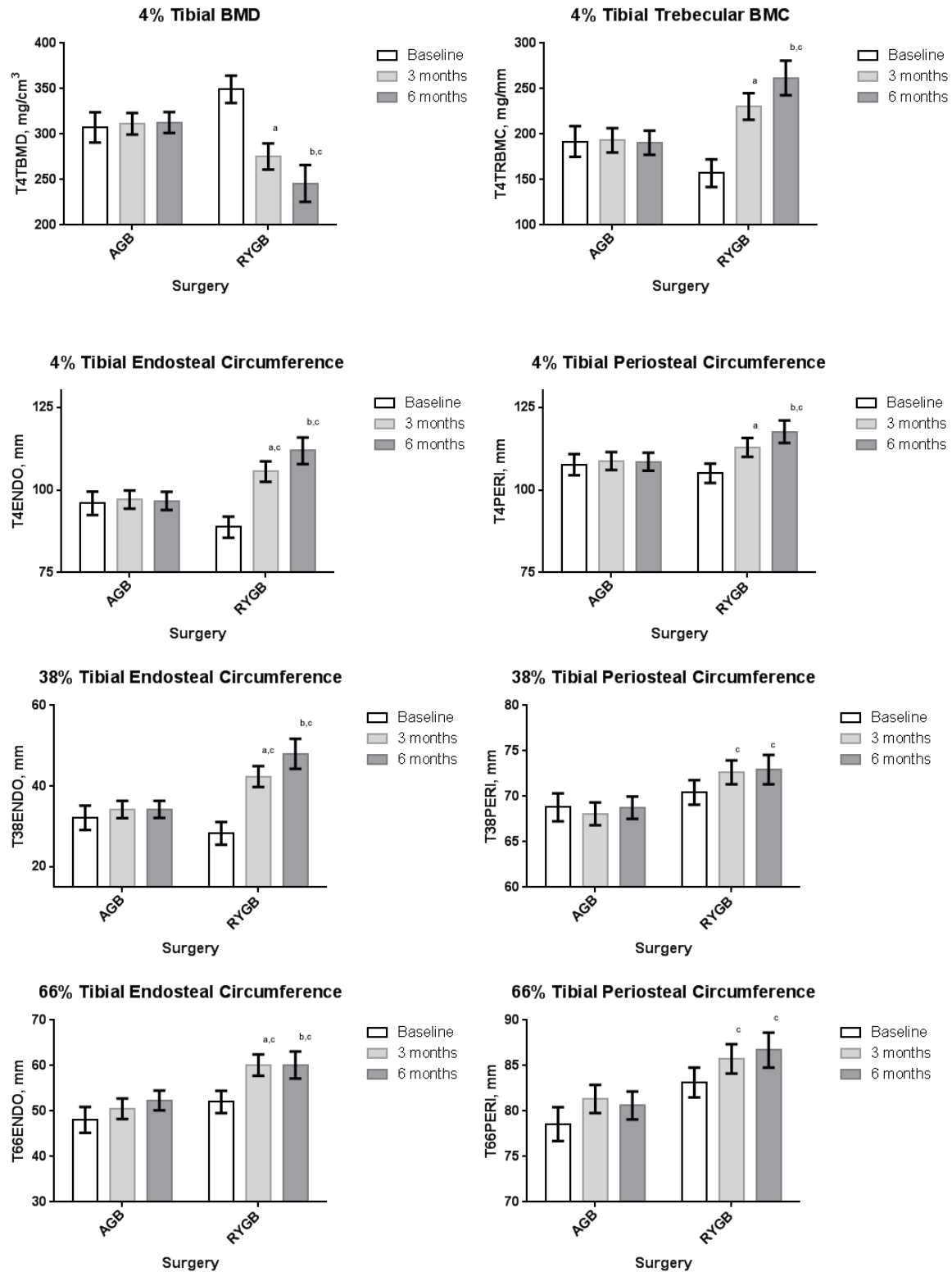
^c – difference between AGB and RYGB surgeries at time points, $p < 0.05$

Figure 4-2. DXA measures adjusted for weight for AGB and RYGB surgeries



- ^a – difference between baseline and 3 months, $p < 0.05$
^b – difference between baseline and 6 months, $p < 0.05$
^c – difference between AGB and RYGB surgeries at time points, $p < 0.05$

Figure 4-3. pQCT Tibial measures adjusted for weight for AGB and RYGB surgeries



^a – difference between baseline and 3 months, $p < 0.05$
^b – difference between baseline and 6 months, $p < 0.05$
^c – difference between AGB and RYGB surgeries at time points, $p < 0.05$

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Chapter 5

Manuscript 3

Effects of weight loss on osteocalcin in premenopausal women undergoing bariatric surgery

Abstract

Purpose: This non-randomized 6-month prospective study compared the effects of adjustable gastric banding (AGB) and Roux-en-Y gastric bypass (RYGB) surgeries on biomarkers of bone turnover, osteocalcin (OC), carboxy-terminal peptide of type I collagen (CTx), and the adipokines, adiponectin and leptin, which have been shown to influence bone metabolism.

Methods: Subjects were 21 Caucasian premenopausal women (Mean age $\pm$ SD 35.0 $\pm$ 5.9 yr) who were accepted for elective bariatric surgery (BarS) at the Carilion Clinic in Roanoke, VA.

Measurements were taken pre-surgery, 3 months and 6 months post-surgery. Dual-energy x-ray absorptiometry (DXA) measures of whole body fat mass, fat-free mass, central body fat, areal bone mineral density (BMD) and bone mineral content (BMC) were performed. Using serum (or plasma) samples obtained from fasting blood draws, ELISA assays were performed for total OC, undercarboxylated OC, CTx, adiponectin, and leptin.

Results: For the entire study sample, significant decreases ($p<0.05$) occurred from baseline, at 3 and 6 months post-surgery for weight, fat-free mass, fat mass, and central body fat (DXA). No changes in BMD or BMC were observed, however CTx ($p<0.05$) increased at 3 months post-surgery, and total OC ($p<0.05$) and undercarboxylated OC ($p<0.01$) both increased at 6 months post-surgery, and leptin ($p<0.05$) decreased at 6 months post-surgery. In comparing results for the two surgical types, analyses were adjusted for weight loss. In the AGB group, CTx increased

(F-ratio: 6.2, $p < 0.04$) at 3 months post-surgery, while all other biomarkers remained unchanged.

In contrast, for the RYGB group, total OC (F-ratio: 5.1, $p < 0.03$), undercarboxylated OC (F-ratio: 21.1, $p < 0.001$), and HMW adiponectin (F-ratio: 2.3, $p < 0.14$) all increased at 6 months, while leptin (F-ratio: 5.7, $p < 0.03$) decreased.

Conclusions: RYGB, which has both restrictive and malabsorptive influences on the gut, was associated with biomarker evidence of increased osteoblastic activity as well as changes associated with increased energy metabolism. In contrast, AGB, which only restricts gastric volume, was associated with biomarker activity that suggested increased osteoclastic activity (resorption) as early as 3 months post-surgery. These results, seen at 6 months post-surgery, demonstrate the complex interactions between bone and energy metabolism that may be an aid to greater weight loss compared to those undergoing simple restrictive surgeries like AGB. More research is needed to better understand the interaction between undercarboxylated OC and the pathways of glucose metabolism as well as additional clinical applications.

Introduction

In recent years, the skeleton has become a focus of investigation for hormonal alterations that affect energy metabolism at the level of the pancreas and adipose tissue [1]. Until recently a relationship had not yet been identified, until leptin, an adipocyte-derived hormone, was shown to be a major regulator of bone turnover [2]. Leptin stimulates bone matrix mineralization, and increases the production of the osteoblast specific protein osteocalcin [3]. Additionally, leptin has also been shown to have an inhibitory effect on bone resorption by inhibiting osteoclast differentiation [4]. Adiponectin, another adipokine related to bone metabolism, has been shown to decrease osteoclastogenesis and increase osteoblastogenesis in mouse models in vivo and in vitro [5], and increase human osteoblast proliferation and enhanced matrix mineralization [6]. In contrast and in line with clinical studies, adiponectin knockout mice have displayed an increase in bone mass [7]. Assuming that there must be a feedback mechanism related to leptin and utilizing a mouse model, Lee et al. [8] identified osteocalcin (OC) as the bone related hormone involved in glucose metabolism. Osteocalcin, a key marker of bone turnover, is a γ -carboxyglutamate protein expressed predominantly by osteoblasts. In rodents, undercarboxylated osteocalcin (unOC) has been shown to play a role in the regulation of glucose homeostasis, and it is likely that a similar relationship exists in humans [9]. In a review by Zhang et al. [10], OC was shown to improve insulin sensitivity through actions on the pancreas and increased production of adiponectin mRNA. Hwang et al. [11] found similar results with plasma OC associated with improved glucose tolerance, increased β -cell function and insulin sensitivity; however, they found no association with plasma adiponectin levels. Few studies to date have examined the relationships of weight loss and OC. Reinehr and Roth [12] studied obese children in a cross-sectional analysis, following these same children after undergoing a 1-year diet and exercise

induced weight loss program; they reported that OC levels were lower in obese children after weight loss and were related both to insulin resistance and leptin. Schafer et al [13] demonstrated that changes in unOC induced by the anti-osteoporosis medications parathyroid hormone and alendronate were associated with changes in metabolism in a group of postmenopausal women, and greater increases in unOC correlated with greater decreases in body weight and fat mass and greater increases in the adiponectin.

The difficulty in losing weight has led to a conservative estimate of over 200,000 bariatric surgeries (BarS) being performed each year [14]. This type of weight loss has led to concerns of bone loss and increased bone turnover. To best understand these changes associated with the skeleton, bone mineral density (BMD) and bone mineral content (BMC) along with biomarkers of bone turnover are often evaluated. For bone formation and resorption, respectively, osteocalcin (OC) and the carboxy-terminal peptide of type I collagen (CTx) are among the most sensitive of the bone-derived biomarkers [15] and both have been documented to increase following BarS [16]. Additionally, adiponectin and leptin, which are produced by the adipocytes, also have demonstrated an anti-osteogenic impact on bone physiology through endocrine and paracrine actions[17]. Limited research has been done with either adipokine in relation to potential influences on bone status and bone turnover markers following bariatric surgery. Thus, the purpose of this study was to examine the effects on various biomarkers associated with the weight loss induced by bariatric surgery on biomarkers of bone turnover in premenopausal women and whether this process may be influenced by adipokines associated with regulation of glucose metabolism; specifically, OC, CTx, adiponectin and leptin.

Methods

Subjects

Subjects were recruited from patients applying for elective BarS at the Carilion Clinic in Roanoke, VA during their initial interview with the surgical staff between March of 2009 and October of 2010. The study protocol was authorized by the Institutional Review Board for the Carilion Clinic and patients provided informed consent. Of the eighty-seven Caucasian premenopausal women that met the inclusion criteria of weighing less than 136 kg (the limitation of this DXA instrument), thirty-five agreed to participate. Additional exclusion criteria were an Epworth Sleepiness Score (ESS) greater than 10, indicating increased risk of obstructive sleep apnea, and subjects taking any medications known to affect bone. It has been shown that individuals with OSA are at 2.7 times the risk of osteoporosis than those without OSA, and females have a higher risk than younger and male counterparts [18]. Health insurance eligibility requirements and pre-surgical protocols delayed surgery for some subjects as much as 8 months. Subsequently, nine subjects either decided against surgery or failed to have surgery before the conclusion of data collection, two were denied surgery by their insurance carriers, and one had an ESS score of 18. One subject failed to return after the 3 month follow-up, and one dropped out before the 3 month follow-up. Therefore data from 21 subjects (Mean age $\pm$ SD 35.0 $\pm$ 5.9 yr) were included in the analyses (n=10 and n=11, for AGB and RYGB, respectively). Based on the initial interview, a required seminar, consultation with the surgeon and personal preferences, the subjects selected a desired surgical procedure, AGB or RYGB, and the desired surgical procedure was conducted by one of three surgeons at the Carilion Clinic. DXA and pQCT measurements for this study were taken prior to surgery and repeated at 3 and 6 months following surgery.

Anthropometrics

Body weight was measured without shoes in relaxed attire with a calibrated electronic scale (ScaleTronix, *Wheaton, IL*) to the nearest 0.1 kg, and height by a calibrated, wall-mounted digital stadiometer (Heightronic, Measurement Concepts, *North Bend, WA*) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The length of the non-dominant radius was measured from the highest point of the radial head to the highest point of the styloid process, to the nearest cm with a retractable tape measure. Likewise, 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 to the nearest cm.

Blood draw/Assays

Patients underwent a blood draw following an overnight fast for at least 8 hours at baseline and then at 3 months and 6 months post-surgery. Serum and plasma were separated, centrifuged, and stored in a -80°C freezer prior to biomarker assay. Based on the lower affinity of unOC for hydroxyapatite (HAP) compare to fully carboxylated OC, unOC was isolated by the HAP binding assay described by Vergnaud et al [19]. Briefly, 250µL of serum were incubated with 7.5 mg HAP (calcium phosphate tribasic type IV, *Sigma Chemical Co., St. Louis, MO*) in an Eppendorf tube, shaken for 1 h at 4°C and then centrifuged at 3000 rpm for 15 min. This supernatant was then used for the unOC assay by standard ELISA. The Serum total osteocalcin (tOC), unOC, high molecular weight (HMW) adiponectin, leptin (*Alpco Diagnostics, Salem, NH*) and CTx (*Immunodiagnostic Systems, Scottsdale, AZ*) were analyzed by ELISA. Intra- and inter-assay CVs were as follows: OC: 4.7-5% and 5.7-8.3%; adiponectin: 3.3-5% and 5.7%; leptin: 3.7-5.5% and 5.8-6.8%; CTx: 1.7-3% and 2.5-10.9%.

DXA measurement

DXA (Hologic QDR 4500-A Elite DXA *Bedford, MA*) scans of the whole body, lumbar spine, non-dominant hip and non-dominant forearm were completed and analyzed by one technician to determine whole body fat mass, fat-free mass, central body fat [20], areal bone mineral density (BMD) and bone mineral content (BMC), as well as site specific areal BMD, BMC and T-score for osteoporotic risk. The coefficient of variance (CV) for test–retest reliability in 15 young adult men and women tested on separate days were < 1.8% for hard and soft tissue parameters measured by DXA [21].

Statistical analyses

Data were analyzed using the NCSS 8 Statistical Software (NCSS, LLC. Kaysville, Utah), a p -value <0.05 was considered statistically significant throughout. Descriptive statistics for all relevant measures were computed using means and standard deviations. One-way ANOVA was used to assess the impact of bariatric surgery across all patients at the three time points (baseline, 3-months, and 6-months post-surgery) and then two-way ANCOVA was used to analyze differences between RYGB and AGB surgery across the three time points with weight as the covariate. In both cases, if significant F ratios were found, post-hoc assessment was performed using the Tukey-Kramer test to isolate the source of these differences.

Results

There were no significant differences in subject characteristics between the two surgical groups at baseline, and these data are displayed in Table 5-1.

Whole body fat and central body fat measures for subjects the two surgical types are displayed in Figure 5-1. The impact of bariatric surgery for all patients at 3 months and 6 months post-surgery indicated significant ($p < 0.05$) mean reductions in weight, fat-free mass, fat mass, and central body fat as measured by DXA. However, no bone measures were significantly changed over the

6 months of follow-up. CTx ($p<0.05$) increased at 3 months post-surgery, tOC ($p<0.05$) and unOC ($p<0.01$) both increased at 6 months post-surgery, and leptin ($p<0.05$) decreased at 6 months post-surgery. Changes in weight, body composition, and biomarkers are displayed in Table 5-2.

In comparing the two surgical types, statistical analyses were adjusted for differences in weight between the two surgical types and accounted for differences in weight loss. At 3 months post-surgery, CTx increased (F-ratio: 6.2, $p<0.04$) for the AGB group, while all other biomarkers were not significantly different. At 6 months post-surgery, tOC (F-ratio: 5.1, $p<0.03$), unOC (F-ratio: 21.1, $p<0.001$), and HMW adiponectin (F-ratio: 2.3, $p<0.14$) increased and leptin (F-ratio: 5.7, $p<0.03$) decreased for the RYGB group and were significantly different from the AGB group, which did not see significant changes. These results are displayed in Figure 5-2.

Discussion

In this study of premenopausal Caucasian women who underwent either AGB or RYGB surgery, both groups lost significant amounts of weight and fat mass. In the context of this weight loss, biomarkers of bone turnover and associated adipokines were examined. Those undergoing AGB surgery only demonstrated a small increase in bone resorption at 3 months post-surgery as evidenced by the increase in CTx. For those undergoing RYGB surgery, both tOC and unOC increased significantly within 6 months post-surgery, with much more dramatic results seen in the unOC. Additionally, there was a significant decrease in leptin, and a trending increase for adiponectin. These results are consistent with Maser et al [22] who found that surgically induced weight loss was associated with increases in OC and unOC and that leptin was negatively correlated with unOC. Additionally, they found that unOC may be a potential short-term compensatory regulator of glucose homeostasis. Leptin has been shown to regulate osteoblast

function through a hypothalamic relay [23] and thus may contribute to the regulation of OC production. Faraj et al [24] found a decrease in fat mass was accompanied by an increase in adiponectin levels in a group of morbidly obese subjects that underwent RYGB surgery. It has been shown in animal studies that hepatic glucose production and increased muscle fatty acid oxidation result from administration of adiponectin [25], and this may contribute to the greater loss of weight in these subjects. In a group of osteoporotic, post-menopausal women that underwent parathyroid hormone (PTH) or alendronate supplementation, Gravenstein et al [26] reported clinical evidence that shows glucose metabolism is also regulated in a leptin-dependent way by OC, where leptin was positively associated with insulin resistance and secretion, while adiponectin and OC were negatively associated with insulin resistance. In contrast, Schafer et al [13] found that increases in unOC at 3 months were associated with decreases in body weight and fat mass and increases in adiponectin, independent of changes in fat mass; however, they did not find an association of ucOC with leptin. In healthy children, higher ucOC levels were associated with higher insulin secretion [27] as well as with higher HMW adiponectin, while children with prediabetes had lower ucOC concentrations and β -cell dysfunction [28]. Additionally in animal studies, it has been suggested that osteocalcin may be of value in the treatment of metabolic diseases because it has been shown to regulate glucose metabolism and affect insulin sensitivity and fat mass [29]. Although the evidence for the roles of leptin and adiponectin in conjunction with unOC seem to be unclear, it is evident that higher levels of circulating unOC are associated with reductions in weight and fat mass.

One strength of this study is that ucOC as well as tOC were measured. Lee et al [8] demonstrated that it was the undercarboxylated form of the protein which has metabolic effects, and thus is of greater interest as a predictor of weight loss. In addition, this was the first study to examine the

effects on these bone turnover biomarkers and associated adipokines after adjusting for differential weight loss. There are several limitations in this study, however. First, the subject population in this study of Caucasian females with a median age of 35 years precludes the projection to other populations. Second, the sample size (n=10 and n=11, for AGB and RYGB, respectively) was relatively small and the subject's smaller percentage losses in weight compared to other studies. Both of these are related to the mechanical limitation of the DXA instrument that allows for a maximum of 136 kg of body weight. Third, the use of the HAP binding assay to obtain a measure unOC only represents an estimate since some of the unOC can nonspecifically bind the chemical substrate HAP.

Conclusions

Bariatric surgery leads to significant changes, like reductions in body weight, hypertension, and type 2 diabetes mellitus as well as increases in bone turnover and risk of osteoporosis [30, 31]. Although OC has traditionally been utilized as a marker of bone turnover, the evidence indicates that serum levels of variants of the molecule, namely unOC, might not always be the consequence of bone formation but may also be an intermediary reflecting a transient metabolic adaptation [32]. Subjects undergoing the restrictive and malabsorptive form of bariatric surgery, RYGB, can have greater negative energy balance compared to those undergoing simple restrictive surgeries like AGB, which leads to greater weight loss and fat loss. In addition, the increased levels of unOC at 6 months post-surgery may also aid in increased energy metabolism through pathways related to the adipokines leptin and adiponectin. More research is needed to better understand the interaction between unOC and the pathways of glucose metabolism as well as additional clinical applications.

Table 5-1. Subject baseline characteristics

	AGB (n=10)	RYGB (n=11)
Age (years)	33.7 ± 6.2	34.9 ± 6.1
Weight (kg)	118.9 ± 12.6	114.2 ± 9.6
BMI (kg/m ²)	43.2 ± 4.2	42.5 ± 2.7
FFM (kg)	58.0 ± 5.1	58.5 ± 6.2
BF (kg)	58.9 ± 8.8	53.8 ± 6.9
Whole body BMC (g/cm)	2391.4 ± 251.2	2461.3 ± 335.6
Whole body BMD (g/cm ²)	1.14 ± 0.08	1.18 ± 0.08
tOC (ng/mL)	10.8 ± 2.4	11.7 ± 5.5
unOC (ng/mL)	1.5 ± 0.9	2.1 ± 1.9
HMW Adiponectin (µg/mL)	8.2 ± 9.8	8.5 ± 6.6
Leptin (ng/mL)	73.9 ± 14.8	64.7 ± 15.2
CTx (ng/mL)	0.4 ± 0.2	0.3 ± 0.2

Data are displayed as mean ± SD. BMI: body mass index, FFM: fat free mass, BF: body fat, BMC: bone mineral content, BMD: bone mineral density, tOC: total osteocalcin, unOC: undercarboxylated osteocalcin, HMW: high molecular weight, CTx: carboxy-terminal peptide of type I collagen.

Table 5-2. Changes in weight and body composition for all bariatric surgery patients

	Baseline (n=21)	3 months (n=21)	6 months (n=21)
Weight (kg)	116.5 ± 11.1	98.1 ± 13.1 ^a	91.8 ± 15.5 ^b
Weight Loss (kg)		18.3 ± 6.5	6.3 ± 5.0 ^c
BMI (kg/m ²)	42.8 ± 3.4	36.1 ± 4.4 ^a	33.8 ± 5.4 ^b
FFM (kg)	58.3 ± 5.6	52.8 ± 5.4 ^a	51.9 ± 5.9 ^b
BF (kg)	56.2 ± 8.1	43.7 ± 9.7 ^a	38.6 ± 11.8 ^b
Central BF(kg)	4.5 ± 1.5	3.0 ± 0.9 ^a	2.7 ± 1.0 ^b
Whole body BMC (g/cm)	2428.0 ± 293.3	2435.0 ± 279.6	2409.6 ± 300.2
Whole body BMD (g/cm ²)	1.16 ± 0.08	1.17 ± 0.09	1.16 ± 0.09
tOC (ng/mL)	11.3 ± 4.3	15.0 ± 6.8	18.3 ± 9.6 ^b
unOC (ng/mL)	1.8 ± 1.5	3.5 ± 1.9	4.8 ± 3.8 ^b
HMW Adiponectin (µg/mL)	8.4 ± 8.1	6.3 ± 5.7	8.9 ± 8.7
Leptin (ng/mL)	69.3 ± 15.3	55.4 ± 20.3	50.5 ± 24.4 ^b
CTx (ng/mL)	0.3 ± 0.1	0.8 ± 0.3 ^a	0.7 ± 0.3

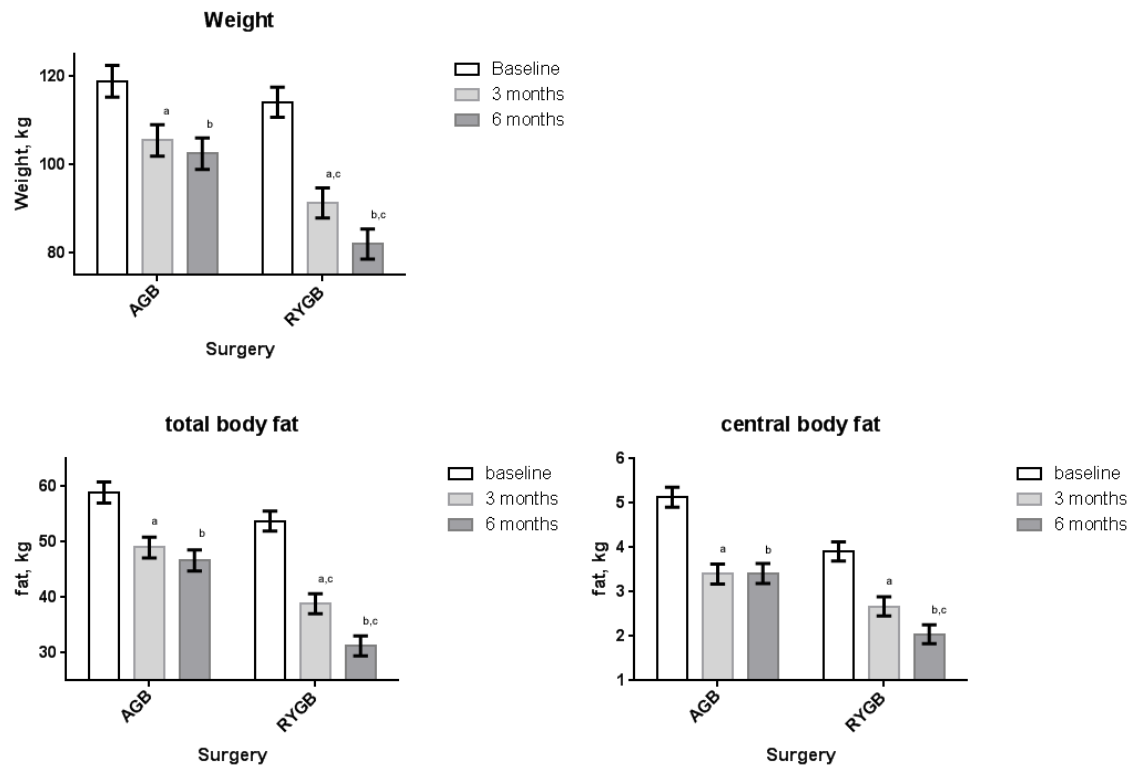
Data are displayed as mean ± SD. BMI: body mass index, FFM: fat free mass, BF: body fat, BMC: bone mineral content, BMD: bone mineral density, tOC: total osteocalcin, unOC: undercarboxylated osteocalcin, HMW: high molecular weight, CTx: carboxy-terminal peptide of type I collagen.

