BONE MINERAL DENSITY IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Background: Polycystic ovary syndrome (PCOS) is a common complex hormonal disorder. Many PCOS symptoms may have implications on bone mineral density (BMD). One way to analyze BMD is quantitated computed tomography (QCT), which may have advantages over other BMD analysis methods. The study analyzed descriptive characteristics of a group of PCOS cases and controls; considered the determinants of BMD (as measured by QCT) from the literature in PCOS cases and controls; and adjusted for these variables via multivariate logistic regression to determine if PCOS case status is an independent predictor of lumbar BMD after controlling for these factors. **Methods:** The study used women from the third implementation of the University of Pittsburgh Cardiovascular Health and Risk Management study (CHARM III). Descriptive information was gathered by survey and clinical visits and blood samples were taken to measure hormones and other biological factors. Lumbar BMD was measured by QCT in a subset of women. Student's T-Test, the Mann-Whitney U-Test and X^2 tests were used to evaluate descriptive characteristics of PCOS cases and controls. BMD measures between PCOS case and controls were compared using Student's T-test. Lumbar BMD comparisons between PCOS cases and controls were also stratified by factors determined from the literature to affect BMD, including age, ethnicity, menstrual period status, BMI, and menstrual history. Correlations of BMD with hormones in cases and controls were considered. Multivariate linear regression models were used to assess the effect of PCOS case-control status on lumbar BMD after

controlling for these factors associated with BMD. **Results:** There was no significant BMD difference between PCOS cases and controls for any univariate comparisons, nor for any multivariate adjusted compositions. **Conclusion:** The deleterious effects of middle age and approaching menopause and the protective effects of heavy BMI in controls may mediate some protective effects of PCOS case status on BMD in this group. **Statement of Public Health Significance:** The current study is one of only a few to use QCT to measure BMD in women with PCOS. Results from this study can serve as the basis of comparison for other studies that use QCT methods to assess BMD.

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PREFACE

I would like to thank my committee for their assistance and knowledge in the development of this document. I owe special thanks to Dr. Evelyn Talbott and to Dr. Jeanne Zborowski. As my advisor, Dr. Talbott provided opportunities to assist in teaching and to participate in research that served as the basis for this thesis and acted as an advocate for often-difficult course scheduling conflicts. Dr. Zborowski provided invaluable insight into the topics of this thesis and was always willing to answer questions and provide support.

I would like to thank my supervisors and coworkers at ChemADVISOR, Inc. for their moral and professional support. I would also like to thank my friends, especially Amy Dressen and Alison McBee, for their mental, moral and technical support and their crisis-aversion tactics.

I dedicate this thesis to my family: my mother Sherry, my father Mike, my sister Meggie, and my (soon-to-be) husband Jason.

1.0 INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common hormonal reproductive disorders in women in the United States, affecting estimates of 4 to 10% of women of reproductive age (Zborowski J, Talbott E, Cauley J, 2001)(Norman RJ, Wu R, Stankiewicz M, 2004). Symptoms of PCOS vary between individuals and range from physiological characteristics such as polycystic ovaries, obesity, central adiposity and hirsutism to biological effects such as infertility, hyperinsulimua, type 2 diabetes, elevated androgens and hypertension. Many of the common morphological and endocrine-related symptoms of PCOS, such as obesity and hyperandrogenism, may have long-term implications on bone density. Obesity and the high waist-to-hip ratio body composition in many women with PCOS (often called "apple shaped") can result in concentrated physical bone stress, which can lead to increased bone density. Androgens have been shown to act directly and indirectly on bone formation and turn over.

Bone density is typically measured via an assessment of bone mineral density (BMD). Quantitative computed tomography (QCT) is one method for measuring BMD. Although QCT is not used as commonly as other methods for assessing bone density, such as dual energy x-ray absorptiometry (DXA), it has been shown to have advantages over other methods in several measures used to diagnose and assess bone-loss conditions. QCT is able to take three-dimensional measures of bone, and can distinguish between trabecular bone (which is responsible for a large amount of bone turnover and formation) and other bone tissues types.

The high prevalence of PCOS makes it a readily available population in which to assess the efficacy of QCT as a method for assessing BMD and an ideal natural model for assessing the effects of PCOS symptoms, such as elevated androgens, on BMD. Accurate methods of analyzing BMD in women with PCOS ensure that the effects of their symptoms on BMD are correctly assessed. Factors determined to affect BMD in women with PCOS can be used to aid in management of PCOS and its long-term effects on BMD. In addition, effects on BMD ascertained from analysis of BMD in women in with PCOS can in turn be applied to the diagnosis and treatment in other common bone-density related diseases, such as osteoporosis. Thus, efforts to accurately assess bone density in women with PCOS can have potentially far-reaching implications.

Despite the promises of QCT analysis, little epidemiologic data exists on QCT specific measures of BMD in women with PCOS. This study examines the complex etiology of PCOS and its relationship to bone density, hormonal parameters, body characteristics, and other health outcomes, using data collected as part of the Cardiovascular Health and Risk Management Study (CHARM). In particular, analysis focused on the previously unanalyzed CHARM III, visit one cohort.

1.1 SPECIFIC AIMS

Specific aims included:

- Analysis of CHARM III Visit One cohort measures of hormonal and body composition features within PCOS cases and controls in order to analyze

- Consider the determinants of BMD as measured by QCT from the literature, and then determine if a significant difference exists between PCOS case and control groups analyzed by these determinates (univariate correlates)
- Determine if PCOS case status is an independent predictor of BMD status after controlling for these factors

2.0 BACKGROUND AND REVIEW OF THE RELEVANT LITERATURE

2.1 POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome (PCOS) is a common endocrine-related disorder, with estimates of its prevalence ranging from approximately 4% to 10% (Zborowski J, Talbott E, Cauley J, 2001) (Norman RJ, Wu R, Stankiewicz M, 2004) in women of reproductive age. The etiology of PCOS is not firmly understood and likely has multiple causes. There is growing evidence of a genetic component to the disease. Although specific genes have yet to be identified, variability has been found in genes which affect androgen production and insulin use (Diamanti-Kandarakis et al., 2006). There is also evidence that PCOS may be related to insulin resistance. For example, modification of certain enzyme receptors can result in hyperinsulinemia and hyperandrogenism, both common symptoms of PCOS. (Vignesh and Mohan, 2007).

2.1.1 Symptoms

2.1.1.1 Oligoovulation and Anovulation

Oligoovulation and anovlation are clinically assessed by the frequency of menstrual periods, specifically, by the presence of oligomenorrhea and amenorrhea. Oligomenorrhea is typically defined as eight or fewer menstrual periods per year, and amenorrhea by the absence of menstrual periods. Estimates of the prevalence of oligo-anovluation range from 60% to 85% of

women diagnosed with PCOS (Trivax and Azziz, 2007) compared to about 4% in the general population (Springhouse, 2005).

2.1.1.2 Polycystic Ovaries

Ultrasound is typically used to asses the presence of polycystic ovaries in women with PCOS. A woman is considered to have polycystic ovaries if each has 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (≥10 mL) (Balen AH, Laven JS, Tan SL, Dewailly D, 2003). Women on oral contraceptives need only have one ovary meet this definition. Recent studies indicate that 70% to 100% of women with PCOS, depending up the criteria set used, will have polycystic ovaries (Trivax and Azziz, 2007).

2.1.1.3 Hyperandrogenmia

Hyperandrogenemia can be assed by either laboratory measures or clinical manifestations such as hirsutism and acne. Biological measures used most frequently are measures of free testosterone and the free androgen index (FAI), though these measures are not without pitfalls. Measures of free testosterone are extremely variable, and normal ranges are not well established. In addition, the use of hormones, including hormonal birth control or hormone replacement therapy, may affect measured levels (Stankiewicz and Norman, 2006). Estimates of the prevalence of hyperandrogenemia range to 70% (Trivax and Azziz, 2007).

2.1.1.4 Other Symptoms Not Resulting in Diagnosis

Women with PCOS have been shown to have increased prevalence of a number of other characteristic symptoms that do not result in diagnosis. PCOS has been shown to be associated with increased obesity, central adiposity, menstrual disorders, insulin resistance and

cardiovascular disorders. Though these conditions do not result in diagnosis, they can result in a number of long term adverse health outcomes, including Type 2 Diabetes Mellitus, cardiovascular disease, obstructive sleep apnea, and infertility (Stankiewicz and Norman, 2006), (Zborowski J, Talbott E, Cauley J, 2001), (Avery and Braunack-Mayer, 2007).

2.1.2 Diagnosis

Two unique factors of PCOS make assessment of PCOS and its related health effects challenging. First, PCOS is a syndrome characterized by a number of symptoms, all of which are not present in every individual. Second, due to the heterogeneous nature of the syndrome, no single symptom is sufficient for diagnosis and several definitions have evolved to diagnose PCOS. This has resulted in a patchwork of definitions and differential diagnosises across studies.

PCOS was first identified in 1935 in a group of seven women with infertility, oligo/amenorrhea and polycystic ovaries. This case series set off a chain of investigation that has led to the current understanding of PCOS. However, even among this group of patients, symptoms differed, with some displaying hirsutism, some obesity, and some acne (Stein and Leventhal, 1935). When Stein and Leventhal removed some of the cysts the women via ovarian wedge resection, the oligo/amenorrhea was reversed in all of them, and fertility was restored in five, strongly suggesting that the symptoms resulted at least in part from the polycystic ovaries. Several of the other symptoms seen in some of the women, namely the hirsutism and acne, are frequently caused by hyperandrogenemia. These symptoms are often used as clinical indicators of elevated androgens in the absence of clinical testing (Stankiewicz and Norman, 2006). Their presence indicates that some of the women studied by Stein and Leventhal the may have also had hyperandrogenism.

Three main symptoms of PCOS seen in the women studied by Stein and Leventhal, oligo/amenorrhea, polycystic ovaries and hyperandrogenemia, have been used in establishing diagnostic criteria for PCOS. In 1990, diagnostic criteria for PCOS were established by the 1990 National Institute of Health/National Institute of Child Health and Human Development consensus statement for the diagnosis of PCOS (the 1990 NIH criteria). In the 1990 NIH criteria, cases must have hyperandrogenemia with the exclusion of other etiologies (such as Cushing disease and adrenal tumors), and ovarian dysfunction (defined as oligomenorrhea or amenorrhea). (Zawadski and Dunaif, 1992).

In 2003, at a conference in Rotterdam sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine, a new criteria for diagnosis of PCOS was proposed by a panel of experts (the 2003 Rotterdam criteria). The 2003 Rotterdam criteria were developed in response to criticism that the 1990 criteria were too restrictive, and did not take into consideration developments in the clinical understanding of PCOS and its symptoms (Rotterdam, 2004). Per the 2003 Rotterdam consensus, women are diagnosed with PCOS if they present with two of the following three criteria (excluding related disorders): hyperandrogenemia, polycystic ovaries, or oligomenorrhea/amenorrhea. Recently, a 2006 task force convened by the Androgen Excess Society suggested a definition consisting of the presence of hirsutism and/or hyperandrogenemia, oligo/anovulation and/or polycystic ovaries, and the exclusion of related disorders (Trivax and Azziz, 2007), though this newer definition has not yet been used extensively in the literature.

The most notable difference between the two most prominent definitions, the 1990 NIH criteria and the 2003 Rotterdam criteria, is the inclusion of polycystic ovaries as a diagnostic criterion in the Rotterdam criteria and the exclusion of this criterion in the NIH criterion. The

2003 Rotterdam criteria created "new" groups of cases not covered by the NIH consensus: women with hyperandrogenemia and polycystic ovaries and women with oligo/anovulation and polycystic ovaries. Some studies suggest this has increased the number of women diagnosed with PCOS. Borekmans et al. estimated an increase of diagnosis of 150% in a cohort based on a large anovulation screening database (Broekmans FJ, Knauff EAH, Valkenburgh O, Laven JS, Eijkemans JS, Fauser BCJM, 2006).

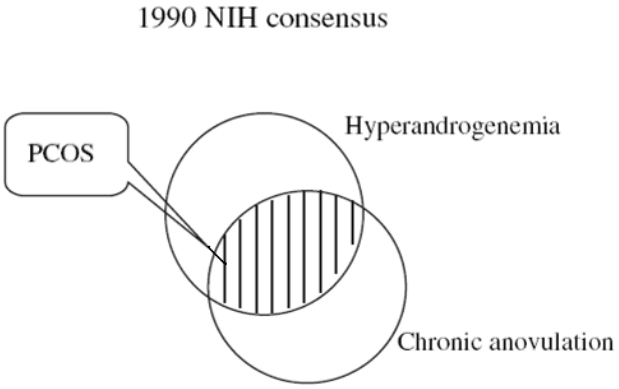


Figure 1: Diagnosis of PCOS by the 1990 NIH Criteria (Reproduced from Broekmans et al, 2006)

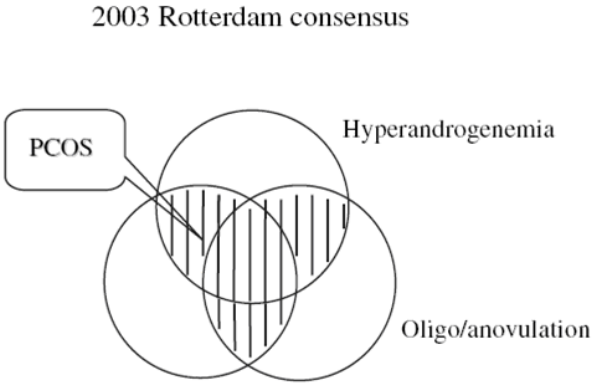


Figure 2: Diagnosis of PCOS by the 2003 Rotterdam Criteria (Reproduced from Broekmans et al , 2006)

2.2 POLYCYSTIC OVARY SYNDROME AND BONE MINERAL DENSITY

Several factors are well known to have effects on bone mineral density, most notably age and menopausal status. As women age and enter menopause, BMD decreases. As individuals age, their rate of bone formation slows, though the exact causes of this process are unknown (Seeman, 2002). As women reach menopause, loss of BMD accelerates. Estrogen is well-known to be protective a protective factor for BMD, though the mechanism of action is not completely understood. Estrogen aids in absorption and conservation of calcium and may help prevent the death of bone-forming cells. Lower levels of estrogen accelerate bone remodeling, resulting in lowered BMD.

