# NUTRITIONAL REGULATION OF ADIPONECTIN AND THE EFFECT OF GENETIC VARIATIONS IN THE ADIPONECTIN GENE ON BONE DENSITY IN THE NEWFOUNDLAND POPULATION

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# NUTRITIONAL REGULATION OF ADIPONECTIN AND THE EFFECT OF GENETIC VARIATIONS IN THE ADIPONECTIN GENE ON BONE DENSITY IN THE NEWFOUNDLAND POPULATION

By

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**Summary**

Adiponectin, as an adipokine, has been shown to be involved in insulin sensitivity and obesity. Recently data mainly from animal models, have suggested that adiponectin may have a role in bone metabolism. This project addresses the association of adiponectin with insulin sensitivity and obesity status, the regulation of positive energy challenge on adiponectin, and the association of DNA sequence variation in adiponectin gene with bone mineral density in the Newfoundland population. This project comprises two studies: 1) a Newfoundland-population-based-genetic-association cohort study and 2) an overfeeding study.

***1) Genetic Association Study of adiponectin with bone density***

A total of 1811 third-generation Newfoundlanders, 403 males and 1408 females, ages 19-73, were recruited to take part in the study. To participate, each subject completed a screening questionnaire, a physical activity form, a dietary form, a menstrual cycle form (females only) and a consent form. Plasma and serum samples were collected. Bone mineral density and percentage body fat were measured by Dual energy X-ray absorptiometry (DEXA). Blood samples were assayed for lipid profile, electrolyte levels, and insulin levels. DNA was extracted from white blood cells. Five single nucleotide polymorphisms (SNPs) in the adiponectin gene (tagging SNPs) were selected and genotyped with the TaqMan SNP genotyping kits performed on an ABI 7000 instrument. Multiple regression analysis was conducted through regression at the SNP markers against gender, age, smoking, and medication status as covariates. Significance of this regression was assessed via a bootstrap with 100,000 resamples. Contrary to previous studies, no association between adiponectin genotypes and bone mineral density was found.

***Regulation of circulating adiponectin by a short-term positive energy challenge***

The response of serum adiponectin in young males aged 19-29 to a 7-day overfeeding course was observed. Subjects were overfed 70% above their baseline with a macronutrient mixture of 50% carbohydrates, 35% fat, and 15% protein. Blood serum and plasma samples were taken prior to the start of the overfeeding, as well as the morning after the last day of the overfeeding period, both after a 12 hour fast. Serum electrolytes, lipid profiles, insulin levels and adiponectin levels were measured. Percentage body fat and bone mineral density values were obtained by DEXA pre- and post-overfeeding. Serum adiponectin levels increased significantly from baseline after a positive-energy challenge in all adiposity categories ($11.60 \pm 6.3$ to $13.96 \pm 4.5$ in underweight/lean individuals; $12.84 \pm 4.6$ to $14.81 \pm 4.05$ in overweight individuals; $10.69 \pm 6.3$ to $12.82 \pm 6.1$ in obese individuals, $p<0.05$ as measured by 2 - Way mixed model ANOVA). This finding contradicts previous studies, which used longer-term overfeeding protocols (Ukkola *et al.*, 2008). This study also shows that baseline adiponectin level is not directly associated with adiposity in response to short-term overfeeding. It also provides evidence that the protective response of adiponectin may be preserved when challenged by a short-term energy balance, irrespective of obesity status.

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**List of Abbreviations**

| | |
|---|---|
| A | - Adenosine |
| ABI | - Applied Biosystems |
| AMP | - Adenosine monophosphate |
| AMPK | - Adenosine monophosphate-dependant protein kinase |
| BMD | - Bone mineral density |
| BMI | - Body mass index |
| BMC | - Bone mineral composition |
| C | - Cytosine |
| CAD | - Coronary artery disease |
| cDNA | - Complementary DNA |
| CHD | - Coronary heart disease |
| cRP | - c-reactive protein |
| DEXA | - Dual X-ray absorptiometry |
| DNA | - Deoxyribonucleic acid |
| DXA | - Dual X-ray absorptiometry |
| ELISA | - Enzyme linked immunosorbent assay |
| G | - Guanine |
| GE | - General Electric |
| HDL | - High-density lipoprotein |
| HDL-c | - High-density lipoprotein cholesterol |
| HOMA-IR | - Homeostasis assessment model – insulin resistance |
| HOMA-β | - Homeostasis assessment model – β-cell function |

| | |
|---|---|
| HWE | - Hardy – Weinberg Equilibrium |
| $K_3EDTA$ | -Ethylenediaminetetraacetic acid tripotassium salt dihydrate |
| LDL | - Low-density lipoprotein |
| MI | - Myocardial infarction |
| mRNA | - Messenger RNA |
| NO | - Nitrous oxide |
| Non-smo | - Non-smoker |
| NTC | - Non-translated controls |
| PCR | - Polymerase chain reaction |
| PDGF | - Platelet-derived growth factor |
| SMO | - Smoker |
| SNP | - Single nucleotide polymorphism |
| T | - Thymine |
| tPA | - Tissue plasminogen activator |
| WBC | - White blood cell |

## 1.0 Introduction

### *1.0.1 Obesity and Society*

Obesity has become a big healthcare problem in today's society. According to the World Health Organization (WHO), more than one billion adults in the world are overweight, with 300 million women and 200 million men being considered clinically obese (WHO, 2008). In some societies, the epidemic is much more rampant than in others (e.g. less than 5% in China and Japan, more than 75% in urban Samoa; WHO, 2006). The obesity epidemic not only affects adults of specific countries, but also has become a great concern in children as well. The WHO and the International Obesity Task Force report 22 million children under age five are estimated to be overweight or obese worldwide (WHO, 2006; Malecka-Tendera & Mazur, 2006). Although prevalence rates in certain subpopulations of differing socioeconomic backgrounds may be slightly higher than others, it is still considered a large problem affecting all peoples and societies (WHO, 2006). Furthermore, in almost all populations and subpopulations, the epidemic is growing. In the United States, it has been reported that in children aged 6-17, obesity and obesity-associated hospital discharge rates have tripled (Dietz, 2004; Rubenstein, 2005). The rate in adults has increased 50% per decade during the 1980s and 1990s (Behn & Ur, 2006). A study of South Australians showed that obesity increased significantly from 8.7% in 1991 to 14.1% in 2003, while the prevalence of severely obese individuals increased significantly from 2.6% to 5.3% (Dal Grande, Gill, Taylor, Chittleborough & Carter, 2005). Moreover, the prevalence of obesity in Canada has more than doubled in Canada between 1985 and 1998 (Vanasse, Demers, Hemiari, & Courteau, 2006).

Obesity often results in numerous chronic and debilitating conditions, including type 2 diabetes, hypertension, cardiovascular problems, insulin resistance, and cancer (Rubenstein,

2005). It is associated with many other debilitating conditions as well, including respiratory difficulties, dyslipidemia, chronic musculoskeletal problems, skin problems and even infertility (WHO, 2006). As a result of these complications, the cost of obesity and obesity-related problem on healthcare systems is substantial. The WHO reports 2-6% of total healthcare costs in several developed countries is attributed to obesity (WHO, 2006). In Canada, it has been estimated that obesity-related illness accounts for $4.3 billion, representing 2.2% of the total healthcare costs in Canada (Katzmarzyk, Janssen, & Ardern., 2003; Vanasse *et al.*, 2005).

### *1.0.2 Obesity and Prevalence*

As previously stated, approximately 1 billion people worldwide are considered to be overweight. Although a serious healthcare issue worldwide, prevalence rates vary in different populations. In the United States, Rubenstein (2005) reported that 35% of U.S. adults are overweight, while 30% are obese. Those that are considered extremely or morbidly obese accounted for 4.7% of U.S. adults, which was an increase from 0.8% in 1960 (Rubenstein, 2005). Similar figures have been reported in later studies (Wyatt, Winters, & Dubbert, 2006). As well, evidence has also shown in the U.S. that obesity rates among non-Hispanic blacks as compared with non-Hispanic whites are considerably higher (Boardman, Saint Onge, Rogers, & Denny, 2005). In Canada in 2003, one-third of Canadian individuals aged 20 years and older were overweight and 15.2% were considered obese (Vanasse *et al.*, 2006). This significantly varied between different regions of the country as well, with Vancouver showing a rate of 6.2%, while some aboriginal populations showed prevalence rates of 47.5% (Vanasse *et al.*, 2006). Some of the highest rates of obesity were found in Newfoundland and Labrador, which showed the greatest relative increase in the number of overweight and obesity-related deaths over 15 years

(Vanasse *et al.*, 2006).

A number of potential causes have been suggested for the increase in obesity prevalence. Lowering in many populations of physical activity has been suggested as a factor (Katzymarzyk *et al.*, 2003; Vanasse *et al.*, 2006). Others attribute the increase to types of foods being eaten, specifically those foods that are high in simple sugars, fats and low in proteins, and a decrease in the amount of vegetables and fresh fruits (Vanasse *et al.*, 2006). Other studies noted relationships between geography and obesity rates, such as industrialization of rural areas in Africa, Latin America and Haiti (Wang & Brownell, 2005), or the increase in likeliness of obesity of African-Americans living in an obese African-American community (Boardman *et al.*, 2005). Socioeconomic factors have also been suggested (Wyatt *et al.*, 2006; Boardman *et al.*, 2005).

However, the role of genetics in human obesity has become of particular interest. Although all experts agree it is not the only factor affecting obese phenotypes, it is agreed that how people respond biologically to their environment and socioeconomic factors directly results from their genes. Since the discovery of the leptin gene in 1995 and its role in obese phenotypes, there is marked increase in the search for potential players in the genetic story of obesity. Many monogenic conditions were identified that lead to obesity, including Prader-Willi Syndrome, Bardet-Biedl syndrome and Cohen syndrome (Clément, 2006). However, aside from these low frequency conditions that lead to severely obese phenotypes, obesity in the general population is a multi-factorial or polygenic disease. Several genes are believed to play a role in the development of obesity, such as the genes of leptin, retinal-binding protein-4, visfatin, adiponectin, TNF-α, IL-6 and adiponectin. Adiponectin, a novel hormone believed to be involved in adiposity, is the focus of this thesis.

### *1.0.3 Definition of Obesity*

Obesity and obesity prevalence are defined by body mass index (BMI; $kg/m^2$), which is an individual's weight in kilograms, divided by their height in meters squared. The WHO divides an individual's BMI in six main categories in an effort to define those individuals who are obese: underweight (BMI<18.5), normal (18.5≤BMI<25), overweight (25≤BMI<30), Class I obese (30≤BMI<35), Class II obese (35≤BMI<40) and Class III obese (40≤BMI); (WHO, 2006). For economic reasons many studies, when assessing prevalence of obesity, often use self-reported data to calculate BMI. However, other studies examined the accuracy of the self-reported approach (Niedhammer, Bugel, Bonenfant, Goldberg, & Leclerc, (2000); Hill & Roberts, (1998); Alvarez-Torices, Franch-Nadal, Alvarez-Guisaola, Hernandez-Mejia, & Cueto-Espinar, (1993)). In addition, using BMI has been found to be inaccurate in many cases, particularly in individuals with differing body sizes (e.g. body builders) because BMI does not indicate an accurate fat distribution throughout the body.

Other techniques exist for examining obesity more accurately in order to assess adiposity. The newer form of evaluating obesity is the use of body fat composition or percentage body fat, which more accurately assesses body fat distribution and obesity. It was originally measured by hydrodensitometry, which determined a person's percentage body fat by submerging the individual completely in water and calculating the water displacement based on the principle that fat components float and non-fat components sink (Bray, 2003). However, the procedure was cumbersome for people and its accuracy was affected by pulmonary residual volume of air in one's lungs. An alternative for measurement of body composition is Dual X-Ray Absorptiometry (DXA) which measures percentage body fat as well as bone mineral composition by using very low energy x-ray beams that are absorbed at different rates in

different tissues (Bray, 2003).

According to Bray (2003), obesity as defined by percentage body fat varies among races, ages, and genders. Obesity is thus classified by Bray (2003), which is shown in Table 1.

Table 1. Body fat for men and women of different ethnic groups according to BMI cut points (adapted from Bray, 2003).

| | **Females (% Body Fat)** | | | **Males (% Body Fat)** | | |
|---|---|---|---|---|---|---|
| **BMI** | **Blacks** | **Asians** | **Whites** | **Blacks** | **Asians** | **Whites** |
| *Age 20-39* | | | | | | |
| 18.5 | 20 | 25 | 21 | 8 | 13 | 8 |
| 25 | 32 | 35 | 33 | 20 | 23 | 21 |
| 30 | 38 | 40 | 39 | 26 | 28 | 26 |
| *Age 40-59* | | | | | | |
| 18.5 | 21 | 25 | 23 | 9 | 13 | 11 |
| 25 | 34 | 36 | 35 | 22 | 24 | 23 |
| 30 | 38 | 41 | 41 | 27 | 29 | 29 |
| *Age 60-79* | | | | | | |
| 18.5 | 23 | 26 | 25 | 11 | 14 | 13 |
| 25 | 35 | 36 | 38 | 23 | 24 | 25 |
| 30 | 41 | 41 | 43 | 29 | 29 | 31 |

### *1.0.4 Identifying obesity candidate genes*

Various techniques exist for identifying potential novel candidate genes that may be linked to different multifactorial diseases. One of these methods is the genetic association study which is designed to determine associations between one or more genetic variants and a specific trait that is normally linked with a disease. The Human Genome Project facilitated this type of study and the generation of over 1.4 million single nucleotide polymorphisms (Lewis, 2002); International SNP MAP Working Group, 2001). These polymorphisms occur throughout the genome in all regions of the DNA (i.e. coding, promoters, introns, splice-sites, etc).

Genetic association studies attempt to identify candidate genes, and often more specifically, single nucleotide polymorphism (SNP) alleles that may potentially co-occur with a diseased state (Lewis, 2002). Such investigations have become a central focus of research aimed at uncovering the underlying etiologies of many multifactorial diseases, such as obesity, cardiovascular disease and asthma (Cordell & Clayton, 2005; Lewis, 2002). However, association studies have sometimes been questioned as not being potentially worthwhile because of the high costs needed in genotyping and sample collection, in addition to the large number of samples needed to conduct an effective analysis (Howson, Barratt, Todd, & Cordell, 2005). Moreover, some researchers claim skepticism because of their lack of reproducibility (Cardon & Bell, 2001), while others have argued cost-benefit of association studies is reasonable with the falling costs of genotyping (Cordell & Clayton, 2005).

Regardless, association studies have been shown to be successful in identifying candidate genes in multifactorial conditions. Reuter, Markett, Melchers and Montag (2012), in an effort to assess the genetic basis for depression, genotyped 800 individuals for the BCL1 rs41423247 and the CHRNA4 rs1044396 single-nucleotide polymorphism alleles. Individuals with the CC

genotype at the BCL1 locus who were also homozygous for the T allele at the CHRNA4 locus had the highest depression scores on Beck's Depression Inventory (Reuter, Markett, Melchers, & Montag, 2012). Other researchers noted that the 373 A9T11 allele gene involved in the production of Interleukin-6 (IL-6), was linked with a reduced susceptibility of chronic periodontitis, a chronic inflammatory disease caused by the interaction between host immune and periodontal bacteria (Komatsu et al., 2005). Association studies were successful in showing connections between CD209 promoter polymorphisms, an immune system surface molecule involved in pathogen recognition and immune activation, and HLA-DQ2 negative celiac disease in a Spanish population (Núñez et al., 2006).

### *1.0.5 Newfoundland as a study population for multi-factorial conditions*

Founder populations are always useful for studying genetic conditions. They have become of particular value in the study of multi-factorial conditions such as obesity, hypertension and immunological disorders, mainly due to clustering of genes resulting from the population being established by a relatively small number of individuals. Some conditions in many founder populations often can occur with an increased frequency which suggests that from a genetic perspective, the extent of linkage disequilibrium in Newfoundland reflects the fact that the ancestry of any given child may only be separated by a few meiotic events. It is reasonable to believe that the probability for finding candidate genes in such populations is much higher.

Particularly, the population of Newfoundland is considered to be of significant use for study, even when compared with other isolated populations (Rahman *et al.*, 2003). It is a very young founder population, where even smaller pockets of genetic isolates exist in the outports of the province. In addition, extended linkage disequilibrium in the Newfoundland population have

been noted. This may reflect complex disease alleles that are relatively high in frequency. Since they have a higher frequency, they tend to persist for a long time (Rahman *et al.*, 2003).

## 1.1 Adiponectin as a Candidate Gene

### *1.1.1 Discovery of Adiponectin*

Adiponectin (i.e. AdipoQ, Adipose Most Abundant Gene Transcript 1, Acrp 30) is a hormone discovered by four different groups at very close periods of time independently. The first group to identify the hormone, Scherer, Williams, Fogliano, Baldini and Lodish (1995), discovered it by creating a full-length cDNA library template by mRNA from 3T3-L1 adipocytes at day 8 of differentiation and screening the library with a digoxyegenin-labeled cDNA fragment. The group found a novel protein, Acrp30, that was exclusively made in adipose tissue and secreted in the serum, and like previously discovered adipocytokines, its secretion was enhanced several fold by insulin (Scherer, Williams, Fogliano,Baldini, & Lodish, 1995).

Several months later in May 1996, Hu, Liang and Spiegelman. (1996), using an mRNA differential display technique, discovered a novel protein expressed exclusively by mature adipose cells AdipoQ. cDNA for the molecule encodes a 247 amino acid polypeptide with a signal sequence at the amino terminus, a collagenous region (Gly-XY repeats), and a globular domain that shares sequence homology with subunits of complement factor C1q, collagen ∀1(X), and brain-specific factor cerebellin (Hu *et al.*, 1996). A third group isolated adiponectin independently from human plasma as gelatin-binding protein 28 (Nakano,Tobe, Choi-Miura, Mazda, & Tomita., 1996).

A fourth group also discovered the molecule at around the same time. Maeda *et al.* (1996) constructed a 3'-directed cDNA library from abdominal subcutaneous and visceral fat

from two female patients, and transfected them into *Escherichia coli*. A partial cDNA sequence, GS3109 was the most abundant transcript in expression profiles of adipose tissue. The group isolated a 4517 BP long clone with a 244-amino acid open reading frame, flowed by a long 3'-untranslated region. As with Hu *et al.* (1996), sequencing exposed a putative signal sequence at the amino-terminus with no transmembrane domains. In addition, short collagen-like motif GXY repeats were located immediately downstream of a short N-terminal non-collagenous sequence. In concordance with the other groups, BLAST database searches showed a cysteine residue within the short non-collagenous segment preceding the collagen-like domain that bears significant sequence similarity with collagen X, VIII and complement protein C1q. However, this domain was a little shorter than other collagen-like domains. Maeda *et al.* (1996) further detected a 4.5kb apM1 mRNA in the adipose tissue, but not in any other tissue (i.e. skeletal muscle, small intestine, placenta, uterus, ovary, kidney, liver, lung brain or heart). A fourth group isolated adiponectin independently from human plasma as gelatin-binding protein 28 (Nakano, Tobe, Choi-Miura, Mazda, & Tomita, 1996).

All four groups found similar results for the novel protein which was later labelled adiponectin. Expression of adiponectin is highly regulated during adipose cell maturation and differentiation and is restricted to adipose tissue *in vivo* (Scherer *et al.*, 1995; Hu *et al.*, 1996; Maeda *et al.*, 1996). Hu *et al.* (1996) showed expression of both 3T3-F442A and 3T3-L1 preadipocytes but not in fibroblastic 3T3-C2 cells. Furthermore, expression of Adiponectin mRNA was found to be significantly reduced in adipose tissue from obese mice and humans (Hu *et al.* 1996).

### *1.1.2 Structure and Genetic Location of Adiponectin*

Adiponectin is encoded on chromosome 3q27, a Type 2 diabetes and metabolic syndrome susceptibility locus. The gene consists of 3 exons and 2 introns occupying a 17-kb region on chromosome 3 (Stumvoll *et al.*, 2002). The protein contains 244 amino acids, a signal peptide, a collagen-like domain at its N-terminus and a globular domain at its C-terminus. The globular domain shares sequence similarities with collagens X, VIII and C1q (Okamoto, Kihara, Funahashi, Matsuzawa, & Libby, 2006; Hu *et al.*, 1996). In plasma, the adipocytokine exists in very high levels (i.e. 3-30:g/ml). In addition, Adiponectin exists in three main different forms: 1) trimers, 2) hexamers, and 3) high-molecular-mass form. Some research also identified a smaller form of adiponectin with globular domain which exists in smaller amounts that cleaves proteolytically from the full-length adiponectin (Fruebis *et al.*, 2001).

### *1.1.3 Adiponectin and Body Fat composition*

Many animal studies have been conducted to delineate the relationship between adiponectin and body fat composition. Among the first groups to identify a relationship between adiponectin and body fat composition was Arita *et al.* (1999). Adiponectin levels were measured in 87 non-obese subjects and 57 obese subjects and found to be significantly lower in obese subjects as compared with non-obese individuals (Arita *et al.*, 1999). A strong negative correlation was noted with adiponectin and body mass indices in both genders (Arita *et al.*, 1999).

Berk *et al.*, (2005), measured changes in adiponectin levels, along with insulin, leptin and glucose, in response to high-fat and low-fat eucaloric diets in 11 obese and 10 lean subjects. Although leptin, insulin and glucose levels were found to be significantly higher in obese groups

when compared with the lean subjects, adiponectin levels were found to be lower (Berk *et al.*, 2005). Insulin-resistant subjects in the obese group were found to have significantly higher adiponectin levels than did the insulin-resistant lean subjects (Berk *et al.*, 2005).

A cohort study of 202 Japanese workers participating in an annual health check revealed subjects with plasma adiponectin levels below 4.0ug/ml had significantly lower HDL-cholesterol levels, higher levels of BMI, systolic and diastolic blood pressure, fasting blood glucose, platelets and total cholesterol as compared with subjects with higher adiponectin levels (Tsukinoki, Morimoto, & Nakayama 2005). The same low-adiponectin levels were also found to be associated with smoking status, a daily diet rich in deep yellow vegetables, eating out, and physical exercise two or more times per week (Tsukinoki *et al.*, 2005).

The relationship between adiponectin concentrations and insulin resistance and body fat composition was assessed in 102 Korean type-2 diabetic subjects and compared with 50 controls (Kim *et al.*, 2005). Post a 12-hour fast, adiponectin levels were found to be significantly lower in men as compared with women. They were also found to be negatively correlated with hip circumference, BMI, HOMA-IR and fasting glucose concentrations, but positively correlated with systolic blood pressure and HDL-level (Kim *et al.*, 2005). Adiponectin was found to be significantly lower in diabetics as compared with non-diabetics, except in obese males (Kim *et al.*, 2005). Similar findings were noted in a study involving 150 South Asians, where subjects were divided into 60 non-diabetic subjects, 60 subjects with impaired glucose tolerance and 30 with diabetes (Wasim *et al.*, 2006). Adiponectin levels were found to be highest in non-diabetic subjects as compared with the other groups, and lowest in those with diabetes (Wasim et al., 2006). An inverse relationship was noted between log adiponectin and resistin levels (Wasim et al., 2006).

Further support for the inverse relationship of adiponectin with insulin, as well as its relationships with adiposity, is provided in a study of 316 men and 353 Yup'ik Inuit of southwest Alaska (Goropashnaya *et al.*, 2008). Adiponectin again was found to be negatively correlated with percentage body fat, sum of skin folds, waist circumference, triglycerides, insulin resistance, fasting insulin and leptin in both groups of men and women, whereas it was positively correlated with HDL-C levels in both sexes, and LDL-c levels in women (Goropashnaya *et al.*, 2008). In addition, insulin sensitive individuals had higher plasma adiponectin concentrations than insulin resistant individuals (Goropashnaya *et al.*, 2008). However, no difference was noted between the Inuit and Caucasian groups (Goropashnaya *et al.*, 2008).

The relationship of adiponectin and insulin has also been noted in children. Nishimura *et al.* (2009) conducted a 3-year prospective cohort study of 268 Japanese boys and 251 Japanese girls aged 9-10 followed over a 3-year period. A significant strong correlation was noted with adiponectin and BMI in both boys and girls before and after the 3-year period of follow-up (Nishimura *et al.*, 2009). More specifically, adiponectin levels decreased in subjects whose BMI increased during the period in which they were followed (Nishimura *et al.*, 2009).

Supportive findings also arose when studying adiponectin levels in pubertal ballet dancers (Donoso *et al.*, 2010). In a prospective follow-up study, 22 female Caucasian ballet dancers of Tanner stage II development were followed throughout puberty. Energy intake in these individuals was deficient according to the physical exercise exerted. These girls were found to have elevated levels of adiponectin throughout puberty, while their percentage body fat and mass was decreased overall (Donoso *et al.*, 2010).

Elevated adiponectin levels associated with increased risk of total and cardiovascular mortality in subjects greater than 69 years of age (Poehls *et al.*, 2008). 3,075 well-functioning

adults aged between 69 and 79 were assessed at baseline and followed for an approximate 6-year period (Poehls *et al.*, 2008). After adjusting for age, sex, hypertension, diabetes, prevalent heart disease, smoking, weight-loss and race, adiponectin remained associated with an increased risk of both total and cardiovascular mortality (Poehls *et al.*, 2008).

Other subject groups were noted to have associated elevated adiponectin levels. Serum adiponectin levels were found to be significantly lower in individuals with hepatocellular carcinoma and liver cirrhosis as compared with healthy controls, whereas patients with chronic hepatitis B Virus infection were found to have similar levels (Liu *et al.*, 2008).

The exact role that adiponectin plays in fat metabolism and metabolic syndrome is still not completely understood. However, its inverse relationship with insulin and with body fat composition has been clearly demonstrated in many studies which seems to add further support to that role.

### *1.1.4 Pathophysiology of Adiponectin*

The complete actions of adiponectin have not been completely described, but much was discovered in terms of potential roles it plays in the body. Given its structure resembles that of other fibrillar collagen molecules, it is believed it may interact with other matrix component proteins, particularly in arteries since the molecule was shown to be associated with many cardiovascular conditions that are often major outcomes in the pathology of the metabolic syndrome (Okamoto *et al.*, 2006). Among these conditions, the role of adiponectin in atherosclerosis was examined. During the early stages of atherosclerosis development, injured endothelial cells release factors that induce circulating monocytes chemotactically to infiltrate the subendothelial space, leading to their differentiation into macrophages (Ouchi *et al.*, 2001).

These macrophages phagocytose modified LDL-c molecules in the injured area and transformed their appearance to foam cells. This scavenging process is aided by the use of two main receptors: the class A and B macrophage scavenging receptors (MSR). Ouchi *et al.* (2001) studied the effects of adiponectin on both the macrophages involved in atherosclerosis and the expression of MSR. Treatment of macrophages with adiponectin for three days significantly reduced their cholesteryl-ester contents by ~50% as compared with the untreated sample. In addition, adiponectin reduced the macrophage to foam cell transition *in vitro*. Furthermore, two days of adiponectin treatment markedly reduced class A MSR protein expression in a dose-dependent manner (Ouchi *et al.* 2001). In a previous study, adiponectin was found to inhibit specifically the I6B- α -NF-6B pathway which is normally stimulated by TNF- α. It was specifically shown to prevent I6B- α from being phosphorylated (Ouchi *et al.*, 2000). Continued immunostaining investigations showed in addition to the suppressed expression of MSR protein, adiponectin was abundant in the endothelium and subendothelial space, but it was not detected in the subendothelial space of atherosclerotic lesions with an intact endothelium (Ouchi *et al.*, 2001).

In the development of atherosclerosis, vascular smooth muscle cell proliferation is a key step. This can often lead to hypertonicity and a resulting decreased arteriole lumen size. In culture, the migration and proliferation of these cells have been shown to be suppressed with the addition of adiponectin through an effect on the direct binding to platelet-derived-growth-factor-BB (Arita *et al.*, 2002). Specifically, plasma adiponectin suppressed PDGF-BB–induced ERK phosphorylation (Arita *et al.*, 2002). It also inhibits growth-factor-stimulated extra-cellular-signal-regulated kinase, further suggesting that adiponectin is a modulator of atherosclerosis through anti-inflammatory and anti-proliferative means (Arita *et al.*, 2002).

Adiponectin is also believed to interact with other key molecules such as interleukin-10 (IL-10). Manigrasso *et al.* (2005) conducted a short-term weight loss program and monitored its effect on circulating adiponectin and IL-10 levels in 15 android obese women. They observed that baseline IL-10 levels were significantly correlated with adiponectin concentrations in android, but not gyenoid in obese women. The effects of short-term weight loss resulted however in no significant change in either adiponectin or IL-10 (Manigrasso *et al.*, 2005). Kobayahsi *et al.* (2004) assessed the three isoforms of adiponectin found in human blood to determine if any changes in distribution occurred in subjects with significant weight reduction, or in subjects that were determined to be obese, non-obese and with coronary artery disease. The high molecular weight form of adiponectin was significantly increased by those who had significant weight reduction, whereas the trimer and hexamer forms significantly declined (Kobayahsi *et al.*, 2004). However, HMW form was decreased in CAD patients, while the trimer isoform increased and the hexamer isoform showed no change. Moreover, after examining the effects of unfractionated, recombinant adiponectin on Human Umbilical Vein endothelial cells that were death induced by mitogen-deprivation, they found that adiponectin decreased the mitogen-induced apoptosis in a dose dependent manner (Kobayashi *et al.*, 2004). Furthermore, the HMW isoform was found to be mainly responsible for the apoptotic effect.

Adiponectin has been shown to be a key regulator of vascular vasomotor function (Okamoto *et al.*, 2006). After analyzing the endothelial function in 202 hypertensive patients, including those 58 not taking medication, Ouchi *et al.* (2003) noted that plasma adiponectin levels significantly correlated with the endothelium-dependent vasodilatation as a result of forearm vasodilatory response to reactive hyperemia. This was found in both the entire group and in the non-medication group. Furthermore, in adiponectin-KO mice which were fed high-

fat/high-sucrose/high-salt diets for four weeks, Ach-induced vasorelaxation was significantly reduced compared with wildtype mice (Ouchi *et al.*, 2003).

Other research suggested close links between hypoadiponectinemia and endothelial dysfunction in men. Forearm blood flow was measured during reactive hyperemia using stain-gauge plethysmography to assess resistant vessel endothelial function physiologically (Shimabukuro *et al.* 2003). Peak forearm blood flow was used as a combination marker of shear stress and local metabolic factors at an early phase of reactive hyperemia, while flow debt repayment marked NO-dependant levels at mid-to-late phase of reactive hyperemia (Shimabukuro *et al.* 2003). Impaired forearm blood flow response and flow debt repayment were correlated with low levels of serum adiponectin (Shimabukuro *et al.* 2003).

Hypoadiponectemia being associated with impaired endothelium-dependent vasodilatation was further confirmed by Tan *et al.* (2004). In a similar study for 73 patients with type 2 diabetes mellitus assessed for endothelium-dependant vasodilatation by high resolution ultrasound, plasma adiponectin levels correlated significantly with endothelium-dependent vasodilatation in both type 2 diabetics and controls, whereas no link showed endothelium-independent vasodilatation (Tan *et al.* 2004). Furthermore, general linear model univariate analysis demonstrated that brachial artery diameter, plasma HDL and adiponectin levels were the main independent determinants of endothelial function.

Another study showed in a similar fashion how circulating adiponectin concentration was significantly associated with insulin sensitivity and fasting serum TG in healthy individuals. This suggested that serum adiponectin concentration is significantly associated with vascular function in healthy people (Fernandez-Real *et al.*, 2004).

In another cross-sectional study, Shetty, Economides, Horton, Mantzoros, & Veves,

(2004) conducted an investigation on the associations between adiponectin and resistin with inflammatory markers, hyperlipidemia and vascular reactivity. They recruited seventy-seven individuals with Type 2 diabetes, who were without any major complications, or who were believed to be at high risk for developing Type 2 diabetes. Adiponectin negatively correlated with tPA, CRP, TG and BMI, but positively correlated with HDL-c, as in previous studies (Shetty *et al.*, 2004). These findings were independent of sex. As a supplemental interventional study, the group was given in a randomized fashion 20mg of atorvastatin or placebo over a 12 week period. However, no association was found between atorvastatin treatment and adiponectin (Shetty *et al.*, 2004).

In almost all studies adiponectin in adults showed it was a predictor of insulin resistance, endothelial function, coronary artery disease, and Type 2 diabetes. Singhal *et al.*, (2005) conducted a similar analysis of insulin resistance, cardiovascular risk factors and endothelial function in young, healthy adolescents. They studied 294 non-smoking adolescents from ages 13-16. Consistent with previous research, adiponectin was significantly associated with HDL-c concentration, but not CRP or blood pressure (Singhal *et al.*, 2005). Insulin resistance was inversely related to adiponectin concentration, but endothelial function was not found to be associated with adiponectin distribution as had been previously demonstrated in adults (Singhal *et al.*, 2005). However, Matsubara *et al.* (2003) took 384 blood samples from a previous cross-sectional study of women residing in Hokkaido, Japan to compare both plasma adiponectin and CRP. Unlike Singhal *et al.* (2004), they found plasma adiponectin and CRP levels in their female subjects to be significantly inversely correlated.

In terms of actual weight reduction and changes in physical appearance, adiponectin has been shown to be significantly associated with changes in BMI, waist and hip circumference

(Yang *et al.*, 2002). Yang *et al.* (2002) observed the plasma adiponectin level changes in 22 individuals that underwent gastric partition surgery. They noted a significant increase of 46% in levels of plasma adiponectin when compared with a 21% drop in BMI. They further noted that changes in plasma adiponectin levels were significantly correlated with changes in plasma adiponectin levels.

Animal models demonstrated other roles for adiponectin. In a study using adiponectin-KO mice, it was found that cardiac hypertrophy resulting from pressure overload increased when adiponectin was deficient. However, when adiponectin was injected via adenovirus, the degree of cardiac hypertrophy lessened (Shibata *et al.*, 2004), which suggests that it may have a role in treatment of conditions leading to hypertrophic cardiomyopathy.

In obese KK-Ay mice, the effects of soy protein diet on body fat composition, plasma glucose, lipid and adiponectin levels and gene expression of those involved in glucose and fatty acid metabolism was assessed (Nagasawa *et al.*, 2002). They found a significant difference in adiponectin gene expression between rats on a soy protein diet and those on a casein-restricted diet (Nagasawa *et al.*, 2002). Adipose adiponectin mRNA expression and plasma adiponectin levels were significantly higher in those rats that were calorie-restricted than controls (Nagasawa *et al.*, 2002). Similarly, adiponectin has been shown to stimulate glucose utilization, fatty acid production, and lactose production in myocytes through the activation of 5'-AMP-activated protein kinase (Yamauchi *et al.*, 2002). Furthermore, blocking AMPK inhibited these effects, suggesting that adiponectin conducted these actions through AMPK (Yamauchi *et al.*, 2006).

Another study also has shown that diet may affect plasma levels of adiponectin. Serum concentrations of adiponectin increased significantly in sixty obese women aged 20-46 years without diabetes, hypertension and hyperlipidemia after two years on a Mediterranean-style diet

and increased physical activity, (Esposito *et al.*, 2006).

### *1.1.5 Adiponectin and Coronary Artery Disease*

Although the initial discovery of adiponectin was about 10 years ago, its role in the metabolic syndrome was recently explored. Arita *et al.,* (1999) was among one of the first groups to assess the plasma levels of adiponectin in obese individuals. Adiponectin was significantly lower in 57 obese subjects than in 87 non-obese individuals. This was contrary to the initial hypothesis since adiponectin is exclusively released by adipose cells. Plasma adiponectin levels and body mass indices in both men and women had a strong negative correlation between them. In addition, plasma adiponectin levels in men were significantly lower than in women among non-obese and obese subjects. However, plasma adiponectin showed no correlation with age when adjusted for BMI.