^a – difference between baseline and 3 months, p<0.05

^b – difference between baseline and 6 months, p<0.05

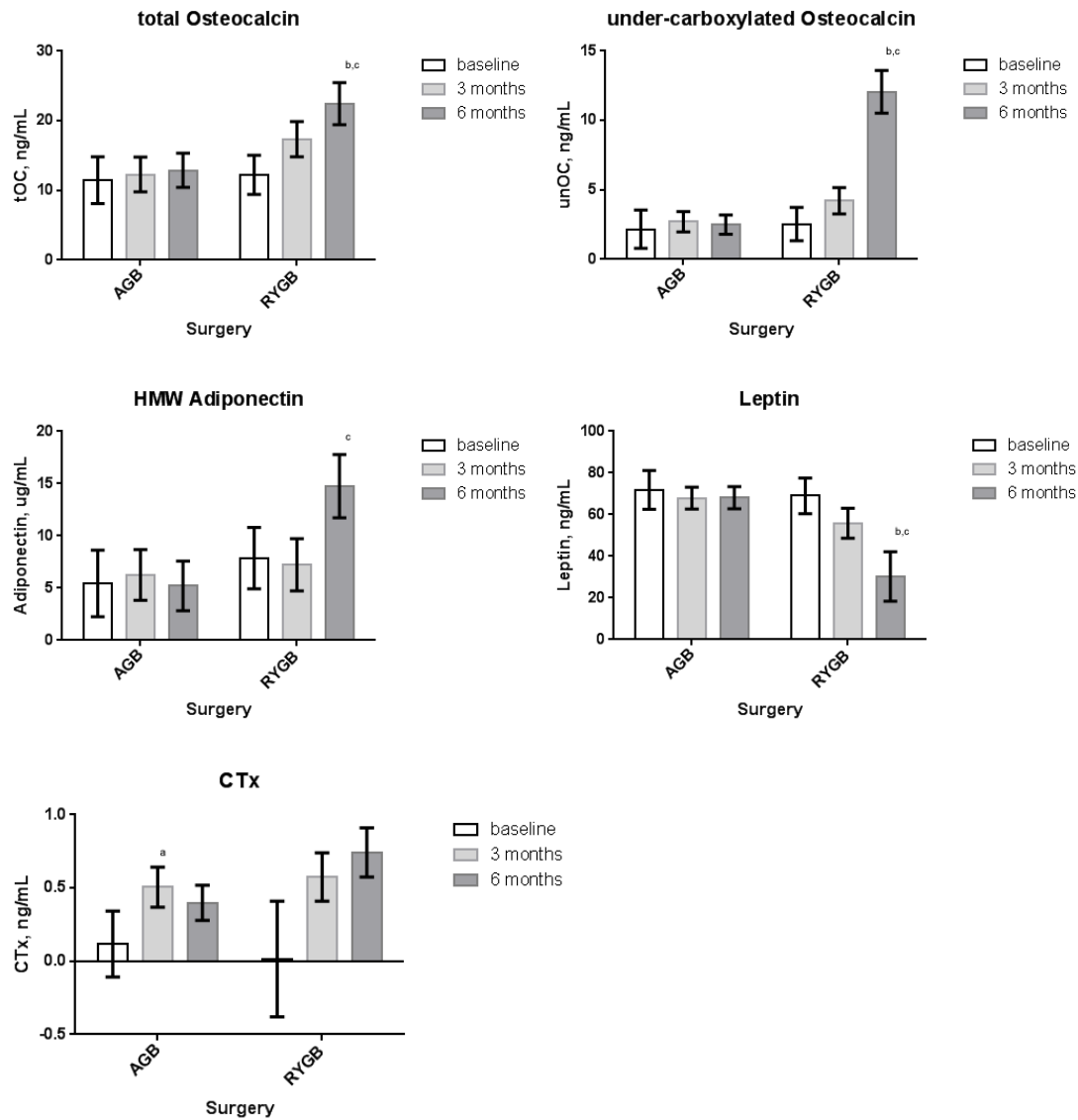
^c – difference between 3 months and 6 months, p<0.05

Figure 5-1. Weight and body fat measures for AGB and RYGB surgeries



^a – difference between baseline and 3 months, $p < 0.01$
^b – difference between baseline and 6 months, $p < 0.01$
^c – difference between AGB and RYGB surgeries at time points, $p < 0.05$

Figure 5-2. Biomarkers for weight adjusted AGB and RYGB surgeries



- ^a – difference between baseline and 3 months, $p < 0.05$
^b – difference between baseline and 6 months, $p < 0.05$
^c – difference between AGB and RYGB surgeries at time points, $p < 0.05$

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Chapter 6

Summary and Recommendations

Summary

Study 1: Influences of physical activity in healthy premenopausal women on appendicular skeleton

In this cross-sectional study, relationships between physical activity (PA) and parameters of bone density and strength were examined by peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA) in a group of healthy premenopausal women. Additionally, a comparison of these two technologies in the context of these measures was performed. Whole body bone mineral density (BMD) and lumbar spine bone mineral content (BMC), showed group differences ($p < 0.05$) between the highest and lowest PA levels (PA-H was higher than PA-L by 4.6% and 12.0%, respectively). Likewise the pQCT tibial 14%, 38%, and 66% loci differed ($p < 0.05$) between the highest and lowest PA levels for cortical area (CoA; PA-H was higher than PA-L by 8.2%, 9.5%, and 12.0%, respectively, PA-H was also higher than PA-M by 7.8% at the 14% locus) and total BMC (ToC; PA-H was higher than PA-L by 9.2%, 9.3%, and 9.6%, respectively). Additionally the 4%, 14% and 66% loci differed ($p < 0.05$) between the highest and lowest PA levels for the polar strength-strain index (SSI_p; PA-H was higher than PA-L by 9.5%, 14.2%, and 15.5%, respectively, PA-H was also higher than PA-M by 11.9% at the 14% locus). Finally the 66% locus differed ($p < 0.05$) between the highest and lowest PA levels for the circular model of cortical thickness (CoTh_c; PA-H was higher than PA-L by 11.5%). The pQCT radial 4% locus differed ($p < 0.05$) between the highest and lowest PA levels for the periosteal circumference (Peri_c; PA-H was higher than PA-L by 9.6%). To

compare the two instruments, the four pQCT tibial loci were utilized as repeated measures and compared to the DXA leg and hip measures. The data were also adjusted for body mass index (BMI). The DXA leg BMD, leg BMC, hip BMD, and hip BMC all showed differences ($p < 0.05$) between the moderate and lowest as well as the highest and lowest PA levels (PA-M was higher than PA-L by 5.7%, 9.4%, 9.2% and 14.7%, respectively; PA-H was higher than PA-L by 7.2%, 14.5%, 9.6%, and 15.2%, respectively). Likewise the pQCT tibial measures of CoA and ToC differed ($p < 0.05$) between the moderate and lowest as well as the highest and lowest PA levels (PA-M was higher than PA-L by 8.2% and 8.6%, respectively; PA-H was higher than PA-L by 13.1% and 13.1%, respectively). Additionally the SSI_p differed ($p < 0.05$) between all levels of PA (PA-M was higher than PA-L by 11.0%, PA-H was higher than PA-L by 19.3%, and PA-H was higher than PA-M by 9.4%). Similarly, the two pQCT radial loci were compared to the DXA arm and radius measures; however, none of these were significantly different.

Both DXA and pQCT revealed differences in parameters of weight-bearing bones in groups where combined planned and unplanned PA averaged greater than ~300 MET-min/d. The areal images from DXA only provide evidence of increased BMC and BMD for the various regions of clinical interest in response to higher levels of PA. In contrast, the pQCT can delineate smaller regions of BMC, BMD, and structural data, and these data are more sensitive than those from DXA.

Study 2: Comparative influences of two forms of bariatric surgery in premenopausal women on changes in DXA and pQCT measures of bone, muscle, and fat

This non-randomized 6-month prospective study compared the effects of adjustable gastric banding (AGB) and Roux-en-Y gastric bypass (RYGB) surgeries on bone, muscle and fat parameters measured with the DXA and pQCT instruments. Examining all subjects, significant

decreases ($p<0.05$) in weight, fat-free mass, fat mass, and central body fat measured by DXA and tibial and radial subcutaneous fat and radial MCSA measured by pQCT at both 3 months and 6 months from baseline were noted. No bone measures were significantly different. In comparing the two forms of bariatric surgery (BarS), the measures were adjusted for weight loss. DXA measures of central body fat ($p<0.05$) and whole body BMC ($p<0.01$) showed significant decreases at both time points for AGB and RYGB surgeries, respectively; however, there was no significant difference between the two surgical types at either time point. All other DXA measures indicated no significant differences. For the pQCT measures at the 4% locus, the total BMD decreased ($p<0.01$) while the trabecular BMC ($p<0.01$), Peri_c ($p<0.05$), and endosteal circumference (Endo_c) increased ($p<0.05$) at both 3 months and 6 months post-surgery for the RYGB surgery. Each of these were significantly different ($p<0.05$) between surgeries at 6 months post-surgery, with the Endo_c also different ($p<0.05$) at 3 months post-surgery. At the 38% locus, there were significant differences between surgeries at 3 months and 6 months post-surgery for the SSI_p , Peri_c and Endo_c measures. The SSI_p increased ($p<0.05$) for AGB at 3 months while both SSI_p ($p<0.05$) and Endo_c ($p<0.01$) increased for RYGB at 3 months as well as 6 months. At the 66% locus, the CoTh_c was significantly different ($p<0.05$) between surgeries at 3 months, muscle density (MD) was different ($p<0.01$) between surgeries at 6 months, and Peri_c and Endo_c were different ($p<0.05$) between surgeries at both 3 months and 6 months post-surgery. Additionally, Endo_c increased ($p<0.05$) for the RYGB surgical group at both time points and MD decreased ($p<0.05$) for the AGB group at 3 months and for the RYGB group at both 3 months and 6 months.

Although this study was of a preliminary nature, it was one of the first to examine the effects of BarS and compare the effects of two different surgical types, AGB and RYGB, which promote

weight loss by altering the digestive process in markedly different ways. In addition, innovative measurements of peripheral bone status as well as the traditional DXA measurements were utilized. In this group of premenopausal women studied 6 months after BarS, there was no significant loss in bone as measured by DXA and pQCT, despite significant losses in weight, fat-free mass, and fat mass. This finding is a positive indication that these women do not appear to be at greater risk of osteoporosis. Differences in the two surgical types were evident with RYGB demonstrating greater changes in bone adaptation. There were discordant results between the DXA and pQCT instruments, which demonstrate the value of utilizing both instruments in evaluating the effects of BarS on hard and soft tissues.

Study 3: Effects of weight loss on osteocalcin in premenopausal women undergoing bariatric surgery

This non-randomized 6-month prospective study compared the effects of AGB and RYGB surgeries on biomarkers of bone turnover, osteocalcin (OC) and carboxy-terminal peptide of type I collagen (CTx), as well as on the adipokines adiponectin and leptin. For the entire study sample, significant decreases ($p < 0.05$) occurred from baseline, at 3 and 6 months post-surgery for weight, fat-free mass, fat mass, and central body fat as measured by DXA. No changes in BMD or BMC were observed, however CTx ($p < 0.05$) increased at 3 months post-surgery, and total OC ($p < 0.05$) and undercarboxylated OC ($p < 0.01$) both increased at 6 months post-surgery, and leptin ($p < 0.05$) decreased at 6 months post-surgery. In comparing results for the two surgical types, analyses were adjusted for weight loss. In the AGB group, CTx increased (F-ratio: 6.2, $p < 0.04$) at 3 months post-surgery, while all other biomarkers remained unchanged. In contrast, for the RYGB group, total OC (F-ratio: 5.1, $p < 0.03$), undercarboxylated OC (F-ratio: 21.1,

$p < 0.001$), and HMW adiponectin (F-ratio: 2.3, $p < 0.14$) all increased at 6 months, while leptin (F-ratio: 5.7, $p < 0.03$) decreased.

RYGB, which has both restrictive and malabsorptive influences on the gut, was associated with biomarker evidence of increased osteoblastic activity as well as changes associated with increased energy metabolism. In contrast, AGB, which only restricts gastric volume, was associated with biomarker activity that suggested increased osteoclastic activity (resorption) as early as 3 months post-surgery. These results seen at 6 months post-surgery demonstrate the complex interactions between bone and energy metabolism that may be an aid to greater weight loss compared to those undergoing simple restrictive surgeries like AGB.

Recommendations

Study 1: Influences of physical activity in healthy premenopausal women on appendicular skeleton

Understanding the relationship between PA and skeletal health is important because of the significant impact of osteoporosis on society. Recent studies have shown that PA has a positive impact on increasing or maintaining bone including in pre- to early-pubertal children [1], young adults [2] and post-menopausal women [3]. Bone geometry and strength as well as bone mineral content and density are each important factors in evaluating risk for osteoporotic fracture. In a study of adolescent males and females, McKay, et al. [4] found that impact PA is positively associated with bone architecture and strength. Additionally in a review in post-menopausal women by Polidoulis, et al. [5], they found that exercise decreases bone loss by maintaining cortical and trabecular volumetric BMD. Although the DXA instrument provides information for the whole body, specific regions, and the clinically relevant regions of interest used for diagnosis of osteoporosis, it cannot provide information about bone geometry and strength. The pQCT not

only provides total BMC and BMD information for each given slice, but also cortical and trabecular BMC and BMD components as well as the areas of each of the bone compartments, muscle, and fat. Additional intervention studies are necessary to determine the types and magnitude of PA that can be discriminated by these instruments as well as optimal exercise routines for improved skeletal health.

Study 2: Comparative influences of two forms of bariatric surgery in premenopausal women on changes in DXA and pQCT measures of bone, muscle, and fat

Rapid weight loss and alterations to nutrient absorption associated with BarS may have potentially detrimental effects on bone mass and therefore increased risk for osteoporosis [6]. Currently, analysis of bone status is not part of the routine care of BarS patients. Williams et al. [7] recommends a pre-operative DXA scan for bariatric patients to assess primary and secondary risks of bone disease, along with repeated scans every 1-2 years. Although DXA may be a good indicator of bone status, it may not provide all the necessary data to make a complete assessment. Yu et al, found that after one year post-surgery spine and hip BMD measured by DXA showed significant decreases while quantitative computed tomography (QCT) measures only demonstrated changes in the spine but not at the femur [8]. This result, along with the findings from this study, implies that DXA findings may overstate the perceived negative effects of BarS on bone integrity. The increases seen in the pQCT data may be a result of the surgical protocol following BarS, which encourages patients to increase physical activity beginning approximately 3 months post-surgery. This physical activity often becomes easier as weight is reduced and body composition changes. Additionally, increased physical activity accompanying weight loss can create a greater lean mass to fat mass ratio. This change in body composition may increase loading on the musculoskeletal system that may counteract the reductions in

loading due to weight loss, and lean mass has been shown to exert a greater effect on BMD than fat mass, with physical activity helping to prevent bone loss and potential osteoporosis [9].

Research with longer duration follow-up focusing on bone changes in BarS patients utilizing both DXA and pQCT may provide evidence supporting the use of these instruments to monitor patients' bone status and may help guide patients and physicians in selecting an appropriate surgical procedure. In addition, interventions that focus on PA may help to determine standards for additional post-surgical protocols to prevent bone loss and greater risk of osteoporosis.

Study 3: Effects of weight loss on osteocalcin in premenopausal women undergoing bariatric surgery

The difficulty in losing weight has led to a conservative estimate of over 200,000 bariatric surgeries (BarS) being performed each year [10]. This type of weight loss has led to concerns of bone loss and increased bone turnover. To best understand these changes associated with the skeleton, bone mineral density (BMD) and bone mineral content (BMC) along with biomarkers of bone turnover are often evaluated. For bone formation and resorption, respectively, osteocalcin (OC) and the carboxy-terminal peptide of type I collagen (CTx) are among the most sensitive of the bone-derived biomarkers [11] and both have been documented to increase following BarS [12]. Additionally, adiponectin and leptin, which are produced by the adipocytes, have also demonstrated an impact on bone physiology [13]. Understanding the connections between bone and energy metabolism may help us to better understand the mechanisms that produce the near immediate alterations in comorbidities like type 2 diabetes mellitus as well as the more dramatic weight loss seen in patients undergoing RYGB surgery. Continued research that examines the links between bone derived hormones, adipokines, and energy metabolism may lead to a better understanding of certain metabolic disorders and the obesity epidemic now

facing the country. These studies may help physicians to better treat those dealing with these issues.

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Appendix A: Virginia Tech Bone Study Detailed Methodology

Recruitment

Subjects were recruited from the resident and student population at Virginia Tech and in the communities surrounding the campus and through flyers (Appendix B) 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

Subject Arrival and Parking

Upon arrival at the scheduled time, the subject comes to 229 Wallace Hall and the researchers fill out a temporary parking pass and instructs the subject to write in their license plate number on the pass and place it in their car.

Informed Consent

Researchers sit down with the subject and explain the informed consent with them. They are given time to read, review and ask questions before signing the form. The subject is reminded of the components of study: DXA and pQCT bone scans, food and physical activity record and blood draw. A copy of the signed informed consent is given to the subject.

DXA/pQCT Measurement Data Sheet (Appendix C)

Date of Birth

The subject's date of birth is recorded on the data sheet.

Pregnancy Test

The subject is given a specimen cup and asked to provide a urine sample for a pregnancy test. After completing the pregnancy test and assuming it is negative, the researchers check off on data sheet that pregnancy test has been completed and the protocol is continued.

Body Weight

The subject is instructed to remove their shoes, any extra jackets or layers and anything from her pockets. The scale is turned on and allowed to zero itself. The subject then steps onto the scale. The weight is recorded to the nearest kilogram and to the nearest pound. Subjects exceeding 136 kilograms of body weight are excluded from the study.

Height

The subject is asked to stand with their back against wall (without shoes) at the wall-mounted stadiometer. The measurement arm is positioned to meet the top of the subject's head. The height is recorded to the nearest centimeter and to nearest inches.

Radius Length

The subject is asked to raise their bare, non-dominant forearm, bent at the elbow 90 degrees in front of their chest and parallel to the floor. A measurement from the highest point of the radial head to the highest point of the styloid process of the radius is taken to the nearest millimeter using a flexible measuring tape and recorded on the form.

Tibia Length

The subject is asked to sit in a chair and cross their bare, non-dominant foreleg over the other leg so that the ankle of the non-dominant leg rests upon the top of the knee of the other leg. A measurement from the highest point of the medial condyle of the tibia to the highest point of the medial malleolus is taken to the nearest millimeter using a flexible measuring tape and recorded on the form.

DXA Scans

Before beginning the bone scans, the subject is asked if they are wearing any items that contain metal, buttons, snaps, zippers, or hard plastic (including underwire bras). If they are, they are removed and the subjects are offered clothing (t-shirt and sweat pants) to wear. The subjects' identifying and measurement 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 are conducted according to the manufactures instructions:

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

DXA Scan Analysis

The analysis is conducted after the subject has completed all requirements and has left the lab. Each of the four scans, whole body, lumbar spine, non-dominant hip and non-dominant forearm, are analyzed 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:

- a. Central abdominal fat analysis
- b. whole body
- c. lumbar spine
- d. total hip
- e. radius

pQCT Scans

The subjects' identifying and measurement 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 (4, 14, 38, and 66% of the tibial length from the distal end) and RADIUS2S (4 and 66% of the radius length from the distal end) scans are conducted.

For the TIBIA4S scans, the pQCT is set up with the leg stabilizer and foot stirrup.

The subject sits in the seat mounted on the pQCT, and it is adjusted left or right for the appropriate non-dominant leg. The bottom of the foot of the non-dominant limb is placed in the stirrup through the gantry of the device and strapped down. The leg stabilizer is 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. The subject is instructed to remain still and keep their leg steady in the device. The scans are then conducted.

For the RADIUS2S scans, the pQCT is set up with the arm and hand rests.

The subject sits in the chair adjacent to the pQCT gantry with the non-dominant arm inserted through the gantry and with the elbow resting at the end of the arm rest. Adjust the hand rest with the hand resting on the holder and the thumb under the holding device so that the arm is parallel to the ground and in the center of the gantry. The subject is instructed to remain still and keep their arm steady in the device. Researchers leave the room during these scans so as not to distract the subject.

pQCT Scan Analysis

The analysis is conducted after the subject has completed all requirements and has left the lab. The scans are initially analyzed using the TIBIA4S Macro and RADIUS2S Macro according to the instructions in the Stratec XCT 3000 Instruction Manual.

After analyses, the output is printed and placed in the subject data file. In addition, the LOOP procedure is conducted on each scan and output to a database file for analysis.

Diet and Physical Activity Record (Appendix D)

Diet Record Instructions

Record the subject code at the top of the form. Give the form to the subject and thoroughly explain the instruction sheet (Appendix E), 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 are 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 are shown a sample food record and how to complete the diet record. Subjects are 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.

The subject is instructed to record all food and beverages consumed over a four day period, preferably 3 weekdays and 1 weekend day.

Arrange a time for subject to return the form. If the subject will not be bringing the form to another visit, give them an addressed, stamped envelope to mail the record to the researchers.

Physical Activity Record Instructions

During the same 4 day period of maintaining the diet record, the subjects will also complete a physical activity log and wear a pedometer for all waking hours. The pedometer will be attached at the hip each morning and 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 at the top of the form. After recording and before attaching for the next day, they will hit the reset button and wait for the pedometer to zero. Subjects will remove the pedometer for running, swimming or any activity more strenuous than walking and then record the activity including the duration and the intensity on the form of each day's diet/physical activity record. The researcher will explain to the subject how to rate their intensity level using a BORG scale (6-20), where 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 the subject that they will be allowed to keep the pedometer following the completion of the study.

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.

Venous Collection

Before subject arrives:

1. Create new subject file.
2. Put a copy of “Blood Draw” in subject’s chart.
3. Take out tourniquet, 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 subject’s 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

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°C freezer with other samples.

Are Your Bones Healthy?

Subjects Needed

**The Bone, Osteoporosis, Nutrition and Exercise Lab
Department of Human Nutrition, Foods and Exercise
Virginia Tech**

We are looking for:

- Women who are 30-45 years old,
- With moderate to high levels of physical activity,
- Non tobacco users,
- Taking no medications regularly that would affect bone metabolism with the exception of contraceptives

We are offering:

- A pedometer for each qualified participant
- Dietary analysis by a nutritionist
- Blood cholesterol and glucose screening
- Body composition (percent body fat)
- Screening for osteoporosis and your bone mineral density

This study will require 2 visits to VT:

- Visit 1 (10-15 minutes)
 - Blood draw
- Visit 2 (45 minutes)
 - DXA (measures bone density)
 - pQCT (it does 3-D bone modeling)

For additional information, please contact Katrina Butner (kbutner@vt.edu) or visit our website at www.sleep.hnfe.vt.edu.

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Appendix C: Virginia Tech Bone Study DXA/pQCT Measurement Data Sheet

<u>DXA/pQCT Measurement Data Sheet</u>	
D.O.B. _____	Pregnancy Test Performed ☐
Height _____ cm/ _____ in kg	Weight _____ lbs./ _____
BMI _____ kg/m ²	
<u>Lengths</u>	
Non-dominant Side _____	
Non-dominant Tibia _____ mm	Non-dominant Radius

Appendix D: 4-Day Diet and Activity Record

Food/Activity Record

Code # _____ Day of Week _____ Date _____
 What time did you get up today? _____ (include am or pm) How many hours did you sleep last night? _____
 If you napped today, please list how long you napped: _____

*Remember: Please do not alter your normal activity or 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.

Physical Activity
 Please list any physical activity you had today. _____ Step Count _____

Type of Activity	Time Spent in Minutes	BORG RPE Scale (6-20 from mild to extremely heavy exertion)

Food You Ate Today - (continue on next page)

Time (include am/pm)	Food Eaten - How Prepared	Amount Eaten	Special Notes

Time (include am/pm)	Food Eaten – How Prepared	Amount Eaten	Special Notes

Appendix E: 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.

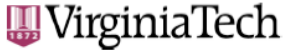
Use your best judgment, and here are some tips:

- 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		

Appendix F: Virginia Tech IRB Approval Letters



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

FROM: David M. Moore 

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

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 submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtain 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

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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 federal 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.

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Appendix G: Virginia Tech Bone Study Informed Consent

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 Guill, 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 80 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

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

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 filling 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.

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 $\sim 13.25 \mu\text{Sv}$ [made up of scans for the Total Body ($2.1 \mu\text{Sv}$), Lumbar Spine ($2.2 \mu\text{Sv}$), Total Proximal Femur ($4.6 \mu\text{Sv}$), and Total Forearm ($0.03 \mu\text{Sv}$) by DXA and for the Tibia ($2.9 \mu\text{Sv}$), Radius ($1.4 \mu\text{Sv}$) by pQCT]. If you are only participating in the radiologic portion of the study, you will receive an additional $8.6 \mu\text{Sv}$ of effective dose (total $\sim 22 \mu\text{Sv}$ 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.

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.