However, many of the long term health effects of PCOS can also manifest in effects on bone mineral density (BMD). The elevated levels of androgens may act as factors which increase BMD in women with PCOS. Body composition characteristics of women with PCOS, including obesity and central adiposity, may also result in increased BMD (Zborowski J, Cauley J, Talbott E, Guzick D, Winters J, 2000). Other characteristics of PCOS, such as oligo/amenorrhea and insulin resistance may manifest in effects on BMD. Some studies have indicated that hyperinsulinemia has a positive correlation with BMD (Yüksel O, Dökmetas HS, Topcu S, Erselcan T, Sencan M, 2001).

2.2.1 Estrogen, Menstrual History and Bone

As previously discussed, estrogen levels are strongly related to BMD. Since estrogen levels are naturally determined by menstrual cycles, menstrual cycles and menstrual history can also affect BMD. In PCOS women with amenorrhea or oligomenorrhea, estrogen levels are more consistent

than in women with normal menstrual cycles which cause levels of estrogen to fluctuate. Constant levels of estrogen can perpetuate amenorrhea or oligomenorrhea since drops in estrogen levels are necessary to trigger ovulation (To and Wong, 2005). A history of abnormal menstruation may affect BMD and some studies indicate that age of onset of menarche and menopause may be associated with BMD; women with early ages of onset of menarche and late onset of menopause are shown to have higher BMD compared to women with late onset of menarche and early onset of menopause (Masaryk et al, 1998). It is well known that diet and exercise induced amenorrhea is associated with low BMD. Estrogen replacement therapy is often used to treat exercise related amenorrhea. However, its efficacy is not certain. A study of dancers with exercise-induced amenorrhea treated with estrogen therapy did not show significant increase in BMD compared to placebos, indicating that factors other than estrogen are important in maintaining and increasing BMD in women with irregular menstrual cycles (Warren, 2003).

2.2.2 Androgens and Bone

The protective effects on estrogen on bone mineral density (BMD) have been well studied, but the effects of androgens on BMD, particularly in women, is less certain. Androgens can be converted into estrogens in the ovary and fat tissues can convert testosterone and androstenedione into 17β-Estradiol and estrone. In addition, osteoblast-like cells in bone are also capable of transforming androgens into estrogens (Zborowski J, Cauley J, Talbott E, Guzick D, Winters J, 2000). Bone forming cells, including osteoblasts, bone marrow stromal cells, osteocytes and osteoclasts, have receptor sites for both androgens and estrogens, though osteoblasts have increased receptors sites for androgens. Clinical trials have shown that androgen therapy combined with estrogen therapy has an additive effect on BMD compared with estrogen-

alone treatment (Notelovitz, 2002). Therefore, androgens can act on bone both directly and through conversion to estrogens. In a cross sectional analysis of 28 female patients aged 11–65 years with androgen insensitivity, lumbar spine BMD was reduced by one standard deviation in 22 of the participants with complete androgen insensitivity when compared to a normal population. However no significant difference was found in lumbar BMD in 6 women with partial androgen insensitivity when compared to a normal population and hip BMD was not significantly different from a normal population in any patient (Marcus R, Leary D, Schneider DL, Shane E, Favus M, Quigley CA, 2000).

Animal models have also indicated that androgens can act directly on bone metabolism and density. Male rats without androgen receptors have decreased bone mass and female rats treated with androgens after oophorectomy have reduced bone loss. However, the model of action of estrogens and androgens on bone in mice cannot be directly transferred to humans since the causal pathways differ. Primate models also indicate that treatment of female cynomogus monkeys with androgens decreased cancellous bone turnover (Kearns and Khosla, 2004).

2.2.3 Body Composition Characteristics and Bone

The physical characteristics of women with PCOS can also affect BMD. As discussed, obesity is prevalent among women with PCOS, though not all women with PCOS are obese. In addition, many women with PCOS have a high wait-to-hip ratio, resulting in a body composition characterized by central adiposity, also known as an “apple shaped” figure. The effects of increased weight and fat mass on BMD have been well-studied and increased BMD and weight have been shown to increase BMD. It has been hypothesized that body composition may partially account for the increased BMD in women with PCOS. Greater central adiposity may

cause more concentrated physical bone stress in the lumbar region, which results in greater BMD (Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R, 1998).

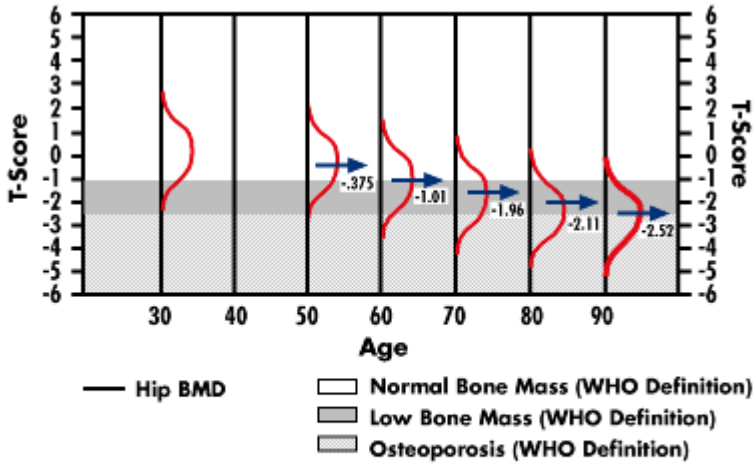
A recent study of 770 post menopausal white women assessed the relationship of total fat mass (TFM) and total lean mass (TLM) to BMD and bone mineral content (BMC) by dual-energy X-ray absorptiometry (DXA); total fat mass was significantly associated with higher BMD and BMC in non-osteoporotic women and total fast mass (TFM) and total lean mass (TLM) were both significantly associated with greater BMD and BMC in osteoporotic women. The results indicated that both TFM and TLM affected bone density (Gnudi S, Sitta E, Fiumi N, 2007).

Androgens may also play a more indirect role in the effect of body composition on BMD. A 16 week double-blind clinical trial investigating estrogen therapy versus estrogen plus methyltestosterone therapy in 40 post-menopausal women (mean age 57) found that total body mass and lower body muscle strength were significantly increased in the group with estrogen plus methyltestosterone compared to the group that received estrogen alone. The results suggest that androgens can alter body composition, which in turn can alter bone density (Dobs AS, Nguyen T, Pace C, Roberts CP, 2002).

2.3 ANALYSIS OF BONE MINERAL DENSITY

Multiple methods currently exist for quantifying BMD. One of the most common is DXA. DXA aims two x-ray beams with different energy levels at bone, and subtracts the absorption of soft tissue to obtain a measure of attenuation of the x-ray signal by the bone. Bone density is positively correlated with the attenuation of the x-rays. DXA is one of the most studied methods

of analyzing BMD, and is the most frequently used method in studies and clinical settings. The WHO uses DXA BMD measures in their definition of osteopenia and osteoporosis, which is the accepted measure for clinical diagnosis of these conditions. Scores of BMD obtained from DXA are compared to normalized populations. Osteoporosis is diagnosed when BMD is greater than or equal to 2.5 standard deviations below that of a reference population and osteopenia is diagnosed when BMD is between 1 and 2.5 standard deviations below a reference population. The standard deviation difference is referred to as either a T-score, when the BMD has been compared to a standard population of healthy young adults, or a Z score when BMD is compared to an age matched population.



The distributions of bone mineral density and the decline with age, for BMD measured at the hip.

Figure 3: T-Score Distribution by Age for Diagnosis of Osteoporosis (Source: http://www.e-radiography.net/radpath/o/osteoporosis.htm)

There are several other methods which can be utilized for analyzing BMD. The most notable for this study's purpose is QCT.

2.3.1 Quantitative Computed Tomography (QCT)

The principle behind QCT is similar to that of DXA. Measures of absorption of a source (a radionucleotide source in peripheral areas, pQCT, and x-rays in other sites, including the lumbar spine) are extrapolated to measures of BMD. However, in QCT, multiple detectors are used and patched together to create a three-dimensional measure of BMD. QCT is not used as frequently as DXA because scanning time is longer, equipment is more expensive, and the dose of radiation required is higher (Damilakis J, Maris TG, Karantanas AH, 2006). The WHO has not developed criteria to calculate T-scores and Z-scores based on QCT BMD measures. However, University of California San Franciso has developed QCT BMD normal age distributions that can be used to extrapolate QCT BMD measures into T-scores and Z-scores (Genant HK, Block JE, Steiger P, Glueer CC Smith R, 1987).

Despite this, QCT has several advantages over DXA in the assessment of BMD. QCT can provide a volumetric measure of BMD, whereas measures from DXA are two dimensional. BMC and volumetric BMD obtained with DXA may be underestimated due to variations in bone size Extrapolations of DXA to three dimensional measures do not fully account for bone size which can result in inaccuracy in measurements. For example, peak volumetric BMD is constant between sexes despite men have larger bones than women (Seeman, 2002). However, bones that are smaller in size (length and/or width) will automatically have smaller volumetric BMD measurements and larger bones larger volumetric BMD measurements when calculated by DXA. A critical review of DXA techniques in measuring BMD indicated that if two women had were screened by DXA for osteoporosis and had the same age and BMD (aged 52, BMD = 0.900 g/cm^3) but different weights and heights (weight of 90 kg and a height of 185 cm and the other a weight of 50 kg and height of 155 cm) the taller would have a Z-score of –1.3 (nearing the -1.5

cut-off for osteopenia) and the larger a Z-score of –0.64 (Neilsen SP, 1999). This is an indicator that diagnosis and analysis based upon a volumetric BMD from DXA can be confounded by bone size.

QCT can also distinguish between the two main types of bone: cortical and trabecular. Cortical bone is dense, found primarily in the shaft of long bones, in the outer shell of bone, at the end of joints and the vertebrae. In contrast, trabecular bone is highly porous and is found in the spine and at all articulating joints. Its unique structure maximizes support while minimizing weight. A high level of bone re-modeling takes place in trabecular bone, particularly in comparison to cortical bone (Zborowski, 2000) and thus analysis of trabecular bone provides important insight into BMD levels. In addition, the ability of QCT to detect trabecular bone allows detection of compartmental changes in bones. This has been shown to be able to aid in predicting spinal fracture risk in post-menopausal women (Ito, 2006). QCT is also more closely correlated with age than DXA, which can be important when assessing risk factors for BMD-related conditions such as osteoporosis which also have a strong age-related component (Angelopoulous et al., 2006 and Murata, 2006). Earlier analysis of bone density has been shown to be preventative in the development of osteoporosis (Bergot C, Laval-Jeantet AM, Hutchinson K, Dautraix I, Caulin F, Genant HK, 2001). These advantages of QCT seen in the study of osteoporosis could be extended to other disease, such as PCOS, which can have an effect on BMD.

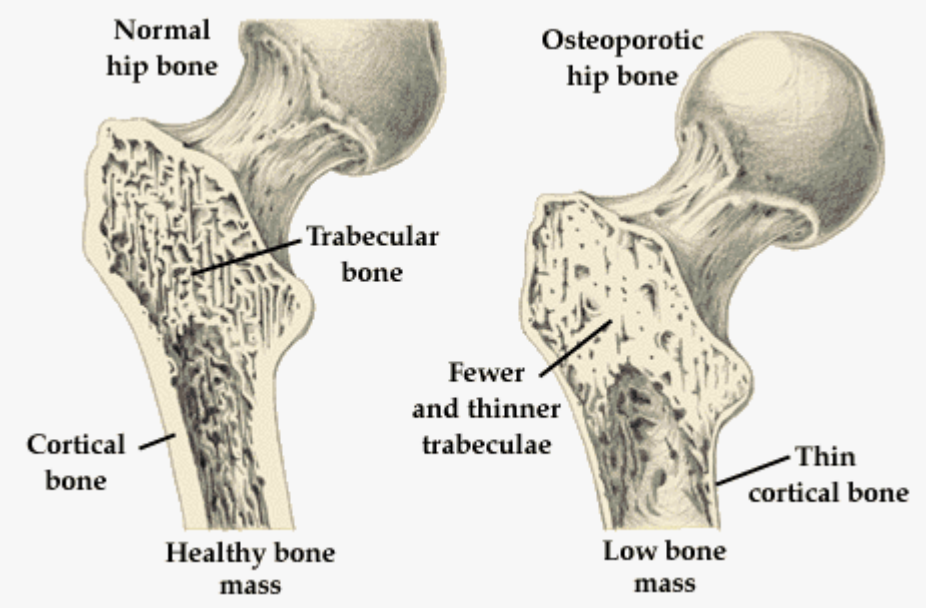


Figure 4: Bone Structure (Source: http://www.cmdrc.com/WomansHealthCareafterThirty/)

2.4 STUDIES OF BONE MASS IN WOMEN WITH PCOS

A handful of studies have investigated the effects of PCOS and its related symptoms on bone, and have used methods for analyzing BMD detailed above. The studies highlight the multifactorial nature of the symptoms of PCOS and their effects on BMD as previously described.