Adiponectin has been further studied in atherosclerotic cardiovascular diseases. Hotta *et al., (*2000), analyzed the plasma adiponectin levels in 183 age and BMI matched Type 2 diabetics (with and without coronary artery disease) and non-diabetic subjects. Patients with defined diabetes were shown to have significantly lower plasma adiponectin levels than controlled non-diabetic women and men when adjusted for age and BMI. The diabetics were further subdivided into two groups: subjects with CAD, and subjects without CAD (Hotta *et al.*, 2000). Diabetic women without CAD had significantly lower adiponectin levels than non-diabetic women without CAD. Plasma adiponectin concentrations were even lower in women with CAD. Adiponectin levels were significantly lower in female and male patients with both CAD and Type 2 diabetes than in those with diabetes alone (Hotta *et al.*, 2000). Furthermore, a reduction in BMI in 13 non-diabetic subjects and 9 diabetics showed plasma adiponectin levels

were significantly increased in both groups. However, Hotta *et al.,* (2000) found no significant changes when assessing the adiponectin levels when comparing men or women factoring for age, BMI status, value of haemoglobin A1c, and other parameters including triglycerides and plasma insulin levels.

Similarly, Kumada *et al.,* (2003) determined whether hypoadiponectinemia was independently associated with CAD. 225 Japanese male patients undergoing coronary angiography age 40 to 69 years with a greater than 75% stenosis of at least one coronary artery (confirmed by angiogram), who had developed MI or who had a previous percutaneous transluminal coronary angioplasty or CABG, had venous blood drawn and analyzed by ELISA for plasma adiponectin levels. Males with hypoadiponectinemia had an increased two-fold prevalence of coronary artery disease shown through multiple logistic regression analysis, independent of diabetes mellitus, dyslipidemia, hypertension, smoking and BMI (Kumada *et al.*, 2003). In addition, as was previously shown, plasma adiponectin levels in CAD patients were significantly lower as compared with controlled subjects.

Other studies have linked plasma adiponectin concentration with cardiovascular events. Mallamaci *et al.* (2002) researched the relationship of plasma adiponectin with BMI, fat mass, and renal function in 36 hypertensive patients as compared with 31 normotensive individuals. The group showed plasma adiponectin levels were higher in hypertensive patients (not enough to reach significance) when compared with normotensive subjects. However, when separated by sex, hypertensive males showed significantly higher levels of plasma adiponectin than normotensive males; women demonstrated no significance (Mallamaci *et al.*, 2002). In terms of kidney function, plasma adiponectin was inversely related to creatinine clearance, and was further confirmed by multiple regression analysis where creatinine clearance was the principle

predictor of plasma adiponectin. Healthy subjects showed a direct relationship with serum HDL and plasma adiponectin, while it was inversely related to serum TG.

Connections between adiponectin and MI have also been made. Pischon *et al.*, (2004) assessed the risk of MI with plasma adiponectin concentrations. In the Nested control case-study containing 18,225 participants, 266 individuals were identified as having an incident of a nonfatal MI or fatal coronary heart disease. While adjusting for age, they noted that adiponectin levels were positively correlated with HDL-c, and physical activity. However, it was negatively correlated with TG, CRP (c-reactive protein; acute-phase reactant and a powerful marker for systemic inflammation), HbA1c and BMI (Pischon *et al.*, 2004). Furthermore, their data showed that high plasma adiponectin concentration was associated with lower risk of myocardial infarct in healthy men after a six year follow up (Pischon *et al.*, 2004). Some case controlled studies examined plasma adiponectin levels in patients with an acute MI and acute coronary syndrome. Thirty-four patients with acute MI were compared with 35 individuals with atypical chest pain at rest or with exercise but with no significant artery stenosis (Kojima *et al.*, 2003). Patients presenting with an acute MI were found to have higher adiponectin levels as compared with controls. In addition, sequential analysis after from 24 hours, three days, seven days, and four weeks after the event showed adiponectin levels dropped significantly 24 hours and three days after the acute MI when compared with admission day levels. However, levels returned to admission day levels on day seven and four weeks later (Kojima *et al.*, 2003). A similar study supported the finds and further noticed that individuals with unstable angina showed significantly lower plasma concentrations of adiponectin (Nakamura *et al.*, 2004). The study additionally showed that adiponectin concentrations in plasma were significantly lower than those in patients with stable angina.

Negative associations in other populations have been shown when comparing plasma adiponectin levels and the future risk of coronary heart disease. Using cases and controls from the case-controlled selected subjects in the strong heart study on American Indians (population at high risk of obesity and Type 2 diabetes and high risk of CHD), plasma adiponectin levels showed no significant association with coronary heart disease (Lindsay *et al.*, 2006). Similarly, 70 Pima Indians who developed Type 2 diabetes were examined and were shown to have significantly lowered plasma adiponectin levels as compared with 70 controls from the same population (Lindsay *et al.*, 2006). These low concentrations of plasma adiponectin correlated strongly with lowered insulin sensitivity, and those with higher adiponectin levels were less likely to develop Type 2 diabetes (Lindsay *et al.*, 2006).

Hypertension has also been linked with variations in adiponectin plasma concentrations. In a study by Adamczak *et al.,* (2003), 33 essential hypertension patients (21 men, 12 women) were compared with 33 normotensive BMI-matched healthy subjects (20 men, 13 women). Essential hypertensive subjects had significantly lower plasma adiponectin levels than normotensive individuals, regardless of gender. Furthermore, plasma adiponectin concentrations were negatively correlated with Mean arterial pressure, systolic pressure, and diastolic pressure (Adamczak *et al.*, 2003).

### *1.1.6 Genetic Associations of Adiponectin*

Much research showed an association between adiponectin and various genetic polymorphisms. In Japanese population, Kondo *et al.* (2002) compared 218 Type 2 diabetics or coronary artery disease patients with 452 age- and BMI-matched non-diabetic control subjects for mutations in the adiponectin gene. The group assessed five mis-sense mutations in the

adiponectin gene: a R112C mis-sense mutation, a G/T polymorphism at position 94, a T-C substitution at nucleotide 517 leading to an amino acid substitution from isoleucine to threonine at position 164, a C-A substitution at nucleotide 687 leading to an arginine-serine substitution at position 221, and an A-C substitution at nucleotide 748 causing a histidine to proline substitution at position 241. No mutations were found in intronic sequences, which suggest there was no mutation to produce alternative splicing for the adiponectin gene (Kondo *et al.*, 2002). Patients carrying the I164T mutation had markedly low plasma adiponectin concentrations as compared with those with other mis-sense mutations. In addition, plasma adiponectin levels in volunteers with the I164T mutation were significantly lower compared to those of the 209 diabetics without mutations. This suggested hypoadiponectinemia was associated with an I164T mutation and not as a consequence of Type 2 diabetes or insulin resistance (Kondo *et al.*, 2002). Previous studies have reported subjects with the R112C mutation as having low plasma adiponectin concentrations, which was further confirmed by Kondo *et al.*, (2002); Takahashi *et al.*, 2000). The current study showed even heterozygotes for the I164T and R112C mutations had significantly lowered plasma concentrations (Kondo *et al.*, 2002). It was later shown that the I164T variant displayed impaired adiponectin secretion from adipose tissue (Okamoto, Kihara, Funahashi, Matsuzawa, & Libby, 2006). The T/G polymorphism at nucleotide 94 is believed to affect plasma adiponectin levels (Stumvoll *et al.*, 2002). Even though studies have shown no statistical significance associated between the polymorphism and plasma adiponectin levels, the G allele has been shown in a Japanese population to decrease adiponectin concentrations in a dose dependent manner (Takahashi *et al.*, 2000). More SNPs were discovered to be associated with Type 2 diabetes, including SNPs at position 45 and 276 (Hara *et al.*, 2006). Some mutations are associated with reduced formation of adiponectin trimers or high-molecular mass

or high-molecular mass multimers, which may affect the action of adiponectin (Waki *et al.*, 2003).

The frequency of the I164T mutation in coronary artery disease patients was significantly higher than in non-CAD patients (Ohashi *et al.*, 2004). In addition, all subjects found to date with the mutation are heterozygotes. Plasma adiponectin levels were low in both coronary artery disease patients and non-CAD patients with the mutation. Ohashi *et al.,* (2004) looked at other single nucleotide polymorphisms in the adiponectin gene, including G/G, G/T and T/T alleles at SNP94 and G/G, G/T and T/T alleles at SNP276; no significant changes were observed.

However, polymorphisms of SNP94 in subjects of the Tubingen Family study did show an association between T/G polymorphisms in Exon 2 (Stumvoll *et al.*, 2002). Unlike the polymorphisms encountered resulting in mis-sense mutations, the T/G exchange in nucleotide 94 shows a much higher prevalence (~25%). Overall, Exon 2 seems to be more polymorphic than Exon 3. 371 non-diabetic subjects (with no family history of type 2 diabetes) that underwent oral glucose tolerance tests showed large BMIs and percentage body fat levels associated with the GG + GT genotypes as opposed to the TT genotype (Stumvoll *et al,* 2002). Furthermore, insulin sensitivity was significantly lower in the GG + GT genotypes as compared with TT. This suggests that the T/G polymorphism in non-type 2 diabetes individuals may mildly increase obesity risk and insulin-resistance (Stumvoll *et al.,* 2002).

Similarly, SNPs 45 and 276 of the adiponectin gene have also been linked with Type 2 diabetes and the metabolic syndrome. Hara *et al.* (2002) detected ten relatively frequent SNPs in the adiponectin gene in both a population of French and Japanese subjects. Of these SNPs, the G/G genotype at position 276 was shown to have an increased risk of type 2 diabetes compared with the T/T genotype. Those with the G/T or G/G genotype at position 45 also showed a

significantly increased risk of type 2 diabetes (Hara *et al.* 2002). In addition, SNPs at position 4034, 3964, and 276 were found to be significantly associated with HOMA-IR. Furthermore, plasma adiponectin levels tended to be lower in subjects with the G allele (Hara *et al.*, 2002).

The same two SNPs were also assessed by Gonzalez-Sanchez *et al.*, (2005) in 747 unrelated Spanish subjects with associated symptoms of the metabolic syndrome. They noted carriers of the G allele at nucleotide 45 had higher odds ratios for impaired glucose tolerance as compared with non-carriers. Similarly, the G/G genotype at position 276 also showed a higher odds ratio for impaired glucose tolerance as compared with the T allele (T/T + G/T; Gonzalez-Sanchez *et al.*, 2005). The group also noted both SNPs are in linkage disequilibrium. In addition, after measuring the serum adiponectin levels in 721 subjects of their cohort, adiponectin levels were higher in women than in men, lower in homozygous carriers of the G allele than in carriers of the G/T or T/T genotypes, and significantly lower in obese subjects carrying the G/G genotype as compared with both heterozygous and T/T homozygotes (Gonzalez-Sanchez *et al.*, 2005). Overall, in both SNPs, the GG haplotype was associated with lower serum adiponectin levels as compared with TT haplotypes.

## 1.2 Adiponectin and Osteoporosis

### *1.2.1 Definition and Diagnosis of Osteoporosis*

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an individual to an increased risk of fracture (Glaser & Kaplan, 1997). The current definition of osteoporosis, although still criticised and debated, is still the same definition which the World Health Organization proposed in 1994. Since then osteoporosis and osteopenia have been defined in terms of the patient's bone mineral density in specific bone sites

around the body at a given age and gender as compared with the general population (Knapp, Blake, Spector & Fogelman, 2004). Specifically, osteoporosis and osteopenia were expressed as standard deviation units called T-scores, with T-score thresholds equal to or less than -2.5 SD and -1.0 SD for osteoporosis and osteopenia respectively. These scores would be calculated by comparing one individual's bone mineral density, usually calculated by dual-X-Ray-absorptiometry (DEXA), with that of a similar population data set. These scans would be taken typically from the highest risk fracture areas in the body and compared with the general population. Specifically, the spine, the hip or the radius are usual measurement areas, with the spine or the hip being most frequently measured (Knapp, Blake, spector, & Fogelman, 2003). This current definition of osteoporosis requires no previous history of fragility fractures for an individual to be diagnosed with the disease. It only serves to identify individuals at high risk for fracture from low-energy-impacts.

In classifying an individual with osteoporosis, variations can occur based on the standards at each center, the methods used, and differences in equipment and population. Goemaere, Zmierczak, Van Pottelberg, and Kaufman (2002) noted that the current working definition for osteoporosis has only been validated in white postmenopausal women. There has been criticism of the current definition of osteoporosis because of its lack of specificity for diagnosing males. Multiple studies note that there is little consensus of using BMD threshold to be applied clinically for identifying men at high risk of fracture, particularly when using DEXA (Goemaere *et al.*, 2002; Seeman, 1999). DEXA use in men has shown low sensitivity, with 80% of men with fractures occurring at BMD-T scores greater than -2.5 (Szulc, Munoz, Duboeuf, Marchand, & Delmas, 2005). As a result, although the T-score approach has shown strong sensitivity and specificity in postmenopausal women, it has been suggested that the diagnosis of

osteoporosis in men should be an integration of clinical history and BMD measurement (Kaufman & Goemaere, 2008). The risk factors of importance on clinical history include previous fracture prior to age 50, elder age, incidence of falls, history of endogenous or exogenous glucocorticoids, hypergonadism, alcoholism, liver disease and family history (Kaufman & Goemaere, 2008). The defined measures for bone mineral density can be found in Table 2.

Table 2. Defining measurements for bone mineral density as defined by WHO.

| | |
|---|---|
| Normal bone density | T score greater than -1 |
| Osteopenia | T score between -1 and -2.5 |
| Osteoporosis | T score less than -2.5 |
| Severe (Established) osteoporosis | T score less than -2.5 with an established fracture |

### *1.2.2 Prevalence of Osteoporosis*

The prevalence of osteoporosis varies in each race and with the methods of calculation. In the United States, one article quotes the rate at approximately 24 million, with 15-20 million of them being women above age 45 (Iqbal, 2000). The rate has been quoted between 10-20% overall. In Denmark, a recent study aiming to assess the number of individuals diagnosed with osteoporosis and osteoporotic fractures found that the estimated prevalence of osteoporosis was 40.8% of women aged greater than or equal to 50 years and 17.7% among men greater than or equal to 50 years old (Vestergaard, Rejnmark, & Mosekilde, 2005). A study from the Netherlands assessing time trends of change for the incidence of osteoporosis in the country, noted the initial increase of incidence in osteoporosis observed in the mid-nineties has been levelling off (Goettsch, deJong, Kramarz, & Herings, 2007). Interestingly, in a paper hosted by the Canadian Association of Orthopaedic Surgeons, the worldwide prevalence of osteoporosis in individuals over 40 was 1-in-3 for women and 1-in-5 for men, with the incidence increasing with age (Guy, 2007; Kanis & Kanis, 1994; Kanis, Melton, Christiansen, Johnston, & Khaltaev, 1994). This increasing incidence has been reported by WHO working group on osteoporosis (Figure 1).

Currently, the gold standard for the diagnosis of low bone mineral density is made by using Dual-X-ray Absorptiometry (DXA; Figure 2). It can be made by radiograph when fractures or structural abnormalities are present (Chun, 2011). However, this is not effective in detecting low-bone mineral density prior to the presentation of clinical findings. In addition, unlike DXA, radiographs fail to take into account demographic factors such as age and gender that influence overall bone mineral density (Chun, 2011). DXA incorporates data obtained from the NHANES III study (National Health and Nutrition Examination Survey), for comparison of

bone-mineral density, to calculated individuals of same age, gender and ethnicity, whose bone mineral density is two-standard deviations below the mean (Chun, 2011).

DXA evolved from the development of single-photon and dual photon absorptiometry. However, unlike the its predecessors, DXA uses a low-energy X-ray beam, high-photon flux that permits faster scanning, improved resolution and image quality (Chun, 2011). As explained in a review by Chun (2011), DXA uses highly collimated beams that pass through soft tissue and bone and are absorbed by a detector on the opposite side of the emitter. Bone-mineral density is calculated by comparison of the intensity of a beam of an excited body part with the intensity of photons of known density. A schematic diagram of how DXA works is displayed in Figure 2, adapted from Chun, 2011.

There is still considerable debate as to the areas scanned that are best assessed to determine osteoporosis. As a result, most centers evaluate at risk sites to determine long-term fracture risk. These sites mainly include hip and the posterior-anterior spine.

The main criticism of DXA is that it only provides an individual with an estimate of bone-mineral density from a 2-dimensional view, such that it may over or under estimate a person's osteoporosis risk and subsequent fracture risk based on the thickness or width of the person's bone in the plane measured. However, as compared with other methods that have been suggested such as MRI, it is more cost-effective, effectively predictive, and still widely used.

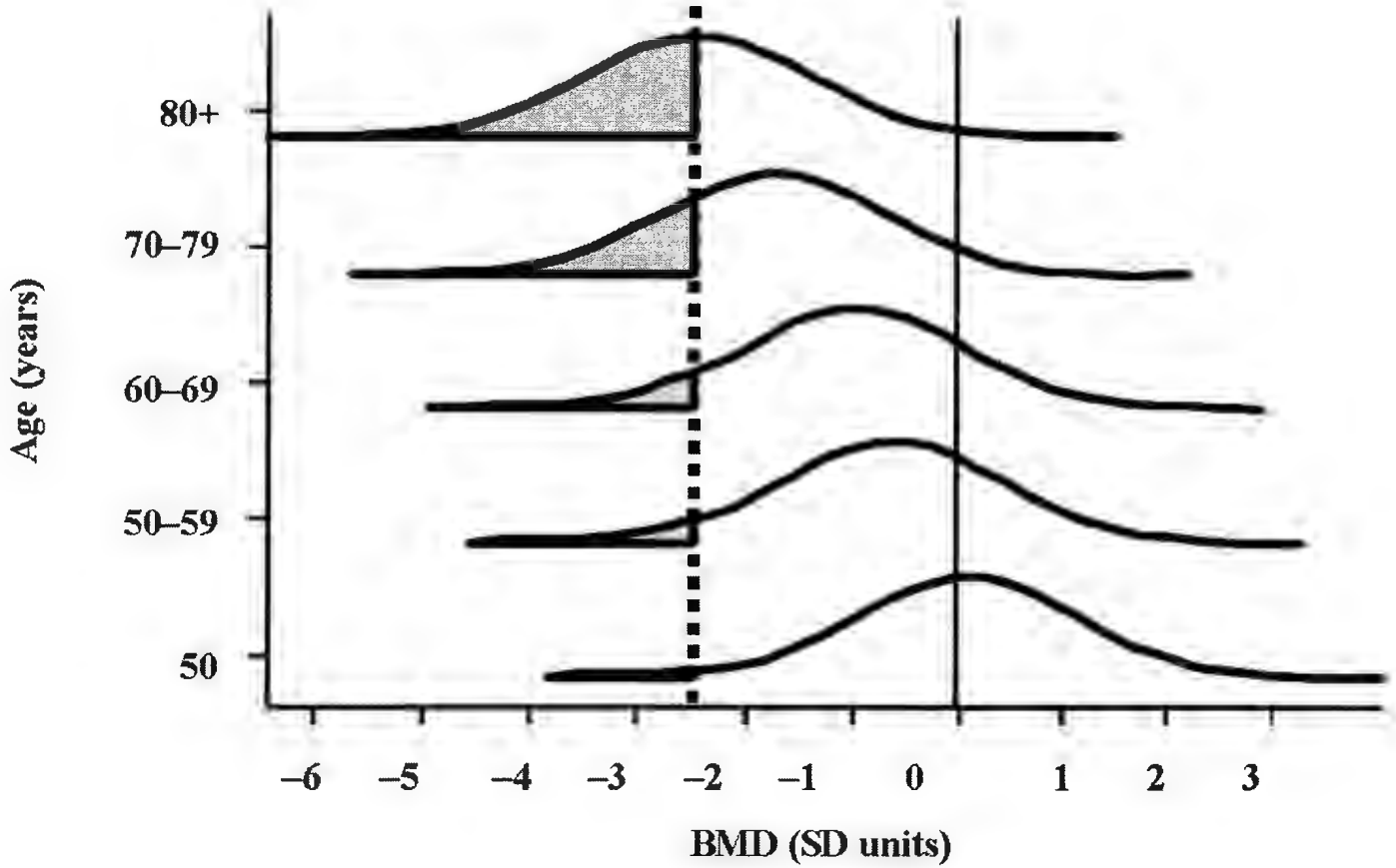


Figure 1. Image adapted from Kanis *et al.* (1994) and adopted from Guy (2007). This figure shows decreasing bone mineral density prevalence with increasing age. This graph specifically represents the bone mineral density change in women.

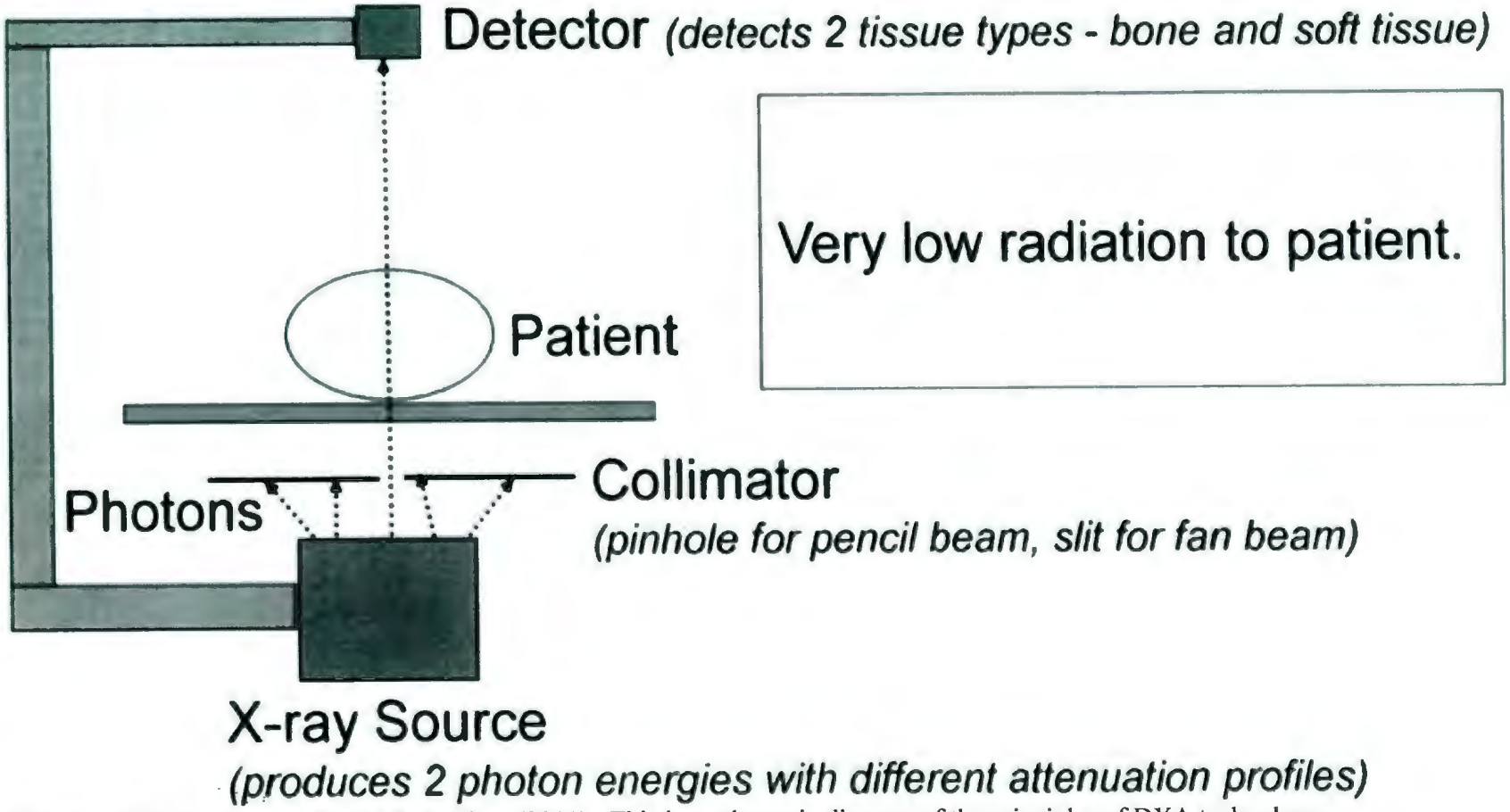


Figure 2. This image was adapted from Chun (2011). This is a schematic diagram of the principles of DXA technology.

### *1.2.3 Adiponectin and Bone Mineral Density*

Adiponectin has been shown to be associated with fat mass, atherosclerosis and anti-inflammatory molecules; however, there has been debate as to whether circulating adiponectin levels show any correlation with bone mineral density. It is uncertain if any correlations that do exist with bone mineral density and fat composition are true direct correlations or are confounded by the direct role that fat mass plays on bone mineral density. The clinically most relevant areas in terms of bone mineral density – specifically, lumbar spine, hip and femoral neck — have been inconsistent in showing a correlation with adiponectin when controlling for other variables.

In a study of 25 premenopausal and 55 postmenopausal women, adiponectin showed no significant correlation with total body bone mineral composition, with an initially negative correlation with L2-L4 bone-mineral density (BMD) (Kontogianni *et al.*, 2004). However, the negative correlation was lost after adjustment for menopausal status (Kontogianni, Dafini, Roustsias, Skopouli, 2004). In a study of 42 men and 38 women in North Carolina, where most of the women were postmenopausal, there was an inverse correlation noted between adiponectin and bone-mineral density at all skeletal sites measured including Areal BMD, L1-L4 spine, total hip, lumbar and thoracic spine, and volumetric BMD (Lenchik *et al.*, 2003). These associations held after adjusting for fat mass, age, menopausal status and other covariates (Lenchik *et al.*, 2003). Another study by Jürimäe, Rembel, Jürimäe, and Rehand, (2005) involved 21 premenopausal and 17 postmenopausal women matched for daily energy expenditure and BMI. Adiponectin showed a significant negative association with total BMD, lumbar spine BMD, independent of body composition, hormonal and physical performance factors exerted on BMD. In a follow-up study by Jürimäe and Jürimäe (2007) with 88 postmenopausal women from

Estonia, adiponectin was found to be significantly related to total bone mineral composition, lumbar spine BMD, and femoral neck BMD. The relationships remained significant when adjusting for body composition, hormonal and insulin resistance values; however, they showed no correlation when adjusted for fat-free mass (Jürimäe and Jürimäe, 2007).

Fat and body mass have been shown to be strong predictors of bone mineral density, particularly in postmenopausal women (Douchi *et al.*, 2000). In a study looking at 296 premenopausal women with regular menses and 230 postmenopausal females, Douchi and colleagues determined that the number of years since menopause, fat mass, total lean body mass and height were significant determinants of bone mineral density in post-menopausal women, whereas only lean body mass and age of menarche were determinants for premenopausal women (Douchi *et al.*, 2000). Subsequently, the group concluded that adiposity status plays a more significant role in bone mineral density in postmenopausal women than it does in premenopausal women.

Other studies have noted correlations between adiponectin levels and other measured areas of bone mineral density. There was a study which assessed adiponectin and leptin levels in 40 Japanese patients with Type 2 diabetes, looking specifically at the distal radius, lumbar spine and femoral neck measurements from DEXA. Tamura *et al.*, (2006) noted no correlations were found with adiponectin and lumbar spine or the femoral neck. However, there was a significant positive correlation found between adiponectin and leptin with the BMD of the distal radius (Tamura *et al.*, 2006). They found no significant correlation between adiponectin and osteocalcin or urine deoxypyridinoline, and other determinants of bone mineral density which were measured in the study (Tamura *et al.*, 2006).

In a large scale cohort study from the United Kingdom involving 1,735 nondiabetic women, increasing serum adiponectin levels were found to be significantly correlated with decreasing bone mineral density, even after controlling for BMI, serum leptin levels, central fat mass, hormone replacement therapy, smoking and exercise (Richards, Valdes, Burling, Perks, & Spector, 2007). In addition, as has been similarly shown by other groups (Douchi *et al.*, 2000), this correlation was strengthened in postmenopausal women, but was lost in premenopausal females (Richards *et al.*, 2007).

Several studies in rodents and human cell lines have attempted to aid in the role adiponectin may play in bone mineral density. Receptors of adiponectin have been identified on both osteoblasts and osteoclasts, further suggesting a potential role in bone mineral density regulation (Shinoda *et al.*, 2006; Bernera *et al.*, 2004). Williams *et al.* (2009), in a series of experiments on rodent and human cell lines and rodent adiponectin-knockout mice, noted an increase in mitogenic activity and proliferation in osteoblastic cell lines treated with adiponectin concentrates, whereas osteoclastic cell lines were significantly inhibited.

However, they noted that adiponectin had no affect on isolated mature osteoclastic cells, suggesting that the inhibitory process may be mediated by an intermediated cell line, possibly a stromal cell (Williams *et al.*, 2009). In addition, in the adiponectin-knockout mice, bone strength increased at all measures of testing – displacement to fracture, work to fracture and displacement to peak load (Williams *et al.*, 2009). Similar findings were previously noted by Oshima *et al.*, (2005). In an experiment involving adiponectin-adenovirus treated mice, an increase in trabecular bone growth was noted and a decrease in plasma osteoclasts were detected (Oshima *et al.*, 2005). In vitro studies showed that adiponectin treated cell lines inhibited macrophage and monocyte differentiation into osteoclasts, while mRNA expression of alkaline phosphatase

enhanced the mineralization activity of the osteoblastic cell line (Oshima *et al.*, 2005). In another study from the same group, adiponectin-deficient mice were found to have an increased bone volume at nine months of age, with normal values at three months of age (Nampei *et al.*, 2004).

These findings of the inhibitory effect of adiponectin on osteoclasts and the proliferative and increased activity on osteoblasts have been similarly replicated. Using the D clone of RAW264 (a highly osteoclastic clone cell line), Yamaguchi *et al.*, (2007) demonstrated that adiponectin inhibited osteoclastic cell formation stimulated by a specific lipopolysaccride the microbe *Actinobacillus actinomycetemcomitans*. Specifically, they showed that adiponectin inhibited the TLR4-mediated NF-kappaB activity in RAW264 cells (Yamaguchi *et al.*, 2007). Lee *et al.* (2009) demonstrated that adiponectin increased osteoblast differentiation in mesenchymal progenitor cells, showing that expression of cyclooxygenase-2 (COX-2) was potently increased by adiponectin, whereas inhibition of COX2 activity completely negated the effect adiponectin had on osteogenesis. Adiponectin has been shown to stimulate osteoblastic activity through the mitogen-activated-protein-kinase pathway and *c-jun* N-terminal kinase pathway, where inhibition of either of these two pathways completely abolishes the effect of adiponectin on osteoblastic proliferation (Luo *et al.*, 2005).

## 2.0 Materials and Methods

### *2.1 Association study*

#### *2.1.1 Recruitment of Volunteers*

The Canadian province of Newfoundland and Labrador recruited 1811 volunteers using several different means including poster distribution, personal contact, television media, and the use of bone density scans as an incentive. Interested volunteers who wished to participate in the study were allowed to do so based on the following criteria: 1) They were between the ages of 19-73; 2) each person is at least a third generation Newfoundland resident with at least one grandparent being born in Newfoundland; 3) no one was pregnant at the time of the study. If the criteria were met, each person was provided with a list of four questionnaires to be answered and returned either prior to or on the day of arrival to the laboratory. These questionnaires included a screening questionnaire, a physical activity form, a dietary form, a menstrual cycle form, and a consent form. All forms were approved by the Human Investigation Committee of Memorial University of Newfoundland, and the physical activity and dietary forms were received from the Heritage Study (Bouchard *et al.*, 1995). The screening questionnaire contained information pertaining to background, volunteer heritage, age, reported height and weight, smoking status, medication status, medical conditions, and family history. The menstrual cycle form was only given to women, which addressed the menopausal status of individuals, as well as period regularity. The overall study design was approved by the Human Ethics Committee.

### *2.1.2 Plasma and Serum Samples extraction*

Plasma and serum blood samples were taken upon arrival to the laboratory after a complete 12 hour fast. To isolate blood plasma, blood was collected in evacuated tubes containing $K_3$EDTA, preventing the blood from coagulating. The plasma samples were immediately centrifuged for 15 minutes at 1300g or stored at 4˚C until they could be centrifuged. The plasma (supernatant) was then stored at -80˚C until it was necessary for further analysis, while the remaining pellet of blood cells was stored at -20˚C.

Serum samples were isolated in tubes containing a clot activator. After collection, the tubes were left to sit at 22˚C (room temperature) for 20 minutes to allow the blood adequate time to fully clot. The tubes were then centrifuged at 25˚C for 10 minutes at 3500rpm. The serum samples were then stored at -80˚C until further analysis, with the remaining pellet being stored at -20˚C.

### *2.1.3 Body composition measurements*

Each volunteer was asked to remove all articles of clothing, particularly all metallic objects, with exception of bottom underwear that contained no metal substances, and was asked to put on a medical gown. Individuals were then weighted and measured for their height using a standard medical scale and ruler (Health-o-meter Inc., Brydgirow, IL USA). Waist and hip measurements were taken using a centimetre measuring tape, with waist being measured around the navel, and hip being measured around the individual's greater trochanter. Body composition measurements, bone mineral composition and density measurements, and muscle and fat weight and percentage measurements for arms, legs, trunk and whole body were conducted using a dual-energy X-ray absorptiometry (DEXA) Lunar prodigy system (GE Medical Systems, Madison,

WI, USA). In addition to total bone density measurements, spine and left hip measurements were conducted. During scans, subjects lay still in a supine position. Analysis of the results was conducted using Lunar prodigy software version 4.0. The classification for lean, normal, overweight and obese followed the Bray 2003 body composition classification for Caucasians. It is displayed in Table 1.

### *2.1.4 Serum biochemical measurements*

Glucose, total cholesterol, high-density lipoprotein (HDL-c), triglycerides (TGs), calcium, magnesium and albumin serum concentrations were measured using Synchron reagents performed on an Lx20 (Beckman Coulter, Inc., CA, USA). Serum insulin levels were obtained using an Immulite immunoassay analyzer (DPC, CA, USA). Low-density lipoprotein-cholesterol (LDL-c) was determined using the following equation Total cholesterol - HDL-c. - 0.45 (triglycerides).

### *2.1.5 DNA Extraction and genotyping*

#### *2.1.5.1 Genomic DNA Extraction*

Extraction of DNA was completed using the Wizard® Genomic DNA purification Kits (Promega, Madision, WI, USA) as per the company's protocol. The DNA was extracted from white blood cells. The isolation protocol involved 3 main steps: 1) Red blood cell lysis, 2) WBC Nuclei lysis and protein precipitation, and 3) DNA precipitation and rehydration. During the first step, red blood cells were lysed by mixing lysis solution obtained from the kit with blood samples of subjects. After mixing, the mixture was incubated for 10 minutes, then centrifuged for 10 minutes at 2000g, discarding the supernatant. As part of the WBC Nuclei lysis and

protein precipitation step, nuclei lysis solution was added, followed by the protein precipitation solution. After vortexing, the mixture was centrifuged again at 2000g for 10 minutes. Finally, as part of the DNA precipitation and rehydration step, the supernatant was retrieved into a new tube containing isopropanol and again centrifuged for 10 minutes at 2000g. The supernatant was discarded and 70% ethanol was added to the pellet for cleaning. The ethanol was aspirated and air dried for 10-15 minutes. Using rehydration solution, the DNA was rehydrated for 1 hour at 65EC.

#### *2.1.5.2 Genotyping*

Five single nucleotide polymorphisms (SNPs) in the gene coding for adiponectin were chosen for investigation. These SNPs were chosen to cover the entire length of the gene. The first was an intronic SNP (public ID, dbSNP: rs182052 chromosome 3q27) which was an A/G SNP at the 5' end of the gene. The second was an A/G SNP chosen within the 3'UTR region of the gene (public ID, dbSNP: rs3774262). The third was a C/T SNP chosen downstream of the gene in the 3' Untranslated region (public ID, dbSNP: rs1063537), as according to SNPbrowserTM 2.0 designed by Applied Biosystems (ABI). The fourth SNP (public ID: rs6773957) was an A/G SNP in the 3'Untranslated region, while the fifth SNP (public ID: rs1063539) was a C/G SNP chosen further downstream in the 3'Untranslated region. D' values for each of the SNPs were also calculated, noting that rs1063537 is in almost perfect linkage with rs377426, while both are weakly linked with rs182052. Descriptions are located in Table 45.