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);

- (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;

- (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
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Researcher (Witness) Signature	Date
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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
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Researcher (Witness) Signature	Date
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Should you have any questions about this research or its conduct, you may contact:

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Appendix H: Virginia Tech Bone Study Raw Data

Table A-1. Virginia Tech Bone Study Raw Data

NAME	Age	Height	Weight	BMI	SBP	DBP	T_CHO	HDL	LDL	TG	T_HDL_R	B_GLU	Blood Glucose
B01	43	169.1	62.4	21.8	118	78	260	101		62		75	4.2
B02	46	166.6	75.3	27.1	122	86	237	50	141	227	4.7	95	5.3
B03	40	166.2	57.7	20.9	132	86	194	88	100	44	2.2	79	4.4
B04	31	162.6	73.0	27.6			176	57		44	3.1	96	5.3
B05	37	166.1	57.0	20.7	122	68	190	36	148	44	5.3	81	4.5
B06	36	159.3	54.7	21.6	98	58	190	62	110	87	3.1	95	5.3
B07	46	165.5	57.2	20.9	104	58	188	69	107	59	2.7	83	4.6
B08	43	157.9	58.7	23.6	118	70	174	88	76	48	2.0	80	4.4
B09	45	167.7	84.1	29.9	116	70	100	38	55	44	2.7	82	4.6
B10	33	161.0	62.2	24.0	122	80	188	68	111	48	2.8	76	4.2
B11	37	171.1	72.4	24.7	112	68	146	72	65	47	2.0	84	4.7
B12	40	160.1	81.2	31.7	138	78	138	54	59	125	2.6	86	4.8
B13	44	160.2	58.5	22.8	104	68	183	88	81	73	2.1	81	4.5
B14	37	165.3	80.7	29.5	180	100	184	40	122	110	4.6	90	5.0
B15	39	168.1	51.6	18.3	100	60	133	76	50	44	1.8	79	4.4
B16	42	164.8	57.9	21.3									
B17	42	161.4	59.3	22.8	106	64	161	46	109	44	3.5	96	5.3
B18	44	165.4	54.9	20.1	112	70	130	67		44	2.0	85	4.7
B19	32	162.1	68.8	26.2	112	80	144	48	89	44	3.0	72	4.0
B20	38	173.8	134.3	44.5	138	80	233	32	184	83	7.3	93	5.2
B21	35	156.4	98.4	40.2	122	74	162	33	114	75	4.9	78	4.3
B22	45	162.8	50.1	18.9									
B23	32	166.3	73.4	26.6	112	68	152	62	64	130	2.4	82	4.6
B24	41	166.3	56.0	20.3	104	70	186	56	109	106	3.3	87	4.8
B25	38	158.1	52.9	21.2	122	80	185	77	99	44	2.4	87	4.8
B26	37	160.1	74.2	28.9	118	78	165	46	98	106	3.6	77	4.3
B27	37	141.1	71.7	36.0			214	90	111	65	2.4	97	5.4
B28	32	177.3	93.2	29.6	124	72	135	42	71	113	3.2	83	4.6
B29	34	160.2	57.1	22.3	108	64	175	74	83	87	2.4	85	4.7
B30	32	163.4	74.0	27.7	116	74	172	55	90	136	3.1	85	4.7
B31	32	177.7	100.2	31.7	106	76	164	34	108	111	4.9	84	4.7
B32	44	159.2	68.0	26.8	134	90	223	57	146	102	3.9	85	4.7
B33	38	167.0	66.7	23.9	118	78	137	49	79	46	2.8	86	4.8
B34	41	178.7	87.8	27.5	138	80	126	38	69	94	3.3	88	4.9
B35	41	158.8	58.9	23.4	104	82	252	72	159	106	3.5	84	4.7
B36	44	172.4	71.0	23.9	102	74	184	41	126	84	4.5	89	4.9
B37	40	160.9	68.4	26.4	118	94	170	50	103	83	3.4	82	4.6
B38	36	171.3	77.9	26.5	118	76	187	49	125	67	3.8	83	4.6
B39	41	153.5	63.6	27.0	122	78	170	53	108	47	3.2	84	4.7
B40	34	159.3	63.4	25.0	106	68	163	46	100	82	3.5	94	5.2
B41	41	158.7	55.5	22.0	108	80	198	70	121	44	2.8	82	4.6

NAME	Age	Height	Weight	BMI	SBP	DBP	T_CHO	HDL	LDL	TG	T_HDL_R	B_GLU	Blood Glucose
B42	35	169.1	66.1	23.1	122	82	134	58	62	66	2.3	77	4.3
B43	38	164.0	87.4	32.5		82	169	39	95	175	4.3	99	5.5
B44	31	155.9	113.2	46.6	128	76	178	62	99	89	2.9	84	4.7
B45	39	170.6	56.8	19.5	118	72	161	70	84	45	2.3	80	4.4
B46	34	163.1	64.2	24.1	120	74	145	46	81	91	3.1	102	5.7
B47	42	164.2	51.8	19.2	110	64	182	52	111	95	3.5	97	5.4
B48	45	151.0	45.0	19.7	118	70	191	74	90	134	2.6	73	4.1
B49	42	163.6	55.2	20.6	120	80	185	58	109	87	3.2	83	4.6
B50	38	164.4	64.2	23.7	114	76	219	40	148	153	5.5	93	5.2
B51	38	171.4	55.6	18.9	110	68	135	81	42	58	1.7	93	5.2
B52	40	163.3	59.9	22.5	118	80	150	40	95	75	3.8	74	4.1
B53	32	159.3	56.2	22.1	120	82	131	58	53	99	2.2	86	4.8
B54	40	169.3	63.6	22.2	112	70	147	76	64	45	1.9	90	5.0
B55	44	157.0	52.8	21.4	118	78	156	58	90	45	2.7	95	5.3
B56	38	160.5	61.3	23.8	104	70	175	51	117	45	3.4	76	4.2
B57	37	179.9	79.4	24.5	120	74	147	58	81	45	2.6	77	4.3
B58	31	178.1	75.9	23.9	122	76	193	59	117	89	3.3	81	4.5
B59	43	177.0	76.1	24.3	110	68	179	36	11	146	5.0	88	4.9
B60	40	160.2	60.6	23.6	110	72	136	57	69	52	2.4	93	5.2
B61	42	164.4	60.8	22.5	102	60	153	56	90	45	2.7	83	4.6
B62	29	185.5	85.7	24.9	102	60	145	60	76	45	2.4	92	5.1
B63	44	174.9	67.8	22.2	104	64	207	68	125	65	3.0	74	4.1
B64	44	160.0	57.8	22.6	120	72	173	71	90	62	2.4	96	5.3
B65	43	167.0	65.5	23.5	122	70	165			45		99	5.5
B66	43	164.5	65.3	24.1	120	78	146	40	89	81	3.6	80	4.4
B67	30	165.1	70.5	25.9	116	74	207	72	111	121	2.9	83	4.6
B68	42	166.6	57.1	20.6	122	70	173	81	83	45	2.1	84	4.7
B69	40	166.8	60.4	21.7	110	70	127	61	42	120	2.1	92	5.1
B70	45	172.2	82.6	27.9	112	70	181	48	106	135	3.8	103	5.7
B71	35	165.3	52.5	19.2	102	58	167	68	83	79	2.5	95	5.3
B72	41	180.1	78.2	24.1	118	78	153	55	79	153	2.8	88	4.9
B73	31	156.8	54.2	22.1	122	68	155	52	83	155	3.0	88	4.9
B74	31	169.1	69.4	24.3	112	80	153	40	102	53	3.8	90	5.0
B75	31	159.9	58.2	22.8	110	66	199	89	96	73	2.2	87	4.8
B76	44	170.9	55.2	18.9	102	60	143	63	62	90	2.3	87	4.8
B77	45	169.1	64.7	22.6	120	82	150	43	97	47	3.5	85	4.7
B78	41	171.0	63.2	21.6	120	70	134	58	70	45	2.3	86	4.8
B79	34	176.7	58.8	18.8	118	70	133	57	61	74	2.3	108	6.0
B80	42	162.1	52.7	20.0	114	64	159	66	79	72	2.4	98	5.4
B81	32	157.3	62.1	25.1	120	70	191	65	112	67	2.9	82	4.6
B82	42	152.3	52.2	22.5	122	78	192	96	88	45	2.0	87	4.8

NAME	BF	C_BF	C_Tot_BF_R	FATM	LBM	Arm 1/3 BMD	Arm Area	Arm BMC	Arm BMD	Arm Fat	Arm Lean	Arm Fat Pct
B01	27.4	20.9	0.76	17315.9	43369.2	0.684	244.46	212.33	0.869	934.6	2098.2	28.8
B02	37.2	37.8	1.02	28190.2	45650.2	0.702	190.98	128.33	0.672	1613.3	1945.4	43.8
B03	23.1	20.9	0.90	13508.2	42416.5	0.723	238.43	214.08	0.898	633.6	2128.9	21.3
B04	29.7	33.1	1.11	21988.8	49552.6	0.759	218.73	165.02	0.754	1325.2	2499.2	33.2
B05	30.4	28.1	0.92	17623.0	38212.6	0.753	242.45	214.48	0.885	929.1	1819.4	31.4
B06	23.8	16.0	0.67	13190.2	39981.6	0.725	216.32	244.80	1.132	675.9	2039.2	22.8
B07	16.3	10.9	0.67	9452.7	45938.8	0.725	226.77	290.25	1.280	429.9	2127.5	15.1
B08	32.2	20.2	0.63	19180.7	38462.1	0.630	232.00	302.68	1.305	1095.5	1593.5	36.6
B09	33.0	32.3	0.98	28043.3	54385.9	0.759	248.08	213.57	0.861	1487.8	2372.2	36.5
B10	25.9	18.1	0.70	16671.7	45152.3	0.719	231.59	208.71	0.901	1174.9	2230.4	32.5
B11	33.9	22.3	0.66	24892.7	46361.8	0.629	217.92	155.66	0.714	1398.9	2026.5	39.1
B12	39.9	41.0	1.03	32847.1	47250.2	0.700	206.26	148.80	0.721	2017.2	2439.0	43.8
B13	26.5	20.0	0.75	15685.7	41491.7	0.673	192.19	137.50	0.715	781.8	1915.6	27.6
B14	34.6	36.8	1.06	28274.0	51290.1	0.705	219.53	172.79	0.787	1645.5	2354.4	39.4
B15	25.2	11.4	0.45	13167.4	36705.7	0.769	219.53	168.57	0.768	796.2	1643.0	30.5
B16	21.3	11.6	0.54	13461.8	43606.8	0.728	196.21	147.05	0.749	614.3	2158.7	21.0
B17	35.0	38.4	1.10	20934.8	37010.0	0.687	178.92	126.05	0.704	1169.8	1648.8	39.7
B18	19.7	16.6	0.84	10952.2	42326.7	0.713	178.12	130.88	0.735	511.1	1874.5	20.3
B19	31.9	34.6	1.08	22064.5	44594.1	0.683	232.40	229.80	0.989	1274.9	1924.6	37.2
B20	47.2	48.1	1.02	63027.8	67625.4	0.752	308.79	298.89	0.968	3059.5	3172.5	46.8
B21	47.2	48.7	1.03	46997.9	50177.1	0.711	223.15	170.58	0.764	3130.5	2437.5	54.6
B22	20.5	9.3	0.45	10372.3	37989.1	0.679	200.63	216.39	1.079	538.4	1608.8	22.8
B23	37.6	39.2	1.04	27823.6	44009.1	0.687	191.39	131.69	0.688	1591.5	1988.0	42.9
B24	22.7	14.9	0.66	12859.1	41546.8	0.651	197.42	153.38	0.777	848.2	1997.3	28.3
B25	30.4	24.5	0.81	16362.1	35321.9	0.631	224.36	269.29	1.200	1072.3	1559.5	37.0
B26	39.7	41.8	1.05	29770.6	43252.9	0.727	202.64	141.21	0.697	2006.3	1924.9	49.3
B27	43.1	44.5	1.03	31294.1	39726.6	0.548	199.03	233.73	1.174	2145.8	1581.2	54.2
B28	39.3	32.4	0.82	36949.6	54657.2	0.810	216.32	163.92	0.758	1963.7	2495.5	42.5
B29	23.6	21.0	0.89	13668.1	42007.5	0.721	205.06	192.32	0.938	734.2	2101.4	24.2
B30	41.2	32.0	0.78	30620.7	41448.2	0.715	189.78	142.77	0.752	1639.6	1735.4	46.6
B31	42.1	40.4	0.96	42605.7	55947.0	0.724	231.19	175.90	0.761	2367.8	2469.6	47.2
B32	33.9	34.4	1.01	32437.7	43612.8	0.728	247.27	305.90	1.237	1199.3	2053.2	33.7
B33	33.5	29.7	0.89	22749.8	42877.5	0.743	236.42	340.48	1.440	1143.9	1790.8	34.9
B34	37.3	31.2	0.84	33102.7	52750.0	0.661	259.74	204.75	0.788	1712.1	2694.3	37.1
B35	32.4	34.1	1.05	19432.1	38406.3	0.711	216.32	233.60	1.080	996.3	1624.3	34.9
B36	31.9	22.3	0.70	23054.4	46940.3	0.705	224.76	164.61	0.732	1510.4	2131.1	39.7
B37	37.3	33.8	0.91	25513.4	41061.2	0.645	204.25	138.81	0.680	1589.8	1959.9	43.1
B38	28.9	28.4	0.98	22827.1	53670.1	0.806	208.27	158.50	0.761	1141.6	2413.6	30.7
B39	36.2	30.1	0.83	23346.2	38958.5	0.753	223.95	167.00	0.746	1503.4	1620.8	45.7
B40	24.4	20.6	0.84	15738.4	46524.0	0.705	196.21	149.51	0.762	747.1	2202.1	24.1
B41	27.7	20.9	0.75	15680.1	38690.7	0.768	207.07	164.32	0.794	877.9	2030.8	28.6

NAME	BF	C_BF	C_Tot_BF_R	FATM	LBM	Arm 1/3 BMD	Arm Area	Arm BMC	Arm BMD	Arm Fat	Arm Lean	Arm Fat Pct
B42	31.1	25.5	0.82	20689.3	43596.1	0.721	213.50	162.24	0.760	1214.6	2344.5	32.6
B43	40.8	34.9	0.86	36186.1	50085.7	0.708	233.60	179.42	0.768	2236.7	2490.4	45.6
B44	51.6	47.2	0.91	59227.9	53094.9	0.690	229.58	164.89	0.718	3678.6	2607.4	57.0
B45	19.8	15.2	0.77	11551.6	44048.1	0.758	219.93	165.84	0.754	616.6	2335.3	19.8
B46	23.6	17.3	0.73	15301.2	47074.0	0.836	207.07	169.63	0.819	910.5	2407.3	26.1
B47	17.6	11.5	0.65	9268.6	41584.1	0.543	161.23	111.14	0.689	441.6	2040.7	17.0
B48	20.1	14.4	0.72	9201.9	34515.2	0.671	207.87	166.82	0.802	532.1	1934.1	20.2
B49	22.0	10.5	0.48	12409.9	41555.7	0.767	209.88	150.40	0.717	642.4	2019.5	22.8
B50	26.5	25.5	0.96	17307.9	45604.7	0.757	219.53	166.18	0.757	990.7	2252.3	29.1
B51	19.1	12.6	0.66	10769.6	43252.0	0.812	197.02	164.42	0.835	585.1	2031.7	21.0
B52	23.7	20.6	0.87	14195.5	43466.4	0.638	190.18	136.03	0.715	1045.2	1695.8	36.3
B53	24.9	22.0	0.88	14023.1	40101.3	0.693	211.09	153.89	0.729	870.7	1740.1	31.5
B54	24.3	20.9	0.86	15742.7	46552.9	0.779	203.45	153.98	0.757	861.6	2235.8	26.5
B55	22.4	12.1	0.54	12070.5	39536.6	0.762	187.37	144.99	0.774	596.6	1884.7	22.7
B56	35.7	38.0	1.06	22178.0	37585.1	0.739	186.96	141.64	0.758	1064.9	1707.0	36.6
B57	31.2	21.7	0.70	25228.5	53244.0	0.767	260.95	194.84	0.747	1351.4	2732.1	31.6
B58	29.8	28.5	0.96	23017.7	51730.4	0.767	201.84	153.08	0.758	1314.2	2426.7	33.7
B59	26.6	29.3	1.10	20472.7	54165.6	0.734	214.71	157.50	0.734	1081.7	2389.1	29.8
B60	25.3	17.7	0.70	15678.2	43670.0	0.718	189.38	142.95	0.755	898.0	1838.4	31.2
B61	27.2	22.4	0.82	16691.6	42530.8	0.658	185.36	137.38	0.741	1004.2	2008.0	31.9
B62	30.8	26.9	0.87	26638.6	56854.6	0.757	232.40	189.95	0.817	1332.5	2719.3	31.4
B63	15.8	8.9	0.56	10856.7	55130.4	0.706	238.03	195.43	0.821	576.3	2869.7	15.8
B64	25.6	27.0	1.05	15018.2	41538.5	0.655	199.83	170.86	0.855	797.6	2036.5	26.5
B65	23.5	16.4	0.70	15683.5	48611.9	0.719	209.88	155.43	0.741	811.0	2561.0	23.0
B66	27.6	23.3	0.84	18199.2	45507.1	0.623	182.94	117.96	0.645	1480.5	1137.5	54.1
B67	32.3	28.7	0.89	23068.2	45904.6	0.743	214.71	162.81	0.758	1149.5	2077.2	33.9
B68	20.8	12.3	0.59	12040.6	43482.3	0.740	195.81	145.25	0.742	565.2	2077.7	20.3
B69	24.2	17.0	0.70	14919.6	44168.9	0.830	226.37	191.61	0.846	740.7	2221.7	23.7
B70	40.9	40.5	0.99	34241.3	46799.8	0.716	214.71	166.18	0.774	1951.0	2303.4	44.1
B71	28.1	26.0	0.93	14999.2	36320.1	0.713	163.24	110.48	0.677	857.6	1436.7	35.7
B72	29.0	22.9	0.79	22849.7	53235.8	0.782	256.92	195.50	0.761	1486.6	2519.6	35.4
B73	31.7	27.2	0.86	17361.0	35342.0	0.733	174.90	127.50	0.729	923.7	1568.5	35.3
B74	27.3	27.4	1.00	19351.9	48964.8	0.675	195.81	148.65	0.759	881.1	2093.4	28.2
B75	20.9	19.2	0.92	12320.8	44481.9	0.747	218.33	209.52	0.960	713.5	2176.5	23.0
B76	25.7	18.2	0.71	14424.6	39834.0	0.678	193.40	135.63	0.701	829.6	1636.4	31.9
B77	30.0	29.8	0.99	19630.7	43361.9	0.792	220.74	186.55	0.845	1066.2	1845.9	34.4
B78	25.6	21.5	0.84	16369.8	45175.1	0.702	219.13	178.44	0.814	755.5	2160.4	24.4
B79	20.5	13.3	0.65	12304.5	44821.0	0.791	248.48	280.27	1.128	500.3	2201.6	16.8
B80	24.5	14.4	0.59	13133.2	38405.6	0.700	198.22	154.27	0.778	716.5	2056.4	24.5
B81	26.6	20.6	0.77	16799.8	43903.7	0.747	185.36	153.07	0.826	988.8	2260.7	29.1
B82	10.6	8.9	0.84	5586.7	44925.0	0.688	203.45	189.63	0.932	298.5	2223.2	11.0

NAME	Leg Area	Leg BMC	Leg BMD	Leg Fat	Leg Lean	Leg Fat Pct	T_BMD	T_BMC
B01	390.01	469.51	1.204	4283.2	7478.3	35.0	1.190	2548.00
B02	336.94	363.08	1.078	4916.4	7382.9	38.8	1.037	1924.78
B03	363.47	445.20	1.225	3438.8	7062.6	31.4	1.228	2522.60
B04	334.93	461.47	1.378	3872.1	7864.6	31.7	1.236	2538.11
B05	348.60	384.79	1.104	4076.9	6055.3	38.8	1.055	2133.71
B06	328.09	384.43	1.172	3119.9	6197.0	32.2	1.190	2266.30
B07	345.38	439.23	1.272	2305.4	7330.0	22.9	1.290	2578.05
B08	300.75	314.84	1.047	4890.6	6799.0	40.7	1.088	1897.30
B09	366.29	478.84	1.307	5574.3	8918.2	37.2	1.247	2670.80
B10	338.95	430.20	1.269	3536.7	6791.6	32.9	1.291	2490.79
B11	367.90	427.77	1.163	6388.8	7797.5	43.7	1.058	2167.13
B12	323.67	359.47	1.111	5759.4	7392.0	42.6	1.118	2181.16
B13	332.51	379.60	1.142	3785.9	7235.6	33.2	1.059	1949.76
B14	337.74	404.32	1.197	4193.9	7868.6	33.6	1.150	2264.75
B15	351.41	432.72	1.231	3696.8	5696.6	37.6	1.224	2458.01
B16	390.01	468.93	1.202	3379.2	7515.3	29.7	1.174	2401.56
B17	323.67	335.59	1.037	4172.8	6107.3	39.3	1.081	1949.69
B18	334.93	416.24	1.243	2598.9	7191.3	25.5	1.203	2317.84
B19	335.33	431.59	1.287	4014.7	7120.8	34.7	1.258	2474.36
B20	402.88	508.79	1.263	12616.4	10657.8	53.0	1.245	2930.69
B21	337.34	458.25	1.358	8586.2	8246.7	49.7	1.153	2370.60
B22	322.06	386.16	1.199	2702.2	6376.8	28.5	1.216	2248.13
B23	351.41	443.46	1.262	5442.3	7731.1	40.0	1.053	2149.71
B24	345.38	381.36	1.104	2853.2	6642.8	28.9	1.176	2242.29
B25	310.00	348.00	1.123	3616.0	5739.7	37.3	1.115	2051.98
B26	324.87	362.37	1.115	5121.5	6593.1	42.4	1.061	1995.62
B27	247.68	220.58	0.891	5690.0	5486.3	49.9	0.978	1593.49
B28	391.62	488.71	1.248	7751.7	8941.8	45.1	1.155	2532.19
B29	328.90	404.41	1.230	3262.4	6863.9	31.0	1.195	2182.22
B30	339.75	412.19	1.213	6829.4	6735.2	48.9	1.197	2283.11
B31	422.98	531.64	1.257	9074.6	9623.9	47.2	1.158	2624.92
B32	301.15	382.42	1.270	4804.3	6674.4	40.5	1.157	2172.16
B33	352.22	392.05	1.113	5343.9	7495.0	40.4	1.152	2225.47
B34	453.95	572.33	1.261	8329.2	9268.9	45.8	1.248	2941.39
B35	308.79	336.41	1.089	3889.2	6114.4	37.6	1.127	2050.30
B36	396.04	442.24	1.117	5754.6	8004.3	40.5	1.024	2209.83
B37	330.50	362.39	1.096	5359.5	6538.8	43.7	1.018	1856.26
B38	383.58	441.46	1.151	4081.2	8263.2	31.9	1.165	2436.45
B39	344.58	407.60	1.183	5061.2	6427.1	42.5	1.169	2264.23
B40	347.79	426.94	1.228	3409.3	7526.5	30.0	1.181	2343.62
B41	343.37	389.55	1.134	3220.5	6098.9	33.2	1.146	2138.85

NAME	Leg Area	Leg BMC	Leg BMD	Leg Fat	Leg Lean	Leg Fat Pct	T_BMD	T_BMC
B42	343.37	381.72	1.112	4230.6	6705.6	37.4	1.065	2197.20
B43	362.67	426.30	1.175	5553.2	7693.1	40.6	1.186	2403.32
B44	357.44	458.53	1.283	12997.4	9673.9	56.2	1.142	2392.79
B45	348.20	456.04	1.310	2671.1	6998.2	26.4	1.254	2641.44
B46	347.39	440.51	1.268	3339.6	7759.0	28.9	1.240	2496.60
B47	332.92	368.25	1.106	2311.7	7218.5	23.4	1.035	1849.41
B48	285.07	321.95	1.129	2032.5	4845.0	28.2	1.092	2020.87
B49	348.20	396.94	1.140	3192.6	6854.8	30.6	1.212	2432.89
B50	343.37	456.61	1.330	3112.7	7372.1	28.4	1.253	2412.09
B51	372.72	490.53	1.316	2616.2	6999.8	25.9	1.230	2471.99
B52	346.59	427.94	1.235	2870.7	7261.9	27.2	1.133	2154.75
B53	319.65	398.64	1.247	3004.5	6514.9	30.3	1.186	2241.40
B54	365.08	454.82	1.246	3419.0	7964.0	28.9	1.232	2450.34
B55	338.95	457.52	1.350	2956.2	6923.4	28.6	1.242	2316.83
B56	355.03	438.15	1.234	4531.5	6297.7	40.2	1.244	2387.07
B57	435.45	502.84	1.155	5835.0	9047.5	37.9	1.084	2517.03
B58	398.05	505.08	1.269	4886.2	8701.8	34.7	1.196	2512.55
B59	408.51	489.81	1.199	3534.0	9370.6	26.4	1.124	2423.63
B60	361.06	454.04	1.258	3608.8	7440.7	31.4	1.274	2548.53
B61	338.95	377.29	1.113	3485.8	6948.1	32.2	1.180	2220.06
B62	446.70	594.11	1.330	6570.2	9507.4	39.4	1.306	3065.01
B63	398.86	536.77	1.346	2664.0	9119.7	21.6	1.217	2779.63
B64	351.81	437.79	1.244	2939.7	7242.7	27.7	1.160	2171.65
B65	362.27	426.07	1.176	4113.1	7826.6	33.3	1.178	2374.53
B66	352.22	444.09	1.261	3703.1	8405.5	29.5	1.196	2313.64
B67	397.65	485.52	1.221	5570.4	7724.8	40.4	1.203	2518.42
B68	340.15	415.42	1.221	2980.0	7037.1	28.6	1.175	2271.69
B69	359.45	485.15	1.350	3859.4	8128.5	30.9	1.312	2620.53
B70	363.88	500.22	1.375	6532.6	7516.5	44.9	1.286	2712.16
B71	347.39	387.31	1.115	3093.8	5675.9	33.8	1.116	2032.63
B72	421.77	541.88	1.285	5359.0	9130.1	35.7	1.175	2791.30
B73	317.24	382.65	1.206	3912.1	5781.6	38.8	1.191	2111.68
B74	371.92	449.19	1.208	4081.4	8106.2	32.3	1.213	2518.10
B75	337.34	427.71	1.268	2788.9	8041.0	24.8	1.211	2257.73
B76	361.06	364.82	1.010	3248.9	6393.0	32.5	1.016	1931.63
B77	348.60	418.70	1.201	4104.6	6909.8	35.9	1.172	2361.61
B78	363.07	422.15	1.163	3761.3	7442.7	32.4	1.220	2507.75
B79	388.40	530.16	1.365	3029.4	8293.5	25.6	1.308	2801.92
B80	316.43	364.10	1.151	2824.0	5973.2	30.8	1.134	2066.13
B81	329.30	428.21	1.300	4172.1	7148.8	35.5	1.283	2376.57
B82	305.17	399.45	1.309	1184.2	7841.1	12.6	1.258	2250.18

NAME	L_BMD	L_BMC	L_T_Score	H_BMD	H_BMC	H_T_Score	R_BMD	R_BMC	R_T_Score
B01	1.008	64.93	-0.41	0.972	33.88	0.25	0.577	7.37	-0.40
B02	1.027	54.72	-0.47	0.724	23.10	-1.78	0.545	6.49	-0.53
B03	1.291	79.49	2.16	0.983	34.38	0.33	0.558	11.89	-0.11
B04	1.028	62.58	-0.39	1.023	31.75	0.67	0.644	8.20	1.31
B05	0.996	57.72	-0.52	0.884	29.57	-0.47	0.691	8.29	2.04
B06	0.946	49.05	-1.19	0.935	29.90	-0.06	0.582	6.02	-0.21
B07	1.203	77.11	1.46	1.015	33.20	0.60	0.608	7.60	0.60
B08	0.873	44.98	-1.67	0.747	22.20	-1.60	0.551	6.15	-0.88
B09	1.236	74.83	1.75	1.179	39.77	1.94	0.656	8.53	1.38
B10	1.100	60.77	0.51	1.060	30.52	0.97	0.672	8.10	1.22
B11	1.023	66.09	-0.42	0.817	28.31	-1.02	0.543	7.31	-0.88
B12	1.021	61.57	-0.31	0.911	26.88	-0.25	0.605	7.13	0.40
B13	0.771	40.81	-2.64	0.819	26.18	-1.01	0.518	6.43	-1.03
B14	1.210	70.27	1.47	0.927	30.40	-0.12	0.593	7.00	0.04
B15	1.194	82.28	1.25	0.949	33.33	0.06	0.632	6.75	1.40
B16	0.948	54.97	-0.88	0.883	32.42	-0.49	0.623	7.73	0.24
B17	1.061	57.57	0.17	0.765	22.38	-1.45	0.539	6.60	-0.42
B18	1.013	60.93	-0.33	0.901	26.15	-0.33	0.562	6.71	0.09
B19	1.163	63.01	0.98	1.052	29.55	0.90	0.638	7.50	0.78
B20	1.095	65.86	0.14	1.095	39.50	1.26	0.625	9.48	1.03
B21	1.020	56.22	-0.50	1.035	30.25	0.76	0.614	6.21	0.95
B22	1.034	61.44	-0.11	1.006	28.65	0.52	0.582	6.05	-0.01
B23	1.050	67.29	-0.09	1.074	32.19	1.08	0.569	6.69	0.11
B24	0.959	55.45	-0.80	0.818	26.82	-1.02	0.547	6.66	-0.79
B25	0.940	47.72	-1.27	0.842	24.21	-0.82	0.535	5.78	-0.82
B26	0.998	52.78	-0.43	0.850	26.54	-0.75	0.591	7.09	0.22
B27	0.844	44.18	-1.80	0.642	16.16	-2.46	0.499	4.39	-1.48
B28	1.059	64.63	-0.16	0.937	34.55	-0.04	0.618	8.72	0.71
B29	1.066	60.80	-0.11	1.009	30.85	0.55	0.628	6.68	0.90
B30	1.025	62.18	-0.21	1.143	33.63	1.64	0.631	7.25	0.97
B31	1.194	79.71	1.44	1.086	39.68	1.18	0.598	8.33	0.36
B32	0.956	50.70	-0.83	0.984	28.75	0.34	0.641	7.21	1.14
B33	0.949	55.94	-1.16	0.914	27.84	-0.23	0.592	6.73	0.23
B34	1.292	88.62	2.22	1.082	71.67	1.15	0.548	9.67	-0.57
B35	1.002	50.65	-0.55	0.925	26.31	-0.14	0.626	6.26	0.86
B36	0.903	50.11	-1.37	0.793	26.61	-1.22	0.593	7.80	0.27
B37	0.919	45.03	-1.08	0.794	24.66	-1.22	0.583	6.87	0.07
B38	0.962	55.54	-0.76	0.872	29.88	-0.57	0.613	7.91	0.63
B39	1.102	53.97	0.45	1.027	31.33	0.69	0.628	8.42	0.90
B40	1.095	62.89	0.42	1.051	32.80	0.89	0.611	8.93	0.58
B41	1.052	57.78	0.07	0.924	28.67	-0.15	0.661	7.66	1.52