Adami et al. studied the effects of amenorrhea on bone mineral metabolism in menopausal women aged 17 to 33. Amenorrhea is often associated with lowered BMD and conditions such as osteoporosis (Broekmans FJ, Knauff EAH, Valkenburgh O, Laven JS, Eijkemans JS, Fauser BCJM, 2006). Participants were recruited and split into four study populations: women with PCOS (n = 51, age = 24.2 ± 4.9, BMI = 23.5 ± 4.8), women with

hypothalamic amenorrhea (n = 26, age = 23.6 ± 1.4, BMI = 23 ± 3.0), women with idiopathic hirsutism (n = 24, age = 22.7 ± 4.4, BMI = 23.9 ± 4.8) and healthy controls (n = 35, age = 29.0 ± 6.5, BMI = 21.7 ± 3.2). PCOS cases were further divided into amenorrhoeic (n = 38, age = 24.2 ± 4.7) and non-amenorrhoeic (n = 13, age = 24.2 ± 5.5) sub-groups. The study evaluated bone mineral metabolism, including BMD, in three case groups with differing endocrine disorders: PCOS, hypothalamic amenorrhea and idiopathic hirsutism. These were compared to a group of healthy controls. The main goal was to asses the importance of normal bone mass in androgen and estrogen production with a secondary goal of analysis of BMD between the groups. Each group underwent an evaluation of bone turnover, endocrine markers (gonadotropins, total and free testosterone, Estradiol, dehydroepiandostendionsulphate (DHEA-S) and andostenedione) and an assessment of BMD via dual x-ray absorptiometry (DXA). Statistical analysis included a two-tailed Student's t-test for between group comparisons, one-way ANOVA for comparisons of more than two groups, and step-wise regression for determination of factors which significantly influenced BMD covariance.

Spine and femoral BMD and bone turnover markers were significantly lower, and bone turnover was significantly higher, in women with hypothalamic amenorrhea compared to the other case groups. In all PCOS cases, BMD was positively correlated with andostenedione and free testosterone ($r = 0.25$, $p < 0.05$). In the total PCOS group, both femoral and spine BMD were measured at normal levels. Differences in BMD did appear when the PCOS cases were split into amenorrhoeic and non-amenorrhoeic sub-groups; the amenorrhoeic sub-group had lower spine and neck BMD (g/cm^3, 1.098 ± 0.124 and 0.938 ± 0.123, respectively) compared to the non- amenorrhoeic sub-group (g/cm^3, 1.180 ± 0.123 and 1.024 ± 0.115, respectively, $p < 0.05$) and idiopathic hirsutism group (g/cm^3, 1.174 ± 0.123 and 1.022 ± 0.139, respectively, $p < 0.05$).

In the PCOS non-amenorrhoeic sub-group, BMD levels were higher than in controls, though differences were not significant.

The higher BMD in the non-amenorrhoeic PCOS sub-group was likely due to the deleterious effects of amenorrhea on BMD. However, overall the results suggest that in PCOS cases, negative effects on BMD resulting from amenorrhea are balanced by higher androgen production in PCOS women. It was also suggested that the increased weight and fat mass of women with PCOS may act as a protective measure for BMD, by increasing bone mineral density through biomechanical force resulting from increased weight. (Adami S, Zamberlan N, Castello R, Tosi F,Gati D, Moghetti P, 1998).

Good et al. further examined the effect of weight and fat on BMD in women with PCOS in 12 non-Hispanic white women with PCOS and a BMI of <= 26 (n = 12, age = 28.5 ± 7.0, BMI = 22.4 ± 2.3) who were matched with 10 healthy control women (n = 10, age = 28.9 ± 8.3, BMI = 22.0 ± 2.2) for age, ethnicity, and weight. Many previous studies have focused on body composition of obese PCOS cases only. Good et al. examined if the same characteristics could be applied to lean cases. The overall goal of the study was to examine BMD and fat distribution in lean women with polycystic PCOS compared with matched controls.

Total body BMD, regional BMD and fat mass were measured. BMD and lean mass were measured by total body DXA. Women were weighed and measured for height and at the waist and hip to obtain a BMI and a waist-hip ratio. In addition, blood samples were assayed for total testosterone, Dehydroepiandrosterone sulfate (DHEA-S), glucose and fasting insulin. Statistical analysis was performed via Student's t-tests and Wilcoxon's rank-sum test for non-normal hormone levels. Correlation coefficients were calculated for the relationship between androgen levels and BMD.

PCOS cases were found to have higher total testosterone (ng/dL, cases = 73.7 ± 21.3, controls = 32.4 ± 11.1, $p < 0.0001$) and bioavailable testosterone (ng/dL, cases = 22.2 ± 13.2, controls = 7.2 ± 3.3, $p = 0.004$) compared to controls. However, there was no statistically significant difference in total body BMD between cases and controls. Significant regional differences were shown, however, with PCOS women having higher BMD in their left arm (g/cm^3, cases = 0.73 ± 0.05, controls = 0.67 ± 0.05, $p = 0.013$), right arm (g/cm^3, cases = 0.77 ± 0.05, controls = 0.72 ± 0.04, $p = 0.014$), and left ribs (g/cm^3, cases = 0.61 ± 0.05, controls = 0.56 ± 0.05, $p = 0.047$). Evaluation of upper body BMD showed a significant correlation between testosterone levels and upper body BMD ($r = 0.62$, $p = 0.02$). There was no statistically significant difference in body fat distribution between cases and controls, consistent with matching on body weight.

The study supported conclusions that difference in BMD between women with PCOS and controls may be regional, with a trend towards higher BMD in the upper bodies on women with PCOS. The results suggest that PCOS can be protective, resulting in increased BMD. This may be related to increased androgens in women with PCOS, supported by a significant positive correlation between total BMD and androgen levels. (Good C, Tulchinksy M, Mauger D, Demers LM, Legros RS, 1999).

Kirchengast and Huber further studied the effects of morphology, body fat and BMD in lean women with PCOS compared to lean controls. Ten women with PCOS and a BMI below 25 were matched with cases based on age, weight and BMI (cases n = 10, age = 23.9, BMI = 20.7 ± 1.4; controls n = 10, age = 22.9, BMI = 20.4 ± 1.6). This study focused on body fat and body composition in lean women, citing reasoning similar to Good et al.

BMD and body composition were measured by DXA and fat distribution was calculated from DXA results. Hormone concentrations were gathered for 17β-estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, DHEA-S, androstendione and sex hormone binding globulin (SHBG). BMI was calculated. Analysis was performed using paired student t-tests; the Wilcoxon test was used for non-normal hormone measures. Regression analysis was performed to asses the impact of weight status and absolute and relative levels of body fat on fat distribution.

In contrast to Good et al., although cases and controls were matched for BMI, significant body composition differences were shown between PCOS cases and controls. PCOS cases had higher total body fat (kg, cases = 21.2 ± 5.4, controls = 14.8 ± 2.5, p = 0.002) and in the upper body (kg, cases = 9.1 ± 3.3, controls = 4.5 ± 1.1, p = 0.001). PCOS cases also had significantly lower lean tissue mass in the total body (kg, cases = 35.6 ± 3.9, controls = 38.7 ± 3.4, p = 0.04) and the upper body (kg, cases = 18.7 ± 2.5, controls = 20.8 ± 1.5, p = 0.03). Significant hormone differences were found only for DHEA-S (ug/ml, cases = 2.47 ± 0.67, controls = 1.39 ± 0.62, p = 004) and testosterone (ng/ml, cases = 0.73 ± 0.21, controls = 0.31 ± 0.27, p = 0.04), with PCOS cases displaying higher levels of both. However, BMD parameters did not differ between cases and controls, though total bone mass was significantly different (g, cases = 2065.7 ± 276.7, controls = 2312.7 ± 237.5, p = 0.03).

The authors concluded that although fat distribution and body morphology differ in lean PCOS case and controls, this seemed to have no effect on BMD between cases and controls. The authors hypothesize that the lack of difference may result from the study's exclusion of obese women. Obese PCOS cases may have accounted for the resulting significant BMD differences seen in other studies by virtue of heavy weight increasing BMD through biomechanical forces.

Good et al. also only used lean cases and controls and found a significant difference in BMD between lean PCOS cases and controls. However, though both studies used thin women, the mean BMIs differed in each study. In Kirchengast and Huber's study population, the mean BMI for cases was 20.7 and 20.4 for controls, lower than the mean BMIs for the thin women used in Good et al, who had mean BMIs of 22.4 for cases and 22.0 for controls. This may mediate the different conclusions between the studies. Both studies found a significant difference in androgens between cases and controls, with PCOS cases having higher DHEA-S and Testosterone. (Kirchengast and Huber, 2001).

Yüksel et al. investigated the relationship between BMD and insulin resistance and hyperinsulinemia in women with PCOS compared to controls. The investigators recruited 28 amenorrhoeic women with PCOS (age = 23.5 ± 0.6, BMI = 26.9 ± 0.8), 11 amenorrhoeic women without PCOS (age = 22.1 ± 1.2, BMI = 25.6 ± 0.9) and 15 healthy control women with normal ovulatory function (age = 23.4 ± 0.7, BMI = 24.3 ± 0.7); all were matched for age and BMI, and all were premenopausal. Again, matching criteria were not described in detail. BMD was measured with DXA at the lumbar spine and femoral neck. Blood samples were used to measure serum levels of insulin, FSH, LH, SHBG, total and free testosterone, androstenedione and estradiol. Insulin resistance was also measured by the insulin tolerance test, used to obtain a plasma glucose disappearance rate (K_{TT}). Statistical analysis consisted of Kruskal-Wallace variance analysis and the Mann-Whitney U-test.

In PCOS cases, insulin levels were significantly higher than in either of the control groups (pmol/l, cases = 120.0 ± 7.8, amenorrheic controls = 73.2 ± 8.4, healthy controls = 62.4 ± 8.0, $p<0.001$ for both comparisons). Femoral neck BMD in the PCOS group (g/cm^3, 0.82 ± 0.2) was lower than in the healthy group (g/cm^3, 0.92 ± 0.02, $p <0.01$) and higher than in the

amenorrhoeic control group (g/cm^3, 0.72 ± 0.02, $p < 0.001$). Similarly, lumbar spine BMD (g/cm^3, 0.95 ± 0.2) in the PCOS group was lower than in the healthy group (g/cm^3, 1.04 ± 0.02, $p <0.01$) and higher than in the amenorrhoeic control group (g/cm^3, 0.88 ± 0.02, $p < 0.001$).In PCOS cases, there was a positive correlation of BMD of the lumbar spine with insulin levels ($r = 0.42$, $p = <0.05$) and negative correlations of BMD with insulin resistance ($r = -0.60$, $p < 0.001$) and SHBG ($r = -0.37$, $p <0.05$).

The results continue to add to the contradictory results of BMD studies in women with PCOS. Unlike previous studies such as Good et al. and Adami et al., which reported higher BMD in PCOS cases or no significant differences in BMD, BMD was lower in PCOS cases compared to healthy controls, though higher than amenorrhoeic controls. The authors acknowledged this inconsistency, noting that it may be due to differing definitions of PCOS in the studies. However, the PCOS group's positive correlation of BMD of the lumbar spine with insulin, and the negative correlation of insulin resistance with BMD are evidence for the protective nature of insulin on BMD. The authors state that this is the first study to indicate preservation of BMD is significantly associated with insulin resistance and hyperinsulinemia. They conclude that the insulin resistance and hyperinsulinemia seen in PCOS cases may add to the postulated protective effect of PCOS on BMD. (Yüksel O, Dökmetas HS, Topcu S, Erselcan T, Sencan M, 2001).

Noyan et al. furthered the investigation of the effects of insulin on BMD by comparing BMD in 29 PCOS cases matched via age and BMI with 17 healthy controls (cases age = 28.2 ± 2.8, BMI = 27.7 ± 6.7; controls age = 29.5 ± 2.0, BMI = 27.0 ± 5.0). Again, matching criteria were not described in detail. Blood samples were taken for FSH, LH, estradiol, DHEA-S, 17 hydroxy-progesterone, free testosterone, SHBG, insulin, and glucose levels. BMD measurements were performed for total body, lumbar spine (L2–L4), and femoral neck with DXA. Insulin

resistance was estimated via fasting insulin level, fasting glucose/insulin ratio and a glucose tolerance test. Statistical analysis was performed via Student's t-test and Mann-Whitney U-test for non normal data. Correlations were calculated for the relationship between hormone levels and BMD measure for PCOS cases.

Free testosterone (pg/ml, cases = 3.14 ± 0.80, controls = 0.99 +- 0.29. p = 0.001) and 17 hydroxy-progesterone (nmol/l, cases = 3.87 ± 3.67, controls = 1.31 ± 0.76, p = 0.04) levels were significantly higher in patients with PCOS compared to the controls . Fasting insulin was significantly higher (uIU/ml, cases = 12.98 /- 9.39, controls = 7.74 ± 2.72, p = 0.021) and fasting glucose/insulin ratio was significantly lower (cases = 11.18 ± 9.99, controls = 12.25 ± 3.70, p = 0.008) in the PCOS group compared to controls. Significant positive or negative correlations were found in PCOS cases between fasting insulin and total BMD ($r = 0.415$, $p < 0.05$), and between fasting insulin/glucose ratio and Lumbar 2, 3 and 4 BMD after adjusting for age and BMI ($r = -0.521$, $p < 0.005$).