Genotyping for each sample was completed using Taqman® validated SNP Genotyping assays from Applied Biosystems, following the company's mandated protocol. For each 96 well

plate completed, a SNP assay mixture was created using 0.25µl of SNP assay solution combined with 2.5µl of DNA Master solution. Sufficient amounts of assay were created to allow for 2.75µl assay to be placed in each 96 well plate. In 91 of the 96 wells, 2.25µL of DNA samples were added to the SNP assay mixture, with two non-translated controls (NTC) and three positive controls, one for each genotype (i.e. homozygous for SNP1, heterozygous, homozygous for SNP 2). Each NTC only had 2.25µl of DNAase, RNAase free distilled water added into each well containing the assay mixture. The remaining concentrated SNP assay mixtures were stored at -20EC. The plates were then sealed with optical caps, vortexed and centrifuged briefly to ensure all the liquid was at the bottom. Plates were then pre-read on an ABI Prism 7000® sequence detection system (SDS). The plates were then placed in Eppendorf Mastercycler to undergo real-time PCR. Settings were set for the reaction to undergo 41 cycles. The first cycle contained four main steps: 1) 2 minutes at 50˚C, 2) 10 minutes at 95˚C, 3) 15 seconds at 95˚C, and 4) 1 minute at 60˚C. Steps three and four were repeated for the remaining 40 cycles. The total length for DNA amplification time would be 2 hours and 15 minutes. Once amplification was completed, the plates were post-read in the ABI prism 7000.

Each SNP assay mixture consisted of 2 probes: VIC and FAM. The probe for allele 1 is labelled at its 5' end with VIC dye, while the 5' end of allele 2 was labelled with the FAM dye. At the 3' end of both probes is a non-fluorescent quencher which allows the reporter dyes (VIC and FAM) to be detected more accurately. Each Taqman probe binds to its complementary sequence on the forward and reverse primer sites. For intact probes, reporter dye proximity to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer (Förster, 1948). Once bound, AmpliTaq Gold® DNA polymerase cleaves only to probes that are hybridized to the target, separating the reporter dye from the quencher dye and

resulting in a fluorescent signal. However, this is only increased if the amplified DNA sequence is complementary to the probe. Through PCR amplification, the generated fluorescence signal is sufficient to determine the allele(s) present. The process is presented diagrammatically in Figure 3 (Obtained from ABI, Adapted from Livak, Flood, Marmaro, Giusti, & Deetz, 1995).

### *2.1.6 Statistical Analysis*

All statistical analysis was conducted using either R statistical software or SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Chi-square analysis with one degree of freedom was used to test for Hardy-Weinberg proportions. Multiple regression analysis using an allele dosage or additive model (i.e. 2 copies of a minor allele doubles the protective effect compared to having just one copy) was used to assess the association between adiponectin SNP variants and body composition and bone mineral density. Smoking status, medication usage, menopausal status, sex, and age were all used as covariates in the regression. The association analysis was conducted through regression at the markers with gender, age, smoking, and medication status as covariates. Significance was assessed via bootstrap with 100,000 resamples. Prior to any analysis, triglycerides, insulin, HOMA-IR and HOMA-B were log transformed to reach a normal distribution. Hypotheses regarding the effect of adiponectin genotypic variants on the parameters measured were two-sided and a P-value of 0.05 was used as the threshold for significance. A power calculation for the data and disequilibrium coefficients was also calculated.

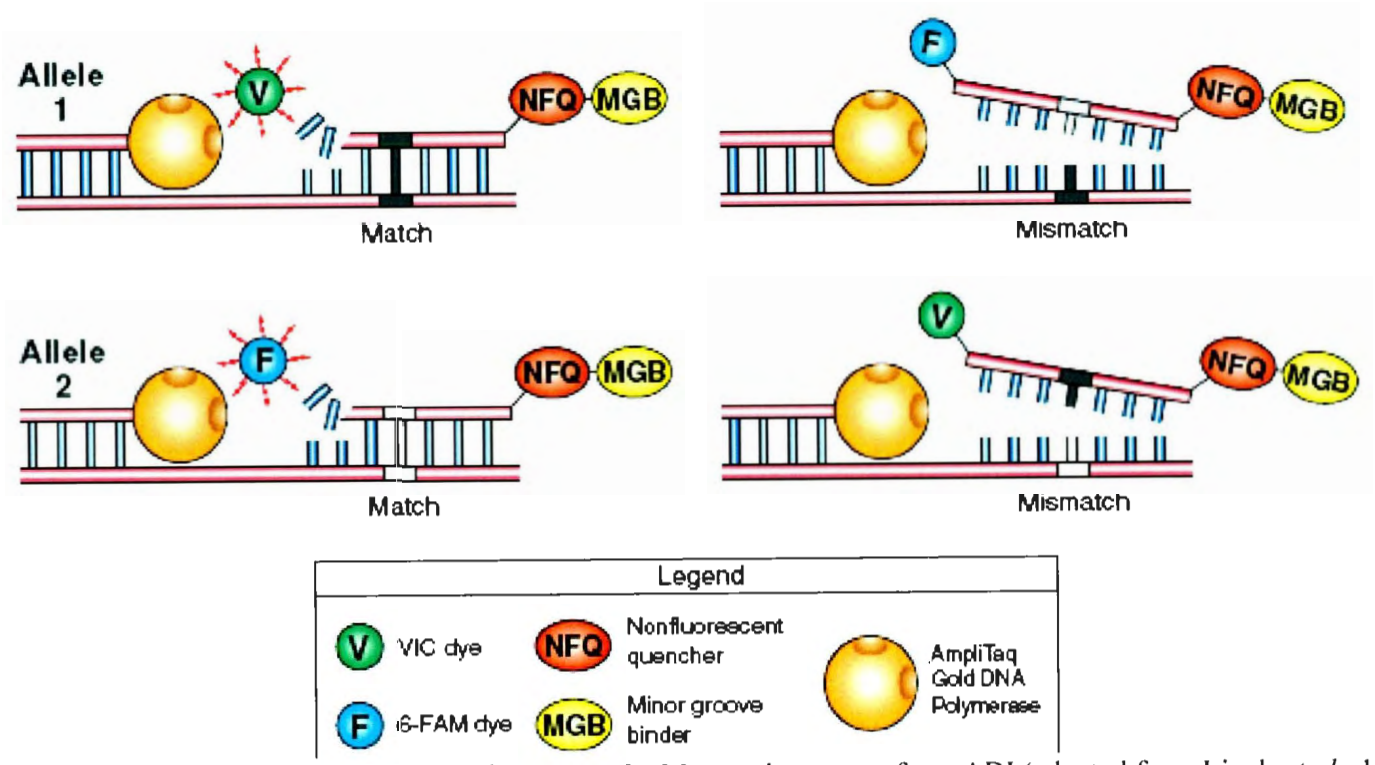


Figure 3. A diagrammatic representation of the TaqMan probe system from ABI (adapted from Livak *et al.*, 1995).

### *2.2 Overfeeding study*

#### *2.2.1 Recruitment of Volunteers*

Similar to the association study recruitment, volunteers were recruited from the St. John's area in the province of Newfoundland and Labrador, Canada. Young males were recruited for the study through solicitation, word-of-mouth, poster distribution and the incentive of free meals for one week. Volunteers who were interested in participating in the study were allowed to do so provided they followed the criteria: 1) subjects were male; 2) their ages ranged between 19-29; 3) they are at least third generation Newfoundlanders with at least one grandparent born in Newfoundland; 4) they are healthy, without any serious metabolic, cardiovascular and/or endocrine conditions; 5) they are not on any medication for lipid metabolism; and 6) they have reported a stable weight ($\forall$5 pounds) within the previous six months. Only healthy young men were recruited for the study because literature showed that the potential risk of injury due to overfeeding in younger individuals is small (Tremblay, Després, Thériault, Fournier, & Bouchard, 1992; Robertson, Henderson, Vist, & Rumsey, 2004). As in the association study, each volunteer completed a screening questionnaire, a physical activity form, and a dietary questionnaire prior to beginning overfeeding. These forms, in addition to the study design, were approved by the Human Investigation Committee of Memorial University of Newfoundland and Labrador.

#### *2.2.2 Plasma and Serum Samples extraction*

The same method was applied for the plasma and serum samples extraction as in the association study. Pre-overfeeding samples were obtained the morning prior to beginning the 7-day overfeeding regime after a 12 hour fast, and post-overfeeding samples were obtained typically on the morning of the eighth day, after a 12 hour fast from their last meal.

### *2.2.3 Body Composition measurements*

A similar protocol was followed as was conducted with the association study. Prior to the 7-day overfeeding regimen, subjects would arrive after a 12 hour fast the morning prior to beginning overfeeding. Pre-overfeeding measurements were obtained in the same manner as the association study, with the same measurements being taken. A second set of post-overfeeding measurements was taken on the eight day, the morning after the final day of overfeeding.

### *2.2.4 Overfeeding protocol*

Multiple studies have employed either long-term or short-term overfeeding intervention methods in an effort to investigate the metabolic and biochemical responses of individuals (Bouchard *et al*, 1990; Jebb *et al.*, 1996; Katezeff & Danforth., 1989; Chin-Chance, Polonsky, & Schoeller, 2000). Although many short-term overfeeding strategies have been implemented, there is limited consistency between studies as to what length of time is standard for short-term overfeeding, with study lengths often ranging between 12 hours to 22 days (Chin-Chance *et al.*, 2000; Jebb *et al*, 1996; Kashiwagi *et al.*, 1985). However, some have argued that overfeeding periods should be long enough to expect an increase in body weight in excess of changes due to bowel contents and edema (Joosen & Westerterp, 2006). In this instance, a 7-day overfeeding intervention was chosen as an adequate length of time to induce changes in adipose tissue gene expression levels, in addition to overall body composition.

The degree of macronutrient intake has also varied greatly. Previous studies have overfed subjects between 30% and 100% above baseline energy intake levels for a given individual (Bouchard *et al.*, 1990; Kashiwagi *et al.*, 1985). As similarly stated by Joosen and Westerterp (2006), pertaining to the length of short-term overfeeding, the same argument can be

made for a degree of overfeeding above baseline. Adequate levels should be achieved to ensure increased adipose tissue gene expression level changes, as well as changes in overall body composition. However, they should be enough to limit the risk to the individuals undergoing the study and for this reason, 70% overfeeding was chosen for this study.

Many types of diet have also been used in overfeeding interventions. Some studies have used high fat diets, others have used high carbohydrate and protein diets, while others have chosen a mixed diet. In an effort to mimic the common North American diet, a mixed diet was chosen for this study.

In terms of overfeeding intervention design, the study can be conducted in an inpatient or outpatient form. Previous studies have argued that the most ideal and reliable studies are those conducted in a controlled environment (i.e. a research institute) during the entire study (Joosen & Westerterp, 2006). However, it is agreed that those types of studies are disadvantaged in that results cannot be effectively extrapolated to other real world situations (Joosen & Westerterp, 2006). Since this study aimed to model North American style eating as frequently as possible, it was decided to undergo the protocol in an outpatient form.

Baseline energy intake measurements were collected in three forms: 1) three 24-hour diet recall questionnaires, 2) completion of a 30-day dietary inventory, and 3) estimation of total energy expenditure by an Actical$^{TM}$ physical activity level monitor (Mini Mitter Co., Inc. Bend, OR, USA). The monitor was placed for the week prior to overfeeding to obtain the energy expenditure measurements. The baseline energy intake measurements were calculated by averaging the three 24 hour recalls, the 30-day dietary inventory and the energy expenditure value obtained by the Actical. Individuals then started on a 7-day regimen of overfeeding (1-7 days, with the 8$^{th}$ day subjects arriving for their fasted post-overfeeding measurements). The macronutrient compositions of the diets were kept stable at 50% carbohydrates, 35% fat, and

15% protein. Meals were purchased from restaurants including McDonald's, Extreme Pita, Tim Horton's, Swiss Chalet, Wendy's, A&W, Dairy Queen, Kentucky Fried Chicken, Subway, and Pizza Hut for which nutritional information of the food was available. Calorie intakes for protein, carbohydrates, fiber, total fat, saturated fat and cholesterol were as follows: 2969 kcal, 106g, 394g, 19g, 105g, 38g, and 304mg per day at baseline and 5471 kcal, 178g, 713g, 33g, 221g, 71g and 735 mg per day during overfeeding. Subjects received their food everyday for breakfast at 9:00 a.m., lunch at 12:00 p.m., and supper at 5:00 p.m. During each meal, someone was present to ensure that all the food was consumed. In addition, physical activity levels were monitored during the week of overfeeding using the Actical™, to ensure the same pattern of physical activity as with the week pre-overfeeding was maintained. The variation in physical activity between baseline measurements and the overfeeding period was controlled below 15%. During overfeeding, subjects were asked to abstain from any alcoholic or additional calorie-containing beverages (outside those provided). The energy values and macronutrient content of the intakes of the volunteers were computed using the Food Processor SQL version 9.5.0.0 (ESHA Research, Salem, OR, USA).

### *2.2.5 Serum biochemical measurements*

Glucose, total cholesterol, high-density lipoprotein (HDL-c), triglycerides (TGs), calcium, magnesium, albumin and insulin serum concentrations were measured in the same manner as the association study. Serum adiponectin levels were measured in duplicate with human adiponectin enzyme immunometric assay (ELISA; Phoenix Pharmaceuticals, Belmont, CA, USA) performed on an Alisei Quality System (SEAC Radim Group, Pomezia, Italy). Each 96 well-immunoplate was pre-coated with Anti-human Adiponectin Capture antibody and had the non-specific binding sites blocked. Reagent preparation involved fifty milliliters of

concentrated assay buffer being diluted 20x with 950 ml of distilled water and then stored at 4EC. The biotinylated anti-human adiponectin detection antibody was then rehydrated with 100Φl of 1x Assay buffer (tubes were centrifuged to dislodge powder from the cap or the tube walls). This was then further diluted to 1:250. 30 µL of Streptavidin-horseradish peroxidase was centrifuged at 4,000 rpm for 5 seconds and further diluted with 1x assay buffer to 1:2000 before use.

Human adiponectin standard preparation was then prepared. Recombinant human adiponectin standard was rehydrated with 1ml of 1x Assay buffer, and the solution was allowed to sit for at least 10 minutes at 22˚C (room temperature) to ensure the 100ng/vial of standard was completely dissolved. Serial dilutions were prepared from the stock solution in the following concentrations: 100ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml and 0.156ng/ml. These were used to make a standard curve for comparison. 100µL of prepared human adiponectin standard was then added in reverse order of serial dilution, and these were used in duplicate. Diluted 100µL samples obtained from study subjects were then added in their designated wells. The immuoplate was then sealed with an acetate plate sealer and incubated for 2 hours at 22˚C (room temperature) on a plate shaker at 350 rpm. The acetate seal was removed and the liquid was discarded from the wells. Each well was washed with 300-350µL of Assay buffer four times. 100µL of biotinylated anti-human adiponectin detection antibody was added into each well and resealed into the immunoplate with the acetate plate sealer and incubated for 2 hours at 22EC on an acetate plate shaker at 350 rpm. A second four time wash was performed with 1x Assay buffer as previously described. Following the second wash, 100µL of streptadivin-hoarseradish peroxidase solution was added into each well and resealed and incubated for 30minutes on a plate shaker at 350rpm. 100 µL of light sensitive substrate

solution was added to each well and incubated from the light at room temperature of 20-30 minutes on the plate shaker. Finally, 100 μL of 2N HCl was added to stop the reaction, inducing a color change from blue to yellow. The intensity of the color is directly proportional to the amount of human adiponectin with known concentration. The plate was allowed to sit for 20 minutes, and finally, the optical density absorbency readings were measured at 450nm on the Alisei Quality System (SEAC Radim Group, Pomezia, Italy).

Human adiponectin in the samples binds to the captured antibody immobilized in the wells. The biotinylated anti-human adiponectin detection antibody then binds to the adiponectin bond to the anti-adiponectin in order to capture antibodies at the bottom of the plate. The Streptavidin-horseradish peroxidase catalyzes and binds to biotin bound to the Fc portion of the anti-adiponectin antibody, inducing the color change from blue to yellow.

### *2.2.6 Genomic Extraction and Genotyping*

The genomic extraction and genotyping followed the same method as the association study.

### *2.2.7 Statistical Analysis*

Data that was obtained that was not normally distributed was logarithmically transformed to approximate a normal distribution for subsequent analysis. These variables included concentrations of triglycerides, insulin, and HOMA-IR and HOMA-β changes at baseline and overfeeding. The Homeostasis model assessment (HOMA) was used as a measure of insulin resistance (HOMA-IR = insulin (μU/ml) x glucose (mmol/L)/22.5)) and β-cell function (HOMA- β = 20*insulin (μU/ml)/(glucose-3.5) (Matthews *et al.*, 1985). To evaluate the effect adipocity had on adiponectin concentrations, subjects were classified according to their adiposity

status. The analysis was conducted using only percentage body fat instead of body mass index (kg/m2). This was done because of increasing inaccuracy with the use of BMI. The men aged 20-39 were classified as normal, overweight and obese as per Bray (2003) classification. The analysis conducted involved comparing physical and biochemical characteristics between the three different body fat categories. The comparisons were completed using a one-factor analysis of variance with a bonferroni correction. The before and after overfeeding changes were tested using a general linear model. The differences among the three groups were conducted using a general linear model by including the baseline covariates in the same model. Multiple comparison adjustments were performed with a post hoc Tukey test. Pearson correlation analysis was performed to screen for potential factors related to fasting serum concentrations of adiponectin. A multiple regression analysis was used to assess factors in prediction fasting serum adiponectin concentrations. These variables in the model specifically included percentage body fat, glucose level, insulin level, triglyceride level, HOMA-IR and HOMA- $\beta$. Baseline data and the change in variables after the 7 day overfeeding were also assessed. Statistical analysis was conducted using SPSSS version 19.0 (SPSS Inc., Chicago, IL USA) for all analysis. Statistical analysis was two-sided and a p-value of $<0.05$ was used to determine statistical significance.

## 3.0 Results

### 3.0.1 Association Study Results

The general descriptive statistic results for the entire cohort used in this analysis are shown in Tables 1-3. A total of 1,811 subjects enrolled in this study, with 403 males and 1,408 female subjects (Table 3). The mean ages for all male and female subjects in the study were 41 and 44 respectively (Table 3). The average height and weight for male subjects were 175.47cm and 84.64 kg respectively, whereas for female subjects they were 162.1cm and 69.65 kg respectively (Table 3). The average male total percentage body fat was 25.7% for all males whereas in females the total percentage body fat was 37.68%. This was significantly different ($p<0.001$). Total bone mineral density, lumbar spine density (L2-L4) and left femoral neck/hip BMD are reported in Table 1. Total BMD, Spine BMD and Left Hip BMD were significantly greater in males when compared with females ($p<0.001$, Table 3). Body-mass index (BMI) was also found to be significantly higher in males than in females ($p<0.001$, Table 3). Waist-Hip ratio were also recorded and were found to be significantly higher in males as compared with females ($p<0.001$).

Baseline laboratory data are also reported in Table 4. Phosphate levels, cholesterol and HDL levels were significantly elevated in females, while glucose, calcium, albumin, GGT, logarithmic-transform for triglycerides, insulin, and HOMA-insulin resistance were more elevated in males. Triglyceride/HDL ratio was also found to be significantly higher in males than in females. The comparison data of bone mineral density and body mass composition between males and females is reported in Table 5. Women displayed significantly higher percentage trunk fat as compared with men. Men however displayed significantly higher arm BMD, lumber BMD and total BMD.

Tables 6-8 compare premenopausal and postmenopausal women. Premenopausal

women had significantly higher total, arm, leg, rib, pelvis, lumbar and left hip BMD, as well as being taller. Premenopausal women had significantly higher logarithmic transform HOMA-β function (Table 7). Postmenopausal women had significantly higher total arm, leg and trunk percentage body fat, weight, waist and hip length. Glucose, phosphate, calcium, cholesterol, triglyceride, GGT, LDL, logarithmic insulin, HOMA-IR and triglyceride levels, and triglyceride/HDL ratio were significantly higher in postmenopausal women.

When separating the cohort into individuals that were taking any medication versus those who were not taking medication, non-medication users were significantly older, with significantly increased total percentage body fat, arm fat, leg fat, trunk fat and BMI (Table 9). However, total arm, leg, trunk, spine, pelvis and left hip BMD were significantly elevated in medication users (Table 9 and 11). Albumin and magnesium levels were significantly elevated in medication users, while glucose and the logarithmic transform of insulin and triglyceride levels, HOMA-IR, and HOMA-β were significantly elevated in non-medication users (Table 10). The cohort was further divided by gender into those individuals taking medication (Table 12-17). In both males and females, the majority of the same relationships held for those individuals taking medications and for those not taking medications (Table 10-12).

The cohort was then divided into subjects who smoked versus those who were non-smokers. A total of 222 smokers were in the cohort versus 1573 non-smokers (Table 18). Non-smokers were found to be significantly older and to have significantly elevated total, spine and left hip BMD, as well as significantly elevated HDL levels (Table 18-20). Smokers, however, were found to have significantly elevated triglyceride, risk factor, and triglyceride/HDL ratio (Table 19). The cohort was further subdivided by gender and smoking status (Table 21-26). Smoking males had significantly elevated total BMD, arm BMC and leg BMC when compared with non-smoking males ($p<0.05$, Table 21 and Table 23). However, non-smoking females were

found to be significantly older and taller with significantly elevated total, spine, leg and left hip BMD (Table 24). They had significantly elevated leg percentage body fat and gynecoid fat, glucose and HDL level (Table 26). Smoking females were found to have significantly elevated triglyceride levels and elevated triglyceride/HDL level (Table 25).

A one-way ANOVA analysis was conducted factoring for Bray percentage body fat categories of lean, overweight and obese for the entire cohort, then factoring for gender (Table 27-35). The normal/underweight group was significantly younger when compared to the overweight and obese groups (Table 27). Total body, spine, rib, pelvis and left hip BMD were significantly elevated in obese subjects as compared with overweight and normal/underweight individuals (Table 27 and 29). Glucose, insulin, HOMA-IR and HOMA-β showed a significant steady increase among the three groups of underweight/normal, overweight and obese individuals (Table 28). Cholesterol, LDL and triglyceride levels were significantly lower in normal/underweight subjects. HDL levels showed a significant steady decrease as individuals increased in percentage body fat (Table 28). When factoring for gender, normal/underweight males had significantly elevated arm, pelvis and total bone mineral density. They also had significantly decreased glucose, cholesterol, triglyceride, and magnesium levels. Insulin, HOMA-IR and HOMA-β showed a significantly steady increase from normal/underweight to overweight to obese males (Table 31). Obese females were found to have significantly elevated total arm, leg, trunk, spine, pelvis, and left hip BMD (Table 33 and 35). Similar laboratory results were shown among female subjects as with male subjects. Glucose, insulin, HOMA-IR and HOMA-β and triglycerides showed a significant steady elevation from normal/underweight to obese females (Table 34). HDL levels showed a significant steady decrease in the three groups (Table 34).

A similar one-way ANOVA analysis was conducted to see if similar findings could be

found when factoring for WHO BMI categories and gender (Table 36-44). Total arm, leg, trunk, pelvis and left hip BMD significantly increased between each group from normal/underweight individuals, overweight, to obese (Table 36 and Table 38). Glucose, insulin, HOMA-IR, HOMA-β and triglycerides showed a significant steady elevation among all three groups (Table 37). HDL levels again showed a steady decrease among the groups (Table 37). The same relationships held when the analysis factored for gender in both females and males (Tables 39-44).

Figure 4 shows the overall sample-size power calculation for the data set, showing that this sample size was adequately powered. It illustrates the power profiles as a function of varying coefficients of determination for a range of heritability estimates. Table 45 shows a summary of the single nucleotide polymorphisms, allelic frequencies and whether if there are any SNPs in Hardy-Weinberg equilibrium. Table 46 shows the linkage disequilibrium (LD) analysis as an estimate of the variants.

Multiple regression analysis for the five SNPs analyzed was conducted. Each SNP frequency was analyzed for bone mineral density and for percentage body fat with and without factoring for specific confounding variables including gender, smoking, age, medication status and smoking. These results have been displayed in Tables 47-51. Significance was noted in total bone mineral density for SNP rs6773957 when factored for age. However, no significance was found in any SNP when assessing for spine or left hip bone mineral density.

Table 3. The descriptive statistics for the entire cohort, male and female subjects.

| | Entire Cohort | | | | Male | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | n'' | Mean | | SD | P | |
| Age | 1811 | 43.68 | ± | 11.87 | 403 | 41.00 | ± | 14.1 | 1408 | 44.47 | ± | 11.0 | 0.000 | Female |
| Weight (kg) | 1808 | 73.00 | ± | 15.45 | 401 | 84.64 | ± | 14.0 | 1407 | 69.65 | ± | 14.2 | 0.000 | Male |
| Height (cm) | 1808 | 165.07 | ± | 8.14 | 401 | 175.47 | ± | 6.4 | 1407 | 162.10 | ± | 5.8 | 0.000 | Male |
| Waist (cm) | 1785 | 92.44 | ± | 14.23 | 389 | 97.86 | ± | 12.2 | 1396 | 90.93 | ± | 14.4 | 0.000 | Male |
| Hip (cm) | 1783 | 102.25 | ± | 11.17 | 388 | 101.31 | ± | 9.1 | 1395 | 102.49 | ± | 11.7 | NS | |
| Percentage Body Fat | 1805 | 34.97 | ± | 9.03 | 400 | 84.42 | ± | 13.9 | 1405 | 68.76 | ± | 14.0 | 0.000 | Male |
| Percentage Trunk Fat | 1805 | 36.93 | ± | 9.39 | 400 | 25.37 | ± | 7.6 | 1405 | 37.68 | ± | 7.4 | 0.000 | Female |
| Total Bone mineral density (BMD) | 1803 | 1.18 | ± | 0.10 | 400 | 30.15 | ± | 8.9 | 1403 | 38.84 | ± | 8.6 | 0.000 | Female |
| Spine BMD | 1806 | 1.21 | ± | 0.16 | 400 | 1.26 | ± | 0.1 | 1406 | 1.16 | ± | 0.1 | 0.000 | Male |
| Left Hip BMD | 1709 | 0.97 | ± | 0.13 | 360 | 1.26 | ± | 0.2 | 1349 | 1.20 | ± | 0.2 | 0.000 | Male |
| BMI | 1212 | 26.74 | ± | 5.09 | 282 | 5.78 | ± | 0.9 | 930 | 4.60 | ± | 0.7 | 0.000 | Male |
| Waist-Hip Ratio | 1793 | 0.90 | ± | 0.08 | 400 | 0.09 | ± | 0.3 | 1393 | 0.00 | ± | 0.2 | 0.000 | Male |

P-values at the end of table indicate if there is a significant difference between both the male and female genders. The last column indicates which gender is greater for that measurement (e.g. average age of the female cohort is significantly higher than that of males). n, n' and n'' refer to the total number of individuals, males and females in the cohort for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 4. The baseline laboratory values, HOMA-IR, and HOMA-β for the entire cohort, male and female subjects.

| | Entire Cohort | | | Male | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SD | n` | Mean | SD | n`` | Mean | SD | P | |
| Glucose | 267 | 5.13 | ± 0.95 | 72 | 0.01 | ± 0.1 | 195 | 0.01 | ± 0.1 | NS | |
| Phosphate | 1793 | 1.19 | ± 0.18 | 400 | 5.30 | ± 1.1 | 1393 | 5.08 | ± 0.9 | 0.000 | Male |
| Calcium | 1713 | 2.35 | ± 0.12 | 369 | 1.13 | ± 0.2 | 1344 | 1.20 | ± 0.2 | 0.000 | Female |
| Albumin | 1717 | 41.30 | ± 3.85 | 374 | 2.36 | ± 0.1 | 1343 | 2.34 | ± 0.1 | 0.002 | Male |
| Cholesterol | 1712 | 5.17 | ± 1.05 | 369 | 42.71 | ± 4.0 | 1343 | 40.92 | ± 3.7 | 0.000 | Male |
| Triglycerides | 1793 | 1.23 | ± 0.79 | 400 | 5.05 | ± 1.1 | 1393 | 5.21 | ± 1.0 | 0.007 | Female |
| Magnesium | 1793 | 0.88 | ± 0.09 | 400 | 1.49 | ± 1.0 | 1393 | 1.15 | ± 0.7 | 0.000 | Male |
| HDL | 1713 | 1.49 | ± 0.38 | 369 | 0.89 | ± 0.1 | 1344 | 0.88 | ± 0.1 | NS | |
| GGT | 1794 | 19.93 | ± 24.11 | 400 | 1.24 | ± 0.3 | 1394 | 1.56 | ± 0.4 | 0.000 | Female |
| LDL | 1207 | 3.12 | ± 0.90 | 291 | 25.77 | ± 21.0 | 916 | 18.07 | ± 24.7 | 0.000 | Male |
| Risk Factor | 1793 | 3.63 | ± 1.03 | 400 | 3.10 | ± 0.9 | 1393 | 3.12 | ± 0.9 | NS | |
| Buche | 1793 | 9229.50 | ± 2710.56 | 400 | 4.20 | ± 1.1 | 1393 | 3.47 | ± 0.9 | 0.000 | Male |
| Corrected BUCHE (SI Units) | 1339 | 5613.57 | ± 5422.55 | 296 | 10134.87 | ± 2557.4 | 1043 | 8973.55 | ± 2704.8 | 0.000 | Male |
| Insulin | 296 | 72.09 | ± 66.48 | 46 | 5609.56 | ± 6195.4 | 250 | 5636.73 | ± 5281.2 | NS | |
| HOMA-β | 1808 | 139.81 | ± 236.64 | 401 | 27.50 | ± 4.4 | 1407 | 26.52 | ± 5.3 | 0.001 | Male |
| HOMA-IR | 1668 | 2.48 | ± 3.15 | 362 | 139.39 | ± 142.9 | 1306 | 139.87 | ± 257.1 | NS | |
| Triglyceride/HDL ratio | 1670 | 0.93 | ± 0.77 | 363 | 2.82 | ± 3.6 | 1307 | 2.39 | ± 3.0 | 0.022 | Male |
| Log Insulin | 1793 | 1.78 | ± 0.25 | 400 | 1.32 | ± 1.0 | 1393 | 0.82 | ± 0.6 | 0.000 | Male |
| Log HOMA-IR | 1671 | 0.29 | ± 0.28 | 363 | 1.81 | ± 0.3 | 1308 | 1.77 | ± 0.2 | 0.001 | Male |
| Log HOMA-β | 1670 | 2.06 | ± 0.26 | 363 | 0.34 | ± 0.3 | 1307 | 0.27 | ± 0.3 | 0.000 | Male |
| Log Triglyceride | 1666 | 0.02 | ± 0.24 | 362 | 2.05 | ± 0.3 | 1304 | 2.07 | ± 0.3 | NS | |
| Waist-Hip Ratio | 1793 | 0.90 | ± 0.08 | 400 | 0.09 | ± 0.3 | 1393 | 0.00 | ± 0.2 | 0.000 | Male |

P-values at the end of table indicate if there is a significant difference between both the male and female genders. The last column indicates which gender is greater for that measurement (e.g. average blood glucose of the female cohort is significantly higher than that of males). n, n` and n`` refer to the total number of individuals, males and females in the cohort for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 5. Baseline cohort bone density and Body composition data for the entire cohort.

| | Entire Cohort | | | | Male | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | n'' | Mean | | SD | P | |
| Total Arm BMD | 1671 | 0.92 | ± | 0.14 | 363 | 78.23 | ± | 58.5 | 1308 | 70.34 | ± | 68.6 | 0.046 | Male |
| Total Leg BMD | 1804 | 1.28 | ± | 0.14 | 400 | 1.07 | ± | 0.1 | 1404 | 0.88 | ± | 0.1 | 0.000 | Male |
| Trunk BMD | 1805 | 0.94 | ± | 0.09 | 400 | 1.43 | ± | 0.1 | 1405 | 1.24 | ± | 0.1 | 0.000 | Male |
| Total Rib BMD | 1805 | 0.68 | ± | 0.08 | 400 | 1.00 | ± | 0.1 | 1405 | 0.92 | ± | 0.1 | 0.000 | Male |
| Total Pelvis BMD | 661 | 1.16 | ± | 0.13 | 176 | 0.75 | ± | 0.1 | 485 | 0.65 | ± | 0.1 | 0.000 | Male |
| Total BMD | 661 | 1.18 | ± | 0.10 | 176 | 1.24 | ± | 0.1 | 485 | 1.13 | ± | 0.1 | 0.000 | Male |
| Arm %Fat | 1802 | 34.95 | ± | 12.22 | 400 | 1.26 | ± | 0.1 | 1402 | 1.16 | ± | 0.1 | 0.000 | Male |
| Arm tissue | 1803 | 8000.14 | ± | 2830.77 | 400 | 20.04 | ± | 8.9 | 1403 | 39.18 | ± | 9.4 | 0.000 | Female |
| Arm Fat weight(g) | 1804 | 2998.27 | ± | 1872.65 | 400 | 9739.40 | ± | 2572.8 | 1404 | 7499.98 | ± | 2704.7 | 0.000 | Male |
| Arm Lean muscle mass (g) | 1804 | 5004.71 | ± | 1762.54 | 400 | 2163.71 | ± | 1558.2 | 1404 | 3232.40 | ± | 1887.9 | 0.000 | Female |
| Arm Bone Mineral Composition | 1804 | 343.59 | ± | 89.57 | 400 | 7573.47 | ± | 1446.2 | 1404 | 4271.87 | ± | 991.7 | 0.000 | Male |
| Leg % Fat | 1803 | 34.86 | ± | 9.85 | 399 | 468.21 | ± | 73.7 | 1404 | 308.03 | ± | 55.5 | 0.000 | Male |
| Leg Tissue weight(g) | 1805 | 22479.56 | ± | 4557.82 | 400 | 21.81 | ± | 6.9 | 1405 | 38.55 | ± | 7.0 | 0.000 | Female |
| Leg Fat weight (g) | 1805 | 8241.91 | ± | 3203.21 | 400 | 25385.61 | ± | 4440.0 | 1405 | 21638.13 | ± | 4229.4 | 0.000 | Male |
| Leg lean muscle mass(g) | 1805 | 14235.58 | ± | 3480.80 | 400 | 5987.16 | ± | 2711.5 | 1405 | 8872.59 | ± | 3024.8 | 0.000 | Female |
| Leg bone mineral composition | 1804 | 975.53 | ± | 201.78 | 400 | 19371.78 | ± | 2792.2 | 1404 | 12769.43 | ± | 1917.0 | 0.000 | Male |
| Trunk %Fat | 1804 | 36.97 | ± | 9.36 | 400 | 1232.89 | ± | 179.2 | 1404 | 901.81 | ± | 137.1 | 0.000 | Male |
| Trunk Tissue weight (g) | 1804 | 35189.55 | ± | 9039.91 | 400 | 30.21 | ± | 8.9 | 1404 | 38.87 | ± | 8.6 | 0.000 | Female |
| Trunk Fat weight (g) | 1805 | 13654.22 | ± | 5777.29 | 400 | 41465.82 | ± | 8842.4 | 1405 | 33396.58 | ± | 8278.6 | 0.000 | Male |
| Trunk lean muscle mass (g) | 1805 | 21555.96 | ± | 5344.13 | 400 | 13264.46 | ± | 5601.8 | 1405 | 13756.06 | ± | 5827.7 | NS | |
| Trunk bone mineral composition | 1805 | 792.99 | ± | 187.17 | 400 | 28193.81 | ± | 4994.1 | 1405 | 19669.24 | ± | 3676.0 | 0.000 | Male |
| Android % fat mass | 1805 | 42.36 | ± | 11.00 | 400 | 960.76 | ± | 208.3 | 1405 | 744.53 | ± | 148.9 | 0.000 | Male |
| Android tissue mass (g) | 1799 | 5432.41 | ± | 1715.68 | 397 | 36.90 | ± | 11.1 | 1402 | 43.88 | ± | 10.5 | 0.000 | Female |
| Android Fat mass (g) | 1800 | 2436.31 | ± | 1230.20 | 398 | 6451.95 | ± | 1758.2 | 1402 | 5141.47 | ± | 1590.9 | 0.000 | Male |
| Android lean muscle mass (g) | 1800 | 2998.08 | ± | 730.59 | 398 | 2546.94 | ± | 1285.7 | 1402 | 2403.00 | ± | 1213.8 | 0.039 | Male |
| Android BMC | 1800 | 53.23 | ± | 11.80 | 398 | 3907.50 | ± | 686.3 | 1402 | 2740.32 | ± | 500.7 | 0.000 | Male |
| Gynecoid % Fat | 1800 | 41.35 | ± | 9.46 | 398 | 59.10 | ± | 13.1 | 1402 | 51.47 | ± | 10.7 | 0.000 | Male |
| Gynecoid tissue mass (g) | 1800 | 11331.66 | ± | 2217.02 | 398 | 29.11 | ± | 7.9 | 1402 | 44.80 | ± | 6.5 | 0.000 | Female |
| Gynecoid fat mass (g) | 1800 | 4841.31 | ± | 1641.42 | 398 | 12518.31 | ± | 2130.5 | 1402 | 10989.31 | ± | 2123.5 | 0.000 | Male |
| Gynecoid lean muscle mass (g) | 1800 | 6489.78 | ± | 1527.99 | 398 | 3849.54 | ± | 1511.1 | 1402 | 5117.45 | ± | 1561.9 | 0.000 | Female |
| Gynecoid BMC | 1800 | 276.69 | ± | 61.98 | 398 | 8674.35 | ± | 1249.5 | 1402 | 5869.53 | ± | 903.0 | 0.000 | Male |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total % body fat | 1800 | 34.97 | ± | 9.01 | 398 | 346.94 | ± | 59.0 | 1402 | 256.52 | ± | 46.1 | 0.000 | Male |
| Total tissue mass (g) | 1805 | 69974.20 | ± | 18512.45 | 399 | 25.39 | ± | 7.5 | 1406 | 37.67 | ± | 7.4 | 0.000 | Female |
| Total fat mass (g) | 1805 | 25675.62 | ± | 10044.01 | 399 | 81317.38 | ± | 13714.4 | 1406 | 66731.12 | ± | 18450.4 | 0.000 | Male |
| Total lean muscle mass (g) | 1805 | 44048.60 | ± | 10152.68 | 399 | 22095.04 | ± | 9144.0 | 1406 | 26667.48 | ± | 10055.3 | 0.000 | Female |
| Total Bone Mineral Composition | 1805 | 2588.08 | ± | 459.69 | 399 | 59222.43 | ± | 7546.6 | 1406 | 39741.99 | ± | 5686.2 | 0.000 | Male |
| L2-L4 (Z-Score) | 1804 | 0.22 | ± | 1.26 | 399 | 84.45 | ± | 13.9 | 1405 | 68.79 | ± | 14.2 | 0.000 | Male |
| L2-L4 BMC | 1226 | 53.64 | ± | 11.04 | 280 | 0.15 | ± | 1.3 | 946 | 0.24 | ± | 1.2 | NS | |
| Femoral Neck (Z-Score) | 1238 | 0.10 | ± | 0.93 | 287 | 62.88 | ± | 11.2 | 951 | 50.86 | ± | 9.3 | 0.000 | Male |
| Neck BMC | 1193 | 4.87 | ± | 0.89 | 270 | 0.00 | ± | 0.9 | 923 | 0.13 | ± | 0.9 | 0.041 | Female |