NAME	L_BMD	L_BMC	L_T_Score	H_BMD	H_BMC	H_T_Score	R_BMD	R_BMC	R_T_Score
B42	1.085	65.53	0.47	1.026	32.24	0.69	0.581	7.62	0.03
B43	1.080	56.01	0.18	1.074	32.31	1.08	0.594	6.87	0.29
B44	1.126	74.46	0.63	1.001	30.63	0.49	0.619	7.84	0.74
B45	1.205	77.82	1.35	0.991	27.14	0.41	0.664	9.96	1.58
B46	0.933	51.82	-1.17	0.889	28.54	-0.43	0.458	4.85	-2.25
B47	1.143	66.80	0.98	0.830	24.85	-0.92	0.572	7.87	-0.12
B48	1.197	72.88	1.27	0.840	30.66	-0.84	0.585	7.75	0.12
B49	1.216	68.11	1.74	1.196	39.62	2.09	0.669	8.42	1.66
B50	0.998	64.28	-0.43	0.960	32.99	0.14	0.626	8.31	0.86
B51	1.059	61.10	0.12	0.948	31.89	0.05	0.519	6.69	-1.11
B52	1.065	57.97	0.02	0.968	29.08	0.22	0.598	6.18	0.36
B53	1.039	64.00	-0.16	0.980	30.81	0.31	0.660	7.77	1.50
B54	1.093	54.93	0.42	1.075	32.75	1.09	0.617	7.51	0.70
B55	1.168	63.91	1.16	0.997	28.52	0.45	0.632	7.62	0.98
B56	1.120	76.66	0.76	0.960	34.65	0.14	0.594	9.84	0.28
B57	0.949	62.45	-1.12	0.914	30.52	-0.23	0.649	7.96	1.29
B58	0.986	65.64	-0.59	0.819	31.20	-1.01	0.550	8.02	-0.54
B59	1.233	76.98	1.55	1.066	29.81	1.01	0.641	8.27	1.15
B60	1.030	54.88	-0.31	0.884	27.56	-0.48	0.539	6.75	-0.74
B61	1.367	94.45	2.88	1.154	44.93	1.74	0.643	9.15	1.19
B62	1.113	77.98	0.71	1.013	36.58	0.58	0.607	8.20	0.52
B63	1.104	53.48	0.54	1.004	32.84	0.51	0.611	7.39	0.59
B64	1.005	66.45	-0.56	0.889	29.42	-0.43	0.576	8.30	-0.06
B65	1.064	69.26	0.13	0.888	32.17	-0.44	0.494	4.87	-1.58
B66	1.253	78.65	1.83	0.971	32.24	0.24	0.621	8.29	0.78
B67	1.021	59.14	-0.18	0.959	28.97	0.14	0.634	8.48	1.02
B68	1.127	64.64	0.77	1.174	40.35	1.90	0.659	8.91	1.48
B69	1.287	73.89	2.19	1.090	33.92	1.22	0.672	7.92	1.73
B70	0.969	51.03	-0.71	0.752	23.98	-1.56	0.546	6.17	-0.61
B71	1.099	86.15	0.38	0.925	36.06	-0.14	0.577	9.81	-0.04
B72	1.012	56.56	0.48	0.980	29.76	0.31	0.601	5.95	0.41
B73	1.022	60.80	-0.36	1.024	34.03	0.67	0.578	8.24	-0.02
B74	0.998	52.15	-0.52	0.930	27.25	-0.09	0.620	7.78	0.76
B75	0.899	57.70	-1.39	0.683	23.85	-2.12	0.490	6.28	-1.65
B76	0.909	54.78	-1.34	0.831	26.07	-0.91	0.657	7.45	1.45
B77	1.150	81.42	0.89	0.967	31.17	0.21	0.560	7.45	-0.34
B78	1.095	67.07	0.31	1.096	38.67	1.26	0.645	9.05	1.22
B79	0.948	56.49	-1.19	0.902	27.65	-0.33	0.581	6.76	0.04
B80	1.132	60.00	0.61	0.964	29.27	0.18	0.685	8.50	1.96
B81	1.003	53.44	-0.54	1.045	30.34	0.84	0.618	6.20	0.72
B82	1.085	65.53	0.47	1.026	32.24	0.69	0.581	7.62	0.03

NAME	Avg_Steps	Steps_Tert	Avg_METx Min	MET_Tert	BLKS_STRS	BLKS_Tert	LY_REC	ACT_WK	VM_ACT_ WK	LOC_ACT	LREC_ACT	LT_ACT
B01	15511	3	1166	3	432	1	2757.6	28665.0	13500.0	1727.3	943.5	18070.7
B02	4508	1	354	1	840	2	1417.5	24885.0	9450.0	9850.0	1029.9	10879.9
B03	10963	3	1009	3	2310	3	2085.6	29160.0	16110.0	8100.0	1024.5	9124.5
B04	7897	2	328	1	1442	2	5614.6	23580.0	5580.0	0.0	1626.3	1626.3
B05	4482	1	280	1	378	1	456.1	221825.0	2430.0	9000.0	0.0	9000.0
B06	7417	2	275	1	1447	2	1647.0	21375.0	2880.0	9000.0	901.9	9901.9
B07	8274	2	828	3	770	2	4989.8	24457.5	7605.0	9372.4	536.5	9908.9
B08	13984	3	576	2	2170	3	2131.4	27675.0	12960.0	9000.0	581.5	9581.5
B09	9897	2	491	2	3220	3	1027.8	16380.0	6300.0	15218.2	320.2	155538.4
B10	7870	2	1105	3	2100	3	5780.8	31545.0	19800.0	1260.0	2871.4	15471.4
B11	5100	1	363	1	58	1	4432.5	27630.0	13770.0	13200.0	971.5	14171.5
B12	3173	1	131	1	350	1	6360.6	23265.0	6210.0	9000.0	0.0	9000.0
B13	7641	2	632	2	397	1	2278.0	23490.0	4230.0	9000.0	231.8	9231.8
B14	6256	1	188	1	840	2	2155.7	22185.0	4860.0	9000.0	271.1	9271.1
B15	15402	3	655	3	1680	3	2576.9	22365.0	3780.0	90000.0	822.2	9822.2
B16	14785	3	782	3	1598	3	2301.9	24771.5	8370.0	9000.0	415.4	9415.4
B17	6286	1	234	1	840	2	1271.3	23207.0	5388.9	9423.5	152.7	9576.3
B18	18878	3	717	3	3080	3	3847.0	26775.0	10530.0	9000.0	775.4	9775.4
B19	11857	3	477	2	2590	3	3035.8	22425.0	8910.0	1035.0	964.5	11314.5
B20	5917	1	244	1	2842	3	0.0	27798.8	13702.5	9030.8	2160.0	11990.8
B21	5983	1	297	1	784	2	1041.1	20070.0	17010.0	14400.0	515.9	14915.9
B22	9795	2	594	2	1330	2	2803.0	27382.5	13005.0	10800.0	998.9	11798.9
B23	5184	1	567	2	728	2	1054.0	26865.0	12600.0	10800.0	605.8	11405.8
B24	9509	2	392	1	560	1	2834.1	24817.5	7425.0	9000.0	348.5	9348.5
B25	11446	3	523	2	2380	3	7038.8	24867.0	9612.0	9000.0	1296.3	10296.3
B26	2593	1	99	1	275	1	259.6	22365.0	4860.0	9000.0	0.0	9000.0
B27	4448	1	133	1	42	1	268.3	21195.0	2430.0	9000.0	0.0	9000.0
B28	9447	2	283	1	1680	3	826.4	22725.0	5130.0	9000.0	1881.7	10881.7
B29	7508	2	508	2	376	1	2627.3	25290.0	8640.0	9000.0	543.4	9543.4
B30	7387	2	222	1	1050	2	367.8	24345.0	8370.0	11000.0	830.8	11830.8
B31	3214	1	246	1	434	1	1475.5	25200.0	8820.0	9000.0	887.3	9887.3
B32	3923	1	162	1	86	1	13110.6	33660.0	21150.0	9276.9	1640.8	10917.7
B33	6891	1	284	1	70	1	446.5	20430.0	540.0	10246.2	456.0	10702.2
B34	15268	3	580	2								
B35	17827	3	677	3	2310	3	5271.9	24705.0	10800.0	9000.0	676.2	9676.2
B36	10694	3	406	2	2240	3	830.8	25695.0	10440.0	9327.3	1088.7	10415.9
B37	9560	2	394	1	784	2	1565.9	24615.0	6660.0	9000.0	1006.0	10006.0
B38	4482	1	170	1	798	2	739.0	24705.0	7110.0	9000.0	685.4	9685.4
B39	5794	1	471	2	229	1	247.5	19530.0	0.0	9000.0	415.4	9415.4
B40	17477	3	664	3	4410	3	2938.3	28350.0	14490.0	10028.6	133.9	10162.5
B41	8737	2	332	1	58	1	249.2	26145.0	12240.0	9000.0	472.3	9472.3

NAME	Avg_Steps	Steps_Tert	Avg_METx Min	MET_Tert	BLKS_STRS	BLKS_Tert	LY_REC	ACT_WK	VM_ACT_ WK	LOC_ACT	LREC_ACT	LT_ACT
B42	3815	1	157	1	714	1	469.5	21870.0	7560.0	12600.0	451.4	13051.4
B43	10094	2	303	1	448	1	129.8	23850.0	6750.0	9000.0	0.0	9000.0
B44					348	1	2120.2	23175.0	5220.0	9600.0	554.5	10154.5
B45	12173	3	836	3	4643	3	5095.0	29295.0	16200.0	14130.0	645.3	14775.3
B46					840	2	7096.2	30487.5	19575.0	10800.0	1654.3	12454.3
B47	13470	3	779	3	2240	3	2616.9	24750.0	8280.0	5400.0	459.7	5859.7
B48	16012	3	633	2	86	1	4945.7	25335.0	9540.0	5400.0	1840.1	7240.1
B49	12069	3	362	1	1890	3	1016.8	27022.5	11925.0	18000.0	1156.2	19156.2
B50	7595	2	424	2	3640	3	2280.3	24480.0	6930.0	0.0	461.2	461.2
B51	14660	3	557	2	1472	2	1384.6	23310.0	5040.0	11897.6	530.9	12428.5
B52	6321	1	769	3	1890	3	3522.1	25065.0	7560.0	7200.0	2026.1	9226.1
B53	7124	1	390	1	1792	3	1021.2	24705.0	8190.0	9000.0	472.8	9472.8
B54												
B55	10913	3	572	2	1540	2	3403.1	22050.0	4680.0	9000.0	714.3	9714.3
B56	7550	2	406	2	201	1	2596.2	25470.0	8550.0	9000.0	141404.0	10414.0
B57	8202	2	741	3	173	1	3633.8	26145.0	10080.0	11652.6	879.8	12532.4
B58	6425	1	460	2	770	2	2176.4	26640.0	11340.0	11025.0	413.2	11438.2
B59	8702	2	878	3	347	1	5176.7	27900.0	12420.0	14400.0	983.9	15383.9
B60	6397	1	794	3	1512	2	6047.3	25650.0	9472.5	10928.6	1561.5	12490.0
B61	9547	2	363	1	1162	2	1629.5	26955.0	12330.0	9000.0	216.2	9216.2
B62	10984	3	643	3	840	2	11911.2	28575.0	14670.0	16200.0	2180.8	18380.8
B63	10064	2	932	3	406	1	6507.7	28192.5	13635.0	15480.0	2705.0	18185.0
B64	12841	3	686	3	373	1	4108.9	26145.0	10710.0	9000.0	5192.3	14192.3
B65	5368	1	671	3	784	2	5397.4	24165.0	6930.0	9000.0	1503.1	10503.1
B66	22222	3	844	3	2212	3	8307.7	29745.0	16020.0	5400.0	2202.5	7602.5
B67	10658	2	644	3	3710	3	6428.1	29475.0	15480.0	11025.0	2087.6	13112.6
B68	17738	3	732	3	1428	2	2428.3	23490.0	6750.0	12232.7	1780.9	14013.5
B69	6526	1	501	2	980	2	6780.3	26415.0	11250.0	16920.0	770.8	17690.8
B70	7614	2	314	1	756	2	1251.4	25200.0	8640.0	9000.0	719.1	9719.1
B71	11442	3	435	2	1470	2	2501.0	23760.0	6120.0	9117.4	1131.4	10248.8
B72	15505	3	705	3	1764	3	2243.7	22140.0	3510.0	9000.0	5295.9	14295.9
B73	6211	1	624	2	714	1	3576.6	27675.0	13050.0	9000.0	1231.5	10231.5
B74	7970	2	517	2	490	1	887.5	24705.0	8190.0	9000.0	0.0	9000.0
B75	6829	1	835	3	840	2	6853.9	26775.0	8280.0	9000.0	20487.5	29487.5
B76	7105	1	461	2	756	2	550.0	28980.0	16380.0	9000.0	2181.0	11181.0
B77					1540	3	51192.0	28980.0	15840.0	22050.0	761.0	22811.0
B78	12873	3	531	2	2212	3	7849.0	26550.0	13680.0	14400.0	478.0	14878.0
B79	7471	2	581	2	2856	3	5397.0	32805.0	22500.0	12600.0	1476.0	14076.0
B80	8114	2	602	2	128	1	4343.0	26550.0	10710.0	9000.0	990.0	9990.0
B81	6451	1	416	2	742	2	3711.0	23335.0	6426.0	5600.0	1359.0	6959.0
B82	9504	2	1702	3	338	1	19212.0	30146.0	15777.0	14400.0	81314.0	95714.0

NAME	4TOT_CNT	4TOT_DEN	4P_SSI	4TRAB_CNT	4TRAB_DEN	4CRT_A	4CRT_THK_C	4PERI_C	4ENDO_C
B01	299.08	283.2	1239.37	225.39	238.10	196.25	1.79	115.21	103.96
B02	259.01	236.4	1112.22	207.83	206.20	217.75	1.96	117.34	105.04
B03	299.13	301.9	1368.78	197.40	239.50	199.50	1.89	111.59	99.73
B04	327.17	358.2	1470.72	188.60	265.30	189.75	1.87	107.13	95.35
B05	194.87	249.7	1058.20	114.77	171.40	167.50	1.79	99.04	87.77
B06	251.4	332.8	1026.33	163.91	272.00	184.00	2.02	97.44	84.75
B07	343.25	278.3	1357.10	276.57	248.40	249.00	2.11	124.49	111.21
B08	216.64	237	846.91	169.95	203.90	203.25	2.02	107.19	94.52
B09	321.83	320.8	1329.87	231.64	271.40	195.75	1.84	112.28	100.73
B10	268.27	335.8	1140.30	170.08	259.10	179.50	1.91	100.20	88.23
B11	289.34	263.4	1197.49	220.27	223.40	203.75	1.82	117.49	106.04
B12	253.2	282.2	1127.60	176.45	228.60	240.50	2.44	106.19	90.85
B13	266.06	316.9	1123.82	162.98	240.80	170.00	1.75	102.71	91.72
B14	288.76	312.8	1221.32	171.90	232.50	185.25	1.82	107.71	96.30
B15	295.3	311.5	1398.44	196.16	244.80	194.50	1.88	109.15	97.31
B16	268.92	263.8	1322.33	193.95	213.30	188.75	1.75	113.19	102.17
B17	225.96	260.9	1018.42	154.81	203.00	173.00	1.75	104.32	93.32
B18	255.95	268	1130.85	194.32	228.10	192.25	1.85	109.55	97.90
B19	338.48	386.4	1301.84	183.97	295.50	179.00	1.80	104.92	93.59
B20	341.5	293.5	1415.25	257.79	249.60	220.75	1.92	120.93	108.86
B21	339.43	362.4	1301.04	232.97	308.70	188.25	1.83	108.48	96.97
B22	291.3	374.7	1127.36	161.26	287.20	190.25	2.06	98.85	85.91
B23	266.89	271.9	1194.14	187.43	220.90	194.50	1.85	111.07	99.46
B24	242.24	252.3	1090.91	183.65	212.20	229.25	2.23	109.85	95.84
B25	245.22	264.6	859.83	180.88	221.70	172.80	1.68	107.91	97.34
B26	245.03	275.4	823.87	199.09	246.40	177.28	1.77	105.74	94.62
B27	205.68	240.1	1108.22	122.05	165.80	158.88	1.61	103.76	93.65
B28	287.4	259.5	1028.27	223.64	222.40	166.56	1.47	117.96	108.73
B29	319.79	335.5	1125.72	213.90	272.80	161.60	1.55	109.45	99.74
B30	303	330.9	1244.07	181.08	244.40	174.08	1.71	107.27	96.54
B31	365.43	367.7	1599.83	221.78	278.40	160.16	1.50	111.75	102.35
B32	261.22	309.7	1017.99	158.32	231.90	147.20	1.50	102.95	93.53
B33	258.02	281.1	837.36	199.99	244.40	154.08	1.50	107.39	97.96
B34	352.02	311.7	1315.44	293.65	287.00	206.00	1.82	119.14	107.73
B35	270.32	317.3	1007.80	173.51	248.30	153.92	1.56	103.47	93.66
B36	299.69	313.2	1371.42	170.59	217.70	164.96	1.58	109.65	99.75
B37	246.48	311.2	995.32	146.61	229.70	151.04	1.59	99.76	89.75
B38	272.63	263.5	1098.40	209.81	223.90	172.80	1.59	114.02	104.06
B39	293.95	323.6	1165.55	191.43	254.60	148.96	1.46	106.84	97.69
B40	317.61	319.4	1222.81	201.76	246.60	167.36	1.57	111.78	101.94
B41	263.56	296.1	1121.57	177.70	231.40	148.48	1.47	105.76	96.54

NAME	4TOT_CNT	4TOT_DEN	4P_SSI	4TRAB_CNT	4TRAB_DEN	4CRT_A	4CRT_THK_C	4PERI_C	4ENDO_C
B42	267.72	244.7	1301.73	176.26	182.50	165.44	1.47	117.26	108.04
B43	293.48	321.3	1278.29	208.32	266.00	183.50	1.81	107.14	95.78
B44	299.62	316.2	1298.75	212.97	261.60	180.75	1.74	109.12	98.16
B45	269.85	287.9	1115.82	208.71	250.90	199.00	1.94	108.53	96.32
B46	304.96	293.9	1451.39	209.28	233.40	190.25	1.75	114.20	103.20
B47	217.76	293.9	954.08	141.45	228.70	174.00	1.92	96.50	84.41
B48	273.65	234.8	1079.66	233.05	213.90	235.75	2.06	121.01	108.08
B49	318.26	299.2	1313.16	222.54	244.20	217.25	1.99	115.62	103.14
B50	299.03	344.4	1249.28	199.15	278.60	184.00	1.87	104.46	92.73
B51	314.88	267.1	1332.40	239.42	230.00	220.25	1.90	121.71	109.75
B52	249.64	283.4	1282.38	157.40	214.30	185.50	1.87	105.20	93.47
B53	276.51	331.5	1052.29	199.14	281.50	188.75	1.96	102.37	90.05
B54	326.99	364.2	1364.95	177.86	266.60	191.00	1.91	106.21	94.24
B55	282.54	317.9	1121.25	197.18	264.10	189.00	1.90	105.68	93.77
B56	293.18	314.9	1160.27	230.71	278.50	196.50	1.92	108.16	96.07
B57	301.5	256.2	1449.58	227.93	215.00	211.75	1.83	121.62	110.14
B58	307.45	294.1	1331.47	225.40	250.20	201.75	1.85	114.62	102.97
B59	311.62	241.1	1241.17	260.44	216.00	226.50	1.86	127.46	115.75
B60	308.44	326.4	1348.83	207.49	263.20	203.75	1.98	108.97	96.51
B61	244.68	258.4	1092.81	191.37	223.30	190.75	1.85	109.07	97.47
B62	328.94	301.2	1563.61	230.68	242.40	208.75	1.88	117.14	105.35
B63	304	287.3	1523.23	214.58	229.60	190.75	1.74	115.31	104.39
B64	290.85	282.9	1302.05	201.57	228.10	195.50	1.81	113.67	102.30
B65	290.95	285.5	1224.04	211.41	237.50	214.00	2.00	113.17	100.59
B66	317.15	290	1530.38	212.75	228.00	202.00	1.81	117.22	105.84
B67	295.4	242.3	1298.40	250.14	219.60	254.75	2.18	123.77	110.08
B68	262.94	344.5	1086.21	158.61	268.30	172.50	1.87	97.94	86.16
B69	368.27	320.3	1493.73	268.16	270.30	207.75	1.81	120.20	108.80
B70	335.93	397.4	1293.66	204.81	317.20	179.00	1.84	103.06	91.50
B71	216.05	244.6	1126.86	150.03	192.00	181.50	1.82	105.35	93.91
B72	328.21	260.4	1516.59	243.21	215.20	217.75	1.81	125.84	114.46
B73	225.21	300.6	927.93	167.89	256.10	176.75	1.94	97.03	84.82
B74	290.11	262.4	1099.23	226.57	226.20	200.50	1.79	117.87	106.64
B75	322.65	375.3	1258.59	198.55	302.20	173.00	1.76	103.94	92.90
B76	249.4	243.9	1090.54	182.24	201.60	200.25	1.86	113.37	101.67
B77	245.97	260.6	964.98	187.05	220.60	192.25	1.87	108.90	97.18
B78	274.89	277.3	1153.32	199.91	228.90	195.75	1.85	111.62	100.00
B79	285.02	330.3	1296.42	147.04	228.20	169.50	1.72	104.14	93.35
B80	227.65	297.8	1128.11	146.16	227.70	173.75	1.89	98.02	86.16
B81	280.31	335.8	1147.99	179.88	266.10	178.00	1.84	102.42	90.85
B82	267.14	320.4	1081.72	162.24	246.90	187.75	1.95	102.36	90.10

NAME	14TOT_CNT	14TOT_DEN	14P_SSI	14CRT_A	14CRT_THK_C	14PERI_C	14ENDO_C
B01	255.97	546.70	1521.02	173.00	2.51	76.71	60.91
B02	209.64	482.50	1159.05	138.25	2.05	73.89	61.02
B03	251.55	602.50	1427.05	183.00	2.89	72.43	54.29
B04	260.37	677.60	1317.76	174.75	2.89	69.49	51.31
B05	199.21	506.30	1108.46	144.50	2.29	70.32	55.94
B06	200.47	693.70	939.66	174.75	3.56	60.26	37.89
B07	259.31	521.70	1540.90	174.50	2.45	79.03	63.66
B08	172.21	484.40	988.93	131.75	2.20	66.84	53.03
B09	272.77	610.90	1394.77	171.75	2.57	74.91	58.76
B10	227.61	599.40	1197.70	172.00	2.86	69.08	51.10
B11	220.76	482.30	1253.07	150.75	2.19	75.84	62.11
B12	200.76	521.10	958.56	151.75	2.45	69.58	54.17
B13	195.13	510.50	1136.18	145.00	2.34	69.31	54.60
B14	204.39	528.20	1139.98	143.75	2.30	69.74	55.29
B15	253.26	632.80	1163.13	166.75	2.67	70.92	54.17
B16	234.86	498.10	1459.87	164.25	2.36	76.97	62.14
B17	185.23	452.60	1132.28	134.50	2.06	71.71	58.76
B18	216.81	479.90	1302.81	147.00	2.14	75.35	61.88
B19	278.43	716.20	1353.12	185.75	3.09	69.89	50.51
B20	266.88	478.50	1602.11	172.50	2.25	83.72	69.58
B21	248.56	546.00	1417.27	163.00	2.39	75.64	60.60
B22	215.97	656.50	949.95	139.25	2.46	64.30	48.83
B23	229.43	565.80	1212.28	168.50	2.68	71.38	54.57
B24	203.25	446.00	1093.43	166.75	2.45	75.68	60.26
B25	187.45	521.40	909.15	120.32	1.97	67.22	54.83
B26	216.73	523.40	1132.69	142.88	2.19	72.14	58.38
B27	183.73	540.60	1001.42	148.32	2.59	65.35	49.06
B28	248.40	486.50	1401.15	145.60	1.97	80.10	67.72
B29	239.54	557.00	1074.30	157.76	2.39	73.52	58.50
B30	226.98	602.60	1152.41	162.24	2.69	68.80	51.91
B31	270.62	566.10	1585.52	178.72	2.57	77.51	61.33
B32	215.22	642.40	1109.26	161.12	2.89	64.89	46.75
B33	203.93	566.70	1012.70	135.68	2.26	67.25	53.07
B34	249.52	435.50	1349.13	148.75	1.88	84.86	73.02
B35	192.23	592.40	972.90	139.36	2.49	63.86	48.23
B36	244.89	519.70	1487.91	172.64	2.50	76.95	61.25
B37	216.64	609.40	1049.32	151.36	2.58	66.84	50.65
B38	226.63	518.10	1196.37	157.92	2.37	74.14	59.27
B39	248.84	522.60	1358.59	164.32	2.35	77.35	62.60
B40	250.28	581.30	1408.98	187.36	2.91	73.56	55.28
B41	228.30	624.50	1214.11	166.08	2.82	67.78	50.07