The results indicated that BMD measurements are not different between the patients with polycystic ovary syndrome and healthy control women, further adding to the conflicting results of BMD measures in women with PCOS. However, as with Yüksel et al., this study indicates that hyperinsulinemia and insulin resistance might play a role in the preservation of BMD. The authors concluded that hyperinsulinemia and insulin resistance could help preserve BMD in women with PCOS. (Noyan Noyan V, Yucel A, Sagsoz N, 2004)

More recently, To and Wong, focused on the effects of oligomenorrhea and amenorrhea on BMD in pre-adolescent girls aged 16 to 19 presenting with oligomenorrhea or amenorrhoea (n = 45, age = 16.9 ± 1.36, BMI= 23.4 ± 4.6) recruited from the gynecological center of a local hospital over nine months. The cases were split into those with normal ovaries (n = 30, age =

16.8 ± 1.21, BMI = 20.9 ± 4.1, and those with polycystic ovaries (n = 15, age = 16.6 ± 1.59, BMI = 23.4 ± 4.6). The study tested whether oligomenorrhea and amenorrhea resulting from polycystic ovaries in adolescent girls was protective against osteoporosis or low BMD; peak bone mass in women is obtained starting in adolescence and disruptions in hormones menstrual disturbances may lower levels of BMD later in life. However, as previously detailed, polycystic ovaries may be protective against lowered BMD

Participants were analyzed for a hormonal profile assessment of body fat by bio-impedance, and for BMD by DXA and quantitative peripheral computed tomography (pQCT). Participants received a diagnosis of polycystic ovaries via ultrasound. An age-matched eumenorrhoeic control group (n = 45, age = 16.9 ± 1.4, BMI = 20.9 ± 4.1) was then enrolled and given the same evaluation. Statistical analysis was performed via Student's t-test and Mann-Whitney U-test for non normal data.

Cases without polycystic ovaries had lower BMD, specifically at the lumbar spine (g/cm^3, cases = 0.881 ± 0.094, controls = 0.964 ± 0/121, p = 0.002) and Ward's triangle (g/cm^3, cases = 0.686, controls = 0.754, p = 0.01), and lower total distal tibial volumetric BMD (g/cm^3, cases = 0.511 ± 0.062, controls = 0.550 ± 0.069, p = 0.013) than the eumenorrheic controls. Cases with polycystic ovaries had no significant difference in volumetric BMD than the eumenorrhoeic controls. The authors use these results to conclude that BMD changes in adolescent girls with oligomenorrhea and amenorrhea could be predicted by diagnosis of polycystic ovaries and by following strict diagnosis criteria. Further, the protective effect of PCOS on BMD was only located in axial skeletal sites. (To and Wong, 2005).

Table 1: Characteristics of Reviewed Published Studies Examining the Relationship of PCOS and BMD

Author	Study Design	Primary Purpose of Study	Study Population *(Mean Age, n total, Mean BMI)*
Adami et al (1998) United States	Case Control	Bone mineral metabolism in women with PCOS, hypothalamic amenorrhea and idiopathic hirsutism to asses the maintenance of normal bone mass in androgen and estrogen production	Women from 17 to 33 • *PCOS cases = 24.2 +/- 4.9, n=51, BMI = 23.5 ± 4.8* • *Idiopathic Hirsutism cases = 22.7 +/- 4.4, n=24, BMI = 23.9 ± 4.8* • *Hypothalamic amenorrhea cases = 23.6 +/1 4.5, n=26, BMI = 23 ± 3.0* • *Controls = 29.0 +/- 6.5, n=35, BMI = 21.7 ± 3.2*
Good et al (1999) United States	Case Control	To examine bone mineral density and fat distribution in women with PCOS compared to matched control women	Non-Hispanic women in good health • *Cases = 28.5 +/- 7.0, n=12, BMI = 22.4 ± 2.3* • *Controls = 28.9 +/ -8.3, n=10, BMI = 22.0 ± 2.2*
Kirchengast and Huber (2001) United States	Case Control	To analyze the body composition, bone density and body fat patterning of lean PCOS women	Caucasian women between 18 and 30 years • *Cases = 23.9, n=10, BMI = 20.7 ± 1.4* • *Controls = 22.9, n=10, BMI = 20.4 ± 1.6*
Yüksel et al (2001) Turkey	Case Control	To evaluate whether there is a relationship between BMD and insulin resistance and hyperinsulinemia in women with polycystic ovary syndrome	Pre-menopausal women • *Cases = 23.5 ± 0.6, n = 28, BMI = 26.9 ± 0.8* • *Amenorrheic Controls = 22.1 +/ 1.2, n=11, BMI = 25.6 ± 0.9* • *Healthy Controls = 23.4 ± 0.7, n = 15, BMI = 24.3 ± 0.7*
Noyan et al (2004) Turkey	Case Control	To compare BMD measures between patients with PCOS and match controls, and to evaluate the relationship between insulin resistance and BMD	Women in good health • *Cases = 28.2 +/- 2.8, n=29, BMI = 27.7 ± 6.7* • *Controls = 29.5 +/- 2.0, n=17, BMI = 27.0 ± 5.0*
To and Wong (2005) China	Case Control	To evaluate if oligomenorrhea or amenorrhea in adolescent girls caused by PCOS is protective against low BMD using DXA and QCT measures	Women between 16 and 19 • *Normal Ovary Cases = 16.8 +/- 1.21 , n=30, BMI = 20.9± 4.1* • *Polycystic Ovary Cases = 16.6 +/- 1.59, n =15, BMI = 23.4± 4.6* • *Controls = 16.9 ± 1.4, n =45 BMI = 20.9 ± 4.1*

BMD = Bone mineral density **PCOS** = Polycystic Ovary Syndrome
DXA= Dual X-ray absorptiometry **QCT** = Quantitated Computed Tomography

Table 2: Results of Reviewed Published Studies Examining the Relationship of PCOS and BMD

Author	Measures Taken	PCOS definition	Significant BMD Results
Adami et al (1998) United States	• BMD by DXA at spine, femoral neck, Ward's triangle • Bone turnover • Endocrine markers	1990 NIH Criteria	In PCOS Amenorrhoeic sub-group, spine and neck BMD significantly lower than in cases with hypothalamic amenorrhea and PCOS cases without amenorrhea • *PCOS Amenorrhoeic sub-group:* o *BMD Spine = 1.098 +/- 0.124 g/cm^3; BMD Neck = 9.38 +/- 0.123 g/cm^3* • *PCOS Non- amenorrhoeic sub-group:* o *BMD Spine = 1.180 +/- 0.123 g/cm^3; BMD Neck = 1.024 +/- 0.115 g/cm^3* • *Idiopathic hirsutism group:* o *BMD Spine = 1.174 +/- 0.123 g/cm^3 BMD Neck = 1.022 +/- 0.139 g/cm^3* • *p<.05 for all*
Good et al (1999) United States	• Biometric measures • Blood sample • Total body/regional BMD and fat analysis by DXA	1990 NIH Criteria	Significant regional higher BMD in PCOS women • *Left arm: Cases = 0.73 +/- 0.05 g/cm^3, Controls = 0.67 +/- 0.05 g/cm3, p = 0.013* • *Right arm: Cases = 0.77 +/-0.05 g/cm^3, controls = 0.72 +/- 0.04 g/cm^3, p = 0.014* • *Left ribs Cases = 0.61 +/- 0.05 g/cm^3, controls = 0.56 +/- 0.05 g/cm^3, p = 0.047*
Kirchengast and Huber (2001) United States	• Total BMD and body composition by DXA • Fat Distribution	Polycystic ovaries and Hyperandrogenism	Significant total bone mass differences between cases and controls • *Cases = 2065.7 +/- 276.7 g, Controls = 2312.7 +/- 237.5 g, p=0.03*
Yüksel et al (2001) Turkey	• BMD by DXA at femoral neck and lumbar spine • Hormone Measures	*Two or three of:* • Polycystic ovaries • Hyperandrogenism • Hirsutism • LH/FSH ratio >2.0	Lumbar spine and femoral BMD in the PCOS group was lower than in the healthy control group • *Femoral BMD: PCOS, 0.82 +/- 0.2 g/cm^3; Healthy Controls,0.92 +/- 0.02 g/cm^3, p <0.01* • *Lumbar Spine BMD: PCOS, 0.95 +/- 0.2 g/cm^3; Healthy Controls, 1.04 +/- 0.02 g/cm^3, p <0.01* Lumbar spine and femoral BMD was higher than in the amenorrheic control group • *Femoral BMD: PCOS, 0.82 +/- 0.2 g/cm^3; Amenorrheic Controls,0.72 +/- 0.0 g/cm^3, p <0.01* • *Lumbar Spine BMD: PCOS, 0.95 +/- 0.2 g/cm^3, Amenorrhoeic Controls, 0.88 +/- 0.02 g/cm^3, p <0.01*
Noyan et al (2004) Turkey	• BMD by DXA at femoral neck, lumbar spine, trochanter, Ward's triangle, and total BMD • Hormone Measures	2003 Rotterdam Criteria	No case/control differences in BMD measurements
To and Wong (2005) China	• BMD by pQCT • Hormone Measures • Ultrasound for ovarian morphology • Estimation of bodyfat	2003 Rotterdam Criteria	PCOS cases without polycystic ovaries had lower BMD from controls • *Lumbar spine: Cases = 0.881 +/- 0.094 g/cm^3; Controls = 0.964 +/- 0.121 g/cm^3, p = 0.002* • *Ward's triangle: Cases = 0.686 g/cm^3; Controls = 0.754 g/cm^3, p = 0.01* • *Total distal tibial volumetric BMD: Cases = 0.512 +/- 0.062 g/cm^3; Controls = 0.550 +/- 0.070 g/cm^3, p = 0.013*

BMD = Bone mineral density
DXA= Dual X-ray absorptiometry
PCOS = Polycystic Ovary Syndrome
QCT = Quantitated Computed Tomography

2.4.1 Limitations of Current Studies

Some inconsistencies and weaknesses are present in the current studies of BMD in women with PCOS. Firstly, there was a noticeable weakness in methods when the studies were compared; some papers were far more detailed in their descriptions of materials and methods. In particular, Good et al., Kirchengast and Huber, and To and Wong provided great detail on methods of BMD and body composition analysis via DXA or QCT, while the other studies did not. There was also no standardization in BMD measurement sites or in BMD measurement techniques across studies. Kirchengast and Huber preformed a whole body scan; Yüksel et al. analyzed the lumbar spine and left femoral neck; Noyan et al. the whole body, spine and femoral neck; Good et al. performed a whole body scan, including 12 subdivisions; Adami et al. scanned the lumbar spine, femoral neck and Ward's triangle; and To and Wong scanned the anterior–posterior lumbar spine, femoral neck, greater trochanter and Ward's triangle. To and Wong was the only study to include measures of volumetric BMD (by QCT), all other studies utilized DXA measures. Comparing the BMD results from different sites is not accurate. Each type of bone has a unique thickness, shape, and volume, which may be distorted by DXA's two-dimensional measures, as previously described. Perhaps most importantly, different bone sites differ in their composition of cortical and trabecular bone. Cortical and trabecular bone have different amounts of bone turn-over and, as previously outlined, give differing insights into BMD levels.

Another troubling inconsistency between papers was the definition of PCOS cases. Each study used a differing combination of symptoms to diagnose PCOS: Adami et al. and Good et al. diagnosed based on the 1990 NIH criteria; Kirchengast and Huber defined PCOS by the presence of polycystic ovaries found via ultrasound and elevated androgens; Yũksel et al. defined PCOS as the presence of two or three of the following: polycystic ovaries diagnosed via ultrasound,

hypernadrogenism, hirsutism, or an LH/FSH ration >2.0; Noyan et al. and To and Wong defined PCOS based on the Rotterdam criteria. The different definitions of PCOS make the comparisons between studies imprecise; as a main objective of all the studies was to investigate the effect of hormonal profiles as seen in women with PCOS on BMD, the different definitions have potentially large implications on comparing the results, and could go a long way towards explaining the conflicting results between studies.

Another issue affecting all the studies was sample size. The largest case group had only 51 women enrolled (Adami S, Zamberlan N, Castello R, Tosi F,Gati D, Moghetti P). Small sample size can usher in a number of statistical issues, mainly in the form of lower precision and/or reliability and lowered power. Only Good et al. analyzed power, acknowledging power was low in their study, and estimating that a sample size of >200 would be needed for a power of ≥90%.

2.4.2 CHARM II Analysis

Another unpublished study of significance has been conducted on the relationship between PCOS status and BMD. In 2000, Zborowski (Zborowski, 2000) analyzed a sub-set of phase II of the University of Pittsburgh Cardiovascular Health and Risk Management study (CHARM II). Ninety-four PCOS cases were selected for a sub-analysis on the basis of elevated androgen levels (testosterone > 51.9 ng/L or androstenedione >250 ng/dL, mean age 41.4 ± 6.2) and 92 controls (mean age 42.0 ± 6.4) were matched for age. BMD (performed via DXA) and measures of body composition were compared in cases and controls. Analysis was stratified by BMI, menopausal status, and history of menopause frequency in the twenties and thirties.