P-values at the end of table indicate if there is a significant difference between both the male and female genders. The last column indicates which gender is greater for that measurement (e.g. average %body fat of the female cohort is significantly higher than that of males). n, n' and n'' refer to the total number of individuals, males and females in the cohort for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 6. The baseline descriptive characteristics for Pre- and Post-menopausal females.

| | Pre-Menopausal | | | | Post-Postmenopausal | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Age | 790 | 38.88 | ± | 9.0 | 548 | 52.75 | ± | 8.1 | 0.000 | Post |
| Weight (kg) | 790 | 68.73 | ± | 13.4 | 547 | 70.61 | ± | 14.7 | 0.015 | Post |
| Height (cm) | 790 | 162.78 | ± | 5.6 | 547 | 160.87 | ± | 5.8 | 0.000 | Pre |
| Waist (cm) | 785 | 88.60 | ± | 13.4 | 545 | 94.18 | ± | 15.1 | 0.000 | Post |
| Hip (cm) | 785 | 101.08 | ± | 11.3 | 544 | 104.50 | ± | 11.8 | 0.000 | Post |
| Total % Body Fat | 790 | 36.48 | ± | 7.5 | 546 | 39.51 | ± | 6.9 | 0.000 | Post |
| Total % Trunk Fat | 790 | 37.23 | ± | 8.7 | 544 | 41.26 | ± | 7.9 | 0.000 | Post |
| Total BMD (g/cm2) | 790 | 1.17 | ± | 0.1 | 546 | 1.14 | ± | 0.1 | 0.000 | Pre |
| Spine BMD | 755 | 1.24 | ± | 0.1 | 526 | 1.16 | ± | 0.2 | 0.000 | Pre |
| Left Hip BMD | 756 | 0.98 | ± | 0.1 | 525 | 0.91 | ± | 0.1 | 0.000 | Pre |

P-values at the end of table indicate if there is a significant difference between both the premenopausal and postmenopausal females. The last column indicates which gender is greater for that measurement (e.g. age of post-menopausal females is significantly higher than that of premenopausal females). Pre- refers to premenopausal subjects, whereas post- refers to post-menopausal subjects. n and n' refer to the total premenopausal and postmenopausal females for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 7. Baseline laboratory values, HOMA-IR, and HOMA-β for pre-menopausal and post-menopausal female subjects.

| | Pre-Menopausal | | | | Post-Postmenopausal | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Glucose | 783 | 4.93 | ± | 0.7 | 543 | 5.29 | ± | 1.1 | 0.000 | Post |
| Phosphate | 755 | 1.18 | ± | 0.2 | 524 | 1.23 | ± | 0.2 | 0.000 | Post |
| Calcium | 754 | 2.32 | ± | 0.1 | 524 | 2.36 | ± | 0.1 | 0.000 | Post |
| Albumin | 755 | 40.89 | ± | 3.8 | 523 | 40.79 | ± | 3.5 | NS | |
| Cholesterol | 784 | 4.97 | ± | 0.9 | 542 | 5.53 | ± | 1.1 | 0.000 | Post |
| Triglycerides | 784 | 1.04 | ± | 0.7 | 542 | 1.30 | ± | 0.7 | 0.000 | Post |
| Magnesium | 755 | 0.88 | ± | 0.1 | 524 | 0.88 | ± | 0.1 | NS | |
| HDL | 784 | 1.58 | ± | 0.4 | 543 | 1.55 | ± | 0.4 | NS | |
| GGT | 489 | 14.55 | ± | 9.1 | 394 | 21.86 | ± | 34.9 | 0.000 | Post |
| LDL | 784 | 2.91 | ± | 0.8 | 542 | 3.39 | ± | 0.9 | 0.000 | Post |
| Risk Factor | 784 | 3.27 | ± | 0.8 | 542 | 3.73 | ± | 1.0 | 0.000 | Post |
| Buche | 616 | 8487.06 | ± | 2572.4 | 370 | 9726.06 | ± | 2714.2 | 0.000 | Post |
| Corrected Buche (SI Units) | 159 | 5750.26 | ± | 5030.0 | 71 | 5383.12 | ± | 5832.2 | NS | |
| Insulin | 736 | 65.19 | ± | 44.7 | 507 | 76.77 | ± | 93.5 | 0.004 | Post |
| HOMA-β | 734 | 146.15 | ± | 180.9 | 507 | 130.08 | ± | 349.2 | NS | |
| HOMA-IR | 735 | 2.13 | ± | 1.7 | 507 | 2.74 | ± | 4.3 | 0.001 | Post |
| Triglyceride/HDL ratio | 784 | 0.73 | ± | 0.6 | 542 | 0.93 | ± | 0.7 | 0.000 | Post |
| Log Insulin | 736 | 1.75 | ± | 0.2 | 507 | 1.79 | ± | 0.3 | 0.002 | Post |
| Log HOMA-IR | 735 | 0.24 | ± | 0.3 | 507 | 0.31 | ± | 0.3 | 0.000 | Post |
| Log HOMA-β | 733 | 2.08 | ± | 0.2 | 506 | 2.04 | ± | 0.3 | 0.011 | Pre |
| Log Triglyceride | 784 | -0.04 | ± | 0.2 | 542 | 0.05 | ± | 0.2 | 0.000 | Post |

P-values at the end of table indicate if there is a significant difference between both the premenopausal and postmenopausal females. The last column indicates which gender is greater for that measurement (e.g. average blood glucose in post-menopausal females is significantly higher than that of premenopausal females). Pre- refers to premenopausal subjects, whereas post- refers to post-menopausal subjects. n and n' refer to the total premenopausal and postmenopausal females for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 8. The baseline cohort bone density and body composition data for pre-menopausal and post-menopausal females.

| | Pre-menopausal | | | | Post-menopausal | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Total Arm BMD | 790 | 0.89 | ± | 0.1 | 545 | 0.86 | ± | 0.1 | 0.000 | Pre |
| Total Leg BMD | 790 | 1.26 | ± | 0.1 | 545 | 1.20 | ± | 0.1 | 0.000 | Pre |
| Trunk BMD | 790 | 0.93 | ± | 0.1 | 545 | 0.90 | ± | 0.1 | 0.000 | Pre |
| Total Rib BMD | 235 | 0.66 | ± | 0.1 | 235 | 0.64 | ± | 0.1 | 0.000 | Pre |
| Total Pelvis BMD | 235 | 1.15 | ± | 0.1 | 235 | 1.11 | ± | 0.1 | 0.000 | Pre |
| Total BMD | 789 | 1.17 | ± | 0.1 | 544 | 1.14 | ± | 0.1 | 0.000 | Pre |
| Arm %Fat | 789 | 37.80 | ± | 9.7 | 545 | 41.32 | ± | 8.2 | 0.000 | Post |
| Arm tissue | 790 | 7375.11 | ± | 2690.8 | 545 | 7635.51 | ± | 2660.6 | NS | |
| Arm Fat weight(g) | 790 | 3095.54 | ± | 1928.7 | 545 | 3421.61 | ± | 1763.8 | 0.002 | Post |
| Arm Lean muscle mass (g) | 790 | 4283.41 | ± | 899.9 | 545 | 4219.37 | ± | 1062.4 | NS | |
| Arm Bone Mineral Composition | 790 | 313.11 | ± | 50.3 | 545 | 298.73 | ± | 59.6 | 0.000 | Pre |
| Leg % Fat | 790 | 37.97 | ± | 6.8 | 545 | 39.49 | ± | 6.9 | 0.000 | Post |
| Leg Tissue weight(g) | 790 | 21916.04 | ± | 4148.5 | 545 | 21126.30 | ± | 4159.6 | 0.001 | Pre |
| Leg Fat weight (g) | 790 | 8860.92 | ± | 3020.3 | 545 | 8864.12 | ± | 2957.1 | NS | |
| Leg lean muscle mass(g) | 790 | 13062.83 | ± | 1782.1 | 545 | 12262.16 | ± | 1867.8 | 0.000 | Pre |
| Leg bone mineral composition | 789 | 919.39 | ± | 133.1 | 545 | 871.94 | ± | 132.0 | 0.000 | Pre |
| Trunk %Fat | 789 | 37.28 | ± | 8.7 | 545 | 41.25 | ± | 7.9 | 0.000 | Post |
| Trunk Tissue weight (g) | 790 | 32368.15 | ± | 7368.6 | 545 | 34675.34 | ± | 9064.4 | 0.000 | Post |
| Trunk Fat weight (g) | 790 | 12828.21 | ± | 5462.2 | 545 | 15045.63 | ± | 6041.1 | 0.000 | Post |
| Trunk lean muscle mass (g) | 790 | 19603.65 | ± | 3369.3 | 545 | 19611.39 | ± | 3944.1 | NS | |
| Trunk bone mineral composition | 790 | 768.54 | ± | 140.2 | 545 | 708.49 | ± | 151.7 | 0.000 | Pre |
| Android % fat mass | 788 | 42.18 | ± | 11.0 | 541 | 46.42 | ± | 9.1 | 0.000 | Post |
| Android tissue mass (g) | 788 | 4941.46 | ± | 1533.3 | 541 | 5396.13 | ± | 1585.2 | 0.000 | Post |
| Android Fat mass (g) | 788 | 2234.88 | ± | 1189.9 | 541 | 2632.75 | ± | 1177.3 | 0.000 | Post |
| Android lean muscle mass (g) | 788 | 2707.61 | ± | 467.7 | 541 | 2766.65 | ± | 526.4 | 0.032 | Post |
| Android BMC | 788 | 52.97 | ± | 10.8 | 541 | 49.38 | ± | 10.6 | 0.000 | Pre |
| Gynecoid % Fat | 788 | 44.48 | ± | 6.6 | 541 | 45.37 | ± | 6.2 | 0.013 | Post |
| Gynecoid tissue mass (g) | 788 | 11046.59 | ± | 2085.9 | 541 | 10841.27 | ± | 2051.6 | NS | |
| Gynecoid fat mass (g) | 788 | 5114.33 | ± | 1573.0 | 541 | 5100.66 | ± | 1471.5 | NS | |
| Gynecoid lean muscle mass (g) | 788 | 5931.97 | ± | 841.3 | 541 | 5735.19 | ± | 915.5 | 0.000 | Pre |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid BMC | 788 | 259.72 | ± 45.4 | 541 | 251.28 | ± 46.5 | 0.001 | Pre |
| Total % body fat | 790 | 36.47 | ± 7.5 | 546 | 39.49 | ± 6.9 | 0.000 | Post |
| Total tissue mass (g) | 790 | 65459.74 | ± 13317.1 | 546 | 68247.68 | ± 24014.5 | 0.007 | Post |
| Total fat mass (g) | 790 | 25520.13 | ± 9772.1 | 546 | 28241.68 | ± 10064.0 | 0.000 | Post |
| Total lean muscle mass (g) | 790 | 39939.21 | ± 5061.7 | 546 | 39178.27 | ± 6065.9 | 0.013 | Pre |
| Total Bone Mineral Composition | 790 | 2486.84 | ± 312.5 | 546 | 2341.51 | ± 322.4 | 0.000 | Pre |
| L2-L4 (Z-Score) | 502 | 0.31 | ± 1.2 | 403 | 0.18 | ± 1.3 | NS | |
| L2-L4 BMC | 506 | 52.64 | ± 9.1 | 404 | 48.42 | ± 9.1 | 0.000 | Pre |
| Femoral Neck (Z-Score) | 488 | 0.16 | ± 0.9 | 398 | 0.09 | ± 0.9 | NS | |
| Femoral Neck BMC | 494 | 4.73 | ± 0.7 | 399 | 4.41 | ± 0.6 | 0.000 | Pre |

P-values at the end of table indicate if there is a significant difference between both the premenopausal and postmenopausal females. The last column indicates which gender is greater for that measurement (e.g. average blood glucose in post-menopausal females is significantly higher than that of premenopausal females). Pre- refers to premenopausal subjects, whereas post- refers to post-menopausal subjects. n and n' refer to the total premenopausal and postmenopausal females for a specific measurement respectively. NS- Not significant if p-value is less than 0.5.

Table 9. The baseline descriptive characteristics for the entire cohort of subjects on medication versus those who were not taking any medication.

| | No Medication | | | | Taking Medication | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n' | Mean | ± | SD | P | |
| Age | 966 | 45.66 | ± | 12.2 | 812 | 41.54 | ± | 10.8 | 0.000 | Non-Med |
| Weight (kg) | 966 | 73.44 | ± | 16.1 | 809 | 72.29 | ± | 14.6 | NS | |
| Height (cm) | 966 | 164.04 | ± | 7.4 | 809 | 166.11 | ± | 8.5 | 0.000 | Med |
| Waist (cm) | 960 | 93.97 | ± | 15.4 | 802 | 90.59 | ± | 12.3 | 0.000 | Non-Med |
| Hip (cm) | 959 | 103.41 | ± | 12.0 | 802 | 100.76 | ± | 10.0 | 0.000 | Non-Med |
| Total %Body Fat | 966 | 36.56 | ± | 8.3 | 807 | 33.21 | ± | 9.3 | 0.000 | Non-Med |
| Total % Trunk Fat | 965 | 38.56 | ± | 8.7 | 806 | 35.16 | ± | 9.6 | 0.000 | Non-Med |
| Total BMD ($g/cm^2$) | 966 | 1.17 | ± | 0.1 | 807 | 1.19 | ± | 0.1 | 0.000 | Med |
| Spine BMD | 927 | 1.21 | ± | 0.2 | 764 | 1.22 | ± | 0.2 | 0.017 | Med |
| Left Hip BMD | 926 | 0.95 | ± | 0.1 | 765 | 0.99 | ± | 0.1 | 0.000 | Med |
| BMI | 966 | 27.25 | ± | 5.4 | 809 | 26.13 | ± | 4.5 | 0.000 | Non-Med |
| Waist-Hip Ratio | 959 | 0.91 | ± | 0.1 | 802 | 0.90 | ± | 0.1 | NS | |

P-values at the end of table indicate if there is a significant difference between all individuals in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average age of individuals not on any medications is significantly higher than that individuals taking medication). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n' refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 10. The baseline laboratory values, HOMA-IR, and HOMA-β for the entire cohort comparing those on medications versus those not taking any medications.

| | No Medication | | | | Taking Medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n` | Mean | ± | SD | P | |
| Glucose | 959 | 5.22 | ± | 1.1 | 803 | 5.02 | ± | 0.7 | 0.000 | Non-Med |
| Phosphate | 919 | 1.18 | ± | 0.2 | 774 | 1.19 | ± | 0.2 | NS | |
| Calcium | 918 | 2.34 | ± | 0.1 | 778 | 2.35 | ± | 0.1 | NS | |
| Albumin | 918 | 40.73 | ± | 3.9 | 774 | 41.93 | ± | 3.7 | 0.000 | Med |
| Cholesterol | 959 | 5.19 | ± | 1.0 | 803 | 5.16 | ± | 1.1 | NS | |
| Triglycerides | 959 | 1.30 | ± | 0.8 | 803 | 1.14 | ± | 0.8 | 0.000 | Non-Med |
| Magnesium | 919 | 0.88 | ± | 0.1 | 774 | 0.89 | ± | 0.1 | 0.001 | Med |
| HDL | 960 | 1.49 | ± | 0.4 | 803 | 1.50 | ± | 0.4 | NS | |
| GGT | 665 | 20.39 | ± | 23.2 | 528 | 19.26 | ± | 25.5 | NS | |
| LDL | 959 | 3.11 | ± | 0.9 | 803 | 3.14 | ± | 0.9 | NS | |
| Risk Factor | 959 | 3.64 | ± | 1.0 | 803 | 3.62 | ± | 1.1 | NS | |
| Buche | 695 | 9414.03 | ± | 2615.2 | 622 | 8993.19 | ± | 2787.7 | 0.005 | Non-Med |
| Corrected Buche (SI Units) | 134 | 6044.15 | ± | 5561.5 | 157 | 5350.06 | ± | 5310.2 | NS | |
| Insulin | 902 | 79.06 | ± | 82.0 | 748 | 63.93 | ± | 40.8 | 0.000 | Non-Med |
| HOMA-β | 901 | 147.24 | ± | 298.1 | 746 | 131.78 | ± | 134.5 | NS | |
| HOMA-IR | 901 | 2.80 | ± | 4.0 | 748 | 2.11 | ± | 1.6 | 0.000 | Non-Med |
| Triglyceride/HDL ratio | 959 | 0.98 | ± | 0.7 | 803 | 0.87 | ± | 0.8 | 0.003 | Non-Med |
| Log Insulin | 902 | 1.81 | ± | 0.3 | 748 | 1.74 | ± | 0.2 | 0.000 | Non-Med |
| Log HOMA-IR | 901 | 0.33 | ± | 0.3 | 748 | 0.24 | ± | 0.3 | 0.000 | Non-Med |
| Log HOMA-β | 900 | 2.08 | ± | 0.3 | 745 | 2.04 | ± | 0.2 | 0.001 | Non-Med |
| Log Triglyceride | 959 | 0.05 | ± | 0.2 | 803 | -0.02 | ± | 0.2 | 0.000 | Non-Med |

P-values at the end of table indicate if there is a significant difference between all individuals in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average glucose level for individuals not on any medications is significantly higher than that of individuals taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n` refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 11. The baseline cohort bone density and body composition data comparing individuals who were on medications against those that were not taking any medications.

| | No Medications | | | | Taking Medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Total Arm BMD | 965 | 0.91 | ± | 0.1 | 807 | 0.94 | ± | 0.1 | 0.000 | Med |
| Total Leg BMD | 965 | 1.26 | ± | 0.1 | 807 | 1.30 | ± | 0.1 | 0.000 | Med |
| Total Trunk BMD | 965 | 0.93 | ± | 0.1 | 807 | 0.94 | ± | 0.1 | 0.043 | Med |
| Total Rib BMD | 350 | 0.67 | ± | 0.1 | 289 | 0.69 | ± | 0.1 | 0.024 | Med |
| Total Pelvis BMD | 350 | 1.15 | ± | 0.1 | 289 | 1.17 | ± | 0.1 | 0.017 | Med |
| Total BMD | 963 | 1.17 | ± | 0.1 | 806 | 1.19 | ± | 0.1 | 0.000 | Med |
| Arm % Fat | 964 | 37.11 | ± | 11.3 | 807 | 32.58 | ± | 12.7 | 0.000 | Non-Med |
| Arm tissue | 965 | 8030.76 | ± | 2942.9 | 807 | 7937.68 | ± | 2729.6 | NS | |
| Arm Fat weight(g) | 965 | 3214.02 | ± | 1910.4 | 807 | 2755.22 | ± | 1813.4 | 0.000 | Non-Med |
| Arm Lean muscle mass (g) | 965 | 4822.06 | ± | 1641.6 | 807 | 5182.48 | ± | 1863.0 | 0.000 | Med |
| Arm Bone Mineral Composition | 964 | 330.02 | ± | 84.2 | 807 | 357.64 | ± | 92.1 | 0.000 | Med |
| Leg % Fat | 965 | 36.22 | ± | 9.3 | 807 | 33.35 | ± | 10.1 | 0.000 | Non-Med |
| Leg Tissue weight(g) | 965 | 22329.96 | ± | 4625.7 | 807 | 22585.39 | ± | 4455.9 | NS | |
| Leg Fat weight (g) | 965 | 8554.68 | ± | 3298.0 | 807 | 7875.93 | ± | 3028.5 | 0.000 | Non-Med |
| Leg lean muscle mass(g) | 964 | 13767.73 | ± | 3110.1 | 807 | 14713.25 | ± | 3757.6 | 0.000 | Med |
| Leg bone mineral composition | 965 | 945.84 | ± | 181.8 | 806 | 1007.95 | ± | 214.1 | 0.000 | Med |
| Trunk % Fat | 965 | 38.57 | ± | 8.7 | 806 | 35.17 | ± | 9.6 | 0.000 | Non-Med |
| Trunk Tissue weight (g) | 965 | 35774.39 | ± | 9899.6 | 807 | 34397.38 | ± | 7792.3 | 0.001 | Non-Med |
| Trunk Fat weight (g) | 965 | 14484.52 | ± | 6067.9 | 807 | 12675.81 | ± | 5209.2 | 0.000 | Non-Med |
| Trunk lean muscle mass (g) | 965 | 21338.93 | ± | 5709.5 | 807 | 21709.15 | ± | 4792.3 | NS | |
| Trunk bone mineral composition | 965 | 765.83 | ± | 176.9 | 807 | 822.32 | ± | 190.8 | 0.000 | Med |
| Android % fat mass | 958 | 44.02 | ± | 10.3 | 806 | 40.56 | ± | 11.3 | 0.000 | Non-Med |
| Android tissue mass (g) | 958 | 5573.05 | ± | 1813.9 | 807 | 5258.10 | ± | 1568.1 | 0.000 | Non-Med |
| Android Fat mass (g) | 958 | 2593.80 | ± | 1270.4 | 807 | 2253.46 | ± | 1139.8 | 0.000 | Non-Med |
| Android lean muscle mass (g) | 958 | 2978.91 | ± | 742.3 | 807 | 3005.77 | ± | 712.5 | NS | |
| Android BMC | 958 | 52.41 | ± | 11.5 | 807 | 54.05 | ± | 11.9 | 0.003 | Med |
| Gynecoid % Fat | 958 | 42.60 | ± | 8.7 | 807 | 40.02 | ± | 10.0 | 0.000 | Non-Med |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) | 958 | 11322.70 | ± 2228.6 | 807 | 11311.72 | ± 2201.4 | NS | |
| Gynecoid fat mass (g) | 958 | 4987.10 | ± 1640.3 | 807 | 4676.02 | ± 1621.2 | 0.000 | Non-Med |
| Gynecoid lean muscle mass (g) | 958 | 6332.86 | ± 1406.8 | 807 | 6637.66 | ± 1622.9 | 0.000 | Med |
| Gynecoid BMC | 958 | 270.61 | ± 58.4 | 807 | 282.82 | ± 64.7 | 0.000 | Med |
| Total % body fat | 966 | 36.53 | ± 8.3 | 807 | 33.20 | ± 9.3 | 0.000 | Non-Med |
| Total tissue mass (g) | 966 | 70661.93 | ± 21410.1 | 807 | 68969.43 | ± 14421.8 | NS | |
| Total fat mass (g) | 966 | 27066.64 | ± 10378.2 | 807 | 24029.29 | ± 9332.7 | 0.000 | Non-Med |
| Total lean muscle mass (g) | 966 | 43117.88 | ± 9565.1 | 807 | 44951.25 | ± 10597.7 | 0.000 | Med |
| Total Bone Mineral Composition | 966 | 2510.60 | ± 414.6 | 807 | 2672.99 | ± 487.3 | 0.000 | Med |
| L2-L4 (Z-Score) | 684 | 0.24 | ± 1.3 | 527 | 0.20 | ± 1.2 | NS | |
| L2-L4 BMC | 689 | 52.92 | ± 11.0 | 534 | 54.63 | ± 11.1 | 0.007 | Med |
| Femoral Neck (Z-Score) | 664 | 0.05 | ± 0.9 | 515 | 0.15 | ± 1.0 | NS | |
| Neck BMC | 671 | 4.75 | ± 0.8 | 527 | 5.03 | ± 1.0 | 0.000 | Med |

P-values at the end of table indicate if there is a significant difference between all individuals in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average glucose level for individuals not on any medications is significantly higher than that of individuals taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n' refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 12. The baseline descriptive characteristics for the male subjects on medications versus those who were not taking any medications.

| | No Medication | | | | Taking medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n` | Mean | ± | SD | P | |
| Age | 158 | 48.93 | ± | 12.2 | 229 | 36.43 | ± | 12.7 | 0.000 | Non-Med |
| Weight (kg) | 158 | 86.98 | ± | 14.8 | 227 | 83.21 | ± | 13.4 | 0.010 | Non-Med |
| Height (cm) | 158 | 174.46 | ± | 6.2 | 227 | 175.83 | ± | 6.1 | 0.032 | Med |
| Waist (cm) | 156 | 101.26 | ± | 12.8 | 223 | 95.75 | ± | 11.1 | 0.000 | Non-Med |
| Hip (cm) | 156 | 102.98 | ± | 9.7 | 223 | 100.09 | ± | 8.6 | 0.002 | Non-Med |
| Total %Body Fat | 158 | 27.52 | ± | 7.0 | 226 | 24.12 | ± | 7.5 | 0.000 | Non-Med |
| Total % Trunk Fat | 158 | 32.98 | ± | 7.8 | 226 | 28.55 | ± | 9.0 | 0.000 | Non-Med |
| Total BMD ($g/cm^2$) | 158 | 1.25 | ± | 0.1 | 226 | 1.27 | ± | 0.1 | NS | |
| Spine BMD | 150 | 1.25 | ± | 0.2 | 207 | 1.26 | ± | 0.2 | NS | |
| Left Hip BMD | 150 | 0.99 | ± | 0.1 | 207 | 1.07 | ± | 0.1 | 0.000 | Med |
| BMI | 158 | 28.59 | ± | 4.7 | 227 | 26.90 | ± | 4.0 | 0.000 | Non-Med |
| Waist-Hip Ratio | 156 | 0.98 | ± | 0.1 | 223 | 0.96 | ± | 0.1 | 0.000 | Non-Med |

P-values at the end of table indicate if there is a significant difference between all males in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average glucose level for males not on any medications is significantly higher than that of males taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n` refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 13. The baseline laboratory values, HOMA-IR, and HOMA-β for male subjects comparing those on medications versus those not taking any medications.

| | No Medication | | | | Taking Medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n' | Mean | ± | SD | P | |
| Glucose | 157 | 5.66 | ± | 1.4 | 227 | 5.07 | ± | 0.7 | 0.000 | Non-Med |
| Phosphate | 151 | 1.10 | ± | 0.2 | 211 | 1.15 | ± | 0.2 | 0.003 | Med |
| Calcium | 151 | 2.36 | ± | 0.1 | 215 | 2.37 | ± | 0.1 | NS | |
| Albumin | 151 | 42.17 | ± | 4.0 | 211 | 43.03 | ± | 3.9 | 0.044 | Med |
| Cholesterol | 157 | 5.00 | ± | 1.0 | 227 | 5.13 | ± | 1.2 | NS | |
| Triglycerides | 157 | 1.61 | ± | 0.9 | 227 | 1.42 | ± | 1.0 | NS | |
| Magnesium | 151 | 0.89 | ± | 0.1 | 211 | 0.90 | ± | 0.1 | NS | |
| HDL | 157 | 1.20 | ± | 0.3 | 227 | 1.27 | ± | 0.3 | 0.012 | Med |
| GGT | 125 | 28.77 | ± | 23.1 | 161 | 23.55 | ± | 19.2 | 0.038 | Non-Med |
| LDL | 157 | 3.06 | ± | 0.8 | 227 | 3.19 | ± | 1.0 | NS | |
| Risk Factor | 157 | 4.30 | ± | 1.0 | 227 | 4.17 | ± | 1.2 | NS | |
| Buche | 112 | 10468.82 | ± | 2511.5 | 172 | 9954.74 | ± | 2496.9 | NS | |
| Corrected Buche (SI Units) | 15 | 7839.65 | ± | 6992.6 | 30 | 4314.27 | ± | 5535.3 | NS | |
| Insulin | 148 | 93.04 | ± | 72.6 | 206 | 68.19 | ± | 44.5 | 0.000 | Non-Med |
| BMI | 158 | 28.59 | ± | 4.7 | 227 | 26.90 | ± | 4.0 | 0.000 | Non-Med |
| HOMA-β | 148 | 140.43 | ± | 105.9 | 205 | 139.84 | ± | 167.2 | NS | |
| HOMA-IR | 148 | 3.64 | ± | 5.3 | 206 | 2.25 | ± | 1.6 | 0.000 | Non-Med |
| Triglyceride/HDL ratio | 157 | 1.45 | ± | 0.9 | 227 | 1.24 | ± | 1.1 | 0.046 | Non-Med |
| Log Insulin | 148 | 1.88 | ± | 0.3 | 206 | 1.77 | ± | 0.2 | 0.000 | Non-Med |
| Log HOMA-IR | 148 | 0.44 | ± | 0.3 | 206 | 0.28 | ± | 0.2 | 0.000 | Non-Med |
| Log HOMA-β | 148 | 2.05 | ± | 0.3 | 205 | 2.04 | ± | 0.3 | NS | |
| Log Triglyceride | 157 | 0.14 | ± | 0.2 | 227 | 0.06 | ± | 0.3 | 0.004 | Non-Med |

P-values at the end of table indicate if there is a significant difference between all males in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average glucose level for males not on any medications is significantly higher than that of males taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n` refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 14. The baseline cohort bone density and body composition data comparing male subjects who were on medications against those that were not taking any medications.

| | No Medication | | | | Taking Medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Total Arm BMD | 158 | 1.06 | ± | 0.2 | 226 | 1.08 | ± | 0.1 | NS | |
| Total Leg BMD | 158 | 1.41 | ± | 0.1 | 226 | 1.45 | ± | 0.1 | 0.003 | Med |
| Total Trunk BMD | 158 | 0.99 | ± | 0.1 | 226 | 1.00 | ± | 0.1 | NS | |
| Total Rib BMD | 72 | 0.75 | ± | 0.1 | 92 | 0.76 | ± | 0.1 | NS | |
| Total Pelvis BMD | 72 | 1.22 | ± | 0.1 | 92 | 1.26 | ± | 0.1 | NS | |
| Total BMD | 158 | 1.25 | ± | 0.1 | 226 | 1.27 | ± | 0.1 | NS | |
| Arm % Fat | 158 | 22.93 | ± | 9.0 | 226 | 18.32 | ± | 8.3 | 0.000 | Non-Med |
| Arm tissue | 158 | 9948.15 | ± | 2968.6 | 226 | 9627.12 | ± | 2311.5 | NS | |
| Arm Fat weight(g) | 158 | 2496.89 | ± | 1710.6 | 226 | 1971.38 | ± | 1431.7 | 0.001 | Non-Med |
| Arm Lean muscle mass (g) | 158 | 7445.60 | ± | 1620.3 | 226 | 7655.76 | ± | 1324.1 | NS | |
| Arm Bone Mineral Composition | 157 | 460.22 | ± | 80.0 | 226 | 473.83 | ± | 69.1 | NS | |
| Leg % Fat | 158 | 22.48 | ± | 6.9 | 226 | 21.40 | ± | 6.8 | NS | |
| Leg Tissue weight(g) | 158 | 25006.44 | ± | 4548.6 | 226 | 25617.38 | ± | 4378.2 | NS | |
| Leg Fat weight (g) | 158 | 6085.91 | ± | 2706.8 | 226 | 5928.77 | ± | 2728.1 | NS | |
| Leg lean muscle mass(g) | 158 | 18853.20 | ± | 2945.7 | 226 | 19688.46 | ± | 2621.9 | 0.004 | Med |
| Leg bone mineral composition | 158 | 1197.58 | ± | 171.1 | 226 | 1254.49 | ± | 178.8 | 0.002 | Med |
| Trunk % Fat | 158 | 33.00 | ± | 7.8 | 226 | 28.63 | ± | 8.9 | 0.000 | Non-Med |
| Trunk Tissue weight (g) | 158 | 43948.82 | ± | 10423.1 | 226 | 39938.62 | ± | 7259.8 | 0.000 | Non-Med |
| Trunk Fat weight (g) | 158 | 15118.35 | ± | 5446.1 | 226 | 12182.83 | ± | 5377.7 | 0.000 | Non-Med |
| Trunk lean muscle mass (g) | 158 | 28811.46 | ± | 6706.3 | 226 | 27755.71 | ± | 3376.6 | 0.043 | Non-Med |
| Trunk bone mineral composition | 158 | 912.47 | ± | 225.4 | 226 | 990.38 | ± | 186.4 | 0.000 | Med |
| Android % fat mass | 156 | 40.19 | ± | 9.4 | 225 | 35.16 | ± | 11.4 | 0.000 | Non-Med |
| Android tissue mass (g) | 156 | 7028.01 | ± | 1830.7 | 226 | 6113.05 | ± | 1606.9 | 0.000 | Non-Med |
| Android Fat mass (g) | 156 | 2959.49 | ± | 1248.9 | 226 | 2311.62 | ± | 1240.5 | 0.000 | Non-Med |
| Android lean muscle mass (g) | 156 | 4055.70 | ± | 787.2 | 226 | 3805.81 | ± | 596.2 | 0.000 | Non-Med |
| Android BMC | 156 | 57.12 | ± | 13.1 | 226 | 60.16 | ± | 12.6 | 0.023 | Med |
| Gynecoid % Fat | 156 | 29.88 | ± | 7.7 | 226 | 28.75 | ± | 8.1 | NS | |
| Gynecoid tissue mass (g) | 156 | 12576.58 | ± | 2069.4 | 226 | 12476.08 | ± | 2192.5 | NS | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gynecoid fat mass (g) | 156 | 3934.74 | ± | 1425.9 | 226 | 3813.27 | ± | 1582.2 | NS | |
| Gynecoid lean muscle mass (g) | 156 | 8636.84 | ± | 1250.4 | 226 | 8676.08 | ± | 1248.6 | NS | |
| Gynecoid BMC | 156 | 344.54 | ± | 55.2 | 226 | 347.96 | ± | 60.5 | NS | |
| Total % body fat | 158 | 27.47 | ± | 7.0 | 226 | 24.14 | ± | 7.5 | 0.000 | Non-Med |
| Total tissue mass (g) | 158 | 83664.01 | ± | 14428.6 | 226 | 79847.25 | ± | 13123.1 | 0.007 | Non-Med |
| Total fat mass (g) | 158 | 24392.73 | ± | 8951.2 | 226 | 20702.66 | ± | 9011.6 | 0.000 | Non-Med |
| Total lean muscle mass (g) | 158 | 59271.32 | ± | 8257.4 | 226 | 59144.72 | ± | 6999.5 | NS | |
| Total Bone Mineral Composition | 158 | 3035.87 | ± | 418.0 | 226 | 3202.64 | ± | 415.4 | 0.000 | Med |
| L2-L4 (Z-Score) | 125 | 0.12 | ± | 1.4 | 154 | 0.18 | ± | 1.3 | NS | |
| L2-L4 BMC | 127 | 63.09 | ± | 11.5 | 159 | 62.68 | ± | 11.1 | NS | |
| Femoral Neck (Z-Score) | 122 | -0.09 | ± | 0.8 | 147 | 0.07 | ± | 1.1 | NS | |
| Neck BMC | 125 | 5.59 | ± | 0.8 | 156 | 5.93 | ± | 1.0 | 0.001 | Med |