NAME	14TOT_CNT	14TOT_DEN	14P_SSI	14CRT_A	14CRT_THK_C	14PERI_C	14ENDO_C
B42	228.91	544.80	1185.07	188.32	2.97	72.66	53.98
B43	216.80	498.40	1199.14	150.50	2.25	73.94	59.79
B44	240.23	566.90	1233.55	152.25	2.32	72.97	58.41
B45	232.27	583.20	1223.74	151.75	2.40	70.74	55.66
B46	265.56	539.20	1521.87	177.25	2.50	78.67	62.94
B47	188.85	563.30	880.66	123.75	2.13	64.91	51.55
B48	227.89	438.30	1067.00	138.00	1.84	80.84	69.29
B49	202.65	480.50	1147.79	137.25	2.07	72.80	59.79
B50	235.08	678.40	1112.97	150.75	2.61	65.99	49.60
B51	225.15	490.50	1383.22	172.75	2.54	75.95	59.98
B52	244.32	536.40	1503.13	192.00	2.88	75.66	57.54
B53	212.19	567.30	1067.00	138.50	2.25	68.56	54.40
B54	266.01	729.30	1360.64	200.50	3.54	67.70	45.43
B55	209.09	576.80	1108.44	147.50	2.47	67.49	51.98
B56	243.33	584.60	1268.18	156.50	2.42	72.32	57.13
B57	234.73	454.50	1508.49	163.00	2.21	80.56	66.65
B58	255.28	635.00	1385.82	183.25	2.97	71.08	52.43
B59	273.77	465.20	1752.44	170.00	2.15	86.00	72.52
B60	253.10	561.50	1467.93	179.25	2.68	75.26	58.41
B61	188.51	487.70	1018.48	130.25	2.06	69.69	56.75
B62	286.93	577.90	1651.59	198.50	2.83	78.99	61.20
B63	257.47	603.00	1396.60	182.50	2.84	73.25	55.43
B64	227.78	537.20	1261.32	158.25	2.42	72.99	57.79
B65	214.47	510.60	1244.80	150.25	2.30	72.65	58.22
B66	225.33	504.70	1284.39	168.00	2.51	74.91	59.16
B67	258.33	522.70	1462.63	159.00	2.21	78.81	64.91
B68	238.84	676.10	1229.95	169.50	2.96	66.63	48.05
B69	251.62	543.20	1403.59	157.00	2.27	76.30	62.04
B70	242.81	704.30	1057.86	160.50	2.82	65.82	48.12
B71	196.90	549.60	1034.04	148.00	2.50	67.10	51.40
B72	271.67	516.50	1695.40	177.50	2.41	81.30	66.18
B73	214.43	650.30	1034.22	150.75	2.70	64.37	47.43
B74	239.75	478.10	1372.93	153.25	2.11	79.39	66.15
B75	258.52	690.80	1327.60	179.00	3.03	68.58	49.53
B76	174.27	421.50	1087.03	134.50	2.05	72.09	59.21
B77	216.32	583.10	1164.39	148.25	2.45	68.28	52.91
B78	225.16	565.00	1308.25	161.25	2.57	70.77	54.60
B79	257.25	628.20	1436.67	193.75	3.13	71.74	52.07
B80	185.13	540.50	956.46	131.75	2.25	65.61	51.46
B81	220.68	621.60	1125.22	151.25	2.58	66.79	50.60
B82	235.46	686.00	1275.29	178.00	3.20	65.68	45.57

NAME	38TOT_CNT	38TOT_DEN	38P_SSI	38CRT_A	38CRT_THK_C	38PERI_C	38ENDO_C
B01	356.18	939.80	1386.29	259.75	4.82	69.01	38.71
B02	314.96	941.60	1368.86	242.25	4.90	64.83	34.05
B03	355.82	945.70	1682.57	285.25	5.56	68.76	33.82
B04	367.21	967.00	1655.56	295.00	5.80	69.08	32.63
B05	306.37	830.80	1521.64	247.00	4.61	68.07	39.12
B06	317.51	1057.50	1057.72	306.00	9.78	61.43	0.00
B07	346.34	935.40	1673.78	291.75	5.86	68.21	31.41
B08	275.39	928.00	1107.37	229.00	5.08	61.07	29.18
B09	347.63	986.20	1600.14	290.75	6.16	66.56	27.86
B10	332.27	1007.60	1184.02	238.25	4.85	64.37	33.91
B11	293.59	940.20	1223.56	235.75	5.04	62.64	31.01
B12	294.49	895.80	1232.38	255.50	5.40	64.27	30.34
B13	305.33	914.80	1308.45	247.75	5.08	64.76	32.87
B14	301.10	958.20	1185.79	234.00	4.95	62.84	31.76
B15	323.48	1051.10	1235.97	254.00	5.76	62.19	25.99
B16	384.81	930.00	1755.47	307.75	5.67	72.11	36.50
B17	309.87	956.40	1363.25	245.00	5.14	63.81	31.51
B18	391.69	942.10	1752.98	310.50	5.72	72.28	36.37
B19	361.74	979.00	1626.61	297.75	6.07	68.14	30.03
B20	406.20	856.00	2123.57	313.50	5.13	77.22	44.98
B21	402.04	1006.40	1730.25	308.00	5.88	70.85	33.91
B22	304.09	977.00	1118.22	322.25	9.95	62.54	0.00
B23	351.99	942.40	1483.37	282.75	5.53	68.51	33.77
B24	326.73	924.90	1394.69	247.75	4.81	66.63	36.41
B25	267.89	929.60	1118.70	212.64	4.67	60.18	30.81
B26	311.05	867.10	1525.61	256.00	4.97	67.14	35.93
B27	255.79	827.50	991.47	194.40	3.88	62.33	37.97
B28	368.35	962.50	1740.65	313.60	6.35	69.35	29.47
B29	359.70	1002.70	1364.91	250.88	4.83	67.14	36.81
B30	352.54	978.00	1535.79	291.52	6.03	67.31	29.44
B31	374.05	995.70	1260.71	227.36	4.06	68.71	43.17
B32	281.02	936.20	1040.72	221.28	4.76	61.42	31.48
B33	333.08	981.50	1276.98	272.48	5.78	65.30	28.99
B34	408.54	991.00	1558.01	297.75	5.42	71.98	37.93
B35	277.90	1001.70	953.60	210.40	4.78	59.05	29.03
B36	353.21	869.10	1605.22	276.96	4.96	71.46	40.33
B37	306.74	826.70	1308.19	228.00	4.12	68.28	42.40
B38	333.72	884.50	1437.53	242.56	4.41	68.86	41.15
B39	360.47	960.70	1280.89	240.64	4.38	68.67	41.12
B40	394.03	906.10	1189.72	391.04	8.03	73.93	23.47
B41	333.14	1079.90	1028.07	196.16	3.93	62.26	37.57

NAME	38TOT_CNT	38TOT_DEN	38P_SSI	38CRT_A	38CRT_THK_C	38PERI_C	38ENDO_C
B42	314.82	906.70	1330.30	237.92	4.62	66.05	37.06
B43	303.24	873.20	1479.22	256.00	5.12	66.06	33.86
B44	357.00	918.30	1579.93	279.75	5.23	69.89	37.01
B45	358.69	1002.60	1675.12	315.00	6.98	67.05	23.18
B46	343.23	909.20	1557.54	290.00	5.68	68.88	33.16
B47	291.15	826.60	1280.06	212.25	3.91	66.53	41.94
B48	309.39	902.00	1361.05	223.00	4.27	65.65	38.83
B49	285.29	881.20	1383.76	235.00	4.84	63.78	33.40
B50	325.76	990.20	1402.03	261.00	5.58	64.30	29.23
B51	371.39	940.20	1463.10	300.00	5.71	70.45	34.55
B52	351.26	990.90	1338.75	262.00	5.20	66.74	34.09
B53	288.50	946.70	1137.19	216.50	4.55	61.88	33.30
B54	362.71	979.60	1455.03	268.75	5.17	68.21	35.71
B55	349.01	1080.50	1511.76	334.25	10.14	63.71	0.00
B56	349.00	957.50	1594.68	295.50	6.09	67.68	29.45
B57	341.16	840.30	1792.89	278.00	4.99	71.43	40.11
B58	376.67	1021.50	1444.00	277.25	5.44	68.07	33.91
B59	404.00	1011.90	1768.17	303.25	5.75	70.83	34.73
B60	360.43	951.60	1696.85	290.75	5.69	68.99	33.25
B61	285.13	946.50	1116.69	218.25	4.65	61.53	32.30
B62	409.69	996.20	1852.78	319.25	6.03	71.89	34.00
B63	350.99	1012.20	1478.94	280.75	5.92	66.01	28.80
B64	342.59	958.30	1374.64	254.50	4.94	67.03	35.98
B65	324.52	894.00	1363.87	250.25	4.76	67.54	37.64
B66	360.29	978.40	1300.76	257.00	4.88	68.03	37.39
B67	341.39	961.00	1379.70	244.50	4.70	66.82	37.31
B68	365.31	1001.50	1702.93	295.75	6.09	67.70	29.45
B69	353.25	1000.70	1534.53	273.75	5.58	66.60	31.56
B70	334.04	994.90	1404.58	265.25	5.60	64.96	29.77
B71	298.47	875.90	1395.30	237.25	4.68	65.44	36.06
B72	409.38	976.50	1818.00	303.25	5.48	72.58	38.18
B73	303.18	980.40	1189.72	233.25	5.00	62.34	30.90
B74	332.03	859.10	1563.34	270.00	5.00	69.69	38.26
B75	362.33	1014.90	1473.52	272.50	5.47	66.98	32.59
B76	285.51	849.70	1406.73	231.00	4.56	64.98	36.32
B77	307.63	1034.10	1070.30	224.25	4.90	61.14	30.34
B78	329.63	951.30	1440.15	299.00	6.61	65.99	24.43
B79	422.62	929.30	2060.95	348.25	6.21	75.60	36.58
B80	309.09	999.50	1263.99	244.00	5.36	62.34	28.64
B81	364.57	1016.90	1410.57	283.00	5.78	67.12	30.80
B82	347.31	948.90	1528.88	273.50	5.37	67.82	34.09

NAME	66TOT_CNT	66TOT_DEN	66P_SSI	66CRT_A	66CRT_THK_C	66PERI_C	66ENDO_C	66T_MA	T_MD	66T_SubQ
B01	392.72	701.90	2525.98	328.00	4.76	83.85	53.94	7039.50	79.21	2985.75
B02	325.10	668.90	1970.74	261.75	3.99	78.15	53.09	6433.75	75.59	3747.75
B03	401.49	752.20	2309.66	306.50	4.53	81.90	53.44	6111.25	77.48	2132.75
B04	423.55	818.10	2369.59	336.50	5.24	80.66	47.73	6396.25	81.33	2684.75
B05	347.03	670.30	2232.65	285.75	4.24	80.66	53.99	5541.50	78.69	3331.25
B06	359.47	876.80	1481.45	262.00	4.56	71.78	43.13	5666.50	78.22	2452.00
B07	386.43	722.60	2304.29	312.50	4.64	81.98	52.85	7197.25	79.43	2217.50
B08	282.22	684.20	1373.97	222.50	3.68	72.00	48.86	6540.00	79.69	5120.25
B09	399.56	821.70	2194.57	324.00	5.25	78.17	45.15	8904.50	76.01	3874.75
B10	393.68	834.90	2127.78	300.50	4.87	76.97	46.36	6798.75	77.76	2619.25
B11	303.52	699.00	1607.14	243.75	3.97	73.87	48.93	7244.00	76.90	4369.75
B12	330.08	716.40	2016.73	273.00	4.38	76.09	48.57	7518.50	78.16	4330.50
B13	351.55	810.00	1503.72	255.75	4.22	73.85	47.33	7556.75	76.92	2524.25
B14	343.17	812.70	1780.49	284.50	4.97	72.84	41.61	7252.50	77.24	3007.50
B15	361.39	840.00	1878.47	302.50	5.33	73.53	40.07	5356.00	77.79	3262.50
B16	406.70	647.40	2872.20	336.00	4.50	88.85	60.60	6222.00	79.80	2633.50
B17	322.77	669.30	1911.93	258.00	3.94	77.85	53.09	5708.50	77.85	2785.75
B18	425.57	785.90	2427.20	342.00	5.16	82.49	50.07	7050.75	71.10	1835.00
B19	368.58	751.80	2048.04	282.00	4.35	78.49	51.16	7037.25	78.47	2810.25
B20	449.85	666.50	2776.01	345.00	4.41	92.10	64.40	8665.75	75.02	9190.25
B21	389.50	689.40	2532.75	311.50	4.43	84.26	56.44	7317.00	74.31	7193.50
B22	338.57	793.80	1587.87	247.50	4.10	73.21	47.43	6059.25	78.80	2901.50
B23	391.75	784.70	2249.39	332.25	5.32	79.21	45.81	7195.75	76.74	3403.75
B24	343.41	703.30	1697.75	254.00	3.83	78.33	54.26	6083.00	78.96	2238.75
B25	302.39	697.10	1495.46	216.00	3.43	73.83	52.31	4916.96	80.51	3070.88
B26	354.91	674.40	2141.57	274.56	3.99	81.32	56.24	5807.20	76.00	3596.64
B27	254.26	277.10	1904.96	238.40	2.39	107.37	92.38	5535.68	73.55	7553.12
B28	411.81	730.00	2163.28	308.16	4.37	84.20	56.72	6532.96	75.59	3721.12
B29	386.67	755.00	2208.83	310.72	4.76	80.23	50.31	6074.56	79.96	2669.92
B30	402.34	851.80	1742.70	258.88	4.02	77.04	51.79	6319.84	75.87	5366.88
B31	400.32	704.00	2582.33	341.28	4.95	84.53	53.45	6700.00	71.96	4988.80
B32	309.21	764.50	1445.27	238.24	4.07	71.29	45.71	5961.44	72.24	3349.76
B33	341.97	688.80	1785.89	256.96	3.84	78.99	54.86	6994.56	76.62	3571.04
B34	431.79	668.90	2656.03	331.50	4.34	90.06	62.82	6300.00	72.96	5773.00
B35	299.23	798.90	1585.94	246.24	4.53	68.61	40.16	6628.64	79.42	2548.96
B36	362.93	670.50	2244.13	285.28	4.10	82.47	56.72	6789.44	75.49	4800.32
B37	336.92	699.40	1360.79	222.72	3.30	77.81	57.05	6130.24	74.62	3046.40
B38	378.29	654.40	2246.18	271.36	3.68	85.23	62.08	6522.08	78.64	2316.16
B39	385.14	729.90	2398.37	300.80	4.46	81.43	53.40	6683.68	71.81	3184.00
B40	398.92	693.90	2084.28	290.72	4.02	85.00	59.76	6709.60	81.43	2313.12
B41	366.62	844.30	1476.73	258.88	4.29	73.87	46.94	5887.20	79.81	2508.32

NAME	66TOT_CNT	66TOT_DEN	66P_SSI	66CRT_A	66CRT_THK_C	66PERI_C	66ENDO_C	66T_MA	T_MD	66T_SubQ
B42	337.27	632.10	2024.17	245.76	3.46	81.89	60.14	5589.92	78.89	3175.36
B43	320.62	640.30	1975.51	264.25	3.95	79.33	54.52	7444.00	77.27	3537.00
B44	401.07	679.50	2035.80	294.50	4.00	86.12	60.96	7316.00	76.37	9904.25
B45	388.34	840.10	1616.95	266.25	4.23	76.22	49.63	6380.50	77.70	1973.75
B46	380.99	704.60	2458.41	311.00	4.57	82.43	53.73	7321.00	79.45	2348.25
B47	315.66	678.10	1615.35	225.25	3.43	76.48	54.95	5273.25	77.62	1729.75
B48	304.54	745.50	1575.33	218.50	3.63	71.65	48.86	4644.25	79.41	1697.50
B49	320.21	666.10	1939.11	245.25	3.71	77.73	54.40	6357.00	79.27	2062.00
B50	374.11	771.80	2142.76	286.50	4.48	78.05	49.91	6886.25	78.87	2408.00
B51	410.60	782.10	2063.04	298.75	4.44	81.22	53.32	6542.50	78.12	1835.00
B52	369.38	604.50	2594.37	300.50	4.00	87.62	62.47	7198.75	76.46	1885.50
B53	349.84	889.60	1423.39	251.00	4.46	70.30	42.28	5866.50	78.73	2269.75
B54	390.61	738.70	2333.48	355.00	5.54	81.51	46.73	7154.00	80.15	2075.75
B55	393.62	896.10	1890.69	323.00	5.74	74.30	38.22	6939.00	79.02	2560.00
B56	391.44	800.10	2257.85	321.50	5.17	78.41	45.91	5928.00	78.25	3167.50
B57	383.27	630.60	2542.97	308.00	4.14	87.39	61.37	6790.50	76.21	3097.00
B58	412.54	817.70	2204.30	331.75	5.26	79.62	46.59	6504.50	79.09	2893.00
B59	411.40	718.30	2680.77	322.75	4.58	84.84	56.05	9328.00	76.91	2775.75
B60	401.06	732.20	2639.81	322.75	4.74	82.97	53.17	6657.25	78.78	2340.50
B61	320.81	712.50	1679.48	246.75	3.92	75.22	50.57	5890.50	78.77	2157.75
B62	462.04	753.40	3107.75	373.00	5.23	87.79	54.95	6476.50	74.52	4391.25
B63	396.22	771.60	2295.02	319.50	4.93	80.33	49.38	7469.25	79.38	1875.00
B64	374.69	801.90	1554.29	266.00	4.19	76.63	50.29	7050.25	80.41	2045.75
B65	351.59	708.10	2074.80	283.00	4.33	78.99	51.80	6290.50	80.69	3158.00
B66	404.79	837.60	2170.26	325.25	5.31	77.93	44.56	7504.00	76.90	2662.50
B67	392.64	766.50	2210.37	296.00	4.47	80.23	52.13	6674.75	79.47	4265.50
B68	394.38	780.90	2425.44	312.00	4.84	79.66	49.25	6053.25	80.38	2335.50
B69	420.89	831.80	2524.15	346.25	5.56	79.74	44.81	6800.50	77.56	2455.00
B70	367.17	822.30	1796.46	275.50	4.54	74.91	46.36	6466.50	77.48	4208.50
B71	296.83	565.40	1863.14	223.50	3.13	81.22	61.55	4862.00	78.07	2060.75
B72	433.53	615.60	3185.42	345.00	4.28	94.07	67.19	7473.00	76.82	3238.00
B73	348.29	762.50	1949.63	276.50	4.48	75.76	47.59	5050.50	77.47	2796.25
B74	371.22	657.60	2260.24	288.75	4.04	84.22	58.87	8367.25	78.73	3022.50
B75	403.61	812.10	2417.14	337.25	5.45	79.03	44.81	6711.50	78.69	2421.25
B76	304.93	619.10	1922.67	246.50	3.67	78.67	55.60	5266.75	78.39	1922.25
B77	355.35	849.10	1794.08	279.25	4.88	72.52	41.83	6521.75	78.85	3268.25
B78	369.40	722.20	2341.97	300.00	4.56	80.17	51.55	5734.75	78.38	2592.75
B79	455.99	856.70	2124.78	337.25	5.14	81.78	49.50	8018.75	78.85	2526.75
B80	316.81	793.50	1571.82	241.75	4.19	70.83	44.49	5131.50	83.40	1965.75
B81	410.47	821.30	2285.73	337.00	5.42	79.25	45.22	5910.00	79.60	3213.50
B82	391.25	837.80	1816.46	327.75	5.54	76.61	41.83	8574.50	81.06	1798.25

NAME	R4TOT_CNT	R4TOT_DEN	R4P_SSI	R4CRT_A	R4CRT_THK_C	R4PERI_C	R4ENDO_C
B01	96.26	336.00	344.77	103.50	1.92	60.00	47.96
B02	26.26	358.50	510.57	500.75	4.83	30.34	
B03	117.58	275.20	487.93	153.00	2.32	73.27	58.71
B04	106.35	396.50	422.63	105.75	2.05	58.06	45.19
B05	83.81	398.60	375.27	86.25	1.90	51.40	39.47
B06	87.36	303.60	282.79	108.25	2.01	60.13	47.49
B07	101.11	372.80	427.21	105.25	2.02	58.38	45.67
B08	65.70	341.80	259.24	83.00	1.93	49.15	37.05
B09							
B10	89.51	346.30	331.17	95.00	1.86	57.00	45.33
B11	95.84	334.50	355.86	108.25	2.02	60.00	47.33
B12	90.67	427.20	315.73	90.25	1.99	51.65	39.16
B13	71.53	243.70	281.62	100.50	1.83	60.73	49.25
B14	79.39	326.70	310.3	96.00	1.95	55.26	42.98
B15	91.43	369.80	316.72	88.50	1.76	55.74	44.66
B16	87.50	246.50	373.91	109.75	1.80	66.79	55.52
B17	85.63	272.30	397.07	118.25	2.10	62.87	49.66
B18	72.86	287.70	252.75	95.00	1.88	56.41	44.59
B19	97.86	402.70	321.49	116.00	2.44	55.26	39.95
B20	134.99	327.00	505.42	130.50	1.98	72.02	59.56
B21	108.09	457.50	374.27	101.00	2.11	54.49	41.23
B22	87.78	377.50	299.83	115.75	2.51	54.05	38.30
B23	94.32	367.70	328.9	103.25	2.05	56.77	43.88
B24							
B25	76.89	313.80	291.98	99.50	2.03	55.49	42.76
B26	8.38	338.60	314.42	209.25	2.81	17.64	
B27	67.83	384.30	211.76	199.50	7.50	47.10	
B28	86.45	355.40	306.21	95.75	1.95	55.29	43.05
B29	99.09	322.00	356.36	114.75	2.06	62.19	49.25
B30	97.70	395.50	333.97	94.50	1.90	55.71	43.78
B31	107.49	390.20	420.32	109.25	2.09	58.84	45.71
B32							
B33	75.01	281.20	265.6	105.00	2.04	57.90	45.08
B34	112.86	280.90	381.11	146.00	2.29	71.05	56.69
B35	84.79	355.90	357.85	111.75	2.36	54.72	39.87
B36	93.34	349.60	404.85	121.00	2.40	57.92	42.83
B37	85.28	317.90	276.2	110.25	2.15	58.06	44.56
B38	88.15	316.20	380.51	161.50	3.31	59.19	38.39
B39	102.29	389.70	412.67	103.00	2.02	57.43	44.77
B40	98.27	262.10	362.16	136.00	2.20	68.65	54.80
B41	98.88	380.30	410.23	98.25	1.92	57.16	45.08

NAME	R4TOT_CNT	R4TOT_DEN	R4P_SSI	R4CRT_A	R4CRT_THK_C	R4PERI_C	R4ENDO_C
B42	100.85	319.40	428.93	119.00	2.11	62.99	49.72
B43							
B44	94.79	282.50	330.03	133.50	2.32	64.93	50.38
B45	104.41	287.80	382.52	148.75	2.49	67.52	51.86
B46	114.44	302.50	498.41	121.00	1.92	68.94	56.86
B47	74.95	260.00	272.79	124.25	2.35	60.19	45.40
B48	118.69	305.90	405.33	128.50	2.03	69.83	57.11
B49	85.38	313.60	318.97	109.50	2.11	58.49	45.22
B50	107.68	376.50	401.11	111.25	2.08	59.95	46.86
B51	90.82	320.10	371.51	104.50	1.95	59.71	47.46
B52	87.00	249.30	282.77	151.50	2.61	66.22	49.82
B53	97.15	325.50	349.49	117.50	2.16	61.25	47.69
B54	95.76	319.70	352.29	108.25	1.96	61.35	49.02
B55	101.84	462.40	349.23	93.50	2.02	52.61	39.91
B56	99.87	326.90	381.12	120.50	2.19	61.96	48.22
B57	103.86	323.30	435.01	110.00	1.91	63.54	51.52
B58	79.57	275.80	333.24	121.00	2.28	60.21	45.88
B59	88.98	263.50	304.78	121.25	2.07	65.15	52.16
B60	94.41	365.20	328.68	114.25	2.30	57.00	42.58
B61	80.59	310.30	270.44	97.50	1.91	57.13	45.15
B62	104.72	369.70	419.33	110.00	2.07	59.66	46.66
B63	115.97	385.00	458.93	115.00	2.09	61.53	48.38
B64	93.14	340.80	356.78	101.50	1.93	58.60	46.46
B65	98.23	319.20	403.74	120.25	2.17	62.19	48.54
B66	60.74	252.30	216.42	90.75	1.84	55.00	43.42
B67	90.45	272.40	388.79	121.00	2.09	64.59	51.49
B68	104.28	413.40	366.91	98.75	1.97	56.30	43.92
B69	108.45	489.60	383.46	87.25	1.86	52.76	41.07
B70	110.55	445.70	359.82	100.75	2.04	55.83	43.02
B71	67.17	250.20	233.43	105.00	2.03	58.09	45.33
B72	114.18	293.20	474.21	128.00	2.01	69.96	57.33
B73	78.93	290.40	282.46	105.00	2.02	58.44	45.78
B74	91.10	295.10	314.78	105.50	1.87	62.29	50.54
B75	84.39	336.50	291.67	100.75	2.02	56.13	43.42
B76	57.77	239.20	248.41	94.00	1.92	55.09	43.05
B77	87.52	312.60	292.23	110.00	2.09	59.32	46.22
B78	83.74	288.00	286.71	102.50	1.88	60.45	48.64
B79	95.87	439.80	413.19	103.75	2.30	52.34	37.89
B80	72.30	270.80	246.18	128.00	2.57	57.92	41.79
B81	107.29	447.00	365.27	98.00	2.02	54.92	42.24
B82	92.90	570.80	295.58	78.50	2.02	45.22	32.54

NAME	R66TOT_CNT	R66TOT_DEN	R66P_SSI	R66CRT_A	R66CRT_THK_C	R66PERI_C	R66ENDO_C	R_MA	R_MD	R_SubQ
B01	87.90	779.60	246.25	67.75	2.21	37.64	23.78	2441.00	73.12	574.00
B02	65.06	856.10	146.27	47.75	1.92	30.90	18.84	2439.50	69.85	1358.75
B03	84.21	757.00	235.55	68.25	2.25	37.39	23.25	2853.25	82.53	2717.25
B04	95.00	911.30	265.69	72.50	2.58	36.20	19.98	2913.00	79.78	1099.25
B05	96.20	751.60	293.81	76.75	2.34	40.11	25.38	2573.50	81.91	856.75
B06	73.88	985.10	183.95	60.00	2.70	30.70	13.73	2236.25	80.08	900.50
B07	83.07	793.00	237.86	71.25	2.51	36.28	20.52	2530.25	80.09	723.25
B08	61.70	689.40	178.05	54.50	2.00	33.54	20.97	2262.75	79.17	1967.75
B09	89.93	810.20	273.95	76.25	2.62	37.35	20.90	3119.25	72.96	1262.25
B10	84.57	894.90	217.89	67.50	2.55	34.46	18.42	2421.50	83.41	1181.50
B11	84.67	785.80	255.55	76.00	2.68	36.80	19.98	2834.50	78.50	1673.50
B12	77.27	712.10	243.49	65.00	2.16	36.93	23.38	3184.25	71.45	1908.50
B13	82.44	865.50	199.83	67.00	2.51	34.60	18.84	2455.00	79.38	698.00
B14	92.40	886.30	249.70	84.75	3.27	36.20	15.65	2979.50	78.62	1794.75
B15	86.96	937.60	144.02	87.50	4.14	34.14	8.12	2236.25	71.04	941.00
B16	96.14	813.00	275.73	77.25	2.52	38.55	22.70	2809.00	80.53	548.00
B17	93.42	927.20	241.88	80.25	3.11	35.58	16.05	2145.25	79.68	1104.25
B18	78.97	766.70	235.58	70.75	2.52	35.98	20.13	2370.00	80.88	528.75
B19	91.59	878.60	255.34	79.00	2.93	36.20	17.81	2513.75	80.16	1539.00
B20	114.84	751.80	401.42	94.00	2.65	43.81	27.17	3457.25	73.51	4167.50
B21	115.03	871.40	331.93	99.00	3.24	40.73	20.36	2785.00	75.15	3015.00
B22	78.59	774.30	220.72	68.75	2.46	35.71	20.29	1990.25	79.80	645.75
B23	72.61	849.30	178.23	65.50	2.69	32.78	15.85	2445.25	74.70	1761.50
B24	79.09	938.80	180.87	61.25	2.47	32.54	17.00	6083.00	80.06	2238.75
B25	84.36	829.10	213.34	70.00	2.51	35.76	19.98	2305.25	76.20	957.75
B26	94.72	966.50	237.46	74.75	2.87	35.09	17.09	2504.25	76.79	2323.00
B27	44.40	388.60	157.50	63.50	2.01	37.89	25.25	2258.75	77.22	4307.00
B28	83.27	730.40	246.83	77.25	2.60	37.85	21.49	2990.50	69.52	1680.75
B29	72.83	813.80	189.77	57.25	2.13	33.54	20.13	6074.56	77.65	2669.92
B30	88.97	885.20	217.48	74.75	2.79	35.54	17.99	2597.75	76.88	2207.50
B31	70.28	844.20	171.02	65.75	2.79	32.34	14.83	2571.25	69.68	2208.25
B32	70.53	844.70	177.40	58.25	2.32	32.39	17.81	5961.44	40.17	3349.76
B33	77.89	792.80	235.92	63.00	2.24	35.14	21.05	2568.75	77.60	1731.75
B34	95.70	686.00	290.67	76.75	2.19	41.87	28.08	2916.50	75.26	1566.75
B35	78.83	1007.40	178.93	65.25	2.96	31.36	12.78	2140.75	80.35	1302.25
B36	91.97	776.20	300.34	81.75	2.72	38.59	21.49	3429.50	79.78	995.50
B37	85.37	822.80	218.19	73.00	2.62	36.11	19.66	2495.25	80.62	1644.00
B38	103.62	774.70	349.70	91.25	2.85	41.00	23.11	3323.75	79.44	1004.50
B39	106.84	821.80	347.23	87.00	2.73	40.42	23.25	2522.00	79.08	2031.75
B40								2962.25	79.82	842.75
B41	94.03	952.30	253.03	79.00	3.10	35.23	15.75	2839.75	81.96	961.75