Cases had significantly higher BMD than controls in all areas but the trochanter before adjustment for any factors; significant differences were noted in the total body BMD ($p = 0.18$), total hip ($p = 0.046$), femoral neck ($p = 0.046$), intertrochanter ($p = 0.028$) and the lumbar spine ($p = 0.002$). After adjustment for BMI, significant differences in BMD remained between cases and control were found in the lumbar spine, with cases have 3.5% higher BMD ($p = 0.049$). In addition, a significant interaction was found for PCOS status and BMI at the lumbar spine. Interestingly, women with PCOS had higher BMD than cases when BMI was ≥ 25; in women with a BMI of < 25, cases had a lower BMD compared to controls. Cases had a greater history of inconsistent menstrual cycles and higher androgens compared to controls, though after adjustment for BMI, any significant differences were removed. Overall, it was concluded that PCOS was linked to BMD primarily through its effects on BMI. However, at the lumbar spine, menstrual cycle history was significantly associated with BMD.

Many participants from the total CHARM II cohort were also included in the CHARM III, visit one cohort analyzed in the study described here-in (cases = 105, controls = 115). Although all of these women were not included the sub-analysis of Zborowski, they form the basis of the current analysis. In addition, consideration of the results from the CHARM II sub-analysis helps remediate some of the problems identified in previous studies: it provides a comparison study in which women were recruited and diagnosed as cases or controls in a manner very similar to the CHARM III visit one study, it provides a large cohort for analysis, and it provides a study for comparison in which women were analyzed for BMD at similar sites. In addition, as previously observed menstrual history and irregularity is a defining characteristic of PCOS but there is little data on its effects on BMD; this study provides background for studying

these factors in the current analysis. It also strengthens the published evidence that PCOS status may have an effect on BMD.

3.0 MATERIALS AND METHODS

3.1 STUDY POPULATION

3.1.1 Parent Studies

The study population analyzed was recruited as part of a series of studies aimed to analyze the link between cardiovascular risk and PCOS case versus control status. The initial parent study was the Cardiovascular Health and Risk Management Study (CHARM) conducted by the University of Pittsburgh Graduate School of Public Health and the Department of Reproductive Endocrinology of Magee-Women's Hospital. Women were identified by analysis of medical records collected between 1972 and 1992 (n = 478), and other minorities were recruited via advertising, mailings, etc. from an additional grant (n = 71). PCOS was diagnosed if women had a history of chronic anovulation and clinical evidence of elevated androgen (identified by the presence of hirsuitism) or biochemical evidence of elevated androgens (testosterone >2nmol/L or LH:FSH ratio >2.0). A total of 244 women agreed to have a clinical assessment, and 244 age, race, and neighborhood matched controls were identified. Findings and methods of studies on this group have been previously published (Talbott EO, Guzick D, Clerici A, 1995).

From September 1996 to August 1999, a follow-up study was conducted, with a goal of recruiting 150 cases and 150 controls from the parent study aged 30 years or older, and an

additional 30 minority cases from the general population. Phase II (CHARM II) met recruitment goals and included a total of 161 PCOS and 174 controls. CHARM II also served as the parent study for the ancillary analysis of PCOS status and BMD described by Zborowski (Zborowski, 2000). CHARM II had a number of study goals, including evaluation of the relationship between PCOS and subclinical vascular disease measured by carotid ultrasound and measured by brachial artery flow, evaluation of the relationship between sub-clinical atherosclerosis and PCOS risk factors such as insulin, triglycerides, testosterone, differences in body composition between PCOS cases and controls, and differences in plasma levels of coagulation factors between PCOS cases and controls. Results have been previously published on this group (Talbott et al., 2000)

3.1.2 Current Study Population

This studied used a sub-set of women from phase three of the CHARM study (CHARM III). This phase began recruitment in 2001 and focused on women from the CHARM II study that were 35 years of age or older, though some minority cases (n= 5) and controls (n=2) aged 28 to 34 were admitted to expand the population of minorities in the study. Inclusion criteria for cases were: aged 35 years or older at the time of entry into phase III, physician confirmed diagnosis of PCOS (previously described), and participated in phases I and II of the study. Controls were selected from the population of the phase one and two cohorts were they were originally matched on age, race and neighborhood to cases. Women were followed for five years with a total of two clinical visits.

Information from the first visit (CHARM III Visit One) conducted between February 2001 and March 2003 supplied the study population for this analysis. CHARM III Visit One had a total of 157 cases (mean age = 46.7 ± 0.5) and 171 controls (mean age = 49.3 ± 0.5).

Additional funds became available and a convenience sample was analyzed for lumbar BMD at the spine by QCT. The sub-set analyzed include cases and controls who were largely 40 and included 104 cases (mean age = 47.1 ± 0.6) and 97 controls (mean age = 48.1 ± 0.6). 74 of the cases and 94 of the controls had been previously analyzed by Zborowski in her DXA analysis. Basic population demographics and key comparisons between CHARM III Visit One data for all women and the sub-set analyzed by QCT are detailed in the "Results" section.

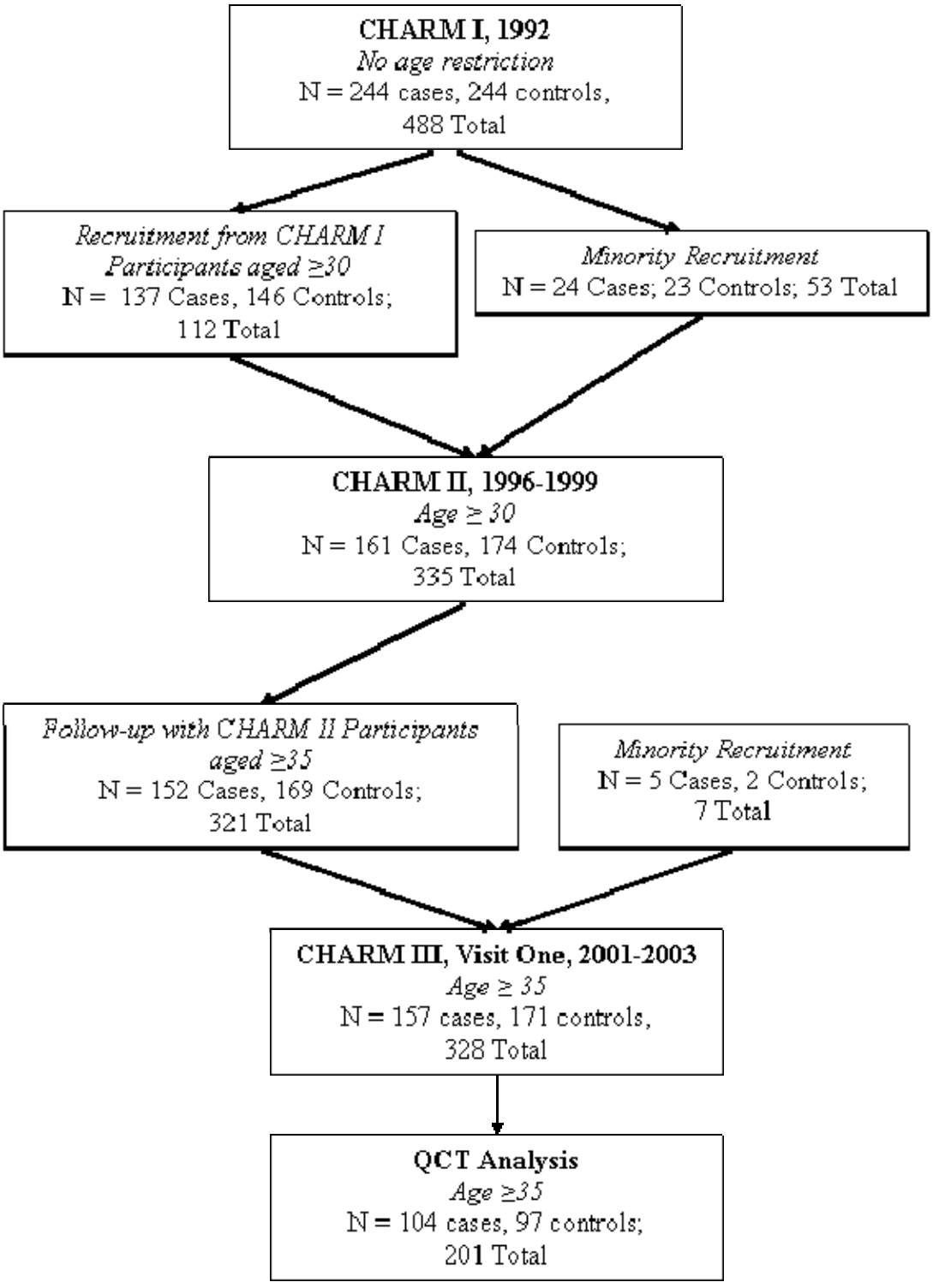


Figure 5: Study flow For CHARM I, CHARM II, and CHARM III Visit One

3.2 MEASURES

3.2.1 Cardiovascular and QCT measures

During their first clinical visit, women in the CHARM III were analyzed for a number of factors related to the overall study goal of analyzing cardiovascular health outcomes, including: b-mode carotid ultrasound in order to measure average wall (intima-media) thickness, degree of plaque (plaque index), and measures of plaque interference with blood flow (ICA/CCA) velocity ratio, pulse wave velocity to measure arterial stiffness and measures of arterial calcification electron beam computed tomography (EBCT).

QCT measures of the lumbar spine were taken during the EBCT testing for a subset of 202 women. Four measurements of the lumbar spine were taken in each woman analyzed ad then averaged for the purposes of analysis. To conduct QCT analysis of the lumbar spine, a lateral scan of the lumbar spine is first taken to find an optimal area to take measurements. Then 10 mm scans are taken in the mid-place of vertebra T11 to L4 while a bone mineral reference phantom containing calibration objects with standardized amounts of calcium hydroxyapatite (the main mineral component of bone) are simultaneously scanned. Data is then extrapolated mathematically to three-dimensional measures (Guglielmi G, Lang TF, 2002). BMD scores were compared to standardized QCT BMD distributions prepared by to the University of California at San Francisco and T-scores and Z-scores were calculated

3.2.2 Risk Factor Assessment

Lifestyle and risk factor assessment information was collected by a clinical interview conducted at the first clinical visit. Questions were asked concerning basic demographic information such as age, ethnicity, residence income level and education level; descriptions of physical characteristics such as weight, height, description of physical characteristics of hyperandrogenism such as hirsutism and acne; risk behaviors such as smoking and alcohol use and physical activity level; medical history, including diabetes, hypertension, breast or uterine cancer, medications; reproductive history, including regulatory and frequency of menstrual cycles, pregnancies, hysterectomies or other surgical treatments, treatment for endocrine disorders and infertility; and treatment for PCOS, such as use of weight loss, oral contraceptive or progestin use and medical procedures. Information was also collected on current medication use, including use of hormone replacement therapy, use of antihypertensives, oral hypoglycemics, insulin and insulin sensitizer use, coronary vasodilators, corticosteroids, and over the counter medications. Dietary and physical information was collected using standardized questionnaires, the 1998 Block food frequency questionnaire, the Paffenbarger questionnaire and the Historical Physical Activity Questionnaire.

3.2.3 Anthropometric and Hormone Measurements

Body weight and height were measured and calculated into BMI. Waist and hip circumference measures were taken and calculated in a waist/hip ratio for an approximation of central adiposity. Blood pressure was taken. Blood samples measured after 12 to 14 hours of fasting for the following hormones: HDLc, LDL, triglycerides, LH, FSH, total testosterone, free testosterone,

cholesterol, fasting glucose and insulin. FSH and estradiol were also drawn for women who reported no period in the last 6 months to determine menopausal status.

3.3 STATISTICAL ANALYSIS

All analysis was performed using SPSS Version 15.0 (SPSS Inc, Chicago, IL). A p-value of 0.05 was considered to be significant for all comparisons. Statistical methods used to analyze each of the study's specific aims are outline below.

3.3.1 Analysis of descriptive characteristics

Descriptive statistics, including demographics, risk factors, body composition, and selected hormones were computed for all of the CHARM III Visit One participants and were then computed separately in PCOS cases and controls; the same descriptive analysis was performed on the CHARM III Visit One QCT measured sub-set. Differences in descriptive characteristics were compared between cases and controls in each cohort using Pearson's $\chi 2$ test (for categorical variables), Student's T-test (for continuous normal variables) and the Mann Whitney U test (for non-normal continuous variables).

Selected demographic, body composition and risk factors were compared between the convenience sample analyzed for QCT measures and those not selected for the QCT analysis. Comparisons were performed using the $\chi 2$ test (for categorical variables), Student's T-test (for normal continuous variables) and the Mann Whitney U test (for non-normal continuous

variables) to check for selection bias and to study the applicability of QCT findings to the entire CHARM III visit one group.

3.3.2 Differences in Determinates of BMD Between PCOS Cases and Controls

In the QCT analyzed sub-set, raw differences in lumbar QCT measures (raw QCT score, 30-year-old matched T-score, Z score) between PCOS cases and controls were assessed using Student's T-test. Given the potentially powerful effects of age, BMI, menopausal status and ethnicity on BMD in the PCOS population, the relationship of each of these variables with BMD and with PCOS case/control status was evaluated in stratified analyses. Stratification included: those ages 48 years or younger compared to those aged over 48 (48 being the median and mean age of the group), those with a BMI <30 compared to those with a BMI ≥30 (the NIH cut-off for obesity), those who were pre-menopausal (defined as having a period in the last 12 months) compared to those who were peri- or post-menopausal (defined as not having a menstrual periods in the last 12 months) and ethnicity (defined as white, black or other). Stratified PCOS comparisons of average lumbar spine BMD between cases and controls were performed using Student's T-test and interactions between these factors and case-control status were assessed by two-way within subject analysis of variance using general linear models. Histograms were created for the continuous factors age and BMI by PCOS case-control status to visually demonstrate differences in these factors by case-control status.