P-values at the end of table indicate if there is a significant difference between all males in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average total mass for all male subjects not on any medications is significantly higher than that of male subjects taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n` refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 15. The baseline descriptive characteristics for the female subjects on medications versus those who were not taking any medications.

| | No Medication | | | | Taking Medication | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n' | Mean | ± | SD | P | |
| Age | 805 | 45.08 | ± | 12.1 | 582 | 43.55 | ± | 9.3 | 0.011 | Non-Med |
| Weight (kg) | 805 | 70.75 | ± | 15.0 | 581 | 68.02 | ± | 12.8 | 0.000 | Non-Med |
| Height (cm) | 805 | 161.97 | ± | 5.7 | 581 | 162.32 | ± | 5.9 | NS | |
| Waist (cm) | 801 | 92.56 | ± | 15.5 | 578 | 88.60 | ± | 12.3 | 0.000 | Non-Med |
| Hip (cm) | 800 | 103.47 | ± | 12.4 | 578 | 100.99 | ± | 10.4 | 0.000 | Non-Med |
| Total %Body Fat | 805 | 38.32 | ± | 7.4 | 580 | 36.72 | ± | 7.4 | 0.000 | Non-Med |
| Total % Trunk Fat | 804 | 39.64 | ± | 8.5 | 579 | 37.72 | ± | 8.6 | 0.000 | Non-Med |
| Total BMD ($g/cm^2$) | 805 | 1.15 | ± | 0.1 | 580 | 1.16 | ± | 0.1 | NS | |
| Spine BMD | 775 | 1.20 | ± | 0.2 | 556 | 1.21 | ± | 0.1 | NS | |
| Left Hip BMD | 774 | 0.95 | ± | 0.1 | 557 | 0.97 | ± | 0.1 | 0.003 | Med |
| Right Hip BMD | 109 | 0.02 | ± | 0.1 | 80 | 0.00 | ± | 0.0 | NS | |
| BMI | 805 | 26.98 | ± | 5.6 | 581 | 25.82 | ± | 4.7 | 0.000 | Non-Med |
| Waist-Hip Ratio | 800 | 0.89 | ± | 0.1 | 578 | 0.88 | ± | 0.1 | 0.000 | Non-Med |

P-values at the end of table indicate if there is a significant difference between all females in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average age for all females not on any medications is significantly higher than that of females taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n' refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 16. The baseline laboratory values, HOMA-IR, and HOMA-β for female subjects comparing those on medications versus those not taking any medications.

| | No Medication | | | Taking Medication | | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | n` | Mean | SD | P | |
| Glucose | 799 | 5.14 | ± 1.1 | 575 | 4.99 | ± 0.7 | 0.004 | Non-Med |
| Phosphate | 765 | 1.20 | ± 0.2 | 562 | 1.21 | ± 0.2 | NS | |
| Calcium | 764 | 2.34 | ± 0.1 | 562 | 2.34 | ± 0.1 | NS | |
| Albumin | 764 | 40.45 | ± 3.8 | 562 | 41.52 | ± 3.6 | 0.000 | Med |
| Cholesterol | 799 | 5.23 | ± 1.0 | 575 | 5.17 | ± 1.0 | NS | |
| Triglycerides | 799 | 1.24 | ± 0.7 | 575 | 1.03 | ± 0.7 | 0.000 | Non-Med |
| Magnesium | 765 | 0.87 | ± 0.1 | 562 | 0.89 | ± 0.1 | 0.002 | Med |
| HDL | 800 | 1.55 | ± 0.4 | 575 | 1.59 | ± 0.4 | NS | |
| GGT | 540 | 18.46 | ± 22.8 | 367 | 17.37 | ± 27.6 | NS | |
| LDL | 799 | 3.11 | ± 0.9 | 575 | 3.12 | ± 0.9 | NS | |
| Risk Factor | 799 | 3.52 | ± 1.0 | 575 | 3.40 | ± 0.9 | 0.027 | Non-Med |
| Buche | 580 | 9211.83 | ± 2594.4 | 449 | 8627.76 | ± 2810.7 | 0.001 | Non-Med |
| Corrected Buche (SI Units) | 118 | 5867.05 | ± 5343.5 | 127 | 5594.73 | ± 5248.3 | NS | |
| Insulin | 751 | 76.23 | ± 83.6 | 541 | 62.31 | ± 39.2 | 0.000 | Non-Med |
| BMI | 805 | 26.98 | ± 5.6 | 581 | 25.82 | ± 4.7 | 0.000 | Non-Med |
| HOMA-β | 750 | 148.41 | ± 323.4 | 540 | 128.79 | ± 120.0 | NS | |
| HOMA-IR | 750 | 2.63 | ± 3.7 | 541 | 2.06 | ± 1.6 | 0.001 | Non-Med |
| Triglyceride/HDL ratio | 799 | 0.88 | ± 0.6 | 575 | 0.72 | ± 0.6 | 0.000 | Non-Med |
| Log Insulin | 751 | 1.79 | ± 0.3 | 541 | 1.73 | ± 0.2 | 0.000 | Non-Med |
| Log HOMA-IR | 750 | 0.31 | ± 0.3 | 541 | 0.23 | ± 0.3 | 0.000 | Non-Med |
| Log HOMA-β | 749 | 2.09 | ± 0.3 | 539 | 2.04 | ± 0.2 | 0.001 | Non-Med |
| Log Triglyceride | 799 | 0.04 | ± 0.2 | 575 | -0.06 | ± 0.2 | 0.000 | Non-Med |

P-values at the end of table indicate if there is a significant difference between all females in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average glucose level for females not on any medications is significantly higher than that of females taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n` refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 17. The baseline cohort bone density and body composition data comparing female subjects who were on medications against those that were not taking any medications

| | No Medication | | | | Taking Medication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n' | Mean | ± | SD | P | |
| Total Arm BMD | 804 | 0.88 | ± | 0.1 | 580 | 0.89 | ± | 0.1 | NS | |
| Total Leg BMD | 804 | 1.23 | ± | 0.1 | 580 | 1.25 | ± | 0.1 | 0.000 | Med |
| Total Trunk BMD | 804 | 0.92 | ± | 0.1 | 580 | 0.92 | ± | 0.1 | NS | |
| Total Rib BMD | 278 | 0.65 | ± | 0.1 | 197 | 0.65 | ± | 0.1 | NS | |
| Total Pelvis BMD | 278 | 1.13 | ± | 0.1 | 197 | 1.13 | ± | 0.1 | NS | |
| Total BMD | 802 | 1.15 | ± | 0.1 | 579 | 1.16 | ± | 0.1 | NS | |
| Arm % Fat | 803 | 39.89 | ± | 9.4 | 580 | 38.11 | ± | 9.3 | 0.001 | Non-Med |
| Arm tissue | 804 | 7646.69 | ± | 2790.5 | 580 | 7278.25 | ± | 2597.1 | 0.013 | Non-Med |
| Arm Fat weight(g) | 804 | 3351.09 | ± | 1917.9 | 580 | 3057.88 | ± | 1856.1 | 0.005 | Non-Med |
| Arm Lean muscle mass (g) | 804 | 4303.09 | ± | 1044.1 | 580 | 4220.38 | ± | 913.0 | NS | |
| Arm Bone Mineral Composition | 804 | 304.28 | ± | 56.9 | 580 | 312.43 | ± | 51.3 | 0.006 | Med |
| Leg % Fat | 804 | 38.91 | ± | 7.0 | 580 | 37.98 | ± | 6.8 | 0.014 | Non-Med |
| Leg Tissue weight(g) | 804 | 21778.05 | ± | 4434.4 | 580 | 21405.18 | ± | 3899.4 | NS | |
| Leg Fat weight (g) | 804 | 9025.01 | ± | 3162.6 | 580 | 8628.93 | ± | 2794.3 | 0.016 | Non-Med |
| Leg lean muscle mass(g) | 803 | 12755.96 | ± | 1931.6 | 580 | 12781.58 | ± | 1885.9 | NS | |
| Leg bone mineral composition | 804 | 895.45 | ± | 136.3 | 579 | 911.96 | ± | 135.6 | 0.026 | Med |
| Trunk % Fat | 804 | 39.64 | ± | 8.5 | 579 | 37.71 | ± | 8.6 | 0.000 | Non-Med |
| Trunk Tissue weight (g) | 804 | 34164.86 | ± | 8979.3 | 580 | 32229.33 | ± | 6877.9 | 0.000 | Non-Med |
| Trunk Fat weight (g) | 804 | 14354.22 | ± | 6186.9 | 580 | 12856.38 | ± | 5131.4 | 0.000 | Non-Med |
| Trunk lean muscle mass (g) | 804 | 19873.27 | ± | 4135.2 | 580 | 19355.70 | ± | 2777.9 | 0.009 | Non-Med |
| Trunk bone mineral composition | 804 | 735.87 | ± | 148.5 | 580 | 756.63 | ± | 147.7 | 0.010 | Med |
| Android % fat mass | 799 | 44.74 | ± | 10.3 | 580 | 42.63 | ± | 10.5 | 0.000 | Non-Med |
| Android tissue mass (g) | 799 | 5287.65 | ± | 1672.7 | 580 | 4923.59 | ± | 1421.6 | 0.000 | Non-Med |
| Android Fat mass (g) | 799 | 2520.64 | ± | 1264.9 | 580 | 2229.11 | ± | 1098.6 | 0.000 | Non-Med |
| Android lean muscle mass (g) | 799 | 2769.10 | ± | 517.1 | 580 | 2694.33 | ± | 470.6 | 0.006 | Non-Med |
| Android BMC | 799 | 51.36 | ± | 10.7 | 580 | 51.63 | ± | 10.7 | NS | |
| Gynecoid % Fat | 799 | 45.07 | ± | 6.4 | 580 | 44.39 | ± | 6.6 | NS | |
| Gynecoid tissue mass (g) | 799 | 11070.31 | ± | 2175.8 | 580 | 10855.11 | ± | 2033.5 | NS | |
| Gynecoid fat mass (g) | 799 | 5186.82 | ± | 1594.1 | 580 | 5007.55 | ± | 1507.5 | 0.035 | Non-Med |
| Gynecoid lean muscle mass (g) | 799 | 5881.19 | ± | 904.3 | 580 | 5845.13 | ± | 898.7 | NS | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid BMC | 799 | 255.80 | ± 46.6 | 580 | 257.39 | ± 45.9 | NS | |
| Total % body fat | 805 | 38.30 | ± 7.4 | 580 | 36.71 | ± 7.4 | 0.000 | Non-Med |
| Total tissue mass (g) | 805 | 68075.52 | ± 21662.1 | 580 | 64722.13 | ± 12582.0 | 0.001 | Non-Med |
| Total fat mass (g) | 805 | 27566.99 | ± 10558.5 | 580 | 25305.07 | ± 9137.6 | 0.000 | Non-Med |
| Total lean muscle mass (g) | 805 | 39935.64 | ± 5891.0 | 580 | 39432.47 | ± 5299.1 | NS | |
| Total Bone Mineral Composition | 805 | 2405.08 | ± 324.0 | 580 | 2466.70 | ± 333.8 | 0.001 | Med |
| L2-L4 (Z-Score) | 559 | 0.26 | ± 1.3 | 373 | 0.21 | ± 1.1 | NS | |
| L2-L4 BMC | 562 | 50.62 | ± 9.5 | 375 | 51.22 | ± 9.2 | NS | |
| Femoral Neck (Z-Score) | 542 | 0.09 | ± 0.9 | 368 | 0.19 | ± 0.9 | NS | |
| Neck BMC | 546 | 4.56 | ± 0.7 | 371 | 4.65 | ± 0.6 | NS | |

P-values at the end of table indicate if there is a significant difference between all females in the cohort who were taking medications and all those that were not on any medications. The last column indicates which group is greater in value for that measurement (e.g. average total bone mineral composition for female subjects not on any medications is significantly higher than that of females taking medications). No-Med refers to subjects who were not taking any medications, whereas Med refers to subjects taking any type of medication. n and n' refer to the total number subjects not taking medication and taking medication respectively. NS- Not significant if p-value is less than 0.5.

Table 18. The baseline descriptive characteristics for all subjects that were smokers versus those that were non-smokers.

| | Non-smokers | | | | Smokers | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | | SD | n' | Mean | | SD | P | |
| Age | 1573 | 43.99 | ± | 11.9 | 222 | 41.79 | ± | 10.9 | 0.009 | Non-SMO |
| Weight (kg) | 1570 | 73.01 | ± | 15.3 | 222 | 72.42 | ± | 16.1 | NS | |
| Height (cm) | 1570 | 165.18 | ± | 8.0 | 222 | 164.17 | ± | 8.4 | NS | |
| Waist (cm) | 1557 | 92.35 | ± | 14.3 | 219 | 92.81 | ± | 13.1 | NS | |
| Hip (cm) | 1555 | 102.20 | ± | 11.2 | 219 | 102.29 | ± | 10.6 | NS | |
| Total %Body Fat | 1568 | 34.96 | ± | 9.1 | 222 | 34.98 | ± | 8.8 | NS | |
| Total % Trunk Fat | 1566 | 36.89 | ± | 9.4 | 222 | 37.34 | ± | 9.2 | NS | |
| Total BMD ($g/cm^2$) | 1568 | 1.18 | ± | 0.1 | 222 | 1.17 | ± | 0.1 | 0.025 | Non-SMO |
| Spine BMD | 1489 | 1.22 | ± | 0.2 | 209 | 1.19 | ± | 0.2 | 0.018 | Non-SMO |
| Left Hip BMD | 1489 | 0.98 | ± | 0.1 | 209 | 0.95 | ± | 0.1 | 0.011 | Non-SMO |
| Waist-Hip Ratio | 1555 | 0.90 | ± | 0.1 | 219 | 0.91 | ± | 0.1 | NS | |

P-values at the end of table indicate if there is a significant difference between all non-smoking subjects and smokers. The last column indicates which group is greater in value for that measurement (e.g. average age for non-smokers is significantly higher than that of smokers). Non-SMO refers to non-smokers. SMO refers to smokers. n and n' refer to the total number of non-smoking and smoking subjects respectively. NS- Not significant if p-value is less than 0.5.

Table 19. The baseline laboratory values, HOMA-IR, and HOMA-β for the entire cohort comparing those who smoke with those who do not smoke.

| | Non-smokers | | | | Smoker | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | ± | SD | n' | Mean | ± | SD | P | |
| Glucose | 1558 | 5.14 | ± | 1.0 | 220 | 5.01 | ± | 0.7 | NS | |
| Phosphate | 1493 | 1.19 | ± | 0.2 | 209 | 1.20 | ± | 0.2 | NS | |
| Calcium | 1494 | 2.35 | ± | 0.1 | 210 | 2.34 | ± | 0.1 | NS | |
| Albumin | 1492 | 41.33 | ± | 3.9 | 209 | 41.04 | ± | 3.9 | NS | |
| Cholesterol | 1558 | 5.18 | ± | 1.0 | 220 | 5.13 | ± | 1.1 | NS | |
| Triglycerides | 1558 | 1.21 | ± | 0.8 | 220 | 1.37 | ± | 0.8 | 0.004 | SMO |
| Magnesium | 1493 | 0.88 | ± | 0.1 | 209 | 0.88 | ± | 0.1 | NS | |
| HDL | 1559 | 1.50 | ± | 0.4 | 220 | 1.43 | ± | 0.4 | 0.006 | Non-SMO |
| GGT | 1049 | 19.95 | ± | 25.3 | 149 | 19.64 | ± | 13.3 | NS | |
| LDL | 1558 | 3.13 | ± | 0.9 | 220 | 3.07 | ± | 0.9 | NS | |
| Risk Factor | 1558 | 3.61 | ± | 1.0 | 220 | 3.78 | ± | 1.1 | 0.023 | SMO |
| Buche | 1154 | 9255.12 | ± | 2696.6 | 175 | 8960.11 | ± | 2854.9 | NS | |
| Corrected Buche (SI Units) | 267 | 5720.11 | ± | 5430.8 | 25 | 5156.03 | ± | 5382.3 | NS | |
| Insulin | 1449 | 72.44 | ± | 69.7 | 209 | 70.44 | ± | 41.4 | NS | |
| BMI | 1570 | 26.72 | ± | 5.1 | 222 | 26.76 | ± | 4.9 | NS | |
| HOMA-β | 1447 | 137.68 | ± | 245.4 | 208 | 157.11 | ± | 176.8 | NS | |
| HOMA-IR | 1448 | 2.51 | ± | 3.3 | 209 | 2.31 | ± | 1.5 | NS | |
| Triglyceride/HDL ratio | 1558 | 0.91 | ± | 0.7 | 220 | 1.07 | ± | 0.9 | 0.002 | SMO |
| Log Insulin | 1449 | 1.78 | ± | 0.3 | 209 | 1.78 | ± | 0.2 | NS | |
| Log HOMA-IR | 1448 | 0.29 | ± | 0.3 | 209 | 0.29 | ± | 0.3 | NS | |
| Log HOMA-β | 1445 | 2.06 | ± | 0.3 | 208 | 2.09 | ± | 0.3 | NS | |
| Log Triglyceride | 1558 | 0.01 | ± | 0.2 | 220 | 0.07 | ± | 0.2 | 0.001 | SMO |
| Waist-Hip Ratio | 1555 | 0.90 | ± | 0.1 | 219 | 0.91 | ± | 0.1 | NS | |

P-values at the end of table indicate if there is a significant difference between all non-smoking subjects and smokers. The last column indicates which group is greater in value for that measurement (e.g. average HDL level for non-smokers is significantly higher than that of smokers). Non-SMO refers to non-smokers. SMO refers to smokers. n and n' refer to the total number of non-smoking and smoking subjects respectively. NS- Not significant if p-value is less than 0.5.

Table 20. The baseline cohort bone density and body composition data comparing all subjects who were smokers against those that who were non-smokers.

| | Non-smoker | | | Smoker | | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | n` | Mean | SD | P | |
| Total Arm BMD | 1567 | 0.92 | ± 0.1 | 222 | 0.92 | ± 0.1 | NS | |
| Total Leg BMD | 1567 | 1.28 | ± 0.1 | 222 | 1.27 | ± 0.2 | NS | |
| Total Trunk BMD | 1567 | 0.94 | ± 0.1 | 222 | 0.93 | ± 0.1 | NS | |
| Total Rib BMD | 572 | 0.68 | ± 0.1 | 78 | 0.70 | ± 0.1 | 0.026 | SMO |
| Total Pelvis BMD | 572 | 1.16 | ± 0.1 | 78 | 1.17 | ± 0.2 | NS | |
| Total BMD | 1565 | 1.18 | ± 0.1 | 221 | 1.17 | ± 0.1 | 0.023 | Non-SMO |
| Arm % Fat | 1566 | 34.98 | ± 12.3 | 222 | 34.81 | ± 11.6 | NS | |
| Arm tissue | 1567 | 7992.12 | ± 2819.3 | 222 | 8023.85 | ± 2990.2 | NS | |
| Arm Fat weight(g) | 1567 | 3000.63 | ± 1893.3 | 222 | 2988.60 | ± 1768.5 | NS | |
| Arm Lean muscle mass (g) | 1567 | 4992.78 | ± 1745.8 | 222 | 5049.31 | ± 1882.6 | NS | |
| Arm Bone Mineral Composition | 1566 | 343.38 | ± 88.6 | 222 | 342.00 | ± 93.8 | NS | |
| Leg % Fat | 1567 | 34.93 | ± 9.9 | 222 | 34.30 | ± 9.4 | NS | |
| Leg Tissue weight(g) | 1567 | 22491.00 | ± 4447.8 | 222 | 22219.86 | ± 5184.2 | NS | |
| Leg Fat weight (g) | 1567 | 8258.51 | ± 3176.2 | 222 | 8043.97 | ± 3256.0 | NS | |
| Leg lean muscle mass(g) | 1567 | 14229.55 | ± 3451.0 | 221 | 14179.43 | ± 3658.0 | NS | |
| Leg bone mineral composition | 1567 | 977.23 | ± 197.2 | 221 | 960.88 | ± 230.9 | NS | |
| Trunk % Fat | 1566 | 36.90 | ± 9.4 | 222 | 37.34 | ± 9.2 | NS | |
| Trunk Tissue weight (g) | 1567 | 35147.13 | ± 9090.2 | 222 | 35181.00 | ± 8390.9 | NS | |
| Trunk Fat weight (g) | 1567 | 13622.31 | ± 5824.1 | 222 | 13734.10 | ± 5322.9 | NS | |
| Trunk lean muscle mass (g) | 1567 | 21548.64 | ± 5374.4 | 222 | 21446.90 | ± 4975.1 | NS | |
| Trunk bone mineral composition | 1567 | 793.97 | ± 185.8 | 222 | 781.67 | ± 188.9 | NS | |
| Android % fat mass | 1559 | 42.31 | ± 11.0 | 222 | 42.73 | ± 11.0 | NS | |
| Android tissue mass (g) | 1560 | 5423.14 | ± 1714.2 | 222 | 5463.14 | ± 1694.8 | NS | |
| Android Fat mass (g) | 1560 | 2430.74 | ± 1232.6 | 222 | 2457.31 | ± 1171.1 | NS | |
| Android lean muscle mass (g) | 1560 | 2994.76 | ± 724.4 | 222 | 3005.35 | ± 777.2 | NS | |
| Android BMC | 1560 | 53.35 | ± 11.7 | 222 | 52.23 | ± 12.0 | NS | |
| Gynecoid % Fat | 1560 | 41.38 | ± 9.5 | 222 | 41.15 | ± 9.1 | NS | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) | 1560 | 11346.60 | ± | 2184.7 | 222 | 11144.01 | ± | 2386.9 | NS | |
| Gynecoid fat mass (g) | 1560 | 4851.39 | ± | 1640.5 | 222 | 4733.90 | ± | 1593.9 | NS | |
| Gynecoid lean muscle mass (g) | 1560 | 6494.81 | ± | 1517.1 | 222 | 6408.30 | ± | 1591.2 | NS | |
| Gynecoid BMC | 1560 | 277.16 | ± | 60.8 | 222 | 272.25 | ± | 69.9 | NS | |
| Total % body fat | 1567 | 34.97 | ± | 9.0 | 222 | 34.96 | ± | 8.8 | NS | |
| Total tissue mass (g) | 1567 | 69984.92 | ± | 18886.9 | 222 | 69405.30 | ± | 15788.4 | NS | |
| Total fat mass (g) | 1567 | 25663.42 | ± | 10093.2 | 222 | 25539.60 | ± | 9509.1 | NS | |
| Total lean muscle mass (g) | 1567 | 44027.23 | ± | 10065.3 | 222 | 43905.84 | ± | 10652.8 | NS | |
| Total Bone Mineral Composition | 1567 | 2592.05 | ± | 452.0 | 222 | 2551.91 | ± | 505.0 | NS | |
| L2-L4 (Z-Score) | 1063 | 0.27 | ± | 1.2 | 153 | -0.09 | ± | 1.3 | 0.001 | Non-SMO |
| L2-L4 BMC | 1074 | 53.94 | ± | 11.0 | 154 | 51.71 | ± | 11.4 | 0.019 | Non-SMO |
| Femoral Neck (Z-Score) | 1035 | 0.13 | ± | 0.9 | 149 | -0.09 | ± | 0.9 | 0.008 | Non-SMO |
| Neck BMC | 1053 | 4.88 | ± | 0.9 | 150 | 4.77 | ± | 0.9 | NS | |

P-values at the end of table indicate if there is a significant difference between all non-smoking subjects and smokers. The last column indicates which group is greater in value for that measurement (e.g. average total bone mineral density for non-smokers is significantly higher than that of smokers). Non-SMO refers to non-smokers. SMO refers to smokers. n and n' refer to the total number of non-smoking and smoking subjects respectively. NS- Not significant if p-value is less than 0.5.

Table 21. The baseline descriptive characteristics for all male subjects that were smokers versus those that were non-smokers.

| | Male | | | | | |
|---|---|---|---|---|---|---|
| | | Non-Smoker | | Smoker | | |
| | n | Mean | SD | Mean | SD | P |
| Age | 350 | 41.49 ± | 14.19 | 39.06 ± | 12.52 | NS |
| Weight (kg) | 348 | 84.35 ± | 13.59 | 87.61 ± | 16.63 | NS |
| Height (cm) | 348 | 175.36 ± | 6.41 | 175.73 ± | 5.54 | NS |
| Waist (cm) | 342 | 97.66 ± | 12.04 | 100.47 ± | 12.69 | NS |
| Hip (cm) | 341 | 101.09 ± | 9.15 | 103.29 ± | 9.22 | NS |
| Total %Body Fat | 347 | 25.37 ± | 7.60 | 25.70 ± | 7.39 | NS |
| Total % Trunk Fat | 347 | 30.18 ± | 8.94 | 30.35 ± | 8.36 | NS |
| Total BMD ($g/cm^2$) | 347 | 1.26 ± | 0.10 | 1.29 ± | 0.10 | 0.05 Smo |
| Spine BMD | 318 | 1.25 ± | 0.16 | 1.29 ± | 0.18 | NS |
| Left Hip BMD | 318 | 1.03 ± | 0.14 | 1.05 ± | 0.16 | NS |
| BMI | 348 | 27.45 ± | 4.33 | 28.28 ± | 4.68 | NS |
| Waist-Hip Ratio | 341 | 0.97 ± | 0.06 | 0.97 ± | 0.06 | NS |

P-values at the end of table indicate if there is a significant difference between all male non-smoking subjects and male smokers. The last column indicates which group is greater in value for that measurement (e.g. average total bone mineral density of male smokers is significantly higher than that of non-smokers. NS- Not significant if p-value is less than 0.5.

Table 22. The baseline laboratory values, HOMA-IR, and HOMA-β for all male subjects comparing those who smoke with those who do not smoke.

| | Male | | | | | |
|---|---|---|---|---|---|---|
| | | Non-Smoker | | Smoker | | |
| | N | Mean | SD | Mean | SD | P |
| Glucose | 347 | 5.30 ± | 1.05 | 5.31 ± | 1.18 | NS |
| Phosphate | 323 | 1.13 ± | 0.18 | 1.13 ± | 0.15 | NS |
| Calcium | 325 | 2.36 ± | 0.12 | 2.35 ± | 0.10 | NS |
| Albumin | 323 | 42.64 ± | 4.05 | 42.98 ± | 3.63 | NS |
| Cholesterol | 347 | 5.08 ± | 1.11 | 4.92 ± | 1.08 | NS |
| Triglycerides | 347 | 1.47 ± | 0.95 | 1.69 ± | 1.11 | NS |
| Magnesium | 323 | 0.89 ± | 0.11 | 0.88 ± | 0.14 | NS |
| HDL | 347 | 1.25 ± | 0.26 | 1.18 ± | 0.26 | NS |
| GGT | 253 | 25.80 ± | 21.57 | 26.40 ± | 17.13 | NS |
| LDL | 347 | 3.15 ± | 0.94 | 2.92 ± | 0.88 | NS |
| Risk Factor | 347 | 4.19 ± | 1.09 | 4.34 ± | 1.33 | NS |
| Buche | 256 | 10190.14 ± | 2587.77 | 9706.14 ± | 2414.46 | NS |
| Corrected Buche (SI Units) | 41 | 5631.58 ± | 6370.22 | 4031.95 ± | 4649.71 | NS |
| Insulin | 315 | 77.98 ± | 59.09 | 83.05 ± | 57.52 | NS |
| HOMA-β | 314 | 134.61 ± | 116.19 | 180.15 ± | 273.09 | NS |
| HOMA-IR | 315 | 2.83 ± | 3.83 | 2.85 ± | 2.07 | NS |
| Triglyceride/HDL ratio | 347 | 1.28 ± | 0.98 | 1.56 ± | 1.20 | NS |
| Log Insulin | 315 | 1.81 ± | 0.25 | 1.84 ± | 0.26 | NS |
| Log HOMA-IR | 315 | 0.34 ± | 0.28 | 0.36 ± | 0.28 | NS |
| Log HOMA-β | 314 | 2.04 ± | 0.26 | 2.09 ± | 0.33 | NS |
| Log Triglyceride | 347 | 0.09 ± | 0.26 | 0.14 ± | 0.28 | NS |

[1]P-values at the end of table indicate if there is a significant difference between all male non-smoking subjects and male smokers.
NS- Not significant if p-value is less than 0.5

Table 23. The baseline cohort bone density and body composition data comparing male subjects who were smokers against those who were non-smokers.

| | Male | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-Smoker | | Smoker | | | |
| | N | Mean | SD | Mean | SD | P | |
| Total Arm BMD | 347 | 1.06 ± | 0.15 | 1.09 ± | 0.14 | NS | |
| Total Leg BMD | 347 | 1.43 ± | 0.13 | 1.46 ± | 0.13 | NS | |
| Total Trunk BMD | 347 | 0.99 ± | 0.09 | 1.01 ± | 0.09 | NS | |
| Total Rib BMD | 143 | 0.75 ± | 0.09 | 0.76 ± | 0.08 | NS | |
| Total Pelvis BMD | 143 | 1.24 ± | 0.14 | 1.26 ± | 0.14 | NS | |
| Total BMD | 347 | 1.26 ± | 0.10 | 1.29 ± | 0.10 | 0.05 | Smo |
| Arm % Fat | 347 | 20.02 ± | 9.04 | 20.50 ± | 8.57 | NS | |
| Arm tissue | 347 | 9667.50 ± | 2389.17 | 10428.39 ± | 3724.51 | NS | |
| Arm Fat weight(g) | 347 | 2133.05 ± | 1486.25 | 2472.59 ± | 2082.53 | NS | |
| Arm Lean muscle mass (g) | 347 | 7531.87 ± | 1384.56 | 7955.91 ± | 1853.77 | NS | |
| Arm Bone Mineral Composition | 346 | 465.50 ± | 73.81 | 490.07 ± | 72.08 | 0.03 | Smo |
| Leg % Fat | 347 | 21.77 ± | 6.86 | 22.23 ± | 7.28 | NS | |
| Leg Tissue weight(g) | 347 | 25293.13 ± | 4266.78 | 26159.91 ± | 5638.82 | NS | |
| Leg Fat weight (g) | 347 | 5940.56 ± | 2647.65 | 6394.67 ± | 3251.28 | NS | |
| Leg lean muscle mass(g) | 347 | 19321.80 ± | 2740.54 | 19765.30 ± | 3173.64 | NS | |
| Leg bone mineral composition | 347 | 1226.74 ± | 174.94 | 1284.39 ± | 204.64 | 0.04 | Smo |
| Trunk % Fat | 347 | 30.25 ± | 8.95 | 30.35 ± | 8.36 | NS | |
| Trunk Tissue weight (g) | 347 | 41354.17 ± | 8938.77 | 42867.83 ± | 8296.14 | NS | |
| Trunk Fat weight (g) | 347 | 13247.18 ± | 5647.12 | 13773.26 ± | 5436.03 | NS | |
| Trunk lean muscle mass (g) | 347 | 28098.27 ± | 5106.25 | 29094.65 ± | 4126.11 | NS | |
| Trunk bone mineral composition | 347 | 959.33 ± | 205.14 | 963.74 ± | 231.38 | NS | |
| Android % fat mass | 344 | 36.93 ± | 11.08 | 37.42 ± | 11.20 | NS | |
| Android tissue mass (g) | 345 | 6428.23 ± | 1734.56 | 6768.04 ± | 1968.06 | NS | |
| Android Fat mass (g) | 345 | 2540.19 ± | 1277.60 | 2697.72 ± | 1385.69 | NS | |
| Android lean muscle mass (g) | 345 | 3890.91 ± | 667.83 | 4070.24 ± | 812.66 | NS | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Android BMC | 345 | 58.74 | ± | 12.94 | 61.17 | ± 13.65 | NS |
| Gynecoid % Fat | 345 | 29.04 | ± | 7.87 | 29.84 | ± 8.85 | NS |
| Gynecoid tissue mass (g) | 345 | 12479.90 | ± | 2066.97 | 12848.39 | ± 2582.78 | NS |
| Gynecoid fat mass (g) | 345 | 3825.99 | ± | 1483.57 | 4073.00 | ± 1758.20 | NS |
| Gynecoid lean muscle mass (g) | 345 | 8660.34 | ± | 1229.79 | 8775.33 | ± 1399.47 | NS |
| Gynecoid BMC | 345 | 345.41 | ± | 57.39 | 360.37 | ± 68.88 | NS |
| Total % body fat | 346 | 25.39 | ± | 7.55 | 25.70 | ± 7.39 | NS |
| Total tissue mass (g) | 346 | 81044.43 | ± | 13332.11 | 84173.26 | ± 16411.87 | NS |
| Total fat mass (g) | 346 | 22006.09 | ± | 9100.46 | 23288.41 | ± 9795.87 | NS |
| Total lean muscle mass (g) | 346 | 59038.43 | ± | 7352.03 | 60884.85 | ± 8737.25 | NS |
| Total Bone Mineral Composition | 346 | 3126.38 | ± | 424.95 | 3223.89 | ± 449.46 | NS |
| TMASS | 346 | 84.17 | ± | 13.48 | 87.40 | ± 16.60 | NS |
| L2-L4 (Z-Score) | 247 | 0.16 | ± | 1.32 | 0.14 | ± 1.52 | NS |
| L2-L4 BMC | 253 | 62.96 | ± | 11.32 | 62.29 | ± 10.71 | NS |
| Femoral Neck (Z-Score) | 238 | -0.01 | ± | 0.93 | 0.05 | ± 1.01 | NS |
| Neck BMC | 249 | 5.76 | ± | 0.90 | 5.89 | ± 0.89 | NS |

P-values at the end of table indicate if there is a significant difference between all male non-smoking subjects and male smokers. The last column indicates which group is greater in value for that parameter (e.g. average total bone mineral density of male smokers is significantly higher than that of non-smokers). Non-SMO refers to non-smokers. SMO refers to smokers. NS- Not significant if p-value is less than 0.5

Table 24. The baseline descriptive characteristics for all female subjects that were smokers versus those that were non-smokers.

| | Female | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-Smoker | | Smoker | | | |
| | N | Mean | SD | Mean | SD | P | |
| Age | 1219 | 44.74 | ± 11.11 | 42.50 | ± 10.36 | 0.01 | Non-Smo |
| Weight (kg) | 1218 | 69.74 | ± 14.24 | 68.45 | ± 13.35 | NS | |
| Height (cm) | 1218 | 162.26 | ± 5.78 | 161.15 | ± 6.04 | 0.02 | Non-Smo |
| Waist (cm) | 1211 | 90.86 | ± 14.60 | 90.94 | ± 12.52 | NS | |
| Hip (cm) | 1210 | 102.48 | ± 11.74 | 102.05 | ± 10.87 | NS | |
| Total %Body Fat | 1217 | 37.68 | ± 7.41 | 37.41 | ± 7.37 | NS | |
| Total % Trunk Fat | 1215 | 38.78 | ± 8.59 | 39.16 | ± 8.51 | NS | |
| Total BMD ($g/cm^2$) | 1217 | 1.16 | ± 0.09 | 1.13 | ± 0.09 | 0.00 | Non-Smo |
| Spine BMD | 1168 | 1.21 | ± 0.15 | 1.17 | ± 0.16 | 0.00 | Non-Smo |
| Left Hip BMD | 1168 | 0.96 | ± 0.12 | 0.93 | ± 0.12 | 0.00 | Non-Smo |
| BMI | 1218 | 26.51 | ± 5.28 | 26.36 | ± 4.88 | NS | |
| Waist-Hip Ratio | 1210 | 0.88 | ± 0.07 | 0.89 | ± 0.07 | NS | |

P-values at the end of table indicate if there is a significant difference between all female non-smoking subjects and female smokers. The last column indicates which group is greater in value for that measurement (e.g. average weight for female non-smokers was significantly higher than that of female smokers). Non-SMO refers to non-smokers. SMO refers to smokers. NS- Not significant if p-value is less than 0.5

Table 25. The baseline laboratory values, HOMA-IR, and HOMA-β for all female subjects comparing those who smoke with those who do not smoke.

| | Female | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-Smoker | | Smoker | | | |
| | n | Mean | SD | Mean | SD | P | |
| Glucose | 1207 | 5.10 ± | 0.96 | 4.93 ± | 0.53 | 0.03 | Non-Smo |
| Phosphate | 1166 | 1.20 ± | 0.18 | 1.22 ± | 0.18 | NS | |
| Calcium | 1165 | 2.34 ± | 0.12 | 2.34 ± | 0.14 | NS | |
| Albumin | 1165 | 40.97 ± | 3.73 | 40.56 ± | 3.80 | NS | |
| Cholesterol | 1207 | 5.21 ± | 1.03 | 5.19 ± | 1.08 | NS | |
| Triglycerides | 1207 | 1.13 ± | 0.70 | 1.29 ± | 0.73 | 0.01 | Smo |
| Magnesium | 1166 | 0.88 ± | 0.08 | 0.88 ± | 0.11 | NS | |
| HDL | 1208 | 1.58 ± | 0.37 | 1.49 ± | 0.38 | 0.01 | Non-Smo |
| GGT | 796 | 18.09 ± | 26.15 | 17.57 ± | 11.21 | NS | |
| LDL | 1207 | 3.12 ± | 0.88 | 3.11 ± | 0.92 | NS | |
| Risk Factor | 1207 | 3.45 ± | 0.93 | 3.63 ± | 1.02 | 0.02 | Smo |
| Buche | 894 | 8989.56 ± | 2674.28 | 8780.21 ± | 2930.57 | NS | |
| Corrected Buche (SI Units) | 225 | 5761.62 ± | 5256.29 | 5370.14 ± | 5587.41 | NS | |
| Insulin | 1130 | 70.84 ± | 72.41 | 67.27 ± | 35.81 | NS | |
| HOMA-β | 1129 | 138.44 ± | 270.94 | 151.28 ± | 143.16 | NS | |
| HOMA-IR | 1129 | 2.43 ± | 3.20 | 2.17 ± | 1.25 | NS | |
| Triglyceride/HDL ratio | 1207 | 0.80 ± | 0.63 | 0.95 ± | 0.69 | 0.00 | Smo |
| Log Insulin | 1130 | 1.77 ± | 0.25 | 1.77 ± | 0.23 | NS | |
| Log HOMA-IR | 1129 | 0.27 ± | 0.28 | 0.27 ± | 0.25 | NS | |
| Log HOMA-β | 1127 | 2.06 ± | 0.26 | 2.10 ± | 0.24 | NS | |
| Log Triglyceride | 1207 | -0.01 ± | 0.23 | 0.05 ± | 0.22 | 0.00 | Smo |

P-values at the end of table indicate if there is a significant difference between all female non-smoking subjects and female smokers. The last column indicates which group is greater in value for that measurement (e.g. average glucose for female non-smokers was significantly higher than that of female smokers). Non-SMO refers to non-smokers. SMO refers to smokers. NS- Not significant if p-value is less than 0.5.