NAME	R66TOT_CNT	R66TOT_DEN	R66P_SSI	R66CRT_A	R66CRT_THK_C	R66PERI_C	R66ENDO_C	R_MA	R_MD	R_SubQ
B42	89.54	858.90	247.44	70.50	2.48	36.20	20.59	2653.50	80.99	1336.75
B43	91.62	722.80	302.21	79.00	2.45	39.91	24.50	3236.50	76.50	1892.00
B44	79.41	692.00	239.48	71.50	2.33	37.97	23.31	2734.00	74.61	4149.00
B45	107.87	856.10	262.07	103.75	3.67	39.79	16.72	2657.50	82.06	699.00
B46	111.84	759.50	398.67	96.25	2.82	43.02	25.32	3028.00	77.46	1292.75
B47	55.86	915.80	127.45	48.50	2.41	27.69	12.53	1999.00	80.24	603.25
B48	97.75	766.70	320.20	78.50	2.42	40.03	24.81	2629.25	80.92	560.50
B49	84.16	678.70	285.24	71.50	2.20	39.47	25.69	2722.50	80.24	580.75
B50	104.03	954.40	283.82	78.00	2.75	37.01	19.74	2736.50	81.00	1057.25
B51	111.08	854.40	359.54	89.00	2.82	40.42	22.70	2770.00	78.35	432.50
B52	86.38	684.20	282.91	73.25	2.23	39.83	25.81	2417.25	79.04	1095.00
B53	93.47	914.10	248.63	81.50	3.14	35.85	16.15	2734.00	82.17	761.00
B54	104.12	865.90	306.19	93.00	3.24	38.87	18.51	2813.75	80.89	1131.00
B55	91.89	947.30	228.15	69.75	2.61	34.91	18.51	2564.25	79.37	572.00
B56	95.70	900.70	255.79	85.00	3.22	36.54	16.34	2313.75	80.90	1540.50
B57	92.69	728.40	297.91	82.25	2.58	39.99	23.78	2817.00	71.83	1566.50
B58	83.15	859.40	232.39	70.00	2.63	34.87	18.33	2521.00	75.65	1467.50
B59	87.61	887.20	237.71	77.00	2.98	35.23	16.53	2893.75	75.65	1340.75
B60	92.10	900.70	262.28	71.75	2.59	35.85	19.58	2685.25	81.30	1250.75
B61	81.36	921.90	214.25	72.75	3.08	33.30	13.96	2572.00	80.19	1058.75
B62	101.11	993.80	245.12	79.00	3.00	35.76	16.91	2454.75	78.65	1760.50
B63	80.68	750.50	205.47	66.25	2.23	36.75	22.77	2927.50	72.89	448.00
B64	83.63	942.30	206.40	67.25	2.70	33.40	16.44	2599.75	80.34	1016.00
B65	87.78	681.80	273.59	69.25	2.05	40.22	27.34	3219.25	82.34	1022.50
B66	81.29	1106.00	137.25	69.50	3.71	30.39	7.09	1947.75	75.44	1634.25
B67	67.70	577.40	235.65	64.25	2.00	38.39	25.81	2573.50	84.25	1362.25
B68	87.68	746.20	267.10	70.75	2.26	38.43	24.24	2558.75	81.18	658.75
B69	113.92	916.90	336.34	98.00	3.40	39.51	18.16	2645.25	81.24	910.50
B70	77.58	845.60	212.53	61.00	2.28	33.96	19.66	2585.00	80.58	1968.00
B71	61.65	731.70	167.34	51.25	1.94	32.54	20.36	1923.00	80.94	1116.00
B72	104.20	712.50	349.45	86.00	2.44	42.87	27.52	3119.25	78.36	1786.25
B73	89.49	1011.10	198.28	73.75	3.14	33.35	13.61	1949.50	81.66	995.75
B74	101.41	769.70	333.37	84.00	2.58	40.69	24.50	2887.50	81.17	1124.50
B75	120.23	939.30	347.94	94.50	3.12	40.11	20.52	2599.25	81.18	1157.00
B76	49.86	722.60	80.07	69.75	4.69	29.45	0.00	1952.25	55.49	560.75
B77	90.20	954.50	186.38	72.00	2.81	34.46	16.82	1999.25	75.65	1034.00
B78	94.19	705.60	317.49	76.00	2.24	40.96	26.88	2755.50	80.10	887.25
B79	96.70	852.00	274.65	82.25	2.86	37.77	19.82	2325.00	78.94	815.50
B80	95.15	923.80	238.33	80.50	3.05	35.98	16.82	2482.50	80.19	601.50
B81	106.28	951.10	276.77	92.75	3.51	37.47	15.45	2702.50	83.90	1272.00
B82	78.32	775.40	212.74	69.00	2.48	35.63	20.05	2655.25	84.58	629.25

NAME	T_IMAT	T66_MarFat	T66_Per_MarFat	R_IMAT	R66_MarFat	R66_Per_MarFat	4BSI
B01	111.00	132.30	59.2	155.50	11.30	26.6	299130.0
B02	101.00	116.00	55.6	141.00			259035.3
B03	109.50	84.30	44.9	143.50	15.50	41.3	299182.9
B04	92.30	38.80	28.3	143.00	9.80	37.0	327126.2
B05	91.00	125.50	57.9	135.70	23.30	52.6	194890.9
B06	83.30	23.50	23.4	127.50	4.80	36.9	251430.4
B07	98.00	61.50	30.8	153.00	10.50	32.0	343213.5
B08	89.70	82.50	51.0	143.80	18.30	54.1	216677.3
B09	30.30	55.30	39.2	172.00	5.50	15.6	321842.6
B10	102.50	45.00	33.3	144.50	6.50	26.0	268304.2
B11	49.50	77.50	33.1	150.70	7.00	22.4	289344.9
B12	95.80	84.00	47.9	152.50	12.30	30.4	253204.0
B13	85.70	58.30	44.2	156.20	2.30	9.4	266037.6
B14	114.00	47.30	40.3	141.30	9.00	38.6	288792.6
B15	106.20	44.50	36.5	159.00	6.00	41.4	295302.0
B16	90.30	130.30	47.5	147.50	16.80	45.7	268944.1
B17	82.00	107.50	51.8	134.20	7.00	34.5	225939.4
B18	90.20	76.00	44.8	135.50	5.00	16.2	255940.0
B19	106.50	41.30	24.9	148.50	8.80	46.3	338486.4
B20	41.00	99.80	35.7	146.80	4.30	9.5	341560.6
B21	98.50	104.00	47.1	157.50	3.00	9.7	339387.6
B22	83.80	5.00	3.8	141.50	9.30	29.1	291329.3
B23	47.80	58.00	41.3		3.00	15.0	266937.8
B24	98.50	101.30	54.5	136.20	6.00	35.7	242271.1
B25	63.50	100.00	22.3	102.20	11.80	42.9	245210.1
B26	100.00	82.70	37.4	102.40	1.30	7.9	245039.9
B27	42.30	260.20	39.1	20.30	21.00	31.0	205717.7
B28		93.90	46.7	122.50	9.00	25.7	287359.9
B29	38.00	68.00	40.5	112.60	3.30	12.7	319825.4
B30	100.80	42.90	8.5	133.30	6.80	30.5	302998.5
B31	186.30	116.30	52.2	142.10			365405.6
B32	105.00	45.90	39.4	116.80	3.80	18.7	261188.6
B33	98.00	85.10	42.2	123.80	12.80	40.3	257982.3
B34	43.50	40.50	14.8	32.80			352065.2
B35	86.50	42.20	34.0	116.80	6.80	55.3	270339.6
B36	110.00	112.00	47.6	116.60	21.00	56.3	299669.8
B37	89.30	46.40	30.2	71.00	11.50	41.8	246470.4
B38	99.50	95.00	38.1	126.20	20.50	49.0	272606.6
B39	42.70	78.40	37.5	111.70	22.30	58.2	293932.4
B40	103.80	55.80	24.0	126.10	15.50	25.1	317560.3
B41	98.50	57.80	46.1	114.90	3.00	16.0	263552.7

NAME	T_IMAT	T66_MarFat	T66_Per_MarFat	R_IMAT	R66_MarFat	R66_Per_MarFat	4BSI
B42	105.00	113.80	48.2	47.20	8.50	28.8	267760.5
B43	37.50	106.00	49.2	156.00	6.50	14.3	293507.6
B44	115.00	75.30	33.5	160.00	8.80	21.6	299599.5
B45	92.00	56.30	42.7	142.00	6.50	23.2	269834.3
B46	25.50	88.00	41.7	150.50	23.80	49.1	304994.7
B47		50.80	26.6	56.70	5.30	37.1	217779.9
B48	97.50	29.80	21.4	124.00	7.50	19.0	273600.7
B49	95.30	86.30	41.0	141.30	22.80	43.2	318274.0
B50	99.50	71.00	42.8	152.80	9.00	37.0	299025.3
B51	101.00	55.00	31.3	147.80	9.50	26.5	314844.1
B52	115.50	139.30	49.1	159.80	27.00	56.5	249604.6
B53	92.00	37.30	39.1	49.80	7.50	33.6	276471.0
B54	100.50	72.30	37.7	152.80	15.80	53.9	326960.6
B55	85.70	32.50	31.0	144.80	4.00	18.8	282533.6
B56	100.50	62.30	44.9	136.50	3.00	11.9	293171.9
B57	51.20	133.80	50.4	148.80	13.80	25.3	301547.4
B58		67.30	49.7	61.20	5.80	22.3	307481.6
B59	218.50	90.80	40.7	69.50	8.80	35.5	311682.0
B60	93.00	74.80	35.8		6.00	22.8	308448.0
B61	96.20	55.80	32.6	136.80	8.50	47.2	244640.2
B62	45.20	107.30	49.4	40.70	3.00	17.9	328910.4
B63	164.00	58.50	34.0	148.20	2.50	6.6	303963.4
B64	95.30	44.00	30.6	192.00	7.80	43.3	290891.9
B65	94.70	93.30	48.1	149.50	10.30	20.3	290995.9
B66	104.70	51.80	37.9	150.00			317115.0
B67	98.70	75.50	43.5	137.00	25.80	45.7	295363.7
B68	29.00	84.80	50.8	132.30	10.50	25.0	262939.6
B69	94.00	59.50	40.3	148.30	11.50	42.1	368264.9
B70	101.80	36.30	27.9	147.30	4.00	15.7	335902.4
B71	92.50	123.50	44.7	126.20	16.50	54.5	216043.0
B72	108.30	166.80	50.5	153.20	27.80	53.0	328169.1
B73	73.30	71.00	44.9	129.30	3.50	26.9	225224.6
B74	48.00	70.30	30.1	153.20	23.50	54.9	290083.2
B75	98.50	79.00	54.1	153.50	12.50	50.0	322664.2
B76	154.80	118.00	51.8	131.70			249448.7
B77	16.80	49.30	40.3		2.50	15.2	245941.3
B78	100.00	86.00	44.6	35.50	32.00	62.7	274943.0
B79	16.30	50.30	37.7	160.50	1.80	6.7	285048.9
B80	89.20	56.00	42.8	130.30	5.30	23.2	227668.1
B81	102.00	62.30	43.6	135.80	6.30	29.3	280309.1
B82	109.50	34.30	28.0	58.50	5.80	21.5	267133.5

Appendix I: Virginia Tech/Carilion Detailed Methodology

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 (Appendix J)

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

DXA and pQCT Bone Scan Procedures

Subject Arrival and Parking

Upon arrival at the scheduled time, the subject comes to 229 Wallace Hall and the researchers fill out a temporary parking pass and instructs the subject to write in their license plate number on the pass and place it in their car.

Informed Consent

Researchers sit down with the subject and explain the informed consent with them. They are given time to read, review and ask questions before signing the form. The subject is reminded of the components of study: DXA and pQCT bone scans, food and physical activity record and blood draw. A copy of the signed informed consent is given to the subject.

DXA/pQCT Measurement Data Sheet (Appendix K)

Date of Birth

The subject's date of birth is recorded on the data sheet.

Pregnancy Test

The subject is given a specimen cup and asked to provide a urine sample for a pregnancy test. After completing the pregnancy test and assuming it is negative, the researchers check off on data sheet that pregnancy test has been completed and the protocol is continued.

Body Weight

The subject is instructed to remove their shoes, any extra jackets or layers and anything from her pockets. The scale is turned on and allowed to zero itself. The subject then steps onto the scale. The weight is recorded to the nearest kilogram and to the nearest pound. Subjects exceeding 136 kilograms of body weight are excluded from the study.

Height

The subject is asked to stand with their back against wall (without shoes) at the wall-mounted stadiometer. The measurement arm is positioned to meet the top of the subject's head. The height is recorded to the nearest centimeter and to nearest inches.

Radius Length

The subject is asked to raise their bare, non-dominant forearm, bent at the elbow 90 degrees in front of their chest and parallel to the floor. A measurement from the highest point of the radial head to the highest point of the styloid process of the radius is taken to the nearest millimeter using a flexible measuring tape and recorded on the form.

Tibia Length

The subject is asked to sit in a chair and cross their bare, non-dominant foreleg over the other leg so that the ankle of the non-dominant leg rests upon the top of the knee of the other leg. A measurement from the highest point of the medial condyle of the tibia to the highest point of the

medial malleolus is taken to the nearest millimeter using a flexible measuring tape and recorded on the form.

DXA Scans

Before beginning the bone scans, the subject is asked if they are wearing any items that contain metal, buttons, snaps, zippers, or hard plastic (including underwire bras). If they are, they are removed and the subjects are offered clothing (t-shirt and sweat pants) to wear.

The subjects' identifying and measurement 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 are conducted according to the manufactures instructions:

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

DXA Scan Analysis

The analysis is conducted after the subject has completed all requirements and has left the lab. Each of the four scans, whole body, lumbar spine, non-dominant hip and non-dominant forearm, are analyzed 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:

- a. Central abdominal fat analysis
- b. whole body
- c. lumbar spine
- d. total hip
- e. radius

pQCT Scans

The subjects' identifying and measurement 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 (4, 14, 38, and 66% of the tibial length from the distal end) and RADIUS2S (4 and 66% of the radius length from the distal end) scans are conducted.

For the TIBIA4S scans, the pQCT is set up with the leg stabilizer and foot stirrup.

The subject sits in the seat mounted on the pQCT, and it is adjusted left or right for the appropriate non-dominant leg. The bottom of the foot of the non-dominant limb is placed in the stirrup through the gantry of the device and strapped down. The leg stabilizer is 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. The subject is instructed to remain still and keep their leg steady in the device. The scans are then conducted.

For the RADIUS2S scans, the pQCT is set up with the arm and hand rests.

The subject sits in the chair adjacent to the pQCT gantry with the non-dominant arm inserted through the gantry and with the elbow resting at the end of the arm rest. Adjust the hand rest with the hand resting on the holder and the thumb under the holding device so that the arm is parallel

to the ground and in the center of the gantry. The subject is instructed to remain still and keep their arm steady in the device. Researchers leave the room during these scans so as not to distract the subject.

pQCT Scan Analysis

The analysis is conducted after the subject has completed all requirements and has left the lab. The scans are initially analyzed using the TIBIA4S Macro and RADIUS2S Macro according to the instructions in the Stratec XCT 3000 Instruction Manual.

After analyses, the output is printed and placed in the subject data file. In addition, the LOOP procedure is conducted on each scan and output to a database file for analysis.

Diet and Physical Activity Record (Appendix D)

Diet Record Instructions

Record the subject code at the top of the form. Give the form to the subject and thoroughly explain the instruction sheet (Appendix E), 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 are 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 are shown a sample food record and how to complete the diet record. Subjects are 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.

The subject is instructed to record all food and beverages consumed over a four day period, preferably 3 weekdays and 1 weekend day.

Arrange a time for subject to return the form. If the subject will not be bringing the form to another visit, give them an addressed, stamped envelope to mail the record to the researchers.

Physical Activity Record Instructions

During the same 4 day period of maintaining the diet record, the subjects will also completed a physical activity log and wear a pedometer for all waking hours. The pedometer will be attached at the hip each morning and 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 at the top of the form. After recording and before attaching for the next day, they will hit the reset button and wait for the pedometer to zero. Subjects will remove the pedometer for running, swimming or any activity more strenuous than walking and then record the activity including the duration and the intensity on the form of each day's diet/physical activity record. The researcher will explain to the subject how to rate their intensity level using a BORG scale (6-20), where 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 the subject that they will be allowed to keep the pedometer following the completion of the study.

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)2D3], 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 l of serum (top layer above gel) into pre-labeled cryovials (from step3) using 1 ml micropipettor and sterile tips.
6. Place the serum vials in the designated box in the -80C 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 K2EDTA (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 each 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 (yellowish, top layer above gel in tubes) from all tubes into a sterile 15 ml (or 50 ml) conical tube and mix gently, by pipetting up and down, to ensure homogeneity of plasma before aliquotting to freeze.
 - b. Aliquot 500-1000 l of EDTA plasma (top layer above gel) into pre-labeled 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 -80C 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.



Research Study

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

Preliminary study to isolate markers with diagnostic and prognostic implications for bariatric surgery patients

Principal Investigator:
T. A. Lucktong, MD

Co-Investigator :
William G. Herbert, PhD

Study Coordinators:
Laura Newsome and Erin Kerr

*For more information
please contact:*

Study Coordinator:
Laura Newsome, MS
Address: 231D War Memorial Hall,
Virginia Tech, Blacksburg, VA, 24061
Phone: (540) 797-8428
E-mail: lnewsome@vt.edu
Fax: (540) 231-8476

Study Eligibility includes:

Caucasian women accepted for elective gastric bypass surgery, exclusive of unusual risk factors or co-morbidities

OSAS Group

- Includes those with moderate to severe sleep apnea
- Epworth Sleepiness Scale (ESS) of 11 or more
 - Embletta (AHI) score ≥ 20
 - Neck circumference of 16 in. or more

Non-OSAS Group

- Includes those with clinically negative results for sleep apnea
- ESS less than 10
 - Embletta (AHI) score ≤ 10

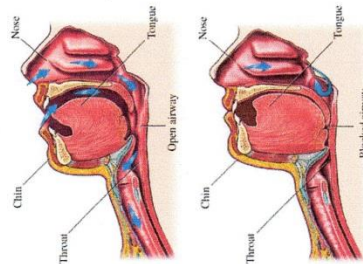
Bone Group

- Includes those with clinically negative results for sleep apnea
- ESS less than 10
 - Free from medications for insulin sensitivity and bone metabolism

Bone Health and Gene expression in Obstructive Sleep Apnea Syndrome (OSAS):

Preliminary study to isolate markers with diagnostic and prognostic implications for bariatric surgery patients

- Obstructive Sleep Apnea Syndrome (OSAS), a nighttime breathing disorder, disrupts sleep patterns, alters a person's physiology and can cause complications related to surgery.
- Undiagnosed OSAS increases risks of cardiovascular complications in major surgeries, both during and post-surgery.
- 70% of bariatric surgery patients may have OSAS, prompting the need for early identification and treatment to minimize surgical risks.



- Since OSAS is not easily or inexpensively diagnosed, testing of patients' blood for a unique protein signature could help to accurately identify those patients that should be diagnosed and treated for OSAS to reduce risks of bariatric surgical complications.



- First-line treatment for most patients is nightly use of nasal continuous positive airway pressure therapy (nCPAP) which may eliminate many of the potential physiological complications associated with surgery.
- We hope to be able to find a connection between certain proteins in the blood with the presence or absence of OSAS in bariatric surgery patients.

- Weight loss after gastric bypass surgery may increase your risk for osteoporotic related fractures.
- Hormonal changes associated with menopause increase the risk for osteoporosis.
- For women under the age of 30, preventing postoperative bone loss may be even more critical, as they have yet to attain peak bone mass.
- Unfortunately, little research has been done to evaluate the impact of rapid weight loss on skeletal health following gastric bypass surgery.



- We will use both conventional and quantitative bone scanning to evaluate bone density and bone quality to increase the understanding of the delicate balance between body mass and bone modeling.

Appendix K: Virginia Tech/Carilion DXA/pQCT Measurement Data Sheet

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

<u>DXA/pQCT Measurement Data Sheet</u>	
D.O.B. _____	Pregnancy Test Performed ☐
Height _____ cm/ _____ in kg	Weight _____ lbs./ _____
BMI _____ kg/m ²	
<u>Lengths</u>	
Non-dominant Side _____	
Non-dominant Tibia _____ mm	Non-dominant Radius _____

Appendix L: Carilion IRB Approval Letters



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,

A handwritten signature in blue ink, appearing to read "Charles Hite".

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

March 3, 2014

T.A. Lucktong, MD
3 Riverside Circle



Approval Date: 3/3/2014 Expiration Date: 3/2/2015
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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:

The Institutional Review Board of Carilion Clinic has received your application for continuance of the above-mentioned study. I would like to inform you that the protocol received an expedited review according to 45 CFR 46.110 and 21 CFR 56.110. This research protocol met the expedited criteria outlined in 63 FR 60364-60367 **(8)(c)** where the remaining research activities are limited to data analysis. The expedited review grants approval for continuance of the study, including the protocol version dated 6/11/10, for a period of twelve (12) months from the review date.

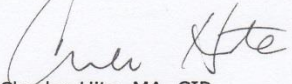
The following documentation must be provided to the Carilion Clinic IRB:

- Continuing Review Application 30 days prior to the expiration date, providing a summary of the project to date and requesting continuation of the original project. If the original project is discontinued the IRB must be notified within seven business days.
- Serious adverse events that are unexpected and related, or unanticipated problems, as outlined in the IRB Guidelines, within seven business days of the investigator becoming aware of them.
- Copies of reports from Data Monitoring Committees or auditing/monitoring reports from a sponsor are to be sent to the IRB Research Compliance Officer within seven business days.
- Any unplanned protocol variance that could adversely affect the safety or welfare of subjects, or the integrity of the research data, within ten days of becoming aware of the variance. Other unplanned variances may be recorded on a log and submitted with continuing review reports. Any changes to the research study must receive IRB approval before those changes can be implemented unless subject safety is directly affected.

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.

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

Sincerely,

A handwritten signature in cursive script, appearing to read "Charles Hite".

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

cc: File
Dan Harrington, MD
Department Chair
Office of Sponsored Projects

Appendix M: Virginia Tech/Carilion Informed Consent

Screening Consent 01.20.10

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.

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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 physical 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

Appendix N: Virginia Tech/Carilion Assay Procedures

Osteocalcin (OC) ELISA Assay Procedure

Two osteocalcin (1-43/49) enzyme-linked immunosorbent assays (ELISA) were purchased from Alpco, Salem, NH. One was used to determine total osteocalcin and the other for the undercarboxylated fraction using the hydroxyapatite binding procedure as outlined by Vergnaud et al. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. (*J Clin Endocrinol Metab* 1997;82: 719-24). Briefly, 250 μ L of serum were incubated with 7.5 mg HAP (calcium phosphate tribasic type IV, *Sigma Chemical Co., St. Louis, MO*) in an Eppendorf tube, shaken for 1 h at 4°C and then centrifuged at 3000 rpm for 15 min. This supernatant was then used for the undercarboxylated OC assay by standard ELISA. The OC ELISAs were analyzed using serum in Carilion Community Basic Research Laboratory, located in Roanoke VA with the assistance of laboratory technicians Frances Philp and Kristin Knight 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:

1. Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
2. Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
3. 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-telopeptide of Type I Collagen (CTx) ELISA Assay Procedure

A human carboxy-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 technicians Frances Philp and Kristin Knight 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:

1. Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
2. Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
3. 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.

Leptin ELISA Assay Procedure

A leptin enzyme-linked immunosorbent assay (ELISA) was purchased from Alpco, *Salem, NH*. The leptin 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, <http://www.alpco.com/pdfs/11/11-LEPHU-E01.pdf>.

The following laboratory equipment was used in for this assay:

1. Shaker, Model 2314FS, Fischer Scientific (*Dubuque, IO*), shaker at room temperature.
2. Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
3. 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.

High Molecular Weight (HMW)Adiponectin ELISA Assay Procedure

An HMW 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-ADPHU-E01.pdf>.

The following laboratory equipment was used in for this assay:

1. Excella E25 Economical Incubator Shaker Series New Brunswick Scientific (*Edison, NJ*), shaker at room temperature.
2. Fischer Scientific Wellwash 4Mk2, Thermo Electron Corporation (*Vantaa, Finland*).
3. 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.