BMD difference between cases and controls were also stratified by period status (≤8 per year versus > 8 per year, the cut-off for oligomenorrhea) in the teens, 20s and 30s to determine if history of menstruation had an effect on BMD in PCOS and controls; age was also compared,

since it is well known to affect BMD. Estradiol levels and BMI were also compared between these groups since, as previously described, these factors may affect history of menstruation.

To examine the relationships of hormones to lumbar BMD in cases and controls, correlations between BMD were assessed separately in cases and controls. Pearson correlation coefficients were calculated for normal variables, and Spearman's ρ for non-parametric variables. Partial correlation coefficients adjusted for age and BMI were also calculated.

3.3.3 Determine if PCOS Status is an Independent Predictor of BMD After Controlling for Univariate Correlates

To control for the effects of the univariate correlates of BMD in PCOS cases and controls examined in the previous analyses, linear regression analysis was performed. A initial univariate linear regression was performed with the raw measure of QCT BMD as the outcome variable and case/control status as the predictor variable. A multivariate regression model was subsequently performed to adjust the effect of PCOS case-controls status for the previously examined determinants from the literature which have been shown to have an effect on BMD: age, BMI and menopausal status. A second multivariate model was performed that that also included ethnicity. Since history of menstrual periods was not shown to be a significant univariate correlate of BMD, its effect was not factored into the adjusted models.

Finally, a multivariate regression was performed to assess the effect of PCOS case-control status on lumbar BMD independent of the effects of other significant predicator factors as determined from a step-wise linear regression. The step-wise regression considered factors in addition to the four considered from the literature, such as a hormone levels investigated by univariate and adjusted correlations in the previous analysis steps. Step-wise linear regression

was performed entering in the effects of: menopausal status, age, BMI, ethnicity, LDL, HDL, cholesterol, triglycerides, glucose, insulin, total testosterone, SHBG, LH, FSH, and progesterone. Estradiol may have an effect on BMD and was significantly correlated with BMD in controls, but it was not measured for all women, and was missing in 59 women (29.4%). A separate stepwise regression was then run also including estradiol, recognizing that the power of the calculation in this group would be lower. Factors were removed at $\alpha > 0.05$. After the significant factors were determined, PCOS case-control status was added to the multivariate model, and a final linear regression model assessing the effects of PCOS case-control status on QCT lumbar BMD independent of these effects was determined.

4.0 RESULTS

4.1 DESCRIPTIVE CHARACTERISTICS IN PCOS CASES AND CONTROLS

Tables 3 shows descriptive characteristics by case-control status for the entire CHARM III Visit One group and Table 4 shows descriptive characteristics for the sub-set analyzed for QCT lumbar BMD. In the entire CHARM III Visit One cohort, cases and controls were similar for systolic and diastolic blood pressure, cholesterol, LDL, fasting glucose, estradiol, smoking status, current oral contraceptive and hormone replacement therapy use, history of oophorectomy and/or hysterectomy and hypertension. Significant differences included: compared to controls, cases had higher BMI, waist/hip ratio, triglycerides, insulin levels and total testosterone measures, and had more women with Type 2 diabetes and had more women with no menstrual period in 12 months; compared to cases, controls were older and had higher levels of HDL, SHBG, FSH and progesterone.

When only women with QCT measures were analyzed, differences in age, triglyceride, LH and history of periods in the last 12 months were removed between cases and controls. However, in the sub-group, cases had higher diastolic blood pressure compared to controls.

Table 3: Descriptive Statistics for All Women in CHARM III Visit One and for Cases and Controls in CHARM III Visit One

	All N = 328[a]	Cases n = 157[a]	Controls n = 171 [a]	p- value Cases vs. Controls
Age, Years	48.0, 47.9 (6.3)	46.65, 46.08 (6.23)	49.31, 49.33 (6.13)	<0.01*
BMI, kg/m^2	30.58, 29.13 (8.14)	33.09, 32.07 (9.19)	28.29, 27.17 (6.24)	<0.01*
Waist/Hip Ratio	0.82, 0.82 (0.08)	0.84, 0.85 (0.09)	0.80, 0.81 (0.08)	<0.01*
Systolic BP, mm Hg	117.7, 116.0 (12.81)	118.7, 120.0 (11.55)	116.9, 112.0 (14.10)	0.07
Diastolic BP, mm Hg	75.77, 78.0 (8.43)	76.38, 78.0 (8.56)	75.1, 76.0 (8.34)	0.23
Cholesterol mg/dl	207.3, 203.0 (39.16)	205.9, 200.5 (42.60)	208.6, 204.5 (35.79)	0.32
LDLc, mg/dl	124.9, 122.7 (35.345)	122.9, 119.9 (38.46)	126.6, 123.6 (32.51)	0.16
HDLc, mg/dl	54.90, 52.75 (14.90)	52.32, 51.54 (15.19)	57.27, 54.7 (14.27)	<0.01*
Triglycerides, mg/dl	142.1, 113.5 (122.9)	161.6, 125.5 (158.2)	124.2, 101.0 (73.78)	0.02*
Fasting Glucose, mg/dl	96.31, 92.0 (23.26)	97.95, 94.78 (22.31)	94.82, 91.0 (24.08)	0.26
Insulin, µU/ml	16.52, 12.75 (10.88)	19.72, 16.65 (12.36)	13.59, 11.1 (8.30)	<0.01*
Total Testosterone nmol/L	1.15, 0.80 (1.51)	1.38, 0.90 (2.05)	0.93, 0.70 (0.66)	<0.01*
SHBG nmol/L	158.8, 115.7 (130.1)	132.45, 85.6 (122.35)	182.9, 152.2 (132.6)	<0.01*
LH µU/ml	18.36, 10.80 (18.53)	13.04, 9.5 (11.42)	23.35, 16.5 (22.22)	<0.01*
FSH µU/ml	27.77, 11.00 (33.7)	16.03, 7.9 (17.10)	38.72, 25.45 (40.98)	<0.01*
Progesterone pg/ml	7.64, 1.70 (15.48)	6.88, 1.90 (12.78)	8.34, 1.50 (17.63)	0.02*
Estradiol pg/ml	90.27, 59.50 (101.6)	79.86, 60.4 (60.62)	98.87, 58.3 (125.40)	0.65
Minorities	65 (19.9%)	29 (18.6%)	36 (21.1%)	0.04*
Current Smoker (yes)	48 (27.1%)	24 (27.3%)	24 (27.0%)	0.55
Currently use Oral Contraceptives (yes)	22 (7.9%)	12 (7.7%)	10 (5.8%)	0.50
Currently use HRT (yes)	51 (42.5%)	20 (40.0%)	31 (18.2%)	0.504
No Period in >12 months	116 (35.4%)	44 (28%)	72 (42.1%)	0.01*
Oophorectomy and/or Hysterectomy (yes)	42 (19.4%)	15 (14.2%)	27 (25.0%)	0.06
Type 2 Diabetes (yes)	23 (10.6%)	19 (16.5%)	43 (3.9%)	0.003*
Hypertension (yes)	71 (21.6%)	41 (26.1%)	30 (17.5%)	0.06

[a] Values are mean, median (SD) for continuous variables; count (%) for categorical variables

Not all categorical variables present for all cases and controls, see counts and row %s.

* Significant at $\alpha = 0.05$ for case versus control status; test of significance if Mann Whitney U or Student's T-Test for continuous variables; Chi-Square of Fischer Exact for categorical variable

Table 4: Descriptive Statistics for All Women in the Lumbar Bone Mineral Density Analyzed Sub-Set of CHARM III Visit One and for Cases and Controls in the Lumbar Bone Mineral Density Asses Sub-Set of CHARM III Visit One

	All N = 201[a]	Cases n Total = 104 [a]	Controls n Total = 97 [a]	p-value Cases vs. Controls
Age, Years	47.62, 46.64 (5.93)	47.09, 46.76 (6.11)	48.2, 48.40 (5.71)	0.19
BMI, kg/m^2	30.21, 28.61 (7.81)	31.97, 31.56 (8.58)	28.34, 26.13 (6.43)	<0.01*
Waist/Hip Ratio	0.82, 0.82 (0.07)	0.84, 0.85 (0.09)	0.80, 0.80 (0.08)	<0.01*
Systolic BP, mm Hg	117.2, 116.0 (12.64)	118.4, 119.0 (11.71)	115.8, 110.0 (13.76)	0.06
Diastolic BP, mm Hg	74.82, 76.01 (8.60)	75.98, 78.0 (8.66)	73.63, 74.00 (8.63)	0.05*
Cholesterol mg/dl	208.8, 203.0 (42.28)	209.2, 202.0 (46.31)	208.4, 204.0 (37.77)	0.08
LDLc, mg/dl	127.1, 122.7 (38.75)	126.5, 120.9 (42.45)	127.81, 123.7 (34.64)	0.64
HDLc, mg/dl	54.08, 52.45 (14.30)	52.44, 50.40 (14.86)	55.83, 53.8 (13.55)	0.04*
Triglycerides, mg/dl	138.0, 103.5 (95.40)	151.2, 115.0 (107.3)	123.9, 98.4 (79.00)	0.10
Fasting Glucose, mg/dl	95.85, 92.69 (23.53)	95.25, 92.69 (13.89)	96.5, 92.69 (30.70)	0.53
Insulin, µU/ml	16.45, 12.75 (10.49)	18.67, 15.4 (11.81)	14.10, 11.3 (8.29)	<0.01*
Total Testosterone nmol/L	1.07, 0.70 (1.71)	1.27, 0.70 (2.34)	0.86, 0.70 (0.32)	0.02*
SHBG nmol/L	165.7, 131.8 (120.1)	136.6, 89.77 (113.7)	197.6, 172.3 (120.2)	<0.01*
LH µU/ml	16.67, 10.0 (16.42)	13.78, 10.1 (11.96)	19.78, 9.7 (19.74)	0.31
FSH µU/ml	23.26, 10.5 (26.19)	16.54, 8.6 (16.88)	30.45, 16.3 (31.98)	0.01*
Progesterone pg/ml	7.98, 1.75 (15.62)	6.95, 1.9 (13.8)	9.06, 1.7 (17.35)	0.25
Estradiol pg/ml	90.55, 56.45 (111.6)	79.30, 55.2 (62.62)	101.2, 59.5 (143.09)	0.63
Minorities	33 (16.5%)	21 (20.4%)	12 (12.4%)	0.32
Current Smoker (yes)	33 (31.4%)	17 (28.8%)	16 (34.8%)	0.53
Currently use Oral Contraceptives (yes)	16 (9.3%)	8 (9.0%)	8 (9.6%)	0.62
Currently use HRT (yes)	33 (47.1%)	17 (50%)	16 (44.4%)	0.58
No Period in >12 months (yes)	62 (30.8%)	27 (26.0%)	35 (36.1%)	0.13
Oophorectomy and/or Hysterectomy (yes)	23 (14.0%)	10 (11.9%)	13 (16.3%)	0.50
Type 2 Diabetes (yes)	10 (5.9%)	7 (8.0%)	3 (3.6%)	0.33
Hypertension (yes)	45 (22.3%)	26 (24.7%)	19 (19.4%)	0.40

[a] Values are mean, median (SD) for continuous variables; count (%) for categorical variables

Not all categorical variables present for all cases and controls, see counts and row %s.

* Significant at a = 0.05 for case versus control status; test of significance if Mann Whitney U or Student's T-Test for continuous variables; Chi-Square of Fischer Exact for categorical variable

Basic differences between the CHARM III Visit One participants with and without QCT lumbar measures were investigated to assess the applicability of the findings of the QCT sub-group analysis to the entire CHARM III Visit One, and to see if the group selected for analysis differed significantly from those not selected for analysis. Table 5 outlines the differences. Participants measured for lumbar QCT were less likely to have had a menstrual period in the last

12 months and less likely to have had an oophorectomy and/or a hysterectomy compared to those who did not have QCT measures.

Table 5: CHARM III Visit 1 Differences in Participants With and Without Quantitated Computed Tomography Measures of Lumbar Bone Mineral Density

	QCT measure Taken?*		
	Yes n =202	**No n = 128**	**p - value****
PCOS Cases	104 (51.5%)	53 (41.4%)	0.09
No Period in <12 Months	62 (30.7%)	54 (42.2%)	0.03**
HRT Use	33 (47.1%)	18 (36.0%)	0.75
OC Use	16 (9.3%)	6 (4.7%)	0.27
Hysterectomy and/or Oophorectomy	23 (14%)	19 (35.8%)	<0.01**
Age	47.6 (5.93)	48.6 (6.10)	0.16
BMI	30.2 (7.81)	31.2 (8.21)	0.39

* Average and SD in continuous variable, Number and % within QCT Measure Group in categorical variables
** Significant at α=0.05

4.2 DIFFERENCES IN DETERMINATES OF BMD BETWEEN PCOS CASES AND CONTROLS

Case-control differences in lumbar BMD were initially assessed by Student's T-test for raw QCT BMD scores, 30-year old matched t-scores and age-matched Z-scores. There were no significant differences at $\alpha = 0.05$ between cases and controls for any of these measures, though differences in T-scores were borderline significant ($p = 0.10$), see Table 6.