Table 26. The baseline cohort bone density and body composition data comparing female subjects who were smokers against those who were non-smokers.

| | Female | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Non-Smoker | | | Smoker | | | |
| | n | Mean | | SD | Mean | | SD | P | |
| Total Arm BMD | 1216 | 0.88 | ± | 0.10 | 0.87 | ± | 0.09 | NS | |
| Total Leg BMD | 1216 | 1.24 | ± | 0.10 | 1.21 | ± | 0.11 | 0.00 | Non-Smo |
| Total Trunk BMD | 1216 | 0.92 | ± | 0.09 | 0.91 | ± | 0.09 | 0.03 | Non-Smo |
| Total Rib BMD | 429 | 0.65 | ± | 0.05 | 0.66 | ± | 0.07 | NS | |
| Total Pelvis BMD | 429 | 1.13 | ± | 0.11 | 1.12 | ± | 0.14 | NS | |
| Total BMD | 1214 | 1.16 | ± | 0.09 | 1.13 | ± | 0.09 | 0.00 | Non-Smo |
| Arm % Fat | 1215 | 39.23 | ± | 9.43 | 38.55 | ± | 9.09 | NS | |
| Arm tissue | 1216 | 7508.64 | ± | 2751.71 | 7395.39 | ± | 2409.93 | NS | |
| Arm Fat weight(g) | 1216 | 3244.01 | ± | 1925.35 | 3123.47 | ± | 1657.10 | NS | |
| Arm Lean muscle mass (g) | 1216 | 4267.02 | ± | 1004.63 | 4289.63 | ± | 889.44 | NS | |
| Arm Bone Mineral Composition | 1216 | 308.47 | ± | 55.46 | 303.30 | ± | 50.07 | NS | |
| Leg % Fat | 1216 | 38.67 | ± | 6.94 | 37.45 | ± | 7.07 | 0.03 | Non-Smo |
| Leg Tissue weight(g) | 1216 | 21675.13 | ± | 4151.68 | 21190.08 | ± | 4540.58 | NS | |
| Leg Fat weight (g) | 1216 | 8907.02 | ± | 2984.00 | 8475.03 | ± | 3125.43 | NS | |
| Leg lean muscle mass(g) | 1216 | 12773.11 | ± | 1906.77 | 12711.14 | ± | 1973.99 | NS | |
| Leg bone mineral composition | 1216 | 905.57 | ± | 135.17 | 875.85 | ± | 147.05 | 0.01 | Non-Smo |
| Trunk % Fat | 1215 | 38.78 | ± | 8.60 | 39.17 | ± | 8.51 | NS | |
| Trunk Tissue weight (g) | 1216 | 33368.66 | ± | 8338.74 | 33171.94 | ± | 7185.04 | NS | |
| Trunk Fat weight (g) | 1216 | 13718.72 | ± | 5876.44 | 13723.86 | ± | 5308.60 | NS | |
| Trunk lean muscle mass (g) | 1216 | 19683.13 | ± | 3741.39 | 19448.06 | ± | 2736.39 | NS | |
| Trunk bone mineral composition | 1216 | 745.98 | ± | 148.73 | 734.08 | ± | 142.58 | NS | |
| Android % fat mass | 1211 | 43.80 | ± | 10.48 | 44.11 | ± | 10.50 | NS | |
| Android tissue mass (g) | 1211 | 5135.03 | ± | 1599.34 | 5122.08 | ± | 1437.70 | NS | |
| Android Fat mass (g) | 1211 | 2397.33 | ± | 1219.47 | 2394.47 | ± | 1104.06 | NS | |
| Android lean muscle mass (g) | 1211 | 2739.91 | ± | 503.82 | 2727.02 | ± | 465.94 | NS | |
| Android BMC | 1211 | 51.71 | ± | 10.72 | 49.89 | ± | 10.30 | 0.04 | Non-Smo |
| Gynecoid % Fat | 1211 | 44.87 | ± | 6.49 | 44.10 | ± | 6.42 | NS | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) | 1211 | 11017.44 | ± | 2108.49 | 10698.55 | ± | 2125.23 | NS | |
| Gynecoid fat mass (g) | 1211 | 5137.30 | ± | 1559.27 | 4906.63 | ± | 1506.20 | NS | |
| Gynecoid lean muscle mass (g) | 1211 | 5877.79 | ± | 900.09 | 5789.64 | ± | 914.39 | NS | |
| Gynecoid BMC | 1211 | 257.46 | ± | 45.72 | 249.22 | ± | 48.81 | 0.03 | Non-Smo |
| Total % body fat | 1217 | 37.66 | ± | 7.40 | 37.38 | ± | 7.37 | NS | |
| Total tissue mass (g) | 1217 | 66812.87 | ± | 19073.46 | 65545.49 | ± | 13161.56 | NS | |
| Total fat mass (g) | 1217 | 26675.16 | ± | 10119.51 | 26127.98 | ± | 9372.13 | NS | |
| Total lean muscle mass (g) | 1217 | 39758.78 | ± | 5702.25 | 39468.14 | ± | 5300.81 | NS | |
| Total Bone Mineral Composition | 1217 | 2438.71 | ± | 325.84 | 2376.27 | ± | 346.97 | 0.02 | Non-Smo |
| L2-L4 (Z-Score) | 816 | 0.30 | ± | 1.21 | -0.16 | ± | 1.28 | 0.00 | Non-Smo |
| L2-L4 BMC | 821 | 51.17 | ± | 9.26 | 48.71 | ± | 9.71 | 0.01 | Non-Smo |
| Femoral Neck (Z-Score) | 797 | 0.17 | ± | 0.94 | -0.13 | ± | 0.81 | 0.00 | Non-Smo |
| Neck BMC | 804 | 4.61 | ± | 0.67 | 4.46 | ± | 0.68 | 0.02 | Non-Smo |

P-values at the end of table indicate if there is a significant difference between all female non-smoking subjects and female smokers. The last column indicates which group is greater in value for that parameter (e.g. average total BMC for female non-smokers was significantly higher than that of female smokers). Non-SMO refers to non-smokers. SMO refers to smokers. NS- Not significant if p-value is less than 0.5.