Appendix O: Virginia Tech/Carilion Study Raw Data

Table A-2. Virginia Tech/Carilion Study Raw Data

Name	Age	Height	Surgery	Time	Weight	BMI	Loss	TBMC	TBMD	TFFM	TBF	TBFP	CBF	CBFP
BL003	42	169.78	1	1	108.1	37.5		1980.7	1.014	57049.9	50232.5	46.0	9779.2	46.1
BL003	43	169.78	1	2	98.1	34.0	10.0	2046.4	1.023	53450.1	44564.3	45.0	2754.3	48.0
BL003	43	169.78	1	3	99.2	34.4	-1.1	2012.8	1.012	55566.8	42649.6	42.6	2801.5	41.5
BL021	40	165.28	1	1	107.7	39.4		2122.2	1.071	54031.2	51738.0	48.0	4416.5	51.7
BL021	41	165.28	1	2	99.1	36.3	8.6	2215.7	1.084	51333.3	45413.0	45.9	2856.3	54.9
BL021	41	165.28	1	3	94.1	34.4	5.0	2120.3	1.078	52717.6	40083.5	42.2	3382.7	45.0
BL026	28	160.00	1	1	114.4	44.7		2242.1	1.095	52126.7	60364.9	52.6	4376.0	57.2
BL026	29	160.00	1	2	107.2	41.9	7.2	2276.5	1.111	51498.4	53829.9	50.0	3710.8	52.6
BL026	29	160.00	1	3	104.1	40.7	3.1	2230.2	1.120	51038.4	51339.8	49.1	4567.2	54.7
BL041	21	164.60	1	1	126.8	46.8		2465.3	1.157	61591.8	62774.6	49.5	5304.7	52.9
BL041	22	164.60	1	2	112.6	41.6	14.2	2520.9	1.191	56732.6	53632.4	47.5	2959.2	49.7
BL041	22	164.60	1	3	108.5	40.0	4.1	2483.5	1.154	56672.8	50677.4	46.1	3314.5	48.8
BL046	32	163.44	1	1	117.1	43.8		2411.6	1.153	58368.5	57358.2	48.6	3893.7	48.0
BL046	32	163.44	1	2	98.0	36.7	19.1	2427.5	1.175	53854.9	42071.1	42.8	2981.2	39.0
BL046	32	163.44	1	3	102.0	38.2	-4.0	2422.5	1.179	57596.9	43429.0	42.0	3315.8	40.2
BL061	33	156.67	1	1	105.3	42.9		2500.4	1.242	52971.8	50267.9	47.5	3859.4	49.8
BL061	33	156.67	1	2	94.8	38.6	10.5	2472.8	1.231	50902.5	42483.2	44.3	3077.4	46.1
BL061	33	156.67	1	3	91.3	37.2	3.5	2457.5	1.213	48807.1	41245.3	44.6	2810.0	44.3
BL065	39	164.61	1	1	141.2	52.1		2586.0	1.217	58919.2	79043.5	56.2	6953.6	56.4
BL065	39	164.61	1	2	131.6	48.6	9.6	2584.4	1.232	60022.0	69111.8	52.5	5792.1	56.2
BL065	39	164.61	1	3	131.0	48.3	0.6	2588.6	1.218	57950.2	70450.4	53.8	4771.5	56.1
BL068	32	176.74	1	1	136.8	43.8		2868.2	1.242	69390.9	65016.7	47.4	4598.7	51.6
BL068	32	176.74	1	2	116.1	37.2	20.7	2893.7	1.264	64339.9	50036.6	42.7	3574.4	46.1
BL068	32	176.74	1	3	114.0	36.5	2.1	2938.1	1.279	61553.6	50759.3	44.0	3625.8	48.4
BL091	36	165.50	1	1	109.4	39.9		2470.2	1.169	54778.0	52272.7	47.7	3618.7	48.5
BL091	37	165.50	1	2	87.9	32.1	21.5	2475.3	1.174	50341.1	36242.5	40.7	2286.6	39.3
BL091	37	165.50	1	3	79.3	29.0	8.6	2469.2	1.183	49323.5	28952.1	35.9	1757.9	33.5
BL092	34	172.40	1	1	122.6	41.2		2267.0	1.038	60642.0	60124.4	48.9	4552.3	53.1
BL092	35	172.40	1	2	109.9	37.0	12.7	2274.6	1.049	56002.4	52718.7	47.5	4030.4	53.7
BL092	35	172.40	1	3	101.7	34.2	8.2	2246.4	1.051	52712.4	47286.4	46.2	3797.9	53.8
BR012	41	167.47	2	1	117.1	41.8		2569.3	1.182	67893.9	48609.0	40.8	3978.3	46.7
BR012	41	167.47	2	2	94.0	33.5	23.1	2561.9	1.161	61184.5	32078.7	33.5	2301.9	40.9
BR012	42	167.47	2	3	87.4	31.2	6.6	2496.4	1.152	62147.2	23780.2	26.9	1965.3	32.6
BR013	45	155.19	2	1	114.5	47.5		2562.2	1.280	50216.5	61427.2	53.8	4120.9	50.8
BR013	46	155.19	2	2	89.8	37.3	24.7	2587.4	1.325	49824.2	37684.3	41.8	2011.9	38.9
BR013	46	155.19	2	3	79.4	33.0	10.4	2575.1	1.289	42961.9	35243.1	43.6	1495.9	42.0
BR020	43	170.07	2	1	109.5	37.9		2769.2	1.245	60252.1	47770.4	43.1	2495.3	48.2
BR020	44	170.07	2	2	85.5	29.6	24.0	2705.5	1.273	51728.7	31243.1	36.5	2518.6	41.6
BR020	44	170.07	2	3	79.1	27.3	6.4	2643.0	1.243	52006.9	25460.5	31.8	2011.3	32.3
BR033	34	157.41	2	1	114.1	46.0		2176.1	1.087	61560.0	50389.9	44.2	4464.9	46.2
BR033	34	157.41	2	2	95.5	38.5	18.6	2088.0	1.062	53768.8	39089.3	41.2	3018.7	43.2
BR033	35	157.41	2	3	94.8	38.3	0.7	2019.5	1.056	54799.4	39063.8	40.7	3083.0	41.9
BR042	37	160.50	2	1	108.7	42.2		1936.1	1.043	50051.5	57149.8	52.4	4291.4	59.7
BR042	37	160.50	2	2	92.0	35.7	16.7	1987.4	1.056	42306.1	48782.0	52.4	3913.1	56.6

Name	Age	Height	Surgery	Time	Weight	BMI	Loss	TBMC	TBMD	TFFM	TBF	TBFP	CBF	CBFP
BR042	37	160.50	2	3	80.8	31.4	11.2	1941.0	1.035	41373.4	38922.4	47.3	2089.8	46.5
BR051	29	173.50	2	1	130.9	43.5		3033.5	1.264	64925.0	63426.2	48.3	3272.6	52.6
BR051	29	173.50	2	2	106.5	35.4	24.4	3045.5	1.243	56972.5	47439.2	44.1	3095.7	50.5
BR051	30	173.50	2	3	92.4	30.7	14.1	3077.8	1.268	56734.4	34422.7	36.5	2308.9	40.1
BR062	31	167.51	2	1	112.2	40.0		2100.6	1.066	61842.4	48309.4	43.0	4390.6	49.6
BR062	32	167.51	2	2	81.9	29.2	30.3	2093.7	1.060	50084.1	30915.5	37.2	2208.4	38.1
BR062	32	167.51	2	3	69.4	24.7	12.5	2114.4	1.069	47220.4	21436.9	30.3	1606.6	29.8
BR074	26	154.23	2	1	95.4	40.1		2248.3	1.170	51098.3	42684.6	44.4	3330.7	51.9
BR074	27	154.23	2	2	75.6	31.8	19.8	2257.1	1.218	45789.2	28892.0	37.6	2826.7	47.6
BR074	27	154.23	2	3	67.6	28.4	8.0	2216.6	1.206	43998.1	22036.8	32.3	2198.9	44.0
BR079	29	169.47	2	1	127.2	44.3		2786.3	1.254	61642.0	62657.5	49.3	4491.0	50.0
BR079	29	169.47	2	2	104.4	36.4	22.8	2753.4	1.259	52839.2	50147.2	47.4	2980.2	48.2
BR079	29	169.47	2	3	91.4	31.8	13.0	2751.1	1.247	50782.6	38980.4	42.1	2661.6	41.8
BR094	35	159.60	2	1	108.0	42.4		2298.6	1.187	53891.4	52416.2	48.3	3726.5	47.1
BR094	35	159.60	2	2	82.0	32.2	26.0	2346.2	1.192	43010.2	37569.4	45.3	2052.4	42.7
BR094	35	159.60	2	3	74.0	29.1	8.0	2276.2	1.181	43674.0	29189.4	38.8	1490.8	34.5
BR095	34	167.90	2	1	118.5	42.0		2594.2	1.151	60284.6	56601.8	47.4	4470.9	54.9
BR095	34	167.90	2	2	97.9	34.7	20.6	2520.6	1.165	52836.8	44021.5	44.3	2484.0	46.3
BR095	34	167.90	2	3	86.5	30.7	11.4	2522.1	1.134	50095.7	35313.7	40.2	1573.5	43.5

Name	TLBMC	TLBMD	LTS	THBMC	THBMD	HTS	TRBMC	TRBMD	RTS	T4TBMC	T4TBMD	T4PSSI
BL003	56.6	0.958	-1.17	23.64	0.801	-1.15	6.73	0.572	-0.14	219.35	263.50	906.49
BL003	59.7	1.018	-0.85	23.03	0.772	-1.39	6.73	0.563	-0.30	224.35	274.90	985.12
BL003	54.6	0.951	-1.15	25.23	0.789	-1.25	5.12	0.568	-0.20	230.06	268.10	979.03
BL021	61.8	1.095	0.36	29.72	0.947	0.04	12.32	0.596	0.63	273.13	293.90	1156.80
BL021	38.5	0.821	-2.14	31.39	0.980	0.31	7.55	0.595	0.30	265.37	285.90	1175.37
BL021	52.6	0.997	-0.66	32.22	0.953	0.09	7.17	0.615	0.67	277.64	292.60	1214.01
BL026	53.9	1.098	0.28	25.65	0.834	-0.88	8.17	0.660	1.50	269.98	317.60	1381.99
BL026	51.7	1.111	0.41	25.22	0.838	-0.85	8.27	0.640	1.12	278.71	310.30	1309.05
BL026	48.4	1.036	-0.39	26.52	0.838	-0.85	8.06	0.643	1.19	269.73	318.60	1379.62
BL041	52.3	1.054	-0.21	31.93	1.091	1.22	8.74	0.701	2.26	363.85	361.90	1427.29
BL041	56.5	1.093	0.25	34.41	1.121	1.47	8.68	0.676	1.80	387.45	370.50	1433.84
BL041	57.2	1.074	0.09	32.29	1.078	1.11	7.48	0.723	2.66	368.02	364.90	1396.32
BL046	58.5	1.050	-0.15	34.53	1.075	1.09	6.77	0.594	0.29	289.06	341.50	1399.18
BL046	60.1	1.022	-0.32	35.75	1.083	1.16	7.41	0.596	0.31	291.45	342.80	1408.05
BL046	62.6	1.098	0.39	33.40	1.073	1.07	7.00	0.593	0.26	286.79	344.10	1416.32
BL061	59.3	1.153	0.85	29.91	1.020	0.64	7.13	0.595	0.31	317.69	348.60	1348.12
BL061	64.8	1.205	1.30	31.76	1.047	0.86	7.07	0.614	0.66	319.43	345.10	1342.44
BL061	67.0	1.187	1.25	30.28	1.032	0.74	7.10	0.617	0.70	321.06	348.10	1359.11
BL065	45.6	1.030	0.00	30.01	1.049	0.88	8.57	0.716	2.54	249.71	363.70	1242.53
BL065	51.5	1.122	0.37	32.03	1.093	1.24	7.72	0.697	2.19	256.05	367.40	1264.80
BL065	55.5	1.163	0.80	30.98	1.061	0.97	8.29	0.709	2.41	255.61	352.00	1314.76
BL068	69.5	1.240	1.44	42.79	1.199	2.10	8.29	0.594	0.28	327.97	282.60	1763.10
BL068	77.6	1.289	1.67	38.68	1.093	1.24	8.42	0.611	0.60	338.25	273.40	1747.49
BL068	75.0	1.223	1.28	39.43	1.098	1.28	8.28	0.609	0.56	333.24	274.80	1739.03
BL091	74.9	1.273	2.05	34.50	1.037	0.78	7.08	0.619	0.73	304.58	304.70	1243.63
BL091	74.7	1.285	2.19	36.30	1.063	0.99	7.19	0.617	0.71	298.66	302.20	1213.66
BL091	76.9	1.272	2.11	34.83	1.043	0.83	7.13	0.602	0.43	308.28	311.50	1255.26
BL092	44.4	0.886	-1.64	32.41	1.023	0.67	7.61	0.542	-0.69	271.00	254.30	1187.90
BL092	47.2	0.896	-1.54	31.19	0.994	0.43	7.74	0.545	-0.63	275.18	254.40	1183.72
BL092	47.7	0.914	-1.39	31.67	0.990	0.39	7.80	0.544	-0.65	273.32	255.00	1194.31
BR012	64.9	1.092	0.40	42.19	1.053	0.91	7.94	0.607	0.53	354.34	293.10	1312.64
BR012	68.8	1.108	0.69	34.56	0.912	-0.24	7.87	0.625	0.85	358.04	294.60	1380.02
BR012	63.6	1.048	0.11	33.37	0.876	-0.54	8.47	0.636	1.06	348.32	292.60	1306.03
BR013	61.4	1.180	1.02	28.57	1.038	0.79	6.52	0.639	1.12	258.33	353.30	1322.70
BR013	62.1	1.158	0.94	27.38	0.991	0.40	6.54	0.641	1.16	252.96	355.50	1257.68
BR013	61.6	1.152	1.03	29.76	0.996	0.44	6.44	0.626	0.87	267.77	319.00	1195.41
BR020	61.7	1.157	0.84	38.44	1.145	1.67	8.33	0.691	2.08	328.08	314.20	1490.63
BR020	63.9	1.140	0.86	37.25	1.090	1.21	8.50	0.703	2.30	323.67	317.70	1432.86
BR020	61.3	1.129	0.84	35.40	1.039	0.80	8.41	0.682	1.92	324.49	313.20	1430.17
BR033	39.2	0.804	-2.77	29.38	0.939	-0.02	5.25	0.660	1.50	240.59	347.20	1246.17
BR033	44.6	0.902	-1.68	28.66	0.901	-0.33	6.75	0.620	0.76	241.24	341.60	1187.14
BR033	44.9	0.859	-2.08	29.19	0.908	-0.28	6.79	0.640	1.13	251.55	291.90	1091.13
BR042	45.8	1.003	-0.48	25.05	0.847	-0.77	5.57	0.622	0.80	207.29	265.20	948.56
BR042	49.6	1.028	-0.38	24.56	0.844	-0.80	5.57	0.625	0.86	217.55	268.50	924.06
BR042	49.4	1.008	-0.67	23.78	0.822	-0.99	5.60	0.641	1.14	213.17	261.00	903.34
BR051	67.2	1.185	0.99	46.46	1.290	2.86	10.36	0.607	0.51	339.50	282.00	1471.40
BR051	67.9	1.194	1.19	47.36	1.258	2.59	10.35	0.611	0.59	327.76	276.90	1520.61
BR051	73.3	1.201	1.27	49.90	1.265	2.65	10.44	0.629	0.93	329.21	277.50	1489.07
BR062	40.7	0.789	-2.77	28.01	0.893	-0.40	5.71	0.503	-1.42	289.03	302.80	1108.97

Name	TLBMC	TLBMD	LTS	THBMC	THBMD	HTS	TRBMC	TRBMD	RTS	T4TBMC	T4TBMD	T4PSSI
BR062	50.6	0.894	-1.41	27.00	0.855	-0.71	5.97	0.535	-0.82	287.11	308.10	1114.43
BR062	51.4	0.892	-1.45	26.94	0.830	-0.92	6.06	0.515	-1.19	434.28	433.40	1482.36
BR074	57.6	1.151	0.91	35.51	1.239	2.44	8.36	0.687	2.00	300.30	354.10	1101.91
BR074	63.5	1.200	1.56	31.47	1.160	1.79	8.25	0.696	2.17	299.64	346.00	996.50
BR074	59.8	1.129	0.88	29.05	1.111	1.39	8.39	0.682	1.91	289.40	344.40	1051.41
BR079	73.6	1.280	2.08	37.47	1.113	1.40	8.72	0.654	1.40	347.02	303.30	1684.39
BR079	71.6	1.236	1.89	32.89	1.033	0.75	8.92	0.660	1.50	343.87	304.30	1726.09
BR079	72.7	1.228	1.65	33.31	1.027	0.69	8.76	0.641	1.14	337.91	293.30	1692.99
BR094	59.9	1.141	0.92	31.24	1.015	0.60	6.72	0.619	0.73	280.69	330.10	1304.85
BR094	58.8	1.105	0.51	32.03	1.002	0.49	6.70	0.620	0.76	283.84	333.10	1267.16
BR094	57.3	1.073	0.23	32.90	0.978	0.29	6.82	0.632	0.99	282.85	328.80	1281.90
BR095	59.1	1.004	-0.36	38.31	1.086	1.18	9.08	0.637	1.07	280.37	269.50	1321.63
BR095	58.6	0.990	-0.47	37.43	1.036	0.77	8.90	0.629	0.93	296.55	265.20	1301.54
BR095	61.0	1.009	-0.22	34.91	0.975	0.27	9.17	0.575	-0.07	270.49	260.80	1260.65

Name	T4TRBMC	T4TRDEN	T4COA	T4COTH	T4PERI	T4ENDO	T14TBMC	T14TBMD	T14PSSI	T14COA
BL003	156.74	213.20	184.25	1.91	102.28	90.26	184.46	460.60	1087.20	138.75
BL003	151.32	214.60	167.25	1.75	101.26	90.29	151.19	406.70	881.24	137.50
BL003	164.31	217.60	180.50	1.84	103.84	92.27	181.43	446.60	1025.84	131.00
BL021	189.58	237.60	190.50	1.86	108.06	96.35	212.15	607.90	1024.57	139.25
BL021	191.75	235.50	195.50	1.92	108.00	95.96	208.35	603.00	1041.43	139.00
BL021	192.50	236.00	191.00	1.85	109.20	97.60	211.47	609.90	1004.26	140.25
BL026	161.70	228.80	169.00	1.73	103.35	92.51	237.13	585.20	1179.53	174.75
BL026	176.05	234.40	184.25	1.83	106.24	94.72	236.26	571.00	1190.19	161.75
BL026	160.44	228.10	169.50	1.74	103.15	92.25	235.69	585.90	1200.85	172.25
BL041	239.83	302.50	207.00	1.95	112.39	100.16	269.29	569.00	1464.43	179.75
BL041	241.28	303.80	214.25	1.98	114.64	102.22	271.90	558.30	1401.79	178.25
BL041	234.82	304.20	209.00	1.96	112.58	100.23	271.16	567.60	1372.61	196.25
BL046	158.44	240.00	187.75	1.93	103.14	90.98	225.71	647.20	1123.61	173.50
BL046	157.13	239.30	188.75	1.94	103.37	91.17	230.90	653.60	1130.76	175.25
BL046	153.82	238.60	194.75	2.03	102.34	89.59	228.65	652.30	1174.47	175.75
BL061	212.06	282.00	177.75	1.75	107.01	96.01	236.64	592.00	1174.66	147.25
BL061	215.50	280.80	185.00	1.81	107.84	96.46	236.59	592.90	1202.23	153.25
BL061	215.24	282.30	187.25	1.84	107.65	96.11	238.04	600.00	1192.49	157.25
BL065	113.80	224.60	202.50	2.37	92.88	77.99	239.87	653.10	1270.82	217.75
BL065	115.02	225.90	161.75	1.84	93.59	82.01	242.32	644.90	1214.15	180.25
BL065	120.92	224.00	171.25	1.91	95.53	83.51	242.47	640.20	1198.04	170.25
BL068	217.67	216.90	212.50	1.85	120.76	109.15	294.09	628.10	1693.27	207.75
BL068	237.63	219.40	228.25	1.92	124.68	112.59	293.90	606.00	1647.20	204.25
BL068	230.47	217.80	223.25	1.90	123.45	111.51	291.95	612.10	1588.33	199.50
BL091	217.92	252.60	222.50	2.11	112.07	98.81	232.35	593.90	1213.12	164.50
BL091	218.36	254.10	195.00	1.85	111.44	99.84	239.10	604.60	1178.38	165.50
BL091	215.54	256.10	193.75	1.83	111.52	100.01	242.19	615.10	1202.73	162.75
BL092	211.55	218.40	211.50	1.93	115.71	103.59	226.73	445.70	1498.96	154.50
BL092	219.36	222.00	208.75	1.89	116.58	104.73	224.61	441.90	1466.99	148.25
BL092	217.02	221.30	216.50	1.97	116.05	103.67	227.98	447.00	1489.17	152.25
BR012	269.50	252.80	206.00	1.75	123.25	112.25	264.15	552.60	1416.64	183.75
BR012	274.92	255.90	217.25	1.85	123.58	111.99	263.06	547.80	1380.60	175.25
BR012	265.07	253.00	207.00	1.77	122.31	111.17	268.07	565.30	1393.54	207.00
BR013	128.85	229.60	163.25	1.81	95.86	84.49	247.50	624.20	1340.86	183.25
BR013	127.16	229.90	157.75	1.77	94.56	83.42	242.55	617.20	1368.48	179.00
BR013	174.44	248.00	184.00	1.90	102.71	90.76	242.69	590.80	1342.41	178.00
BR020	228.38	252.80	196.50	1.81	114.55	103.21	279.73	610.10	1625.93	187.75
BR020	223.14	255.00	196.50	1.83	113.15	101.65	277.95	613.60	1623.21	190.00
BR020	228.82	255.90	198.00	1.83	114.10	102.62	278.65	609.10	1654.37	192.50
BR033	115.49	219.60	156.75	1.79	93.32	82.09	243.81	688.70	1303.77	186.00
BR033	121.91	223.30	162.75	1.84	94.21	82.64	241.06	688.70	1228.48	180.00
BR033	177.26	238.30	182.00	1.85	104.06	92.42	229.91	628.60	1251.55	176.50
BR042	152.84	219.50	173.75	1.86	99.12	87.41	200.21	639.60	960.81	150.25
BR042	156.98	220.00	165.75	1.74	100.91	90.00	199.44	642.30	878.29	193.50
BR042	160.04	219.20	173.50	1.82	101.31	89.91	202.73	658.20	972.16	148.00
BR051	255.43	237.00	205.50	1.75	123.00	112.02	294.06	507.20	1680.18	178.50
BR051	258.04	238.70	212.25	1.83	121.95	110.48	294.89	508.00	1648.14	178.00
BR051	259.28	239.50	209.50	1.80	122.11	110.80	295.27	506.00	1628.40	179.75
BR062	195.15	242.80	202.25	1.96	109.52	97.23	204.07	475.70	1144.88	147.25

Name	T4TRBMC	T4TRDEN	T4COA	T4COTH	T4PERI	T4ENDO	T14TBMC	T14TBMD	T14PSSI	T14COA
BR062	185.96	242.70	184.00	1.79	108.22	96.95	202.16	478.50	1216.50	231.50
BR062	123.30	288.90	512.75	5.38	112.21	78.41	204.68	407.90	1185.64	147.75
BR074	181.42	278.90	190.75	1.97	103.23	90.88	249.55	665.50	1206.63	169.25
BR074	192.18	281.60	185.25	1.88	104.32	92.49	251.60	662.10	1251.11	175.00
BR074	188.83	281.70	185.75	1.92	102.76	90.69	251.03	656.70	1234.03	167.75
BR079	241.37	244.10	211.00	1.85	119.90	108.28	279.65	496.70	1708.60	171.50
BR079	232.22	238.40	199.50	1.76	119.16	108.13	286.13	513.90	1750.57	178.25
BR079	238.40	236.60	207.75	1.81	120.33	108.95	284.87	507.10	1731.23	173.00
BR094	172.40	250.20	183.75	1.89	103.37	91.52	242.27	643.50	1285.74	165.75
BR094	173.95	253.90	197.50	2.03	103.47	90.69	247.42	627.20	1137.98	215.50
BR094	179.45	254.80	183.75	1.87	103.97	92.20	247.31	647.00	1289.62	170.25
BR095	198.87	219.20	195.75	1.80	114.33	103.02	242.89	541.60	1448.88	187.00
BR095	222.61	224.10	205.75	1.82	118.53	107.07	239.22	523.70	1437.13	180.50
BR095	202.90	219.50	196.50	1.81	114.16	102.77	243.80	545.10	1443.87	186.25

Name	T14COTH	T14PERI	T14ENDO	T38TBMC	T38TBMD	T38PSSI	T38COA	T38COTH	T38PERI
BL003	2.16	70.94	57.35	322.15	754.90	863.09	253.00	4.22	73.23
BL003	2.24	68.35	54.26	289.76	851.00	1367.26	261.75	5.40	65.41
BL003	2.01	71.45	58.81	290.23	870.90	1389.82	248.75	5.11	64.71
BL021	2.37	66.22	51.34	317.49	934.50	1306.78	245.00	4.91	65.34
BL021	2.38	65.89	50.94	315.37	933.70	1357.84	249.75	5.08	65.15
BL021	2.40	66.01	50.94	318.01	933.30	1333.71	248.25	4.99	65.44
BL026	2.79	71.36	53.82	326.52	881.30	1552.94	265.00	5.07	68.23
BL026	2.52	72.11	56.27	330.08	868.60	1507.23	271.00	5.11	69.10
BL026	2.76	71.10	53.76	331.28	888.70	1467.97	251.50	4.68	68.44
BL041	2.61	77.12	60.73	389.23	712.20	1798.20	686.50	13.19	82.87
BL041	2.54	78.23	62.29	375.62	887.50	1927.64	324.00	5.99	72.93
BL041	2.87	77.48	59.48	380.31	840.50	1558.45	398.25	7.85	75.41
BL046	3.07	66.20	46.93	321.10	867.20	681.56	345.25	8.04	68.21
BL046	3.08	66.63	47.30	323.55	948.80	1368.79	267.25	5.57	65.46
BL046	3.10	66.37	46.86	333.33	915.10	1198.56	257.25	4.93	67.66
BL061	2.32	70.88	56.33	328.46	1018.50	1344.38	276.25	6.30	63.66
BL061	2.43	70.81	55.57	331.78	1016.20	1382.07	271.75	6.02	64.05
BL061	2.51	70.61	54.86	329.19	1019.20	1325.68	276.00	6.27	63.71
BL065	3.91	67.93	43.34	337.90	901.70	1369.87	309.50	6.36	68.62
BL065	3.05	68.72	49.57	343.97	939.20	1585.30	278.50	5.51	67.84
BL065	2.83	68.99	51.19	342.93	935.70	1395.37	249.00	4.69	67.86
BL068	3.10	76.71	57.22	407.44	935.60	1737.22	304.75	5.32	73.98
BL068	2.97	78.07	59.40	402.04	930.70	1557.24	279.50	4.76	73.68
BL068	2.92	77.42	59.05	403.90	932.80	1930.50	334.00	6.13	73.77
BL091	2.66	70.12	53.38	359.53	967.80	1627.19	285.75	5.65	68.33
BL091	2.66	70.50	53.76	359.42	964.20	1528.75	273.50	5.27	68.44
BL091	2.62	70.34	53.88	364.18	969.20	1383.01	264.00	4.97	68.72
BL092	2.11	79.96	66.72	365.44	876.40	1829.77	289.75	5.16	72.39
BL092	2.02	79.92	67.26	364.56	879.00	1818.71	293.25	5.27	72.19
BL092	2.07	80.06	67.05	368.14	883.90	1868.37	293.50	5.26	72.35
BR012	2.66	77.50	60.81	375.60	900.70	1724.49	298.00	5.37	72.39
BR012	2.51	77.69	61.91	375.93	904.80	1725.60	315.75	5.87	72.26
BR012	3.06	77.20	57.95	377.97	895.10	1543.48	254.75	4.29	72.84
BR013	3.00	70.59	51.77	406.19	937.00	1730.19	339.25	6.27	73.81
BR013	2.93	70.28	51.86	390.50	961.80	1959.69	325.75	6.31	71.43
BR013	2.83	71.85	54.08	381.25	981.30	1594.76	275.25	5.12	69.87
BR020	2.80	75.91	58.33	400.00	951.20	1789.96	315.50	5.79	72.69
BR020	2.86	75.45	57.49	400.84	957.80	1605.89	290.00	5.15	72.52
BR020	2.88	75.82	57.71	396.84	953.90	1645.93	296.00	5.33	72.30
BR033	3.30	66.70	45.95	353.09	967.40	1332.28	276.00	5.46	67.73
BR033	3.20	66.32	46.22	351.49	973.00	1338.92	259.75	5.04	67.38
BR033	3.03	67.80	48.77	343.71	977.80	1359.93	263.00	5.27	66.46
BR042	2.78	62.72	45.22	293.36	866.00	1247.21	245.75	4.94	65.25
BR042	3.84	62.47	38.34	301.37	803.70	955.09	225.00	4.02	68.65
BR042	2.77	62.21	44.84	293.49	871.50	1351.30	240.50	4.82	65.05
BR051	2.28	85.35	71.01	423.70	861.60	2317.47	341.75	5.60	78.61
BR051	2.27	85.41	71.12	424.23	863.10	2339.98	353.25	5.87	78.59
BR051	2.29	85.63	71.23	423.14	860.50	2132.63	316.75	5.05	78.61
BR062	2.22	73.42	59.50	325.11	812.80	1400.59	253.00	4.44	70.90