Table 6: Differences in Unadjusted Measures of Lumbar Bone Mineral Density Measured by Quantitated Computed Tomography in PCOS Cases and Controls

٦	Case-Control Status	N	Mean	Std. Deviation	p Value
Unadjusted					
Lumbar Bone Mineral Density	Case	104	162.09	38.06	0.14
	Control	97	154.31	36.68	
QCT Z-Score	Case	105	0.73	1.34	0.16
	Control	97	0.48	1.17	
QCT T-score (30 yr old matched)	Case	105	-0.21	1.50	0.10
	Control	97	-0.54	1.41	

Cases and controls were then stratified by factors previously described known to have effects on BMD and compared using Student's T-test. Stratification included: those ages 48 years or younger compared to those aged over 48 (48 being the median and mean age of the group), those with a BMI <30 compared to those with a BMI ≥30 (the NIH cut-off for obesity), those who were pre-menopausal compared to those who were peri- or post-menopausal (defined as having <12 periods per year) and ethnicity (defined as white, black or other) (Tables 7 to 10). No stratification resulted in significant differences between cases and controls, though differences were noted in the different strata. Women > 48 years of age had lower BMD compared to those ≤ 48; women with a BMI of ≥ 30 had a BMI greater than those with a BMI <30, women with a period in the last 12 months had a higher BMD than those without a period, and black women had a higher BMD than white women.

Table 7: Differences between PCOS Cases and Controls in Lumbar Bone Mineral Density Measured by QCT Stratified by Mean Age

	Case-Control Status	N	Mean	Std. Deviation	p Value
Age ≤ 48					
Lumbar Bone Mineral Density	Case	59	175.19	35.56	0.64
	Control	46	172.03	32.23	
QCT Z-Score	Case	60	0.81	1.39	0.53
	Control	46	0.65	1.19	
QCT T-score (30 yr old matched)	Case	60	0.32	1.42	0.51
	Control	46	0.14	1.24	
Age > 48					
Lumbar Bone Mineral Density	Case	45	144.92	34.54	0.34
	Control	51	138.33	33.15	
QCT Z-Score	Case	45	0.61	1.27	0.24
	Control	51	0.32	1.15	
QCT T-score (30 yr old matched)	Case	45	-0.90	1.33	0.33
	Control	51	-1.16	1.28	

Table 8: Differences between PCOS Cases and Controls in Lumbar Bone Mineral Density Measured by QCT Stratified by BMI

	Case-Control Status	N	Mean	Std. Deviation	p Value
BMI < 30					
Lumbar Bone Mineral Density	Case	47	155.3	37.3	0.72
	Control	62	152.8	35.4	
QCT Z-Score	Case	47	0.45	1.27	0.99
	Control	62	0.45	1.09	
QCT T-score (30 yr old matched)	Case	47	-0.51	1.43	0.72
	Control	62	-0.60	1.36	
BMI ≥ 30					
Lumbar Bone Mineral Density	Case	56	168.1	38.3	0.19
	Control	35	157.1	39.2	
QCT Z-Score	Case	57	0.96	1.37	0.14
	Control	35	0.53	1.32	
QCT T-score (30 yr old matched)	Case	57	0.52	1.54	0.13
	Control	35	-0.44	1.51	

Table 9: Differences between PCOS Cases and Controls in Lumbar Bone Mineral Density Measured by QCT Stratified by Period Status in Past 12 Months

	Case-Control Status	N	Mean	Std. Deviation	p Value
Period in the Last 12 Months					
Lumbar Bone Mineral Density	Case	77	172.70	33.77	0.53
	Control	62	169.22	30.52	
QCT Z-Score	Case	78	1.00	1.27	0.27
	Control	62	0.78	1.06	
QCT T-score (30 yr old matched)	Case	78	0.21	1.35	0.41
	Control	62	0.03	1.17	
No Period in the Last 12 Months					
Lumbar Bone Mineral Density	Case	27	131.85	33.51	0.64
	Control	35	127.91	31.70	
QCT Z-Score	Case	27	-0.05	1.25	0.99
	Control	35	-0.05	1.19	
QCT T-score (30 yr old matched)	Case	27	-1.41	1.29	0.62
	Control	35	-1.57	1.22	

Table 10: Differences between PCOS Cases and Controls in Lumbar Bone Mineral Density Measured by QCT Stratified by Ethnicity

	Case-Control Status	N	Mean	Std. Deviation	p Value
White Women					
Lumbar Bone Mineral Density	Case	83	156.51	35.16	0.21
	Control	85	149.66	34.67	
QCT Z-Score	Case	84	0.57	1.26	0.20
	Control	85	0.33	1.10	
QCT T-score (30 yr old matched)	Case	84	-0.41	1.41	0.14
	Control	85	-0.72	1.33	
Black Women					
Lumbar Bone Mineral Density	Case	17	186.92	41.38	0.99
	Control	12	187.28	34.76	
QCT Z-Score	Case	17	1.55	1.41	0.95
	Control	12	1.52	1.15	
QCT T-score (30 yr old matched)	Case	17	0.71	1.59	0.98
	Control	12	0.73	1.34	

Interaction between case/control status and each of the stratification criteria were tested by two-way between subject analysis of variance using general linear models. The results revealed no significant interaction effect between case control status and menopausal status as measured by whether a menstrual period had occurred in the last 12 months (interaction p =

0.96). There was no significant main effect of case control status (p = 0.46), but there was a main effect of menopausal status (p = <0.01); see Figure 6.

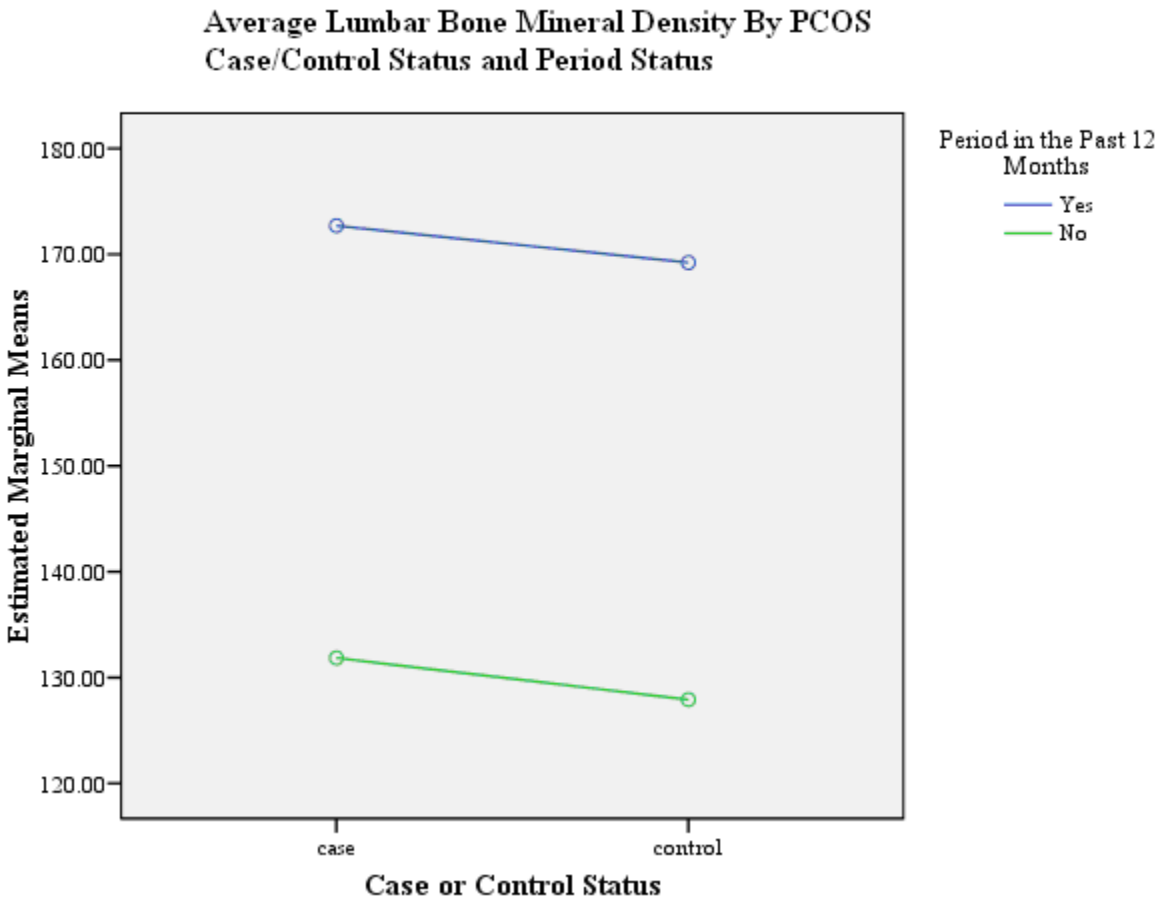


Figure 6: Interaction of PCOS Case-Control Status and Period in Last 12 Months on Average Lumbar Bone Mineral Density

There was no significant interaction between case-control status and age group (≥48 years old and >48 years old) p = 0.723. There was no significant main effect of case-control status (p = 0.31) but there was a significant main effect of age group (p = <0.01); see Figure 7.

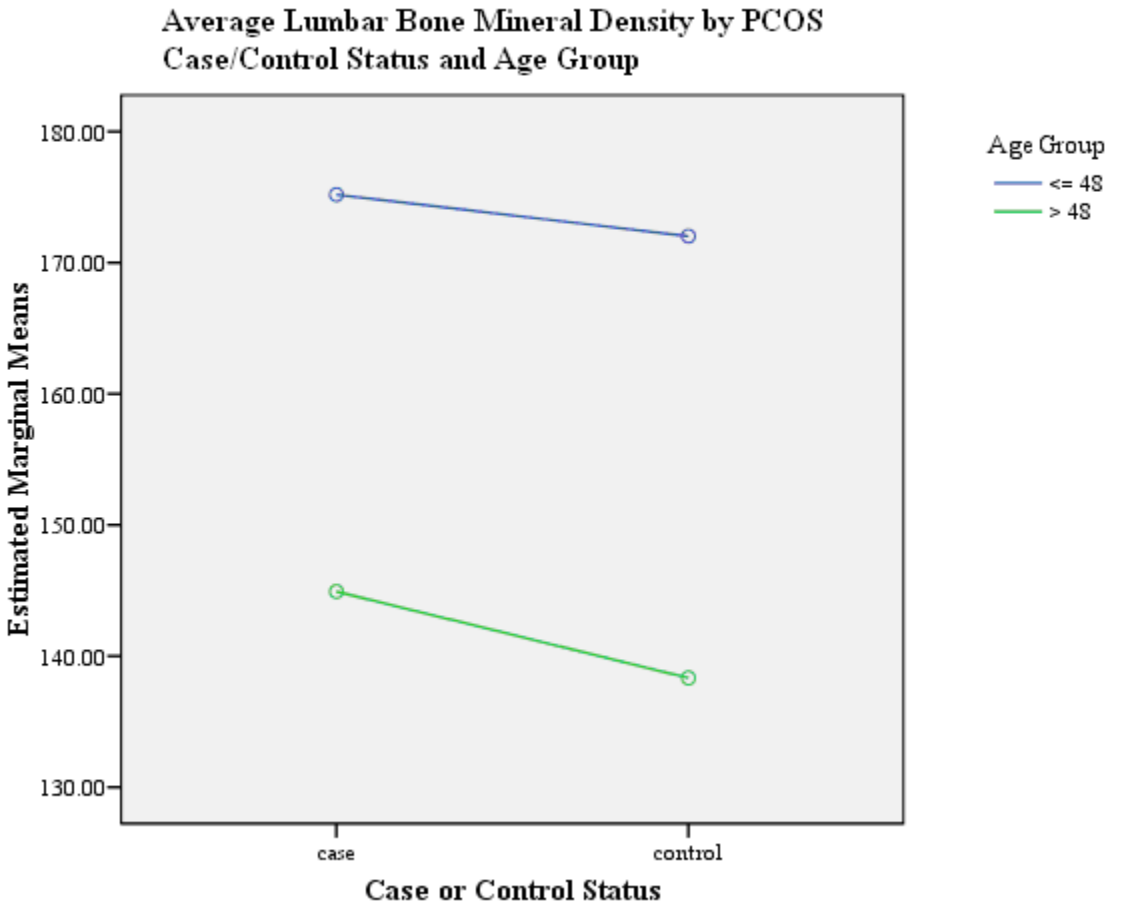


Figure 7: Interaction of PCOS Case-Control Status and Age Group on Average Lumbar Bone Mineral Density

There was a minor interaction effect between case and control status and ethnicity (black and white), though it was not significant ($p = 0.622$). Again, there was no main effect of case-control status ($p = 0.66$) but there was a main effect of ethnicity ($p = <0.01$); See Figure 8.