Table 27. One-way ANOVA analysis comparing descriptive characteristics of the entire cohort factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 81 - 587 | 78 - 544 | 87 - 668 |
| Age[1,2] | 41.25 ± 12.45 | 45.34 ± 10.92 | 44.44 ± 11.35 |
| Weight (kg) [1,2,3] | 63.18 ± 10.41 | 70.18 ± 10.78 | 83.93 ± 15.58 |
| Height (cm) [1,2] | 166.03 ± 8.11 | 164.33 ± 7.84 | 164.79 ± 8.36 |
| Waist (cm) [1,2,3] | 81.93 ± 8.99 | 90.54 ± 9.42 | 103.27 ± 13.63 |
| Hip (cm) [1,2,3] | 93.52 ± 6.19 | 100.72 ± 6.32 | 111.25 ± 10.88 |
| Total %Body Fat[1,2,3] | 26.51 ± 6.90 | 35.42 ± 5.28 | 42.12 ± 6.23 |
| Total % Trunk Fat[1,2,3] | 27.32 ± 7.02 | 37.79 ± 4.72 | 44.77 ± 5.56 |
| Total BMD ($g/cm^2$) [2,3] | 1.17 ± 0.10 | 1.17 ± 0.10 | 1.21 ± 0.10 |
| Spine BMD[2,3] | 1.20 ± 0.16 | 1.20 ± 0.15 | 1.24 ± 0.16 |
| Left Hip BMD[3] | 0.97 ± 0.14 | 0.96 ± 0.12 | 0.99 ± 0.13 |
| BMI[1,2,3] | 22.84 ± 2.74 | 25.91 ± 2.91 | 30.87 ± 5.03 |
| Waist-Hip Ratio[1,2,3] | 0.88 ± 0.07 | 0.90 ± 0.07 | 0.93 ± 0.08 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 28. One-way ANOVA analysis comparing laboratory values, HOMA-β, and HOMA-IR for all cohort subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 81 - 587 | 78 - 544 | 87 - 668 |
| Glucose[1,2,3] | 4.91 ± 0.69 | 5.12 ± 0.91 | 5.32 ± 1.12 |
| Phosphate[1,3] | 1.21 ± 0.18 | 1.19 ± 0.18 | 1.16 ± 0.18 |
| Calcium | 2.35 ± 0.12 | 2.34 ± 0.13 | 2.34 ± 0.12 |
| Albumin[1,3] | 41.95 ± 3.80 | 41.55 ± 3.96 | 40.54 ± 3.66 |
| Cholesterol[1,2] | 4.97 ± 1.01 | 5.29 ± 1.07 | 5.26 ± 1.04 |
| Triglycerides[1,2,3] | 1.00 ± 0.61 | 1.22 ± 0.78 | 1.44 ± 0.87 |
| Magnesium | 0.88 ± 0.10 | 0.89 ± 0.09 | 0.88 ± 0.09 |
| HDL[1,2,3] | 1.59 ± 0.40 | 1.50 ± 0.36 | 1.40 ± 0.35 |
| GGT[2] | 17.72 ± 21.48 | 18.97 ± 16.04 | 22.27 ± 30.44 |
| LDL[1,2] | 2.92 ± 0.88 | 3.23 ± 0.92 | 3.20 ± 0.87 |
| Risk Factor[1,2,3] | 3.27 ± 0.91 | 3.67 ± 1.03 | 3.92 ± 1.04 |
| Buche[1,2,3] | 8453.92 ± 2745.59 | 9207.80 ± 2453.22 | 9948.12 ± 2719.64 |
| Corrected Buche (SI Units)[1] | 4638.03 ± 4825.42 | 7074.40 ± 5159.52 | 5669.19 ± 6172.78 |
| Insulin[1,2,3] | 52.87 ± 33.73 | 67.62 ± 77.61 | 91.15 ± 71.74 |
| HOMA-β[2,3] | 119.12 ± 185.77 | 124.69 ± 294.12 | 168.69 ± 219.83[2,3] |
| HOMA-IR[1,2,3] | 1.72 ± 1.43 | 2.30 ± 3.82 | 3.24 ± 3.40 |
| Triglyceride/HDL ratio[1,2,3] | 0.70 ± 0.57 | 0.91 ± 0.75 | 1.14 ± 0.86 |
| Log Insulin[1,2,3] | 1.66 ± 0.22 | 1.75 ± 0.23 | 1.89 ± 0.24 |
| Log HOMA-IR[1,2,3] | 0.16 ± 0.24 | 0.26 ± 0.25 | 0.41 ± 0.27 |
| Log HOMA-β[1,2,3] | 1.99 ± 0.24 | 2.05 ± 0.25 | 2.13 ± 0.26 |
| Log Triglyceride[1,2,3] | -0.06 ± 0.22 | 0.02 ± 0.24 | 0.09 ± 0.24 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 29. One-way ANOVA analysis comparing bone density and body composition data comparing all cohort subjects factoring for Bray percentage body fat categories of lean/normal individuals, overweight and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 81 - 587 | 78 - 544 | 87 - 668 |
| Total Arm BMD[1,3] | 0.90 ± 0.13 | 0.91 ± 0.13 | 0.96 ± 0.15 |
| Total Leg BMD[3] | 1.28 ± 0.14 | 1.27 ± 0.13 | 1.29 ± 0.13 |
| Total Trunk BMD[1,3] | 0.92 ± 0.09 | 0.93 ± 0.09 | 0.96 ± 0.09 |
| Total Rib BMD[1,3] | 0.66 ± 0.08 | 0.67 ± 0.08 | 0.69 ± 0.08 |
| Total Pelvis BMD[1,3] | 1.14 ± 0.13 | 1.15 ± 0.13 | 1.19 ± 0.13 |
| Total BMD[1,3] | 1.17 ± 0.10 | 1.17 ± 0.10 | 1.21 ± 0.10 |
| Arm % Fat[1,2,3] | 25.68 ± 10.24 | 35.47 ± 9.03 | 42.80 ± 10.22 |
| Arm tissue[1,2,3] | 6529.74 ± 1725.72 | 7504.79 ± 1661.16 | 9703.25 ± 3433.69 |
| Arm Fat weight(g) [1,2,3] | 1702.94 ± 745.78 | 2720.50 ± 803.14 | 4376.67 ± 2238.68 |
| Arm Lean muscle mass (g) [1,3] | 4825.14 ± 1765.29 | 4789.79 ± 1585.63 | 5331.26 ± 1848.53 |
| Arm Bone Mineral Composition | 342.67 ± 87.19 | 339.90 ± 82.80 | 347.11 ± 96.49 |
| Leg % Fat[1,2,3] | 27.93 ± 8.43 | 34.96 ± 7.72 | 40.95 ± 8.29 |
| Leg Tissue weight(g) [1,2,3] | 19969.64 ± 3185.90 | 21581.43 ± 3399.51 | 25408.23 ± 4756.08 |
| Leg Fat weight (g) [1,2,3] | 5744.40 ± 1725.16 | 7778.74 ± 1822.75 | 10827.97 ± 3116.14 |
| Leg lean muscle mass(g) [3] | 14224.95 ± 3464.48 | 13789.86 ± 3399.91 | 14584.73 ± 3504.63 |
| Leg bone mineral composition[1,3] | 956.41 ± 199.95 | 947.74 ± 200.12 | 1013.89 ± 197.65 |
| Trunk % Fat[1,2,3] | 27.33 ± 7.02 | 37.83 ± 4.64 | 44.84 ± 5.35 |
| Trunk Tissue weight (g) [1,2,3] | 29662.71 ± 6650.47 | 33828.17 ± 6189.72 | 41186.06 ± 9268.12 |
| Trunk Fat weight (g) [1,2,3] | 8333.69 ± 2937.65 | 13066.08 ± 2708.24 | 18848.83 ± 4906.23 |
| Trunk lean muscle mass (g) [1,3] | 21329.24 ± 5195.10 | 20835.74 ± 5015.93 | 22332.93 ± 5627.83 |
| Trunk bone mineral composition[2] | 776.48 ± 182.98 | 796.25 ± 170.12 | 803.41 ± 200.95 |
| Android % fat mass[1,2,3] | 31.57 ± 9.30 | 43.41 ± 6.01 | 51.18 ± 6.15 |
| Android tissue mass (g) [1,2,3] | 4294.84 ± 1137.18 | 5169.92 ± 1129.92 | 6669.00 ± 1749.80 |
| Android Fat mass (g) [1,2,3] | 1413.76 ± 720.95 | 2281.76 ± 632.73 | 3481.33 ± 1128.94 |
| Android lean muscle mass (g) [1,3] | 2882.37 ± 672.53 | 2891.53 ± 661.47 | 3189.17 ± 794.38 |
| Android BMC[1,3] | 51.65 ± 10.88 | 51.95 ± 11.18 | 55.56 ± 12.41 |
| Gynecoid % Fat[1,2,3] | 35.06 ± 8.96 | 41.71 ± 7.57 | 46.72 ± 7.61 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) [1,2,3] | 9999.27 | ± | 1569.53 | 10912.80 | ± | 1494.39 | 12866.21 | ± 2285.87 |
| Gynecoid fat mass (g) [1,2,3] | 3572.34 | ± | 1017.09 | 4621.92 | ± | 901.45 | 6158.80 | ± 1570.41 |
| Gynecoid lean muscle mass (g) [1,3] | 6426.25 | ± | 1563.39 | 6287.82 | ± | 1467.24 | 6708.95 | ± 1515.35 |
| Gynecoid BMC[1,2,3] | 260.28 | ± | 60.30 | 269.96 | ± | 60.69 | 296.44 | ± 59.07 |
| Total % body fat[1,2,3] | 26.52 | ± | 6.88 | 35.42 | ± | 5.26 | 42.10 | ± 6.21 |
| Total tissue mass (g) [1,2,3] | 60035.19 | ± | 10266.83 | 67712.67 | ± | 21841.82 | 80550.52 | ± 15518.30 |
| Total fat mass (g) [1,2,3] | 16368.56 | ± | 4380.51 | 24295.86 | ± | 3907.63 | 35018.28 | ± 8741.91 |
| Total lean muscle mass (g) [1,3] | 43665.57 | ± | 10039.22 | 42569.74 | ± | 9626.79 | 45546.01 | ± 10442.37 |
| Total Bone Mineral Composition[1,3] | 2547.76 | ± | 473.73 | 2564.79 | ± | 454.04 | 2639.99 | ± 444.00 |
| L2-L4 (Z-Score) [2] | 0.36 | ± | 1.25 | 0.20 | ± | 1.20 | 0.14 | ± 1.30 |
| L2-L4 BMC[1,3] | 53.02 | ± | 10.51 | 52.36 | ± | 11.12 | 55.12 | ± 11.17 |
| Femoral Neck (Z-Score) [2] | 0.21 | ± | 1.01 | 0.10 | ± | 0.87 | 0.02 | ± 0.92 |
| Neck BMC[3] | 4.88 | ± | 0.98 | 4.75 | ± | 0.83 | 4.95 | ± 0.85 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 30. One-way ANOVA analysis comparing descriptive characteristics of all male subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 19 - 138 | 13 - 104 | 14 - 154 |
| Age[1] | 38.08 ± 14.70 | 44.21 ± 13.10 | 41.40 ± 13.12 |
| Weight (kg) [1,2,3] | 75.66 ± 9.23 | 84.10 ± 9.12 | 93.35 ± 14.72 |
| Height (cm) | 176.37 ± 6.47 | 175.24 ± 5.87 | 174.99 ± 6.63 |
| Waist (cm) [1,2,3] | 88.25 ± 7.41 | 97.21 ± 6.54 | 107.04 ± 11.37 |
| Hip (cm) [1,2,3] | 94.87 ± 6.09 | 100.66 ± 5.45 | 107.61 ± 8.96 |
| Total %Body Fat[1,2,3] | 17.40 ± 4.33 | 25.44 ± 2.06 | 32.66 ± 3.80 |
| Total % Trunk Fat[1,2,3] | 20.90 ± 6.14 | 31.04 ± 3.57 | 38.07 ± 3.98 |
| Total BMD ($g/cm^2$) [2] | 1.24 ± 0.10 | 1.26 ± 0.09 | 1.28 ± 0.10 |
| Spine BMD | 1.24 ± 0.17 | 1.25 ± 0.16 | 1.27 ± 0.16 |
| Left Hip BMD | 1.04 ± 0.17 | 1.02 ± 0.12 | 1.04 ± 0.13 |
| BMI[1,2,3] | 24.33 ± 2.72 | 27.39 ± 2.72 | 30.46 ± 4.36 |
| Waist-Hip Ratio[1,2,3] | 0.93 ± 0.07 | 0.97 ± 0.04 | 0.99 ± 0.05 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 31. One-way ANOVA analysis comparing laboratory values, HOMA-β, and HOMA-IR for all male subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 19 - 138 | 13 - 104 | 14 - 154 |
| Glucose[2] | 5.08 ± 0.78[2] | 5.39 ± 0.83 | 5.43 ± 1.33 |
| Phosphate | 1.15 ± 0.17 | 1.13 ± 0.18 | 1.11 ± 0.18 |
| Calcium | 2.37 ± 0.14 | 2.36 ± 0.10 | 2.36 ± 0.11 |
| Albumin | 43.03 ± 3.95 | 42.83 ± 3.68 | 42.33 ± 4.06 |
| Cholesterol[1,2] | 4.73 ± 1.02[1,2] | 5.32 ± 1.12 | 5.18 ± 1.10 |
| Triglycerides[1,2] | 1.16 ± 0.74[1,2] | 1.55 ± 0.99 | 1.76 ± 1.07 |
| Magnesium[1] | 0.88 ± 0.10[1] | 0.91 ± 0.12 | 0.89 ± 0.11 |
| HDL[2] | 1.31 ± 0.27[2] | 1.25 ± 0.27 | 1.18 ± 0.24 |
| GGT[1,2] | 19.19 ± 10.00[1,2] | 29.25 ± 21.49 | 28.91 ± 25.93 |
| LDL[1,2] | 2.87 ± 0.93[1,2] | 3.35 ± 0.98 | 3.18 ± 0.89 |
| Risk Factor[1,2] | 3.74 ± 1.02[1,2] | 4.37 ± 1.15 | 4.50 ± 1.06 |
| Buche[1,2] | 8956.38 ± 2968.32[1,2] | 10419.81 ± 1844.09 | 10978.93 ± 2246.65 |
| Corrected Buche (SI Units)[1] | 2173.02 ± 4388.43[1] | 9203.20 ± 5692.65 | 6936.48 ± 6649.55 |
| Insulin[1,2] | 54.27 ± 35.25[1,2] | 76.12 ± 44.77 | 99.52 ± 73.12 |
| HOMA-β[1,3] | 101.21 ± 80.94 | 125.49 ± 89.83 | 180.38 ± 195.17[1,3] |
| HOMA-IR[2] | 1.79 ± 1.14[2] | 2.67 ± 1.66 | 3.76 ± 5.39 |
| Triglyceride/HDL ratio[1,2] | 0.97 ± 0.76[1,2] | 1.36 ± 1.03 | 1.60 ± 1.09 |
| Log Insulin[1,2,3] | 1.68 ± 0.21[1,2] | 1.82 ± 0.22[3] | 1.92 ± 0.24 |
| Log HOMA-IR[1,2,3] | 0.19 ± 0.22[1,2] | 0.36 ± 0.25[3] | 0.46 ± 0.28 |
| Log HOMA-β[1,2,3] | 1.93 ± 0.23[1,2] | 2.03 ± 0.23[3] | 2.15 ± 0.28 |
| Log Triglyceride[1,2] | -0.01 ± 0.24[1,2] | 0.11 ± 0.25 | 0.17 ± 0.26 |
| Waist-Hip Ratio[1,2,3] | 0.93 ± 0.07[1,2] | 0.97 ± 0.04[3] | 0.99 ± 0.05 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 32. One-way ANOVA analysis comparing bone density and body composition data comparing all male subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 19 - 138 | 13 - 104 | 14 - 154 |
| Total Arm BMD[2] | 1.04 ± 0.15 | 1.07 ± 0.14 | 1.09 ± 0.15 |
| Total Leg BMD | 1.44 ± 0.13 | 1.43 ± 0.12 | 1.44 ± 0.14 |
| Total Trunk BMD | 0.98 ± 0.10 | 1.00 ± 0.09 | 1.01 ± 0.09 |
| Total Rib BMD | 0.74 ± 0.09 | 0.76 ± 0.07 | 0.77 ± 0.10 |
| Total Pelvis BMD[1,2] | 1.20 ± 0.14 | 1.27 ± 0.13 | 1.27 ± 0.14 |
| Total BMD[2] | 1.24 ± 0.10 | 1.26 ± 0.09 | 1.28 ± 0.10 |
| Arm % Fat[1,2,3] | 11.87 ± 4.59 | 19.42 ± 3.46 | 27.94 ± 7.44 |
| Arm tissue[1,2,3] | 8699.95 ± 1569.65 | 9516.17 ± 1493.79 | 10863.14 ± 3329.31 |
| Arm Fat weight(g) [1,2,3] | 1091.99 ± 450.01 | 1947.84 ± 512.42 | 3292.69 ± 1891.48 |
| Arm Lean muscle mass (g) | 7601.46 ± 1360.47 | 7568.33 ± 1182.18 | 7570.51 ± 1669.57 |
| Arm Bone Mineral Composition | 470.26 ± 70.32 | 472.97 ± 62.97 | 464.47 ± 82.84 |
| Leg % Fat[1,2,3] | 15.38 ± 3.38 | 20.83 ± 2.65 | 28.41 ± 4.96 |
| Leg Tissue weight(g) [1,2,3] | 23171.30 ± 3341.92 | 25042.81 ± 3207.53 | 27661.52 ± 4901.53 |
| Leg Fat weight (g) [1,2,3] | 3782.80 ± 1058.30 | 5492.18 ± 1078.12 | 8349.99 ± 2640.43 |
| Leg lean muscle mass(g) | 19388.46 ± 2713.80 | 19448.34 ± 2710.60 | 19311.53 ± 2921.90 |
| Leg bone mineral composition | 1220.25 ± 180.37 | 1231.88 ± 171.30 | 1246.07 ± 184.09 |
| Trunk % Fat[1,2,3] | 20.90 ± 6.14 | 31.20 ± 3.33 | 38.09 ± 4.08 |
| Trunk Tissue weight (g) [1,2,3] | 36139.76 ± 6831.18 | 41432.40 ± 5313.07 | 46384.27 ± 9446.53 |
| Trunk Fat weight (g) [1,2,3] | 7938.99 ± 3160.62 | 13305.91 ± 2595.19 | 18107.39 ± 4178.68 |
| Trunk lean muscle mass (g) | 28200.70 ± 4771.24 | 28126.41 ± 3283.26 | 28257.37 ± 6068.01 |
| Trunk bone mineral composition | 976.57 ± 186.57 | 987.34 ± 172.55 | 929.90 ± 244.20 |
| Android % fat mass[1,2,3] | 26.55 ± 9.78 | 38.25 ± 5.91 | 45.57 ± 5.38 |
| Android tissue mass (g) [1,2,3] | 5286.37 ± 1159.95 | 6362.77 ± 1126.89 | 7599.61 ± 1821.37 |
| Android Fat mass (g) [1,2,3] | 1511.36 ± 911.66 | 2483.70 ± 656.63 | 3550.17 ± 1109.29 |
| Android lean muscle mass (g) [2] | 3782.26 ± 537.80 | 3878.97 ± 625.69 | 4049.43 ± 811.10 |
| Android BMC | 58.97 ± 12.20 | 57.76 ± 12.65 | 60.26 ± 14.21 |
| Gynecoid % Fat[1,2,3] | 22.74 ± 7.35 | 28.58 ± 4.65 | 35.45 ± 4.66 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) [1,2,3] | 11422.44 | ± | 1648.74 | 12251.14 | ± | 1502.41 | 13731.95 | ± 2251.86 |
| Gynecoid fat mass (g) [1,2,3] | 2770.93 | ± | 1266.53 | 3590.45 | ± | 659.75 | 5037.11 | ± 1280.01 |
| Gynecoid lean muscle mass (g) | 8673.33 | ± | 1220.05 | 8652.94 | ± | 1248.52 | 8694.93 | ± 1276.53 |
| Gynecoid BMC[2] | 335.24 | ± | 55.18[2] | 348.28 | ± | 57.10 | 357.50 | ± 62.17 |
| Total % body fat[1,2,3] | 17.41 | ± | 4.31 | 25.48 | ± | 2.07 | 32.63 | ± 3.71 |
| Total tissue mass (g) [1,2,3] | 72525.26 | ± | 9082.39 | 80701.21 | ± | 8898.20 | 89787.93 | ± 14492.41 |
| Total fat mass (g) [1,2,3] | 13326.88 | ± | 4068.20 | 21390.89 | ± | 3093.87 | 30550.55 | ± 7144.35 |
| Total lean muscle mass (g) | 59198.47 | ± | 6829.57 | 59310.50 | ± | 6539.28 | 59237.39 | ± 8697.89 |
| Total Bone Mineral Composition | 3131.92 | ± | 440.48 | 3179.77 | ± | 388.32 | 3119.46 | ± 447.33 |
| L2-L4 (Z-Score) | 0.14 | ± | 1.33 | 0.20 | ± | 1.34 | 0.13 | ± 1.36 |
| L2-L4 BMC[2] | 59.80 | ± | 11.09 | 63.82 | ± | 11.05 | 64.78 | ± 10.84 |
| Femoral Neck (Z-Score) | 0.06 | ± | 1.12 | 0.00 | ± | 0.74 | -0.06 | ± 0.91 |
| Neck BMC | 5.80 | ± | 1.01 | 5.70 | ± | 0.78 | 5.81 | ± 0.90 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 33. One-way ANOVA analysis comparing descriptive characteristics for all female subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 56 - 449 | 59 - 440 | 73 - 514 |
| Age[1,2] | 42.22 ± 11.52 | 45.61 ± 10.33 | 45.34 ± 10.61 |
| Weight (kg) [1,2,3] | 59.35 ± 7.28 | 66.89 ± 8.22 | 81.10 ± 14.71 |
| Height (cm) [1,2] | 162.86 ± 5.49 | 161.75 ± 5.75 | 161.74 ± 6.10 |
| Waist (cm) [1,2,3] | 80.03 ± 8.55 | 89.03 ± 9.32 | 102.15 ± 14.05 |
| Hip (cm) [1,2,3] | 93.11 ± 6.18 | 100.73 ± 6.51 | 112.32 ± 11.17 |
| Total % Body Fat[1,2,3] | 29.32 ± 4.79 | 37.78 ± 2.08 | 44.96 ± 3.35 |
| Total % Trunk Fat[1,2,3] | 29.30 ± 6.03 | 39.39 ± 3.34 | 46.78 ± 4.23 |
| Total BMD ($g/cm^2$) [1,3] | 1.14 ± 0.08 | 1.15 ± 0.08 | 1.18 ± 0.09 |
| Spine BMD[1,3] | 1.19 ± 0.16 | 1.19 ± 0.15 | 1.23 ± 0.16 |
| Left Hip BMD[1,3] | 0.95 ± 0.12 | 0.94 ± 0.12 | 0.97 ± 0.12 |
| BMI[1,2,3] | 22.39 ± 2.58 | 25.57 ± 2.85 | 30.99 ± 5.21 |
| Waist-Hip Ratio[1,2,3] | 0.86 ± 0.07 | 0.88 ± 0.07 | 0.91 ± 0.07 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 34. One-way ANOVA analysis comparing laboratory values, HOMA-β, and HOMA-IR for all female subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight individuals and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 56 - 449 | 59 - 440 | 73 - 514 |
| Glucose[1,2,3] | 4.86 ± 0.65[1,2] | 5.06 ± 0.92[3] | 5.29 ± 1.04 |
| Phosphate[1,3] | 1.23 ± 0.17 | 1.21 ± 0.17 | 1.17 ± 0.17[1,3] |
| Calcium | 2.35 ± 0.12 | 2.34 ± 0.13 | 2.34 ± 0.12 |
| Albumin[1,3] | 41.64 ± 3.70 | 41.24 ± 3.97 | 40.03 ± 3.38[1,3] |
| Cholesterol[1,2] | 5.05 ± 1.00[1,2] | 5.28 ± 1.06 | 5.29 ± 1.02 |
| Triglycerides[1,2,3] | 0.95 ± 0.56[1,2] | 1.14 ± 0.70[3] | 1.34 ± 0.78 |
| Magnesium | 0.89 ± 0.09 | 0.88 ± 0.08 | 0.87 ± 0.08 |
| HDL[1,2,3] | 1.68 ± 0.39[1,2] | 1.56 ± 0.35[3] | 1.47 ± 0.35 |
| GGT | 17.19 ± 24.31 | 16.20 ± 12.93 | 20.16 ± 31.47 |
| LDL[1,2] | 2.94 ± 0.86[1,2] | 3.20 ± 0.90 | 3.20 ± 0.87 |
| Risk Factor[1,2,3] | 3.13 ± 0.82[1,2] | 3.51 ± 0.93[3] | 3.74 ± 0.97 |
| Buche[1,2,3] | 8304.98 ± 2662.52[1,2] | 8903.00 ± 2494.98[3] | 9639.71 ± 2775.00 |
| Corrected Buche (SI Units) | 5079.87 ± 4785.40 | 6684.62 ± 5001.34 | 5426.15 ± 6095.61 |
| Insulin[1,2,3] | 52.46 ± 33.31[1,2] | 65.53 ± 83.65[3] | 88.75 ± 71.24 |
| HOMA-β | 124.32 ± 206.24 | 124.50 ± 325.49 | 165.34 ± 226.47 |
| HOMA-IR[1,2,3] | 1.70 ± 1.51[1,2] | 2.21 ± 4.19[3] | 3.09 ± 2.56 |
| Triglyceride/HDL ratio[1,2,3] | 0.62 ± 0.47[1,2] | 0.81 ± 0.63[3] | 1.00 ± 0.72 |
| Log Insulin[1,2,3] | 1.66 ± 0.22[1,2] | 1.74 ± 0.23[3] | 1.88 ± 0.24 |
| Log HOMA-IR[1,2,3] | 0.15 ± 0.24[1,2] | 0.24 ± 0.25[3] | 0.40 ± 0.27 |
| Log HOMA-β[1,3] | 2.01 ± 0.24 | 2.05 ± 0.26 | 2.13 ± 0.25[1,3] |
| Log Triglyceride[1,2,3] | -0.07 ± 0.21[1,2] | 0.00 ± 0.23[3] | 0.06 ± 0.23 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 35. One-way ANOVA analysis comparing bone density and body composition data comparing for all female subjects factoring for Bray Percentage body fat categories of lean/normal individuals, overweight and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 56 - 449 | 59 - 440 | 73 - 514 |
| Total Arm BMD[1,3] | 0.85 ± 0.08 | 0.87 ± 0.09 | 0.92 ± 0.12 |
| Total Leg BMD[1,3] | 1.23 ± 0.11 | 1.23 ± 0.10 | 1.25 ± 0.10 |
| Total Trunk BMD[1,3] | 0.90 ± 0.08 | 0.91 ± 0.08 | 0.95 ± 0.09 |
| Total Rib BMD[1,3] | 0.63 ± 0.05 | 0.65 ± 0.06 | 0.67 ± 0.05 |
| Total Pelvis BMD[1,3] | 1.11 ± 0.11 | 1.11 ± 0.10 | 1.16 ± 0.11 |
| Total BMD[1,3] | 1.14 ± 0.08 | 1.15 ± 0.08 | 1.18 ± 0.09 |
| Arm % Fat[1,2,3] | 29.93 ± 7.34 | 39.26 ± 4.75 | 47.27 ± 5.74 |
| Arm tissue[1,2,3] | 5862.73 ± 1115.95 | 7029.37 ± 1305.63 | 9354.38 ± 3390.76 |
| Arm Fat weight(g) [1,2,3] | 1890.72 ± 717.83 | 2903.13 ± 749.25 | 4702.71 ± 2233.88 |
| Arm Lean muscle mass (g) [1,2,3] | 3971.84 ± 637.26 | 4133.05 ± 721.60 | 4657.73 ± 1283.19 |
| Arm Bone Mineral Composition | 303.74 ± 43.91 | 308.45 ± 48.62 | 311.81 ± 68.29 |
| Leg % Fat[1,2] | 31.79 ± 5.11 | 38.30 ± 3.69 | 44.71 ± 4.54 |
| Leg Tissue weight(g) [1,2,3] | 18985.61 ± 2394.39 | 20763.28 ± 2893.33 | 24731.80 ± 4501.01 |
| Leg Fat weight (g) [1,2,3] | 6347.29 ± 1414.52 | 8319.20 ± 1518.36 | 11571.84 ± 2854.17 |
| Leg lean muscle mass(g) [1,3] | 12637.94 ± 1646.42 | 12449.36 ± 1781.21 | 13165.77 ± 2165.15 |
| Leg bone mineral composition[1,3] | 875.32 ± 119.63 | 880.58 ± 137.85 | 944.05 ± 139.85 |
| Trunk % Fat[1,2,3] | 29.31 ± 6.03 | 39.40 ± 3.33 | 46.87 ± 3.79 |
| Trunk Tissue weight (g) [1,2,3] | 27672.00 ± 5166.03 | 32030.80 ± 4881.51 | 39625.58 ± 8631.37 |
| Trunk Fat weight (g) [1,2,3] | 8455.01 ± 2858.41 | 13009.40 ± 2734.07 | 19071.40 ± 5087.07 |
| Trunk lean muscle mass (g) [1,3] | 19217.29 ± 3053.91 | 19112.49 ± 3608.61 | 20554.45 ± 4058.28 |
| Trunk bone mineral composition[1,2] | 714.98 ± 130.49 | 751.08 ± 134.59 | 765.44 ± 168.67 |
| Android % fat mass[1,2,3] | 33.11 ± 8.58 | 44.63 ± 5.35 | 52.85 ± 5.31 |
| Android tissue mass (g) [1,2,3] | 3990.10 ± 940.30 | 4887.33 ± 929.16 | 6390.56 ± 1629.23 |
| Android Fat mass (g) [1,3] | 1383.77 ± 649.52 | 2233.92 ± 618.09 | 3460.74 ± 1135.02 |
| Android lean muscle mass (g) | 2605.78 ± 420.78 | 2657.60 ± 403.75 | 2931.76 ± 579.68 |
| Android BMC[1,3] | 49.40 ± 9.37 | 50.58 ± 10.36 | 54.15 ± 11.46 |
| Gynecoid % Fat[1,2,3] | 38.84 ± 5.22 | 44.82 ± 3.91 | 50.09 ± 4.39 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) [1,2,3] | 9561.85 | ± | 1255.12 | 10595.75 | ± | 1306.37 | 12607.17 | ± 2233.72 |
| Gynecoid fat mass (g) [1,2,3] | 3818.65 | ± | 777.15 | 4866.27 | ± | 768.59 | 6494.43 | ± 1492.48 |
| Gynecoid lean muscle mass (g) [1,3] | 5735.61 | ± | 841.70 | 5727.51 | ± | 809.04 | 6114.71 | ± 981.39 |
| Gynecoid BMC[1,2,3] | 237.23 | ± | 39.51 | 251.40 | ± | 44.61 | 278.17 | ± 43.95 |
| Total % body fat[1,2,3] | 29.31 | ± | 4.79 | 37.77 | ± | 2.08 | 44.94 | ± 3.32 |
| Total tissue mass (g) [1,2,3] | 56224.19 | ± | 7094.96 | 64642.65 | ± | 22849.36 | 77782.89 | ± 14737.00 |
| Total fat mass (g) [1,2,3] | 17296.65 | ± | 4042.30 | 24982.49 | ± | 3764.23 | 36356.87 | ± 8737.95 |
| Total lean muscle mass (g) [1,3] | 38926.14 | ± | 4599.27 | 38612.83 | ± | 4744.63 | 41443.93 | ± 6788.34 |
| Total Bone Mineral Composition[1,3] | 2369.51 | ± | 312.98 | 2419.44 | ± | 329.90 | 2496.33 | ± 327.03 |
| L2-L4 (Z-Score) [2] | 0.44 | ± | 1.22 | 0.19 | ± | 1.16 | 0.14 | ± 1.29 |
| L2-L4 BMC[3] | 50.74 | ± | 9.27 | 49.38 | ± | 9.01 | 52.20 | ± 9.51 |
| Femoral Neck (Z-Score) [2] | 0.26 | ± | 0.96 | 0.12 | ± | 0.90 | 0.04 | ± 0.93 |
| Neck BMC[1,3] | 4.56 | ± | 0.74 | 4.50 | ± | 0.64 | 4.70 | ± 0.65 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 36. One-way ANOVA analysis comparing descriptive data for all subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 112 - 807 | 91 - 648 | 37 - 352 |
| Age[1,2] | 41.65 ± 12.37 | 45.00 ± 11.27 | 45.70 ± 11.09 |
| Weight (kg) [1,2,3] | 61.59 ± 7.44 | 75.60 ± 8.39 | 94.05 ± 14.23 |
| Height (cm) | 164.87 ± 7.63 | 165.51 ± 8.53 | 164.79 ± 8.52 |
| Waist (cm) [1,2,3] | 81.98 ± 7.94 | 94.99 ± 7.82 | 111.49 ± 12.01 |
| Hip (cm) [1,2,3] | 94.35 ± 6.10 | 103.90 ± 5.78 | 117.17 ± 10.67 |
| Total %Body Fat[1,2,3] | 30.39 ± 7.88 | 36.68 ± 7.86 | 42.26 ± 7.46 |
| Total % Trunk Fat[1,2,3] | 31.14 ± 8.34 | 39.73 ± 7.32 | 45.01 ± 6.19 |
| Total BMD ($g/cm^2$) [1,2,3] | 1.14 ± 0.08 | 1.19 ± 0.10 | 1.26 ± 0.10 |
| Spine BMD[1,2] | 1.18 ± 0.15 | 1.24 ± 0.16 | 1.26 ± 0.15 |
| Left Hip BMD[1,2,3] | 0.94 ± 0.13 | 0.98 ± 0.13 | 1.03 ± 0.13 |
| BMI[1,2,3] | 22.62 ± 1.84 | 27.54 ± 1.48 | 34.58 ± 4.14 |
| Waist-Hip Ratio[1,2,3] | 0.87 ± 0.07 | 0.92 ± 0.07 | 0.95 ± 0.07 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 37. One-way ANOVA analysis comparing laboratory, HOMA-β and HOMA-IR data for all subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 112 - 807 | 91 - 648 | 37 - 352 |
| Glucose[1,2,3] | 4.87 ± 0.56 | 5.18 ± 0.93 | 5.62 ± 1.39 |
| Phosphate[1,2] | 1.21 ± 0.18 | 1.18 ± 0.17 | 1.15 ± 0.19 |
| Calcium | 2.35 ± 0.13 | 2.35 ± 0.12 | 2.34 ± 0.12 |
| Albumin[1,2,3] | 41.75 ± 3.88 | 41.23 ± 3.77 | 40.43 ± 3.77 |
| Cholesterol[1,2] | 5.05 ± 1.02 | 5.30 ± 1.05 | 5.24 ± 1.07 |
| Triglycerides[1,2] | 0.97 ± 0.53 | 1.35 ± 0.89 | 1.60 ± 0.86 |
| Magnesium | 0.88 ± 0.09 | 0.88 ± 0.08 | 0.88 ± 0.11 |
| HDL[1,2,3] | 1.62 ± 0.37 | 1.44 ± 0.36 | 1.31 ± 0.31 |
| GGT[1,2] | 16.67 ± 19.24 | 22.75 ± 32.39 | 21.46 ± 12.64 |
| LDL[1,2] | 2.98 ± 0.88 | 3.24 ± 0.91 | 3.20 ± 0.89 |
| Risk Factor[1,2,3] | 3.21 ± 0.80 | 3.86 ± 1.09 | 4.16 ± 1.02 |
| Buche[1,2,3] | 8543.13 ± 2599.16 | 9512.07 ± 2743.74 | 10399.85 ± 2426.79 |
| Corrected Buche (SI Units) | 4887.44 ± 4745.27 | 6339.19 ± 5847.78 | 6557.08 ± 6450.26 |
| Insulin[1,2,3] | 51.68 ± 28.67 | 70.64 ± 40.35 | 118.84 ± 119.52 |
| HOMA-β[2] | 123.57 ± 169.35 | 141.55 ± 139.78 | 171.46 ± 425.60 |
| HOMA-IR[1,2,3] | 1.64 ± 1.04 | 2.38 ± 1.50 | 4.51 ± 6.14 |
| Triglyceride/HDL ratio[1,2,3] | 0.65 ± 0.46 | 1.05 ± 0.86 | 1.34 ± 0.89 |
| Log Insulin[1,2,3] | 1.66 ± 0.20 | 1.79 ± 0.22 | 1.99 ± 0.24 |
| Log HOMA-IR[1,2,3] | 0.16 ± 0.22 | 0.31 ± 0.24 | 0.54 ± 0.28 |
| Log HOMA-β[1,2,3] | 2.01 ± 0.24 | 2.06 ± 0.25 | 2.18 ± 0.26 |
| Log Triglyceride[1,2,3] | -0.06 ± 0.21 | 0.05 ± 0.25 | 0.15 ± 0.22 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 38. One-way ANOVA analysis comparing percentage body fat and bone-mineral composition for all subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Entire Cohort | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 112 - 807 | 91 - 648 | 37 - 352 |
| Total Arm BMD[1,2,3] | 0.87 ± 0.09 | 0.94 ± 0.14 | 1.02 ± 0.16 |
| Total Leg BMD[1,2,3] | 1.24 ± 0.12 | 1.30 ± 0.14 | 1.34 ± 0.13 |
| Total Trunk BMD[1,2,3] | 0.90 ± 0.08 | 0.96 ± 0.09 | 1.00 ± 0.09 |
| Total Rib BMD[1,2,3] | 0.64 ± 0.06 | 0.70 ± 0.08 | 0.72 ± 0.09 |
| Total Pelvis BMD[1,2,3] | 1.11 ± 0.11 | 1.18 ± 0.13 | 1.23 ± 0.13 |
| Total BMD[1,2,3] | 1.14 ± 0.08 | 1.19 ± 0.10 | 1.26 ± 0.10 |
| Arm % Fat[1,2,3] | 30.24 ± 10.74 | 35.91 ± 11.43 | 43.90 ± 11.40 |
| Arm tissue[1,2,3] | 6280.25 ± 1271.45 | 8260.80 ± 1458.19 | 11478.64 ± 3824.78 |
| Arm Fat weight(g) [1,2,3] | 1979.06 ± 824.20 | 3016.53 ± 1009.10 | 5304.78 ± 2631.70 |
| Arm Lean muscle mass (g) [1,2,3] | 4303.82 ± 1283.50 | 5248.92 ± 1682.31 | 6173.85 ± 2093.88 |
| Arm Bone Mineral Composition[1,2] | 320.20 ± 69.82 | 359.13 ± 91.26 | 369.46 ± 111.06 |
| Leg % Fat[1,2,3] | 32.11 ± 8.76 | 35.40 ± 9.86 | 40.04 ± 9.91 |
| Leg Tissue weight(g) [1,2,3] | 19625.44 ± 2722.39 | 23128.98 ± 3067.86 | 27772.60 ± 4920.53 |
| Leg Fat weight (g) [1,2,3] | 6559.47 ± 1985.21 | 8473.47 ± 2481.59 | 11618.47 ± 3725.22 |
| Leg lean muscle mass(g) [1,2,3] | 13065.65 ± 2822.04 | 14660.11 ± 3469.91 | 16132.33 ± 3847.26 |
| Leg bone mineral composition[1,2,3] | 905.48 ± 169.43 | 1010.00 ± 208.95 | 1073.79 ± 200.25 |
| Trunk % Fat[1,2,3] | 31.15 ± 8.33 | 39.81 ± 7.15 | 45.05 ± 6.23 |
| Trunk Tissue weight (g) [1,2,3] | 28735.19 ± 3952.21 | 36774.53 ± 4722.32 | 46869.61 ± 9910.64 |
| Trunk Fat weight (g) [1,2,3] | 9229.55 ± 2936.52 | 14916.09 ± 3022.83 | 21365.74 ± 5027.77 |
| Trunk lean muscle mass (g) [1,2,3] | 19493.47 ± 3518.26 | 21858.66 ± 4317.85 | 25637.79 ± 7475.18 |
| Trunk bone mineral composition[1,2,3] | 747.86 ± 153.96 | 852.17 ± 181.86 | 791.75 ± 226.61 |
| Android % fat mass[1,2,3] | 35.42 ± 10.14 | 45.71 ± 7.86 | 52.19 ± 6.58 |
| Android tissue mass (g) [1,2,3] | 4207.27 ± 846.49 | 5708.13 ± 939.13 | 7743.59 ± 1663.94 |
| Android Fat mass (g) [1,2,3] | 1546.54 ± 665.87 | 2646.70 ± 660.95 | 4093.94 ± 1092.02 |
| Android lean muscle mass (g) [1,2,3] | 2664.19 ± 511.18 | 3061.01 ± 625.91 | 3652.85 ± 858.01 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Android BMC[1,2] | 50.33 | ± | 10.17 | 55.30 | ± | 11.84 | 56.31 | ± 13.58 |
| Gynecoid % Fat[1,2,3] | 39.05 | ± | 8.99 | 41.81 | ± | 9.41 | 45.76 | ± 8.95 |
| Gynecoid tissue mass (g) [1,2,3] | 9902.99 | ± | 1332.68 | 11657.44 | ± | 1391.26 | 14028.17 | ± 2317.63 |
| Gynecoid fat mass (g) [1,2,3] | 3970.35 | ± | 1100.76 | 4978.53 | ± | 1243.02 | 6590.74 | ± 1822.18 |
| Gynecoid lean muscle mass (g) [1,2,3] | 5930.92 | ± | 1243.03 | 6677.92 | ± | 1488.66[3] | 7440.27 | ± 1650.51 |
| Gynecoid BMC[1,2,3] | 247.23 | ± | 49.65 | 291.24 | ± | 59.96[3] | 318.45 | ± 58.41 |
| Total % body fat[1,2,3] | 30.38 | ± | 7.85 | 36.68 | ± | 7.85 | 42.23 | ± 7.44 |
| Total tissue mass (g) [1,2,3] | 58987.97 | ± | 17559.30 | 72237.12 | ± | 8180.48 | 90672.72 | ± 14267.45 |
| Total fat mass (g) [1,2,3] | 18421.37 | ± | 5156.29 | 27174.21 | ± | 5451.30 | 39324.99 | ± 9270.84 |
| Total lean muscle mass (g) [1,2,3] | 40005.15 | ± | 7704.82 | 45063.38 | ± | 9690.49 | 51347.75 | ± 11253.87 |
| Total Bone Mineral Composition[1,2] | 2445.10 | ± | 396.19 | 2697.95 | ± | 475.28 | 2719.88 | ± 468.91 |
| L2-L4 (Z-Score) | 0.24 | ± | 1.18 | 0.28 | ± | 1.31 | 0.12 | ± 1.31 |
| L2-L4 BMC[1,2,3] | 50.74 | ± | 9.73 | 54.61 | ± | 11.43 | 57.90 | ± 11.22 |
| Femoral Neck (Z-Score) | 0.10 | ± | 0.95 | 0.05 | ± | 0.88 | 0.19 | ± 0.97 |
| Neck BMC[1,2,3] | 4.63 | ± | 0.81 | 4.95 | ± | 0.91 | 5.21 | ± 0.86 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 39. One-way ANOVA analysis comparing descriptive data for all male subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 15 - 124 | 25 - 184 | 6 - 92 |
| Age[1,2] | 36.51 ± 15.13 | 42.55 ± 13.21 | 43.53 ± 13.13 |
| Weight (kg) [1,2,3] | 71.73 ± 6.95 | 84.49 ± 7.00 | 102.43 ± 12.07 |
| Height (cm) [2] | 176.36 ± 6.68 | 175.55 ± 6.36 | 174.17 ± 5.89 |
| Waist (cm) [1,2,3] | 87.28 ± 8.83 | 97.65 ± 6.88 | 112.40 ± 8.95 |
| Hip (cm) [1,2,3] | 94.03 ± 7.58 | 101.05 ± 5.34 | 111.44 ± 7.44 |
| Total %Body Fat[1,2,3] | 18.70 ± 6.48 | 26.49 ± 5.45 | 32.11 ± 4.92 |
| Total % Trunk Fat[1,2,3] | 21.99 ± 8.01 | 32.01 ± 6.51 | 37.43 ± 4.54 |
| Total BMD ($g/cm^2$) [1,2,3] | 1.20 ± 0.09 | 1.27 ± 0.09 | 1.33 ± 0.09 |
| Spine BMD[1,2] | 1.20 ± 0.14 | 1.28 ± 0.17 | 1.28 ± 0.15 |
| Left Hip BMD[1,2] | 1.00 ± 0.16 | 1.04 ± 0.14 | 1.08 ± 0.12 |
| BMI[1,2,3] | 23.05 ± 1.70 | 27.39 ± 1.33 | 33.73 ± 3.19 |
| Waist-Hip Ratio[1,2,3] | 0.93 ± 0.07 | 0.97 ± 0.05 | 1.01 ± 0.05 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 40. One-way ANOVA analysis comparing laboratory, HOMA-β and HOMA-IR data for all male subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 15 - 124 | 25 - 184 | 6 - 92 |
| Glucose[1,2] | 5.02 ± 0.70 | 5.34 ± 1.02 | 5.62 ± 1.40 |
| Phosphate[1,2] | 1.18 ± 0.18 | 1.12 ± 0.16 | 1.09 ± 0.18 |
| Calcium | 2.36 ± 0.15 | 2.37 ± 0.10 | 2.36 ± 0.10 |
| Albumin | 42.87 ± 4.03 | 42.81 ± 3.95 | 42.44 ± 3.82 |
| Cholesterol[1,2] | 4.75 ± 1.04 | 5.21 ± 1.10 | 5.13 ± 1.13 |
| Triglycerides[1,2,3] | 1.12 ± 0.71 | 1.55 ± 1.03 | 1.89 ± 1.00 |
| Magnesium | 0.88 ± 0.11 | 0.89 ± 0.08 | 0.90 ± 0.16 |
| HDL[1,2,3] | 1.34 ± 0.27 | 1.23 ± 0.25 | 1.13 ± 0.23 |
| GGT | 22.75 ± 19.57 | 28.29 ± 24.61 | 24.96 ± 13.78 |
| LDL[1] | 2.87 ± 0.92 | 3.25 ± 0.96 | 3.14 ± 0.91 |
| Risk Factor[1,2] | 3.63 ± 0.92 | 4.36 ± 1.14 | 4.64 ± 1.02 |
| Buche[1,2] | 9239.51 ± 2850.01 | 10310.09 ± 2505.82 | 11044.03 ± 1754.12 |
| Corrected Buche (SI Units) | 3891.23 ± 5050.08 | 5563.67 ± 6078.65 | 10096.55 ± 7978.38 |
| Insulin[1,2,3] | 57.15 ± 43.16 | 73.59 ± 48.16 | 112.92 ± 75.79 |
| HOMA-β[2] | 116.21 ± 99.44 | 137.94 ± 181.10 | 168.68 ± 87.09 |
| HOMA-IR | 1.88 ± 1.49 | 2.53 ± 1.64 | 4.53 ± 6.59 |
| Triglyceride/HDL ratio[1,2,3] | 0.90 ± 0.69 | 1.37 ± 1.06 | 1.78 ± 1.08 |
| Log Insulin[1,2,3] | 1.70 ± 0.20 | 1.80 ± 0.24 | 1.99 ± 0.22 |
| Log HOMA-IR[1,2,3] | 0.21 ± 0.22 | 0.33 ± 0.26 | 0.54 ± 0.27 |
| Log HOMA-β | 1.98 ± 0.24 | 2.02 ± 0.28 | 2.17 ± 0.232 |
| Log Triglyceride[1,2,3] | -0.02 ± 0.24 | 0.11 ± 0.27 | 0.22 ± 0.22 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 41. One-way ANOVA analysis comparing body fat and bone-mineral composition all male subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Male | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 15 - 124 | 25 - 184 | 6 - 92 |
| Total Arm BMD[1,2,3] | 0.97 ± 0.10 | 1.08 ± 0.13 | 1.16 ± 0.16 |
| Total Leg BMD[1,2] | 1.39 ± 0.13 | 1.44 ± 0.12 | 1.47 ± 0.14 |
| Total Trunk BMD[1,2] | 0.94 ± 0.09 | 1.01 ± 0.08 | 1.03 ± 0.09 |
| Total Rib BMD[1,2] | 0.70 ± 0.05 | 0.77 ± 0.06 | 0.80 ± 0.13 |
| Total Pelvis BMD[1,2,3] | 1.16 ± 0.11 | 1.27 ± 0.12 | 1.33 ± 0.15 |
| Total BMD[1,2,3] | 1.20 ± 0.09 | 1.27 ± 0.09 | 1.33 ± 0.09 |
| Arm % Fat[1,2,3] | 13.00 ± 6.06 | 20.44 ± 6.74 | 28.71 ± 8.13 |
| Arm tissue[1,2,3] | 8017.69 ± 1215.76 | 9627.86 ± 1174.76 | 12283.04 ± 3693.87 |
| Arm Fat weight(g) [1,2,3] | 1108.94 ± 602.98 | 2041.47 ± 666.38 | 3829.85 ± 2215.40 |
| Arm Lean muscle mass (g) [1,2,3] | 6901.48 ± 1069.22 | 7586.42 ± 1167.76 | 8453.28 ± 1866.74 |
| Arm Bone Mineral Composition[1,2] | 435.37 ± 71.68 | 478.76 ± 63.51 | 491.64 ± 80.78 |
| Leg % Fat[1,2,3] | 17.09 ± 5.63 | 22.29 ± 5.43 | 27.20 ± 6.75 |
| Leg Tissue weight(g) [1,2,3] | 22322.70 ± 2758.82 | 25286.35 ± 2943.34 | 29712.40 ± 5195.79 |
| Leg Fat weight (g) [1,2,3] | 4036.05 ± 1446.14 | 5954.52 ± 1780.34 | 8682.17 ± 3240.89 |
| Leg lean muscle mass(g) [1,2,3] | 18286.45 ± 2414.21 | 19331.80 ± 2367.88 | 20914.55 ± 3318.91 |
| Leg bone mineral composition[1,2] | 1160.41 ± 168.40 | 1251.54 ± 162.81 | 1293.27 ± 193.77 |
| Trunk % Fat[1,2,3] | 22.02 ± 8.12 | 32.10 ± 6.43 | 37.44 ± 4.57 |
| Trunk Tissue weight (g) [1,2,3] | 33928.49 ± 3790.51 | 41425.63 ± 4300.85 | 51705.23 ± 10138.70 |
| Trunk Fat weight (g) [1,2,3] | 7826.85 ± 3369.93 | 13739.68 ± 3536.20 | 19642.96 ± 3900.78 |
| Trunk lean muscle mass (g) [1,2,3] | 26101.55 ± 2929.48 | 27685.89 ± 2923.87 | 32029.65 ± 7694.28 |
| Trunk bone mineral composition[1,3] | 909.25 ± 171.70 | 1017.09 ± 179.49 | 917.52 ± 271.32 |
| Android % fat mass[1,2,3] | 27.71 ± 11.26 | 38.36 ± 8.00 | 46.28 ± 5.57 |
| Android tissue mass (g) [1,2,3] | 5053.93 ± 1081.29 | 6380.99 ± 1032.67 | 8485.09 ± 1738.39 |
| Android Fat mass (g) [1,2,3] | 1517.41 ± 974.75 | 2523.73 ± 809.66 | 3985.45 ± 1062.26 |
| Android lean muscle mass (g) [1,2,3] | 3544.55 ± 472.78 | 3857.27 ± 473.40 | 4499.65 ± 882.62 |
| Android BMC[1] | 56.31 ± 11.18 | 60.59 ± 13.29 | 59.86 ± 14.62 |
| Gynecoid % Fat[1,2,3] | 24.27 ± 8.47 | 29.40 ± 5.72 | 35.09 ± 6.82 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid tissue mass (g) [1,2,3] | 11011.88 | ± | 1555.65 | 12429.35 | ± | 1344.63 | 14734.36 | ± 2232.27 |
| Gynecoid fat mass (g) [1,2,3] | 2863.57 | ± | 1384.58 | 3770.49 | ± | 904.16 | 5342.04 | ± 1489.38 |
| Gynecoid lean muscle mass (g) [1,2,3] | 8172.81 | ± | 1104.92 | 8654.52 | ± | 1070.04 | 9392.35 | ± 1427.44 |
| Gynecoid BMC[1,2,3] | 314.78 | ± | 50.24 | 355.59 | ± | 53.40 | 372.92 | ± 62.46 |
| Total % body fat[1,2,3] | 18.69 | ± | 6.37 | 26.52 | ± | 5.45 | 32.10 | ± 4.89 |
| Total tissue mass (g) [1,2,3] | 68707.16 | ± | 6802.73 | 80988.91 | ± | 6798.51 | 98833.64 | ± 11927.88 |
| Total fat mass (g) [1,2,3] | 13516.45 | ± | 5023.62 | 22391.27 | ± | 5285.41 | 32971.78 | ± 7511.57 |
| Total lean muscle mass (g) [1,2,3] | 55190.83 | ± | 6093.14 | 58597.72 | ± | 6030.06 | 65861.91 | ± 7671.97 |
| Total Bone Mineral Composition[1,2] | 2966.17 | ± | 420.03 | 3223.26 | ± | 405.37 | 3195.86 | ± 426.92 |
| L2-L4 (Z-Score) | -0.07 | ± | 1.15 | 0.31 | ± | 1.46 | 0.11 | ± 1.31 |
| L2-L4 BMC[1,2] | 57.30 | ± | 9.44 | 64.34 | ± | 11.60 | 66.89 | ± 10.01 |
| Femoral Neck (Z-Score) | -0.16 | ± | 1.11 | 0.01 | ± | 0.86 | 0.15 | ± 0.85 |
| Neck BMC[1,2] | 5.46 | ± | 0.90 | 5.82 | ± | 0.90 | 6.09 | ± 0.76 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 42. One-way ANOVA analysis comparing descriptive data for all female subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 92 - 683 | 56 - 461 | 31 - 259 |
| Age[1,2] | 42.58 ± 11.57 | 46.02 ± 10.23 | 46.55 ± 10.12 |
| Weight (kg) [1,2,3] | 59.75 ± 5.88 | 72.02 ± 5.90 | 91.10 ± 13.79 |
| Height (cm) [1,2] | 162.78 ± 5.69 | 161.48 ± 5.41 | 161.46 ± 6.64 |
| Waist (cm) [1,2,3] | 81.05 ± 7.40 | 93.97 ± 7.94 | 111.23 ± 12.90 |
| Hip (cm) [1,2,3] | 94.40 ± 5.81 | 104.98 ± 5.54 | 119.18 ± 10.94 |
| Total %Body Fat[1,2,3] | 32.51 ± 6.04 | 40.72 ± 4.03 | 45.84 ± 4.19 |
| Total % Trunk Fat[1,2,3] | 32.81 ± 7.25 | 42.78 ± 4.97 | 47.71 ± 4.12 |
| Total BMD ($g/cm^2$) [1,2,3] | 1.13 ± 0.08 | 1.16 ± 0.08 | 1.23 ± 0.09 |
| Spine BMD[1,2,3] | 1.18 ± 0.15 | 1.22 ± 0.15 | 1.25 ± 0.15 |
| Left Hip BMD[1,2,3] | 0.94 ± 0.12 | 0.95 ± 0.11 | 1.01 ± 0.12 |
| BMI[1,2,3] | 22.55 ± 1.85 | 27.60 ± 1.53 | 34.89 ± 4.40 |
| Waist-Hip Ratio[1,2,3] | 0.86 ± 0.07 | 0.90 ± 0.07 | 0.93 ± 0.07 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 43. One-way ANOVA analysis comparing laboratory, HOMA-β and HOMA-IR data for all female subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 92 - 683 | 56 - 461 | 31 - 259 |
| Glucose[1,2,3] | 4.84 ± 0.53 | 5.12 ± 0.89 | 5.62 ± 1.39 |
| Phosphate[2] | 1.22 ± 0.17 | 1.20 ± 0.17 | 1.18 ± 0.18 |
| Calcium | 2.35 ± 0.12 | 2.34 ± 0.12 | 2.34 ± 0.13 |
| Albumin[1,2,3] | 41.56 ± 3.82 | 40.64 ± 3.54 | 39.74 ± 3.50 |
| Cholesterol[1] | 5.10 ± 1.01 | 5.33 ± 1.04 | 5.28 ± 1.05 |
| Triglycerides[1,2,3] | 0.95 ± 0.49 | 1.27 ± 0.82 | 1.50 ± 0.79 |
| Magnesium | 0.89 ± 0.09 | 0.88 ± 0.08 | 0.87 ± 0.09 |
| HDL[1,2,3] | 1.67 ± 0.37 | 1.52 ± 0.36 | 1.37 ± 0.31 |
| GGT[1] | 15.38 ± 18.95 | 20.40 ± 34.95 | 20.14 ± 11.96 |
| LDL[1,2] | 3.00 ± 0.87 | 3.24 ± 0.89 | 3.22 ± 0.89 |
| Risk Factor[1,2,3] | 3.14 ± 0.75 | 3.66 ± 1.00 | 3.99 ± 0.97 |
| Buche[1,2,3] | 8421.96 ± 2536.47 | 9193.07 ± 2783.85 | 10174.45 ± 2598.53 |
| Corrected Buche (SI Units) | 4994.94 ± 4717.88 | 6660.67 ± 5781.31 | 5872.02 ± 6027.23 |
| Insulin[1,2,3] | 50.77 ± 25.40 | 69.31 ± 36.88 | 121.19 ± 131.72 |
| HOMA-β[2] | 124.80 ± 178.45 | 142.73 ± 120.80 | 172.55 ± 493.23 |
| HOMA-IR[1,2,3] | 1.60 ± 0.94 | 2.31 ± 1.44 | 4.52 ± 5.99 |
| Triglyceride/HDL ratio[1,2,3] | 0.60 ± 0.38 | 0.92 ± 0.74 | 1.18 ± 0.76 |
| Log Insulin[1,2,3] | 1.66 ± 0.20 | 1.79 ± 0.21 | 1.99 ± 0.25 |
| Log HOMA-IR[1,2,3] | 0.15 ± 0.21 | 0.30 ± 0.23 | 0.54 ± 0.28 |
| Log HOMA-β[1,2,3] | 2.01 ± 0.24 | 2.08 ± 0.24 | 2.18 ± 0.28 |
| Log Triglyceride[1,2,3] | -0.07 ± 0.20 | 0.03 ± 0.24 | 0.12 ± 0.22 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 44. One-way ANOVA analysis comparing body fat and bone-mineral composition all female subjects factoring for WHO BMI categories of lean/normal individuals, overweight and obese individuals.