Name	T14COTH	T14PERI	T14ENDO	T38TBMC	T38TBMD	T38PSSI	T38COA	T38COTH	T38PERI
BR062	3.80	72.87	48.99	321.06	806.20	1505.78	268.00	4.82	70.74
BR062	2.02	79.41	66.70	298.53	765.50	1323.51	262.75	4.78	70.01
BR074	2.83	68.65	50.85	348.50	994.30	1255.68	362.00	10.56	66.37
BR074	2.92	69.10	50.76	347.77	997.90	1371.29	265.50	5.39	66.18
BR074	2.77	69.31	51.92	331.88	1004.20	1380.57	271.00	5.91	64.45
BR079	2.22	84.11	70.14	436.73	918.50	2320.34	354.00	6.08	77.30
BR079	2.34	83.64	68.97	441.01	929.40	2386.07	360.50	6.27	77.22
BR079	2.25	84.02	69.89	441.54	910.40	1696.57	312.50	5.02	78.07
BR094	2.76	68.78	51.46	347.33	894.60	1633.63	293.50	5.63	69.85
BR094	3.66	70.41	47.43	347.47	915.00	1726.68	276.25	5.26	69.08
BR094	2.82	69.31	51.62	349.50	916.70	1706.34	274.75	5.19	69.22
BR095	2.83	75.07	57.33	382.92	896.20	1751.60	308.25	5.51	73.27
BR095	2.68	75.76	58.92	383.08	897.10	1739.32	308.00	5.50	73.25
BR095	2.82	74.97	57.27	386.49	901.40	1776.36	303.75	5.37	73.40

Name	T38ENDO	T66TBMC	T66TBMD	T66PSSI	T66COA	T66COTH	T66PERI	T66ENDO	T66MA	T66MD
BL003	46.73	307.55	625.10	1911.13	254.50	3.82	78.63	54.63	7228.25	77.10
BL003	31.46	307.99	631.40	1781.86	272.50	4.18	78.29	52.01	6890.25	77.83
BL003	32.59	310.26	639.00	1789.77	280.50	4.35	78.11	50.76	7098.50	77.80
BL021	34.51	350.41	769.70	1876.46	291.00	4.81	75.64	45.43	6173.00	75.21
BL021	33.25	355.24	767.70	1952.85	282.00	4.55	76.26	47.66	6264.50	75.63
BL021	34.09	352.90	764.30	1882.75	268.00	4.27	76.17	49.34	6139.50	75.35
BL026	36.41	352.95	608.50	2061.73	251.50	3.36	85.37	64.25	7232.00	72.87
BL026	37.01	361.75	635.50	2243.04	297.50	4.16	84.58	58.44	6714.75	76.02
BL026	39.03	363.90	625.00	2421.43	295.50	4.06	85.54	60.03	6408.00	78.11
BL041		444.40	730.00	2691.97	372.25	5.24	87.46	54.52	8062.00	72.95
BL041	35.32	481.50	634.40	2319.13	840.75	15.54	97.66		7903.50	66.25
BL041	26.11	440.54	742.30	2351.42	327.50	4.54	86.36	57.82		
BL046	17.73	338.57	712.80	1966.33	277.75	4.37	77.26	49.79	7962.00	75.00
BL046	30.44	344.42	714.60	1983.02	269.50	4.16	77.83	51.68		
BL046	36.67	344.49	701.60	2003.35	278.75	4.28	78.55	51.65	7980.50	76.91
BL061	24.11	398.90	893.40	2093.93	330.50	5.85	74.91	38.18	7670.75	76.87
BL061	26.23	403.44	885.70	1901.85	321.00	5.50	75.66	41.11	7363.50	75.79
BL061	24.30	397.41	887.10	1797.12	295.75	4.98	75.03	43.74	7213.00	75.22
BL065	28.64	361.40	712.80	1965.81	313.25	4.85	79.82	49.34	8046.25	70.67
BL065	33.21	364.09	709.70	2160.17	309.50	4.73	80.29	50.57	7787.25	72.67
BL065	38.43	361.12	720.80	1868.53	287.25	4.38	79.35	51.83		
BL068	40.54	453.86	797.70	2351.36	336.00	4.85	84.56	54.11		
BL068	43.78	456.18	813.10	2338.18	356.75	5.30	83.96	50.66	7895.75	69.27
BL068	35.27	456.32	808.00	2134.64	336.75	4.89	84.24	53.53		
BL091	32.83	392.98	813.60	2026.47	289.75	4.56	77.91	49.28	8478.00	76.08
BL091	35.32	393.68	816.30	2030.75	299.75	4.77	77.85	47.89	7970.75	74.41
BL091	37.47	398.93	817.50	2044.15	294.25	4.61	78.31	49.34	7735.25	77.96
BL092	39.99	400.06	694.20	2335.14	312.00	4.37	85.10	57.63	8836.50	75.36
BL092	39.07	400.34	694.70	2323.83	310.00	4.34	85.10	57.84	3800.25	65.57
BL092	39.32	404.87	703.80	2540.44	321.75	4.55	85.02	56.44	7526.50	79.20
BR012	38.67	409.25	674.20	2506.40	335.25	4.60	87.34	58.44	10584.00	75.39
BR012	35.41	412.79	679.80	1990.34	301.75	4.04	87.36	61.96		
BR012	45.88	408.65	658.10	2008.48	252.50	3.23	88.34	68.05	9178.25	72.72
BR013	34.42	400.97	682.80	2276.37	314.75	4.36	85.91	58.52	6766.00	67.89
BR013	31.76	405.60	691.30	2268.51	311.25	4.30	85.87	58.84	6800.00	68.46
BR013	37.73	395.99	724.90	2492.00	345.25	5.19	82.85	50.26	6442.75	66.76
BR020	36.32	455.52	803.40	2106.81	322.50	4.61	84.41	55.43	9362.25	71.50
BR020	40.18	453.26	804.00	2341.65	340.50	4.97	84.17	52.97	7537.75	74.24
BR020	38.83	453.88	803.70	2720.16	372.75	5.59	84.24	49.12	7756.00	72.18
BR033	33.44	392.88	839.00	1774.82	307.25	5.05	76.71	44.98	9816.25	76.38
BR033	35.71	390.42	840.10	1869.48	299.75	4.92	76.42	45.54	10111.75	70.84
BR033	33.35	388.61	860.70	1629.58	280.50	4.61	75.32	46.36		
BR042	34.19	325.26	640.00	1894.26	269.75	4.01	79.92	54.75	5715.50	76.93
BR042	43.42	320.69	631.00	1844.92	267.75	3.97	79.92	54.98	4794.00	77.08
BR042	34.78	336.93	609.30	2088.77	324.50	4.74	83.36	53.59	5000.00	76.05
BR051	43.42	453.93	645.00	2759.91	378.50	4.79	94.04	63.93	9299.50	69.12
BR051	41.68	457.73	659.30	2844.37	332.75	4.14	93.40	67.40	8253.50	66.45
BR051	46.90	457.69	655.00	2948.62	348.25	4.35	93.71	66.37	7884.50	70.78
BR062	42.98	345.65	588.80	2227.83	284.25	3.85	85.89	61.68	8649.25	76.92

Name	T38ENDO	T66TBMC	T66TBMD	T66PSSI	T66COA	T66COTH	T66PERI	T66ENDO	T66MA	T66MD
BR062	40.46	348.08	592.20	2267.34	278.25	3.75	85.94	62.36	6902.25	75.26
BR062	39.99	346.01	647.70	2159.11	280.50	4.05	81.94	56.47	6197.00	75.01
BR074	0.00	363.60	789.60	2065.74	311.25	5.22	76.07	43.31	8496.50	75.50
BR074	32.30	367.66	803.60	1726.24	273.75	4.42	75.82	48.05	6993.75	76.64
BR074	27.34	366.18	799.10	1889.82	313.25	5.28	75.89	42.69	6898.75	77.10
BR079	39.07	431.55	656.10	2672.87	302.75	3.84	90.92	66.79	5175.25	62.93
BR079	37.85	427.89	653.30	2021.89	300.50	3.82	90.73	66.74		
BR079	46.56	433.24	658.90	2968.04	334.25	4.32	90.90	63.73	7093.75	76.39
BR094	34.51	379.36	714.10	1619.79	449.75	7.91	81.71	32.00	6694.25	75.04
BR094	36.06	383.04	724.10	1933.86	267.00	3.84	81.53	57.38	5553.50	77.05
BR094	36.58	383.59	720.70	2084.19	272.50	3.92	81.78	57.13	5449.75	79.66
BR095	38.67	396.65	638.70	2604.67	324.00	4.34	88.34	61.09	7667.75	67.86
BR095	38.67	395.88	653.50	2489.34	317.75	4.31	87.25	60.16	7612.00	72.00
BR095	39.63	399.11	649.20	2541.43	319.25	4.29	87.89	60.94	8331.50	73.21

Name	T66SubQ	R4TBMC	R4TBMD	R4PSSI	R4TRBMC	R4TRDEN	R4COA	R4COTH	R4PERI	R4ENDO
BL003	4510.75	63.65	236.60	220.92	36.36	163.00	97.75	1.87	58.14	46.39
BL003	4208.25	78.59	432.40	272.44	12.21	116.20	92.75	2.28	47.79	33.44
BL003	4130.25	67.03	183.50	197.68	54.15	159.60	146.75	2.44	67.75	52.40
BL021	4755.00	98.39	396.70	383.26	33.53	203.80	104.75	2.13	55.83	42.43
BL021	4410.25	96.74	276.20	375.23	52.90	189.30	125.50	2.10	66.34	53.14
BL021	3835.25	99.97	272.60	371.42	55.58	191.00	123.00	2.00	67.89	55.35
BL026	6538.25	107.66	458.60	387.32	33.53	227.70	96.00	2.00	54.31	41.76
BL026	6650.25	104.26	484.40	371.79	28.59	218.70	91.00	1.99	52.01	39.51
BL026	6325.50	96.73	388.80	348.65	41.99	232.60	98.25	1.98	55.91	43.49
BL041	11520.75	112.41	421.80	416.62	41.54	235.00	106.00	2.06	57.87	44.91
BL041	10100.75	114.15	563.70	400.41	24.22	238.00	95.00	2.18	50.45	36.75
BL041		122.59	415.20	453.09	44.13	241.50	153.00	2.97	60.91	42.28
BL046	7656.50	88.63	311.00	339.89	47.29	209.20	110.00	2.06	59.85	46.90
BL046		88.38	344.20	320.32	38.04	201.80	100.25	1.98	56.80	44.35
BL046	6476.25	97.01	320.40	338.02	49.91	213.50	109.75	1.98	61.68	49.25
BL061	6934.00	107.39	337.20	389.88	58.29	239.10	112.50	1.97	63.26	50.88
BL061	6031.25	116.28	333.90	429.04	67.18	248.80	121.25	2.03	66.15	53.41
BL061	6149.50	113.61	327.60	428.78	61.52	235.90	132.00	2.24	66.01	51.95
BL065	10261.00	104.27	463.90	385.88	28.30	216.00	119.50	2.67	53.14	36.37
BL065	10282.50	99.47	468.10	376.33	26.39	206.20	113.50	2.61	51.68	35.27
BL065		99.93	464.80	349.63	29.19	215.80	89.25	1.95	51.98	39.75
BL068		93.41	307.80	344.83	47.99	202.50	115.75	2.10	61.76	48.57
BL068	6132.50	96.24	299.80	331.34	51.43	206.50	118.25	2.08	63.51	50.48
BL068		96.03	295.70	362.31	52.74	206.00	117.75	2.05	63.88	51.00
BL091	7359.75	92.19	358.70	295.35	50.56	264.70	99.50	1.96	56.83	44.49
BL091	5807.50	98.50	337.10	330.28	63.09	270.20	119.00	2.22	60.60	46.66
BL091	4840.75	95.44	395.20	296.76	48.30	277.60	96.50	1.97	55.09	42.69
BL092	6707.50	79.25	218.00	280.51	59.38	181.70	144.50	2.41	67.59	52.46
BL092	6027.75	80.52	210.50	293.13	66.80	188.60	169.50	2.80	69.33	51.74
BL092	5621.50	79.04	216.80	253.85	57.87	178.10	141.25	2.34	67.68	52.97
BR012	5362.50	110.67	382.00	383.85	53.49	256.90	102.00	1.87	60.34	48.57
BR012		115.99	333.80	442.28	67.68	250.00	126.50	2.13	66.08	52.70
BR012	3231.25	120.11	432.80	447.13	46.83	261.60	108.50	2.06	59.05	46.08
BR013	11866.00	89.30	310.60	307.35	49.04	218.00	99.75	1.84	60.11	48.57
BR013	8867.25	90.59	347.40	316.19	41.96	222.90	97.00	1.89	57.24	45.36
BR013	7881.50	88.55	458.20	342.57	24.92	216.20	102.00	2.45	49.28	33.86
BR020	4068.75	103.58	303.30	352.81	57.19	212.20	120.75	2.04	65.51	52.67
BR020	3279.50	105.22	461.50	391.42	27.37	191.70	91.75	1.93	53.53	41.38
BR020	2810.50	113.29	278.90	390.09	78.65	228.50	154.00	2.41	71.45	56.30
BR033	5789.50	90.05	329.80	347.57	37.54	185.90	102.00	1.94	58.57	46.36
BR033	3834.00									
BR033		86.81	359.80	343.06	33.00	189.90	97.00	1.99	55.06	42.58
BR042	8149.75	77.27	380.20	272.29	28.79	207.90	87.25	1.97	50.54	38.18
BR042	7245.00	77.48	629.90	242.61	6.68	121.50	66.75	2.03	39.32	26.59
BR042	6296.50	76.84	350.90	276.88	32.44	205.30	89.50	1.93	52.46	40.34
BR051	6499.75	119.34	393.20	467.56	41.83	198.00	106.50	1.91	61.76	49.76
BR051	5969.50	119.74	380.70	466.27	46.05	203.50	106.50	1.87	62.87	51.13
BR051	5795.25	124.20	403.60	505.27	42.52	201.30	109.50	1.95	62.19	49.91
BR062	4765.50	69.75	255.30	260.93	44.28	190.90	109.75	2.11	58.60	45.33

Name	T66SubQ	R4TBMC	R4TBMD	R4PSSI	R4TRBMC	R4TRDEN	R4COA	R4COTH	R4PERI	R4ENDO
BR062	3410.25	74.44	249.40	290.81	52.18	201.90	123.00	2.27	61.25	46.96
BR062	2525.25	80.59	249.50	281.46	57.42	205.30	127.25	2.25	63.71	49.60
BR074	3712.75	102.93	364.00	349.69	48.13	233.90	100.75	1.88	59.61	47.82
BR074	3254.00	102.63	390.60	374.17	42.46	234.60	102.25	2.00	57.46	44.91
BR074	2764.00	104.45	385.80	347.49	45.31	240.10	114.75	2.24	58.33	44.28
BR079	9563.25	100.65	342.10	358.04	50.68	228.00	107.50	1.97	60.81	48.44
BR079		106.86	356.80	364.81	49.45	227.30	102.50	1.85	61.35	49.76
BR079	6696.50	101.15	445.60	356.28	29.97	210.30	86.75	1.82	53.41	41.98
BR094	8397.25	83.35	313.60	303.26	40.95	207.80	111.25	2.19	57.79	44.06
BR094	6915.75	80.69	355.50	309.01	34.89	218.40	170.75	4.27	53.41	26.59
BR094	5716.50	94.12	341.00	360.47	44.49	224.70	132.25	2.61	58.89	42.50
BR095	9330.75	88.60	299.60	360.15	38.54	172.80	107.50	1.96	60.96	48.64
BR095	7280.75	87.01	271.90	391.50	42.48	170.80	116.75	2.05	63.41	50.54
BR095	6124.25	97.31	269.90	400.55	54.24	187.20	125.00	2.05	67.31	54.40

<u>Name</u>	<u>R66TBMC</u>	<u>R66TBMD</u>	<u>R66PSSI</u>	<u>R66COA</u>	<u>R66COTH</u>	<u>R66PERI</u>	<u>R66ENDO</u>	<u>R66MA</u>	<u>R66MD</u>	<u>R66SubQ</u>
BL003	93.82	657.20	313.61	120.00	4.05	42.35	16.91			
BL003	94.77	418.90	312.46	106.75	2.32	53.32	38.75	3056.25	68.79	2769.50
BL003	87.09	718.30	243.63	77.50	2.48	39.03	23.45	3190.50	77.26	2843.00
BL021	81.12	627.60	235.70	100.00	3.36	40.30	19.17			
BL021	104.69	794.60	322.88	90.25	2.84	40.69	22.84	2825.50	79.06	2500.75
BL021	98.30	781.70	302.40	92.25	3.06	39.75	20.52	3196.75	69.81	1914.50
BL026	99.93	807.50	314.49	88.25	2.92	39.44	21.12	2879.50	78.62	2668.00
BL026	101.17	738.50	330.09	92.25	2.83	41.49	23.71	2796.25	77.69	2256.00
BL026	104.24	863.30	265.05	93.25	3.24	38.95	18.59	2744.50	78.66	2237.50
BL041	93.66	730.30	283.52	100.50	3.42	40.15	18.67			
BL041	128.90	659.30	391.87	116.00	2.86	49.57	31.61	3152.00	72.08	3635.00
BL041	103.21	716.70	328.15	127.75	4.50	42.54	14.29	3086.00	66.46	3498.25
BL046	111.17	716.10	293.83	96.75	2.72	44.17	27.11	3365.50	71.49	3133.00
BL046	112.29	653.80	366.22	92.75	2.38	46.46	31.51	2950.25	78.73	2353.25
BL046	109.69	802.10	363.53	98.25	3.10	41.45	22.00	3125.25	80.38	2546.25
BL061	111.02	886.40	350.99	105.75	3.82	39.67	15.65	3319.50	77.31	3049.75
BL061	71.11	675.60	201.76	77.00	2.79	36.37	18.84			
BL061	101.34	805.90	271.67	106.00	3.82	39.75	15.75	3151.50	71.45	2275.00
BL065	97.42	867.90	262.92	92.50	3.47	37.56	15.75	2818.00	77.21	5176.25
BL065	110.40	622.00	317.65	118.75	3.19	47.23	27.17			
BL065	88.20	866.80	219.57	75.75	2.81	35.76	18.08			
BL068	86.77	814.80	221.46	71.25	2.47	36.58	21.05	3611.75	69.58	3093.75
BL068	101.09	846.00	300.33	88.00	3.00	38.75	19.90	3378.00	72.79	2396.75
BL068	86.76	812.70	225.64	68.50	2.34	36.63	21.92	3049.50	73.28	2479.25
BL091	101.01	888.00	220.62	111.25	5.13	37.81	5.61	3088.00	79.81	2908.50
BL091	83.89	919.40	206.77	65.75	2.54	33.86	17.90	2760.50	79.44	2106.50
BL091	83.59	827.60	172.48	66.25	2.34	35.63	20.90	2746.25	65.02	1514.00
BL092	93.76	846.60	270.90	92.50	3.53	37.31	15.14	3227.00	78.62	3392.50
BL092	93.18	864.80	244.39	83.25	3.06	36.80	17.55	2888.75	80.75	3060.50
BL092	85.14	849.20	224.43	66.50	2.37	35.49	20.59	2786.75	80.50	2753.75
BR012	86.79	737.10	230.58	77.25	2.53	38.47	22.56	4087.25	76.55	2887.75
BR012	86.79	748.20	250.66	79.75	2.68	38.18	21.34			
BR012	95.41	743.90	248.74	83.50	2.62	40.15	23.71	4276.75	73.73	948.75
BR013	105.37	889.20	282.67	93.25	3.31	38.59	17.81	3089.25	72.11	3152.00
BR013	96.24	948.20	234.76	78.75	2.99	35.71	16.91	2411.50	76.36	1900.00
BR013	83.99	865.80	200.10	70.00	2.63	34.91	18.42	2592.50	69.09	1436.00
BR020	124.05	957.90	288.29	99.75	3.34	40.34	19.34	3394.50	76.72	1720.75
BR020	102.56	882.20	247.52	81.25	2.75	38.22	20.97	3120.00	73.53	1309.25
BR020	127.18	961.60	349.28	108.50	3.74	40.77	17.28	2981.00	79.61	1281.50
BR033	70.73	870.50	164.95	59.00	2.42	31.95	16.72	3775.50	71.17	2733.00
BR033	85.16	724.80	230.48	76.75	2.51	38.43	22.63	2605.25	75.28	2982.75
BR033	81.82	861.20	175.05	117.25	5.50	34.55		2970.25	78.47	2272.50
BR042	72.38	699.30	198.45	67.25	2.34	36.06	21.34	2861.25	74.49	3677.00
BR042	77.93	762.20	235.07	67.00	2.36	35.85	21.05	2342.00	75.85	3367.25
BR042	75.88	742.10	215.10	69.25	2.46	35.85	20.36	2372.00	76.08	2643.25
BR051	107.99	686.70	380.54	108.25	3.13	44.45	24.81	3631.50	76.67	2217.25
BR051	108.07	686.20	396.30	92.50	2.53	44.49	28.58	3084.25	75.13	1825.25
BR051	108.99	685.50	413.50	97.50	2.69	44.70	27.80	3054.75	76.27	1557.50
BR062	93.73	760.50	253.43	82.25	2.65	39.36	22.70	3246.00	77.48	2174.00

Name	R66TBM	R66TBM	R66PSSI	R66COA	R66COTH	R66PERI	R66ENDO	R66MA	R66MD	R66SubQ
BR062	83.52	801.20	228.12	68.25	2.38	36.20	21.27	2429.00	78.25	1583.50
BR062	82.08	794.90	214.26	65.00	2.24	36.02	21.92	2414.25	78.40	1173.50
BR074	128.20	710.30	434.10	131.50	3.63	47.63	24.81	3866.25	81.49	1715.50
BR074	112.81	786.10	376.96	102.25	3.14	42.47	22.77	3156.75	77.72	1295.25
BR074	109.85	737.30	303.27	97.25	2.83	43.27	25.50	3163.50	78.73	1069.75
BR079	112.76	633.50	391.85	103.75	2.67	47.30	30.55	3050.50	75.64	5183.25
BR079	93.83	731.60	282.03	75.25	2.28	40.15	25.81	2669.00	73.93	4264.25
BR079	95.71	647.80	330.69	87.00	2.46	43.09	27.63	2446.50	77.03	3625.75
BR094	71.30	838.80	162.15	60.50	2.41	32.68	17.55	2733.00	70.46	2581.00
BR094	84.00	815.50	206.54	80.25	3.04	35.98	16.91	1940.25	76.17	2065.75
BR094	75.11	860.80	194.81	57.50	2.19	33.11	19.34	2169.00	80.18	1852.25
BR095	99.65	627.70	337.99	107.25	3.06	44.66	25.44	3470.00	71.02	4629.50
BR095	94.83	599.30	327.47	84.25	2.24	44.59	30.49			
BR095	93.35	662.10	295.45	88.50	2.61	42.09	25.69	2823.50	73.09	3162.25

Name	tOC	unOC	Adipo	Lep	CTx
BL003					
BL003	13.23	4.55	3.01	68.86	0.43
BL003	9.11	3.26	2.00	72.56	0.25
BL021	9.37	2.12	3.58	81.22	0.42
BL021	11.85	3.47	3.25	77.48	0.61
BL021	13.63	2.83	3.93	82.06	0.40
BL026	8.08	1.75	2.98	107.76	0.18
BL026	12.36	1.88	10.22	78.47	0.61
BL026	12.23	4.37	8.47	97.04	0.56
BL041	12.05	2.59	17.63	74.88	0.46
BL041	14.59	7.11	2.10	78.99	0.78
BL041	14.81	4.14	5.11	86.20	0.62
BL046			31.01	75.93	
BL046	7.79	1.17	5.22	55.76	
BL046	9.87	1.07	7.17	71.48	
BL061	11.41	2.66	4.73	56.99	
BL061	12.13	1.41	27.42	71.78	
BL061	9.24	0.79	4.27		
BL065	10.68	0.54	6.62	72.97	
BL065	7.18	0.64	7.74	65.25	
BL065	7.91	0.54	7.54	62.80	
BL068	7.81	0.59	4.31	67.46	
BL068	8.92	0.91	3.61	75.64	
BL068	13.62	1.50	9.61	60.55	
BL091	11.58	0.44	2.79	65.76	
BL091	18.18	2.02	3.33	54.53	
BL091	30.42	5.20	3.78	29.49	
BL092	15.37	0.95	0.57	61.78	
BL092	14.80	2.98	2.93	61.23	
BL092	7.31	0.41	2.12	61.61	
BR012					
BR012	17.50	5.56	1.49	31.59	1.22
BR012	26.87	13.22	3.72	16.24	1.18
BR013	15.68	3.90	13.78	92.80	0.46
BR013	22.69	6.50	13.82	68.36	1.06
BR013	24.67	9.81	20.12	50.72	0.85
BR020	5.64	1.92	1.62	73.41	0.26
BR020	17.40	6.14	4.62	26.72	1.06
BR020	22.24	9.55	5.20	19.99	0.84
BR033	20.15	0.61	15.04	53.54	0.15
BR033	10.95	3.57	2.85	25.48	0.42
BR033	43.18	11.17	18.92	29.88	0.51
BR042	8.67	3.34			
BR042	39.97	5.39	1.82	81.29	0.64
BR042	3.05	0.84	6.21	48.00	0.66
BR051					
BR051	10.02	3.17	8.41	59.92	
BR051	19.72	9.25	41.09	22.42	
BR062	21.56	6.19	15.96	65.81	

Name	tOC	unOC	Adipo	Lep	CTx
BR062	15.15	4.38	5.00	30.47	
BR062	21.52	5.04	10.32		
BR074	9.51	1.30	2.74	59.28	
BR074	10.12	1.27	4.66	12.42	
BR074	20.02	2.98	5.22	26.82	
BR079	9.58	0.41	16.09	52.20	
BR079	14.85	2.13	7.40	54.24	
BR079	28.95	7.21	6.04	35.55	
BR094	7.40	1.22	3.15	60.13	
BR094	19.24	3.87	3.80	55.93	
BR094	23.07	3.53	7.82	56.10	
BR095	8.53	0.95	6.92	80.81	
BR095	17.33	2.88	10.25	49.24	
BR095	22.37	4.75	7.58	30.25	