| | Female | | |
|---|---|---|---|
| | Normal/Lean | Overweight | Obese |
| | 92 - 683 | 56 - 461 | 31 - 259 |
| Total Arm BMD[1,2,3] | 0.85 ± 0.08 | 0.89 ± 0.10 | 0.97 ± 0.12 |
| Total Leg BMD[1,2,3] | 1.21 ± 0.10 | 1.24 ± 0.10 | 1.29 ± 0.09 |
| Total Trunk BMD[1,2,3] | 0.89 ± 0.07 | 0.93 ± 0.08 | 0.98 ± 0.09 |
| Total Rib BMD[1,2,3] | 0.63 ± 0.05 | 0.66 ± 0.06 | 0.69 ± 0.05 |
| Total Pelvis BMD[1,2,3] | 1.09 ± 0.10 | 1.13 ± 0.11 | 1.20 ± 0.11 |
| Total BMD[1,2,3] | 1.13 ± 0.08 | 1.16 ± 0.08 | 1.23 ± 0.09 |
| Arm % Fat[1,2,3] | 33.37 ± 8.10 | 42.05 ± 5.58 | 49.31 ± 6.44 |
| Arm tissue[1,2,3] | 5964.81 ± 997.72 | 7709.38 ± 1176.11 | 11193.32 ± 3844.14 |
| Arm Fat weight(g) [1,2,3] | 2137.03 ± 758.01 | 3400.22 ± 845.83 | 5829.90 ± 2576.52 |
| Arm Lean muscle mass (g) [1,2,3] | 3832.20 ± 539.53 | 4315.69 ± 606.90 | 5363.39 ± 1488.67 |
| Arm Bone Mineral Composition[1,2,3] | 299.29 ± 44.55 | 311.16 ± 45.04 | 326.33 ± 85.51 |
| Leg % Fat[1,2,3] | 34.84 ± 6.05 | 40.61 ± 5.25 | 44.57 ± 6.12 |
| Leg Tissue weight(g) [1,2,3] | 19135.75 ± 2413.15 | 22258.09 ± 2669.04 | 27049.76 ± 4614.27 |
| Leg Fat weight (g) [1,2,3] | 7017.61 ± 1706.60 | 9470.25 ± 1937.00 | 12635.43 ± 3289.48 |
| Leg lean muscle mass(g) [1,2,3] | 12116.41 ± 1579.83 | 12794.33 ± 1540.90 | 14425.93 ± 2268.69 |
| Leg bone mineral composition[1,2,3] | 859.20 ± 121.86 | 912.97 ± 132.75 | 994.96 ± 133.24 |
| Trunk % Fat[1,2,3] | 32.80 ± 7.22 | 42.86 ± 4.65 | 47.75 ± 4.17 |
| Trunk Tissue weight (g) [1,2,3] | 27792.34 ± 3174.06 | 34906.52 ± 3434.23 | 45196.34 ± 9247.14 |
| Trunk Fat weight (g) [1,2,3] | 9484.22 ± 2778.51 | 15371.10 ± 2651.68 | 22000.92 ± 5240.02 |
| Trunk lean muscle mass (g) [1,2,3] | 18293.76 ± 1923.57 | 19535.76 ± 1916.82 | 23389.23 ± 5932.19 |
| Trunk bone mineral composition[1,2,3] | 718.56 ± 130.76 | 784.73 ± 133.10 | 746.66 ± 190.12 |
| Android % fat mass[1,2,3] | 36.80 ± 9.28 | 48.62 ± 5.55 | 54.29 ± 5.55 |
| Android tissue mass (g) [1,2,3] | 4054.58 ± 695.47 | 5437.13 ± 747.90 | 7485.42 ± 1554.42 |
| Android Fat mass (g) [1,2,3] | 1551.80 ± 594.19 | 2693.23 ± 586.51 | 4136.09 ± 1102.88 |
| Android lean muscle mass (g) [1,2,3] | 2505.41 ± 321.29 | 2743.28 ± 322.54 | 3353.68 ± 608.69 |
| Android BMC[1,2] | 49.25 ± 9.60 | 53.05 ± 10.34 | 54.82 ± 12.56 |
| Gynecoid % Fat[1,2,3] | 41.71 ± 5.99 | 46.74 ± 4.90 | 49.54 ± 6.04 |
| Gynecoid tissue mass (g) [1,2,3] | 9703.00 ± 1183.69 | 11340.72 ± 1283.62 | 13774.02 ± 2303.87 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gynecoid fat mass (g) [1,2,3] | 4169.96 | ± | 908.81 | 5453.28 | ± | 997.23 | 7032.05 | ± 1722.25 |
| Gynecoid lean muscle mass (g) [1,2,3] | 5526.59 | ± | 730.74 | 5887.80 | ± | 676.35 | 6745.83 | ± 1056.30 |
| Gynecoid BMC[1,2,3] | 235.05 | ± | 38.55 | 265.16 | ± | 39.34 | 298.65 | ± 42.29 |
| Total % body fat[1,2,3] | 32.49 | ± | 6.03 | 40.72 | ± | 4.03 | 45.80 | ± 4.15 |
| Total tissue mass (g) [1,2,3] | 57237.66 | ± | 18318.13 | 68716.33 | ± | 5702.59 | 87798.58 | ± 13948.25 |
| Total fat mass (g) [1,2,3] | 19304.69 | ± | 4663.43 | 29054.00 | ± | 4176.32 | 41572.18 | ± 8805.81 |
| Total lean muscle mass (g) [1,2,3] | 37270.39 | ± | 3788.07 | 39662.96 | ± | 3797.46 | 46226.42 | ± 7056.01 |
| Total Bone Mineral Composition[1,2,3] | 2351.26 | ± | 309.72 | 2485.71 | ± | 308.23 | 2550.39 | ± 353.80 |
| L2-L4 (Z-Score) | 0.29 | ± | 1.18 | 0.26 | ± | 1.25 | 0.12 | ± 1.31 |
| L2-L4 BMC[2,3] | 49.46 | ± | 9.26 | 50.66 | ± | 8.67 | 54.57 | ± 9.73 |
| Femoral Neck (Z-Score) | 0.15 | ± | 0.91 | 0.07 | ± | 0.89 | 0.20 | ± 1.02 |
| Neck BMC[1,2] | 4.47 | ± | 0.69 | 4.59 | ± | 0.62 | 4.90 | ± 0.65 |

P-values less than 0.5

NS- Not significant values are less than 0.5

[1] Normal/Underweight individuals are significantly different as compared with overweight individuals

[2] Normal/Underweight individuals are significantly different as compared with obese individuals

[3] Overweight individuals are significantly different as compared with only obese individuals

Table 45. Summary of single nucleotide polymorphisms, allele frequencies, and Hardy-Weinberg equilibrium (HWE). HWE was estimated using chi-squared analysis with one-degree of freedom.

| Position (Base Pair Number) | RS number | Variant | Location | MAF/Minor allele count | HWE (P-value) |
|---|---|---|---|---|---|
| 188043476 | rs182052 | A/G | Intron | 0.412/902 | 1 |
| 188054508 | rs3774262 | A/G | Intron | 0.142/321 | 0.011 |
| 188056399 | rs6773957 | A/G | 3'UTR | 0.489/1070 | 0.877 |
| 188056769 | rs1063537 | C/T | 3' UTR | 0.142/311 | 0.007 |
| 188058086 | rs1063539 | C/G | 3'UTR | 0.146/320 | 0.021 |

Table 46. Linkage disequilibrium analysis for the 5 SNPS assessed on the adiponectin gene. These are the measures of linkage disequilibrium (D' and R2) among the five SNPs in adiponectin.

| SNP1 x SNP2 | D | D' | r | R^2 | n |
|---|---|---|---|---|---|
| rs1063537 x rs1063537 | 0.08427 | 0.999192 | 0.999192 | 0.998384 | 1796 |
| rs1063537 x rs3774262 | 0.083679 | 0.994929 | 0.980436 | 0.961255 | 1706 |
| rs1063537 x rs182052 | -0.0193 | 0.625157 | -0.14113 | 0.019919 | 1705 |
| rs1063537 x rs1063539 | 0.080878 | 0.968486 | 0.920604 | 0.847512 | 1634 |
| rs1063537 x rs6773957 | 0.058224 | 0.998831 | 0.414554 | 0.171855 | 1706 |
| rs3774262 x rs3774262 | 0.086301 | 0.99919 | 0.99919 | 0.99838 | 1707 |
| rs3774262 x rs182052 | -0.01945 | 0.613495 | -0.14055 | 0.019754 | 1705 |
| rs3774262 x rs1063539 | 0.082408 | 0.960927 | 0.92692 | 0.859181 | 1633 |
| rs3774262 x rs6773957 | 0.059793 | 0.998831 | 0.420681 | 0.176973 | 1705 |
| rs182052 x rs182052 | 0.221745 | 0.999764 | 0.999764 | 0.999529 | 1706 |
| rs182052 x rs1063539 | -0.01548 | 0.457365 | -0.10862 | 0.011799 | 1634 |
| rs182052 x rs6773957 | -0.071 | 0.573115 | -0.31174 | 0.097182 | 1704 |
| rs1063539 x rs1063539 | 0.091439 | 0.999184 | 0.999184 | 0.998368 | 1634 |
| rs1063539 x rs6773957 | 0.055524 | 0.869198 | 0.379514 | 0.144031 | 1634 |

Table 47. A multivariate regression analysis of total bone mineral density for each SNP frequency, factoring for 4 specific confounding variables. The values listed are the p-values for each regression.

| SNP | No confounders | Gender | Age | Medication Status | Smoking Status |
|---|---|---|---|---|---|
| rs1063537 | 0.3936 | 0.4602 | 0.2655 | 0.6576 | 0.83125 |
| rs3774262 | 0.4905 | 0.8213 | 0.4598 | 0.8969 | 0.55384 |
| rs182052 | 0.7817 | 0.1589 | 0.9134 | 0.5447 | 0.20145 |
| rs1063539 | 0.8648 | 0.3016 | 0.6531 | 0.29 | 0.13557 |
| rs6773957 | 0.6446 | 0.3812 | 0.04078* | 0.5075 | 0.7464 |

Table 48. A multivariate regression analysis of spine bone mineral density between discs L2-L4 for each SNP frequency, factoring for 4 specific confounding variables. The values listed are the p-values for each regression.

| SNP | No confounders | Gender | Age | Medication Status | Smoking Status |
|---|---|---|---|---|---|
| rs1063537 | 0.4053 | 0.2378 | 0.5428 | 0.45211 | 0.835004 |
| rs3774262 | 0.701 | 0.5171 | 0.8097 | 0.56177 | 0.89 |
| rs182052 | 0.5128 | 0.8618 | 0.858 | 0.50072 | 0.74536 |
| rs1063539 | 0.5259 | 0.1339 | 0.593 | 0.08479 | 0.6365 |
| rs6773957 | 0.4268 | 0.8832 | 0.513 | 0.56067 | 0.6071 |

Table 49. A multivariate regression analysis of left hip-femoral neck bone mineral density for each SNP frequency, factoring for 4 specific confounding variables. The values listed are the p-values for each regression.

| SNP | No confounders | Gender | Age | Medication Status | Smoking Status |
|---|---|---|---|---|---|
| rs1063537 | 0.9671 | 0.3241 | 0.5186 | 0.9922 | 0.820159 |
| rs3774262 | 0.7457 | 0.4231 | 0.5274 | 0.6941 | 0.515642 |
| rs182052 | 0.7272 | 0.1801 | 0.6292 | 0.3146 | 0.103945 |
| rs1063539 | 0.2219 | 0.1152 | 0.9731 | 0.7782 | 0.335048 |
| rs6773957 | 0.8978 | 0.8844 | 0.0863 | 0.5421 | 0.258928 |

Table 50. A multivariate regression analysis of total percentage body fat measured by DEXA for each SNP frequency, factoring for 4 specific confounding variables. The values listed are the p-values for each regression. The significant values are bolded.

| SNP | No confounders | Gender | Age | Medication Status | Smoking Status |
|---|---|---|---|---|---|
| rs1063537 | 0.8309 | 0.577 | 0.08669 | 0.7474 | 0.4357 |
| rs3774262 | 0.3624 | 0.6629 | 0.06179 | 0.8522 | 0.6245 |
| rs182052 | 0.2103 | 0.95496 | 0.1347 | 0.7434 | 0.1837 |
| rs1063539 | 0.8978 | 0.9001 | **0.01256** | 0.5719 | 0.7156 |
| rs6773957 | 0.6297 | 0.5078 | 0.3079 | 0.9649 | 0.5824 |

Table 51. A multivariate regression analysis of total percentage trunk fat measured by DEXA for each SNP frequency, factoring for 4 specific confounding variables. The values listed are the p-values for each regression. The significant values are bolded.

| SNP | No confounders | Gender | Age | Medication Status | Smoking Status |
|---|---|---|---|---|---|
| rs1063537 | 0.9926 | 0.5444 | 0.1581 | 0.6729 | 0.709 |
| rs3774262 | 0.5287 | 0.6884 | 0.1185 | 0.7963 | 0.9214 |
| rs182052 | 0.112 | 0.95179 | 0.1974 | 0.68655 | 0.2302 |
| rs1063539 | 0.9451 | 0.99 | **0.03455** | 0.471 | 0.7846 |
| rs6773957 | 0.6704 | 0.8219 | 0.4482 | 0.8987 | 0.7748 |

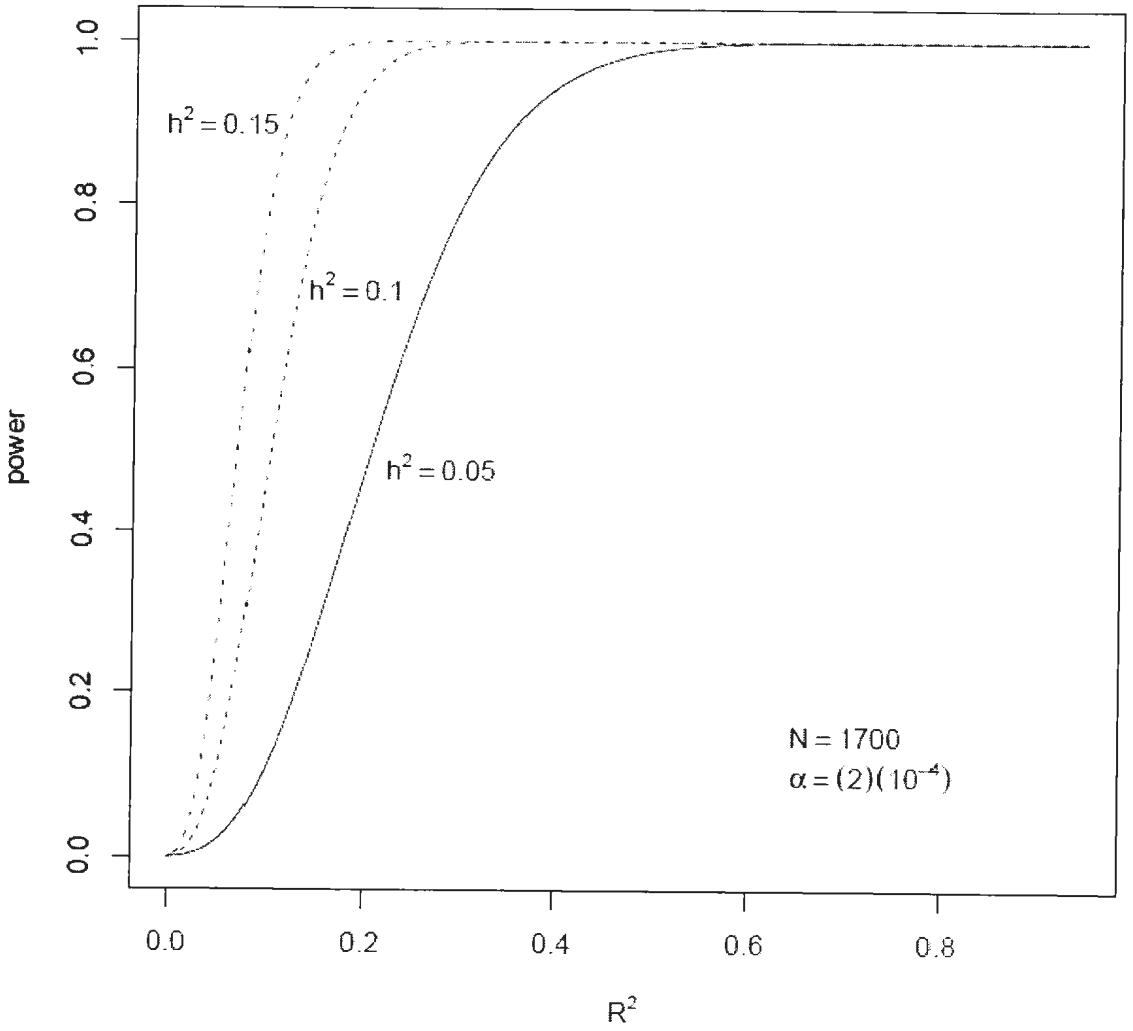


Figure 4. Power calculation for total sample size used for the data.

### 3.0.2 Overfeeding study results

The results from the overfeeding study are displayed in tables 52 through to 55. Table 52 displays the physical and biochemical characteristics of the 64 males overfed a positive energy balance of 30% greater than their baseline intake. The study cohort was divided into 3 groups – normal weight, overweight and obese. Percentage body, android and trunk fat were significantly increased after 7 days of overfeeding. There was also a significant increase was also noted in HDL and LDL cholesterol after 7 days of overfeeding. There was also a significant increase in triglycerides and insulin all groups after 7 days of overfeeding in all groups. Insulin resistance and β-cell function significantly increased in all three groups after 7 days of overfeeding. Adiponectin increased significantly after 7 days of overfeeding.

Partial correlations of baseline variables that were related to baseline fasting serum adiponectin, after controlling for age are located in Table 53. Significant partial correlations are labeled. Only HDL in all subjects was found to be negatively correlated significantly after correcting for age. Table 54 shows the partial correlations of changes in variables due to overfeeding related to baseline fasting serum adiponectin, after control for age. No significant results were found. Table 55 shows partial correlations of changes in variables due to overfeeding related to changes in fasting serum adiponectin, after control for age. Again, no significance was found between any of the variables assessed.

Table 52. Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

| | Normal Weight[2] (n = 23 - 25) | | Overweight[2] (n = 13-14) | | Obese[2] (n = 22 - 25) | |
|---|---|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** | **Before** | **After** |
| Age (y) | 23.87 ± 3.7 | NA | 21.97 ± 3.1 | NA | 23.16 ± 2.4 | NA |
| Height (cm) | 178.89 ± 6.6 | NA | 179.62 ± 4.8 | NA | 179.55 ± 7.1 | NA |
| Weight (kg) [4,6,7] | 72.26 ± 9.6 | 74.42 ± 9.6 | 77.81 ± 4.3 | 79.39 ± 4.3 | 91.39 ± 15.4 | 93.93 ± 16.0 |
| BMI (kg/m$^2$) [4,6,7] | 22.58 ± 2.7 | 23.26 ± 2.9 | 24.13 ± 1.3 | 24.63 ± 1.5 | 28.23 ± 4.2 | 29.103 ± 4.3 |
| Percent Body Fat (%) [3,6,7,8] | 14.82 ± 3.4 | 15.60 ± 3.4 [9] | 22.54 ± 0.8 | 22.82 ± 1.1 [9] | 31.15 ± 4.9 | 31.01 ± 4.8 |
| Percent Trunk Fat (%) [3,6,7,8] | 16.78 ± 3.7 | 17.78 ± 3.8 [9] | 25.39 ± 1.9 | 25.79 ± 2.2 [9] | 35.07 ± 5.4 | 34.97 ± 5.3 |
| Percent Android Fat (%) [3,6,7] | 19.38 ± 4.4 | 20.16 ± 5.0 | 28.84 ± 2.6 | 29.45 ± 2.7 | 40.00 ± 7.2 | 40.77 ± 6.8 |
| Total Cholesterol (mmol/L) [6] | 4.41 ± 0.9 | 4.68 ± 0.9 | 4.63 ± 0.9 | 4.72 ± 1.1 | 4.59 ± 0.7 | 4.86 ± 0.8 |
| HDL-Cholesterol (mmol/L) [6] | 1.38 ± 0.3 | 1.48 ± 0.3 | 1.38 ± 0.3 | 1.43 ± 0.3 | 1.12 ± 0.2 | 1.34 ± 0.3 |
| LDL-Cholesterol (mmol/L) | 2.61 ± 0.7 | 2.64 ± 0.7 | 2.82 ± 0.7 | 2.83 ± 0.9 | 2.81 ± 0.7 | 2.83 ± 0.6 |
| Triacylglycerol (mmol/L) [6,7] | 0.94 ± 0.3 | 1.22 ± 0.8 | 0.92 ± 0.3 | 1.00 ± 0.5 | 1.35 ± 0.7 | 1.57 ± 0.9 |
| Glucose (mmol/L) | 4.98 ± 0.4 | 5.03 ± 0.5 | 5.03 ± 0.4 | 5.09 ± 0.6 | 5.24 ± 0.7 | 5.11 ± 0.5 |
| Insulin (pmol/L) [4,6,7] | 44.22 ± 23.8 | 64.02 ± 23.7 | 51.54 ± 17.03 | 68.69 ± 43.6 | 80.19 ± 53.3 | 108.22 ± 76.7 |
| HOMA-IR [4,6,7] | 1.43 ± 0.8 | 2.09 ± 0.9 | 2.35 ± 2.68 | 2.95 ± 2.9 | 2.80 ± 2.3 | 3.67 ± 2.8 |
| HOMA-β [4,6,7] | 85.45 ± 39.38 | 126.34 ± 49.9 | 101.49 ± 25.1 | 140.84 ± 101.7 | 134.95 ± 63.0 | 190.28 ± 107.3 |
| Adiponectin (μg/mL) [6] | 11.60 ± 6.3 | 13.96 ± 4.5 | 12.84 ± 4.6 | 14.81 ± 4.05 | 10.69 ± 6.3 | 12.82 ± 6.1 |

[1] All values are means ± SDs. HOMA - IR, Homeostasis model assessment of insulin resistance; HOMA - β of β cell function; NA, not applicable. Adiposity status and response to overfeeding analyzed by 2 - factor mix model ANOVA (SPSS, version 17.0 Chicago, IL, USA) for repeated measures.

[2] Subjects were classified on the basis of %BF as either normal weight (8 - 20.9%), overweight (21 - 25.9%) or obese (> 26%) according to criteria recommended by Bray {Bray, 2003 #48}.

[3] Significant difference between normal weight, overweight and obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[4] Significant difference between normal weight vs obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[5] Significant difference between obese vs normal weight and obese vs overweight subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[6] Significant difference due to overfeeding (2 - Way mixed model ANOVA, $P < 0.05$).

[7] Significant difference due to adiposity status (2 - Way mixed model ANOVA, $P < 0.05$).

[8] Significant overfeeding by adiposity status interaction (2 - Way mixed model ANOVA, followed by a Bonferroni corrected test when significant, $P < 0.05$).

[9] Significant difference within group (paired $t$-test, $P < 0.05$).

Table 53. Partial correlations of baseline variables related to baseline fasting serum adiponectin, after control for age[1]

| | All Subjects (n = 59 - 64) | | Normal weight[2] (n = 23 - 25) | | Overweight[2] (n = 14) | | Obese[2] (n = 22 - 25) | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| Weight | - 0.246 | 0.050[3] | -0.284 | NS | -0.425 | NS | -0.177 | NS |
| BMI | - 0.289 | 0.023[3] | -0.322 | NS | -0.188 | NS | -0.296 | NS |
| Percent Body Fat | - 0.127 | NS | -0.094 | NS | 0.338 | NS | -0.309 | NS |
| Percent Trunk Fat | - 0.155 | NS | -0.096 | NS | 0.235 | NS | -0.403 | 0.041[3] |
| Percent Android Fat | - 0.140 | NS | 0.064 | NS | 0.066 | NS | -0.343 | NS |
| Total Cholesterol | 0.057 | NS | 0.340 | NS | 0.050 | NS | -0.313 | NS |
| HDL-Cholesterol | 0.401 | 0.001 | 0.178 | NS | 0.222 | NS | 0.357 | NS |
| LDL-Cholesterol | 0.020 | NS | 0.328 | NS | -0.039 | NS | -0.242 | NS |
| Triacylglycerols | -0.175 | NS | 0.089 | NS | 0.167 | NS | -0.339 | NS |
| Glucose | -0.225 | NS | 0.196 | NS | 0.026 | NS | -0.427 | 0.042[3] |
| Insulin | -0.225 | NS | 0.126 | NS | -0.192 | NS | -0.499 | 0.015[3] |
| HOMA-IR | -0.229 | NS | 0.141 | NS | -0.170 | NS | -0.505 | 0.014[3] |
| HOMA-β | -0.204 | NS | 0.042 | NS | -0.292 | NS | -0.399 | NS |

[1] HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for potential factors related to fasting adiponectin.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray {Bray, 2003 #48}.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

Table 54. Partial correlations of changes in variables due to overfeeding related to baseline fasting serum adiponectin, after control for age[1]

| | All Subjects (n = 59 - 64) | | Normal weight[2] (n = 23 - 25) | | Overweight[2] (n = 14) | | Obese[2] (n = 22 - 25) | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| Weight | -0.043 | NS | 0.015 | NS | -0.103 | NS | -0.049 | NS |
| BMI | -0.045 | NS | 0.011 | NS | -0.103 | NS | -0.050 | NS |
| Percent Body Fat | 0.066 | NS | 0.155 | NS | -0.415 | NS | -0.027 | NS |
| Percent Trunk Fat | 0.101 | NS | 0.158 | NS | -0.248 | NS | 0.183 | NS |
| Percent Android Fat | -0.123 | NS | -0.191 | NS | -0.244 | NS | -0.057 | NS |
| Total Cholesterol | 0.053 | NS | 0.146 | NS | 0.326 | NS | -0.069 | NS |
| HDL-Cholesterol | -0.086 | NS | 0.050 | NS | -0.073 | NS | -0.115 | NS |
| LDL-Cholesterol | 0.168 | NS | 0.241 | NS | 0.439 | NS | -0.040 | NS |
| Triacylglycerols | -0.142 | NS | -0.035 | NS | -0.430 | NS | -0.435 | 0.038[3] |
| Glucose | -0.045 | NS | -0.264 | NS | 0.095 | NS | 0.002 | NS |
| Insulin | -0.125 | NS | -0.438 | 0.032[3] | 0.313 | NS | 0.112 | NS |
| HOMA-IR | -0.084 | NS | -0.175 | NS | 0.059 | NS | 0.230 | NS |
| HOMA-β | -0.081 | NS | -0.298 | NS | 0.210 | NS | 0.099 | NS |

[1] HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for the potential changes in factors due to overfeeding related to fasting adiponectin.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray {Bray, 2003 #48}.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

Table 55. Partial correlations of changes in variables due to overfeeding related to changes in fasting serum adiponectin, after control for age[1]

| | All Subjects (n = 59 - 64) | | Normal weight[2] (n = 23 - 25) | | Overweight[2] (n = 14) | | Obese[2] (n = 22 - 25) | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| Weight | -0.098 | NS | -0.125 | NS | -0.022 | NS | -0.159 | NS |
| BMI | -0.097 | NS | -0.124 | NS | -0.021 | NS | -0.157 | NS |
| Percent Body Fat | 0.005 | NS | -0.040 | NS | 0.347 | NS | 0.067 | NS |
| Percent Trunk Fat | 0.027 | NS | 0.036 | NS | 0.074 | NS | -0.061 | NS |
| Percent Android Fat | 0.208 | NS | 0.286 | NS | 0.200 | NS | 0.072 | NS |
| Total Cholesterol | 0.113 | NS | -0.010 | NS | -0.336 | NS | 0.436 | 0.038[3] |
| HDL-Cholesterol | 0.258 | NS | 0.197 | NS | 0.212 | NS | 0.296 | NS |
| LDL-Cholesterol | 0.001 | NS | -0.149 | NS | -0.452 | NS | 0.409 | 0.050[3] |
| Triacylglycerols | 0.018 | NS | -0.101 | NS | 0.078 | NS | 0.271 | NS |
| Glucose | 0.201 | NS | 0.298 | NS | -0.055 | NS | 0.239 | NS |
| Insulin | 0.193 | NS | 0.316 | NS | -0.440 | NS | 0.165 | NS |
| HOMA-IR | 0.084 | NS | 0.141 | NS | -0.189 | NS | -0.040 | NS |
| HOMA-β | 0.076 | NS | 0.152 | NS | -0.354 | NS | 0.029 | NS |

[1] HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for potential changes in factors due to overfeeding related to the potential changes in adiponectin due to overfeeding.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray {Bray, 2003 #48}.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

## 4.0 Discussion

### *4.0.1 Adiponectin and Bone Mineral Density*

Adiponectin, since its initial discovery as an adiopkine released by fat cells, has been shown to be involved in insulin regulation, body fat composition, and has an anti-inflammatory role. Most recently, adiponectin has been suggested as having a direct role in bone mineral density regulation and in bone metabolism, but this relationship has not been completely understood. This study aimed to further validate this relationship and to show whether specific genotypes of adiponectin were associated with differences in bone mineral density.

Previous studies (Lenchik *et al.*, 2003; Jürimäe *et al.*, 2007; Kontogianni *et al.*, 2004) suggested an association of serum adiponectin with bone mineral density. However, few studies to date have explored the effect of genetic variations in the adiponectin gene on the variations of bone density at the population level. This genetic association study shows no association of any SNP genotype or haplotype with any phenotype of bone mineral density. Although mouse models showed knock out and transgenic mice of adiponectin that may have a significant influence of adiponectin on BMD, this does not support that any genetic variations in the adiponectin gene play an important role in determining bone mineral density in the Newfoundland population.

There were a total of 1811 subjects in this study, with 403 males and 1408 females. The power calculation, shown in Figure 4, demonstrates this study had adequate power to show a direct effect from adiponectin on bone mineral density. It is possible, given the lack of variation in SNPs within the cohort, that a larger sample-size is needed to see a potential genotypic effect. However, according to the calculation, there were sufficient subjects obtained for the analysis.

In addition, although three of the five single nucleotide polymorphisms were not in HWE, these were still used in the analysis as it was difficult to select ideal tagging SNPs with reasonable allele frequencies. Moreover, although these SNPs were not in HWE, they could still provide valuable information with the two tagging SNPs in HWE with the other three.

The findings are similar to an analysis of SNP polymorphisms in the Chinese population. Zhang *et al.* (2007) found no significant association of adiponectin genotypes with peak BMD in Chinese men and women. However, a significant within-family association with peak femoral BMD was noted in the Thr394Thr polymorphism in the PPGAGC1 gene.

A study in 249 Korean women and 80 Korean men looked at two polymorphisms using the Taqman probe (Lee *et al*, 2006). Genotyping of the T45G polymorphism in exon 2 and the G276T polymorphisms in intron 2 in the adiponectin gene was performed. Within the female cohort, subjects with G alleles at the T45G locus had significantly lower lumbar spine BMD than those subjects with the TT genotype (Lee *et al.*, 2006). However, no association was found with the other polymorphism. The results do not support this finding as in this study no association was found. Their small sample size does not provide adequate power to properly assert an association.

In another study of 1329 postmenopausal Korean women, the two genes for the adiponectin receptors ADIPOR1 and ADIPOR2 were assessed (Kim *et al.*, 2012). ADIPOR1 rs16850799 and rs34010966 polymorphisms were significantly associated with femur neck BMD (Kim *et al.*, 2012). ADIPOR2 SNPs and haplotypes were not associated with BMD at any site. Our study did not assess the adiponectin receptor gene; however, this may be focus for further research.

There is the possibility that a subgroup in the general population may have its BMD phenotype be affected by the genetic variations in the adiponectin gene. In order find such a relationship, there needs to be a much larger sample size with homogeneous conditions, both genetic and environmental factors. This is often difficult to obtain but highly recommended if possible.

### *4.0.2 Adiponectin and its response to Positive Energy Challenge*

This study displayed a new major finding that over short-term positive energy challenge, there is a significant initial increase in serum adiponectin levels. This was found in all categories of normal and underweight individuals, overweight and obese individuals, as measured by DEXA. To our knowledge, this is the first study to present this finding. This is an especially interesting finding since several cross-sectional studies have shown that circulating adiponectin is decreased in individuals with increasing adiposity phenotypes (Kumada *et al.*, 2003, Vilarrassa *et al.* 2005).

One study has shown a negative correlation between adiposity or obesity with adiponectin when overfeed for longer periods of time. Ukkola *et al.* (2008) in a 100 day study of 24-sedentary young males, 12 identical twin pairs, each overfed 84,000 kcal over baseline. Serum adiponectin concentration correlated positively with body weight at baseline, but not with other indicators of adioposity. Serum adiponectin levels, however, decreased significantly over 100-day period of overfeeding (Ukkola *et al.* 2008). Another study by Astrand *et al.* (2010) also helps support our findings that there is a difference in adiponectin secretion with long-term and short-term overfeeding. In a four week positive energy challenge on 18 young normal-weight adults (12 male and 6 female), there was no significant increase in circulating adiponectin (Astrand *et al.*, 2010). This lack of difference may be explained by the relatively small sample size and not controlling for gender (Astrand *et al.* 2010)

Interestingly, in a study of prepubertal obese children from Warsaw, Poland, circulating adiponectin levels were 25% lower in obese children as compared with controls (Gajewksa *et al.* 2011). However, after implementation of a three-month lifestyle modification program aimed at

weight reduction in these children, serum adiponectin levels substantially increased (Gajewksa *et al.*, 2011). A similar finding was reported by Medina-Bravo *et al.* (2011) when assessing the relationship between adipocity and visceral adipose tissue measured by CT in children and adolescents. An inverse correlation was associated with visceral adipose tissue and adiponectin levels in this group (Medina-Bravo *et al.*, 2011). There has been some recent evidence that shows adiponectin is also released by myocytes, as well as adipocytes, and there has been some suggestion that its regulation may be related to PPAR-$\gamma$ (Amin *et al.* 2010). This evidence may partly explain the increase of serum adiponectin both short-term overfeeding, as well as with exercise and weight reduction. However, the mechanism of the changes in adiponectin levels is not known at present.

There were other studies that explored the relationship of overfeeding with adiponectin. Brøns *et al.* (2009) conducted 5-day high-fat diet (60% fat, 32.5% carbohydrates and 7.5% protein) on 26 normal-weight young men with daily overfeeding set to 50% greater than their baseline intake. Although the group used a different macronutrient composition as part of their study as compared with ours, they still observed a significant increase in adiponectin concentration (Brøns *et al.* 2009). Age and gender of the Brøns *et al.* (2009) subjects were comparable to the normal-weight subgroup in our cohort. These findings, along with our own, provide strong evidence that the increase of adiponectin concentration is driven by the increase in caloric intake irrespective of macronutrient composition. In addition, our results show for the first time in the short-term, that the ability of increased synthesis and secretion of adiponectin is preserved in subjects spanning all obesity categories.

In this cohort, adiponectin concentrations significantly increased within the entire cohort

across normal-weight, overweight and obese subject phenotypes. However, when comparing between each group, no significant difference was found in serum adiponectin pre- and post overfeeding within the obese group. This finding would indicate that adiposity is not a significant factor in serum adiponectin's response to short-term overfeeding. In addition, further stratification of the adiponectin serum levels in low, medium and high serum adiponectin concentrations did not reveal any significant difference in pre- and post-overfeeding serum adiponectin groups. This gave further support to the notion that adiponectin levels during short-term overfeeding are not affected by adiposity status.

Despite the evidence showing long-term positive energy challenge being associated with decreased levels of adiponectin, our results indicate that there may be a difference in action and regulation of adiponectin with short-term overfeeding when compared with long-term overfeeding. No studies in humans have been published yet addressing the biochemical role of adiponectin during metabolism.

Many overweight and most obese individuals have insulin resistance. To counteract the high insulin resistance, the synthesis and secretion of adiponectin from the body may increase physiologically, such as in obesity. However, as can be seen with the findings and this study of Ukkola *et al.* (2007), there seems to be a paradox that has still yet not been properly explained. The low adiponectin level in obese and type 2 diabetic patients suggest that their ability for adiponectin secretion is low. However, the data does not show that adiposity status is a contributing factor to adiponectin concentration. Circulating adiponectin levels were, however, inversely correlated with BMI and weight for the entire cohort along with percentage trunk fat, fasting glucose, fasting insulin and HOMA-IR in obese subjects. Some studies advocate that the

inverse relationship between adiponectin and adiposity phenotypes is likely solely dependent upon the development of insulin resistance (Pellme, 2003; Cnop, 2003). To address this concern the presented data was controlled for fasting insulin and/or HOMA-IR. After this control, no negative partial correlations between adiponectin and adiposity-related phenotypes were found. This finding also supports the belief that the inverse relationship between adiposity and circulating adiponectin is likely dependent on changes in insulin resistance rather than solely the change in obesity status (Cnop, 2003; Pellme, 2003). These findings provide a key stepping-stone for future study in the elements involved in adiponectin secretion.

### *4.0.3 Study Limitations*

It is important to identify the limitations in this study. This study only assessed young males aged 19-29. Females, nor those males later in age were not included. This limits the ability to extrapolate the results to other groups. In addition, this genetic analysis, although it had 1800 subjects, was likely still not large enough to establish any genotypic associations. Furthermore, only total circulating adiponectin was measured in this investigation. Wang *et al.*, (2008) suggested that high molecular weight adiponectin is the main physiologically active isoform of adiponectin, but other isoforms do exist. Low, medium and high molecular weight adiponectin also may respond to nutritional regulation and exercise in the same manner as total circulating adiponectin (Christiansen, 2010).

### *4.0.4 Conclusion*

Our CODING study cohort revealed no significant association with bone-mineral density and adiponectin genotypes. Although this finding was supported by other studies, it is acknowledged that this sample size may not have been large enough to show any link. However, the overfeeding cohort does demonstrate that endogenous adiponectin concentration significantly increases within the entire cohort and all three adiposity groups in response to short-term overfeeding. Results further show this occurs despite the macronutrient feeding regime used. These novel findings suggest adiponectin may act as a protective mechanism during periods of weight gain and insulin resistance independent of adiposity status and diet composition.

## 5.0 References

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