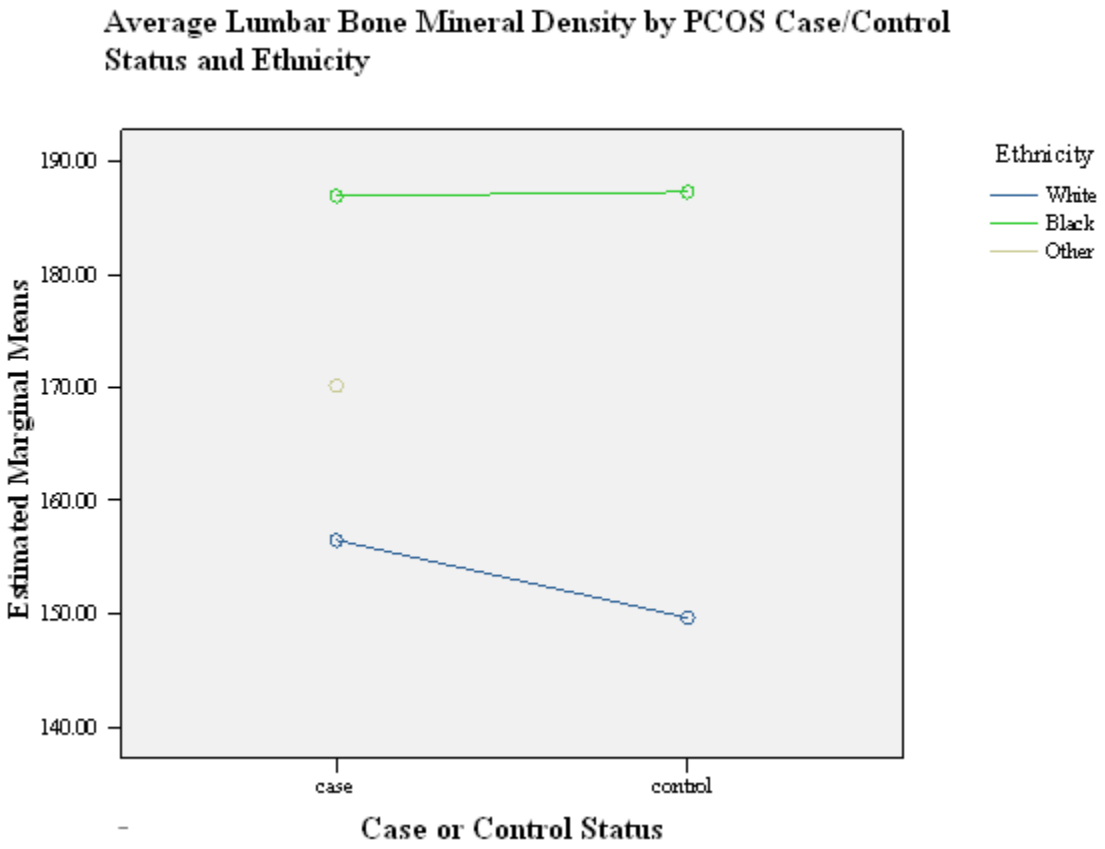


Figure 8: Interaction of PCOS Case-Control Status and Ethnicity on Average Lumbar Bone Mineral Density

There was a larger interaction effect between and case and control status and BMI (≥30 versus <30) though this was not also significant (p = 0.429). One again, there was no main effect of case-control status (p = 0.21). There was also no main effect of BMI (p = 0.11); see Figure 9.

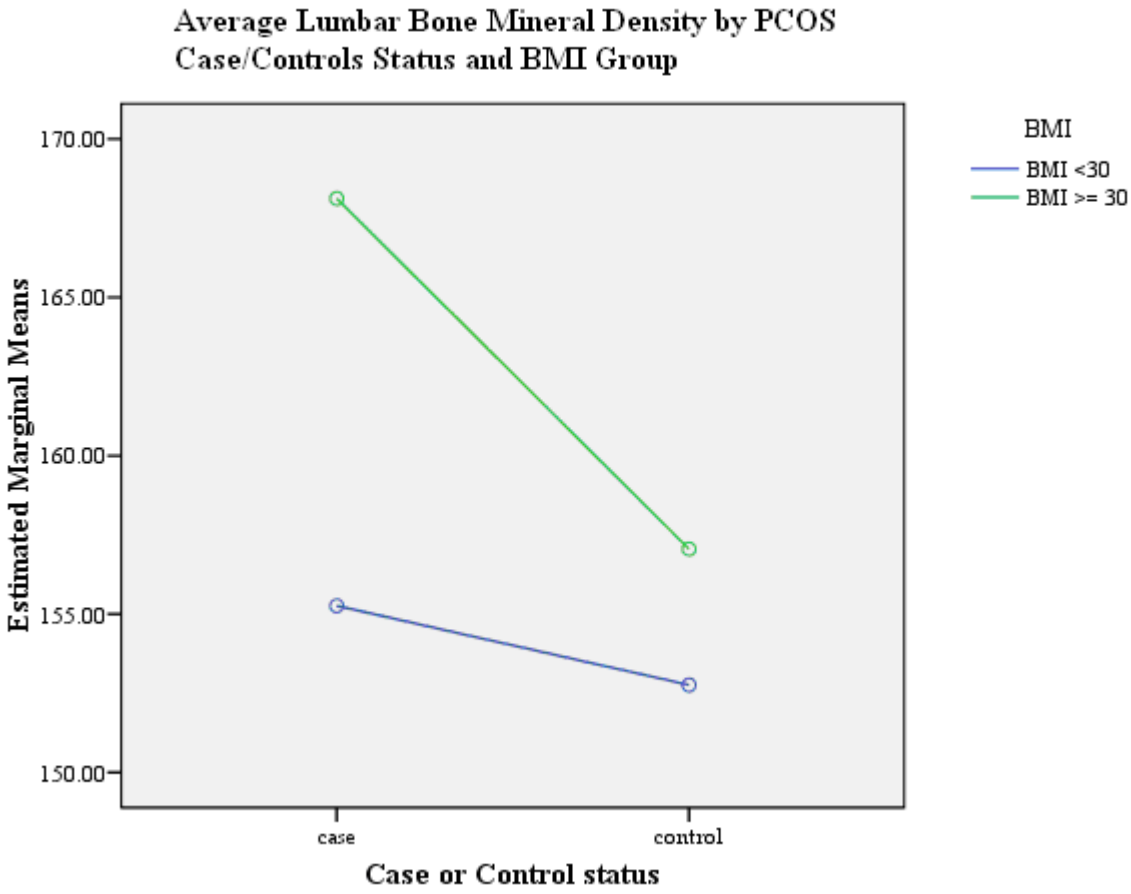


Figure 9: Interaction of PCOS Case-Control Status and BMI on Average Lumbar Bone Mineral Density

Effect of menstrual history in the teens, twenties and thirties on BMD was measured by stratifying case-control comparisons. QCT lumbar BMD measures, BMI, estradiol, and age were compared between PCOS cases and controls further sub-divided into those who had ≤8 periods per year compared to those who had > 8 periods per year. There were no significant differences in any comparison, except for BMI in comparison stratified by number of periods in the teenage years; See Tables 11 to 13. Control women with >8 periods per year had significantly higher BMI than control women with ≤8 periods per year.

Table 11: Differences in selected characteristics in PCOS Cases and Controls by Number of Periods in the Teenage Years

	Cases n = 66		p-value	Controls n = 72		p - value
	=8 periods per year n=25	>8 periods per year n=41		=8 periods per year n=10	>8 periods per year n=62	
Age (years)	50.4±1.1	45.4±0.9	0.05*	48.0±1.8	48.1±0.8	0.78
BMI (kg/m^2)	28.5±1.3	34.7±1.5	0.04*	22.3±0.5	29.7±0.8	**<0.01***
QCT Lumbar Average (g/cm^3)	159.7±6.6	157.9±4.7	0.92	157.0±9.6	153.2±4.1	0.78
Estradiol (pg/ml)	66.2±9.1	86.7±11.2	0.29	80.7±19.8	96.1±17.6	0.80

Average and SD in continuous variable, Number and % within QCT Measure Group in categorical variables
* = Significant at $\alpha = 0.05$

Table 12: Differences in selected characteristics in PCOS Cases and Controls by Number of Periods in the Twenties

	Cases n = 66		p-value	Controls n = 72		p - value
	=8 periods per year n=23	>8 periods per year n=43		=8 periods per year n=9	>8 periods per year n=63	
Age (years)	49.0±1.5	46.4±0.8	0.40	48.2±0.8	47.3±1.1	0.98
BMI (kg/m^2)	28.9±1.4	34.2±1.5	0.21	26.0±2.2	29.0±0.8	0.22
QCT Lumbar Average (g/cm^3)	165.8±6.6	154.3±4.6	0.22	146.6±8.2	155.2±4.2	0.32
Estradiol (pg/ml)	62.2±8.3	87.8±11.0	0.23	94.1±22.9	93.9±17.2	0.48

Average and SD in continuous variable, Number and % within QCT Measure Group in categorical variables
* = Significant at $\alpha = 0.05$

Table 13: Differences in selected characteristics in PCOS Cases and Controls by Number of Periods in the Thirties

	Cases n = 64		p-value	Controls n = 71		p - value
	=8 periods per year n=17	>8 periods per year n=47		=8 periods per year n=6	>8 periods per year n=65	
Age (years)	47.1±1.7	47.8±0.7	0.22	47.1±1.5	48.2±0.8	0.62
BMI (kg/m^2)	32.3±2.7	32.3±1.3	0.79	27.8±3.1	28.7±0.8	0.64
QCT Lumbar Average (g/cm^3)	168.7±7.3	154.3±4.4	0.12	152.5±10.0	154.0±4.0	0.90
Estradiol (pg/ml)	66.1±12.6	84.2±10.0	0.31	105.5±28.2	92.2±16.8	0.26

Average and SD in continuous variable, Number and % within QCT Measure Group in categorical variables
* = Significant at $\alpha = 0.05$

Lastly, the relationship between hormone measures and lumbar QCT was analyzed. Correlation and partial correlation adjusted for BMI and for age and BMI were performed separately in cases and controls to explore associations between hormones and raw QCT BMD measures, QCT T-scores and QCT Z-scores.

Unadjusted correlations in cases showed a significant relationship between lumbar BMD and age, LH, FSH and progesterone in raw BMD scores; between HDL and total testosterone in

Z-scores; and between age, LH, FSH, estradiol, and progesterone in t-scores. When adjusted for age and BMI, no significant relationships remained in any measure. See Table 14.

Table 14: Correlations Between BMD and Selected Factors in PCOS Cases

	Unadjusted			Adjusted for Age and BMI		
	QCT Lumbar Average	**QCT Z-score (age-matched)**	**QCT T-score (30 yr old matched)**	**QCT Lumbar Average**	**QCT Z-score (age-matched)**	**QCT T-score (30 yr old matched)**
Age	-0.48 **0.00***	-0.12 0.24	-0.47 **0.00***	.	.	.
BMI	0.14 0.15	0.17 0.08	0.14 0.15	.	.	.
Cholesterol mg/dl	-0.11 0.25	-0.04 0.66	0.12 0.23	0.01 0.96	0.00 0.98	0.01 0.96
LDLc, mg/dl	-0.02 0.82	0.01 0.89	-0.02 0.86	0.07 0.57	0.06 0.65	0.07 0.57
HDLc, mg/dl	-0.19 0.06	-0.20 **0.04***	-0.20 **0.04***	-0.14 0.28	0.12 0.34	-0.14 0.28
Triglycerides, mg/dl	-0.03 0.75	0.03 0.73	-0.03 0.77	-0.02 0.88	-0.02 0.88	-0.02 0.88
Fasting Glucose, mg/dl	-0.01 0.93	0.12 0.22	0.02 0.87	0.11 0.39	0.13 0.28	0.11 0.39
Insulin, µU/ml	0.11 0.27	0.16 0.11	0.13 0.19	-0.07 0.58	-0.07 0.57	-0.07 0.58
Estradiol pg/ml	0.23 0.06	0.15 0.23	0.26 **0.03***	0.01 0.94	0.00 0.97	0.01 0.94
Total Testosterone nmol/L	0.15 0.13	0.23 **0.02***	0.17 0.08	-0.10 0.41	-0.09 0.46	-0.10 0.41
SHBG nmol/L	-0.13 0.20	-0.16 0.11	-0.11 0.26	-0.13 0.31	-0.14 0.27	-0.13 0.31
LH µU/ml	-0.27 **0.01***	-0.06 0.58	-0.24 **0.01***	0.05 0.67	0.07 0.57	0.05 0.67
FSH µU/ml	-0.39 **0.00***	-0.13 0.18	-0.39 **0.00***	-0.08 0.51	-0.06 0.65	-0.08 0.51
Progesterone pg/ml	0.27 **0.01***	0.16 0.11	0.27 **0.00***	0.04 0.73	0.01 0.91	0.04 0.74

Values are Correlation Coefficient, p = value
* Significant at $\alpha = 0.05$

Unadjusted correlations in controls showed a significant relationship between lumbar BMD and age, LH, FSH and estradiol in raw BMD scores, Z-scores and t-scores. When adjusted for age and BMI, significant relationships remained between estradiol, LH and FSH and a significant relationship was added to testosterone for raw BMD; significant relationships remained between estradiol and FSH and a significant relationship was added to testosterone for Z-scores; and significant relationships remained between estradiol, LH and FSH and a significant relationship was added to testosterone and progesterone for T-scores. See Table 15.

Table 15: Correlations Between BMD and Selected Factors in PCOS Controls

	Unadjusted			Adjusted for Age and BMI		
	QCT Lumbar Average	**QCT Z-score (age-matched)**	**QCT T-score (30 yr old matched)**	**QCT Lumbar Average**	**QCT Z-score (age-matched)**	**QCT T-score (30 yr old matched)**
Age	-0.48 **0.00***	-0.12 0.24	-0.47 **0.00***			
BMI	0.14 0.15	0.17 0.08	0.14 0.15			
Cholesterol mg/dl	-0.11 0.25	-0.04 0.66	-0.12 0.23	0.01 0.96	0.00 0.98	0.01 0.96
LDLc, mg/dl	-0.02 0.82	0.01 0.89	-0.02 0.86	0.07 0.57	0.06 0.65	0.07 0.57
HDLc, mg/dl	-0.19 0.06	-0.20 **0.04***	-0.20 **0.04***	-0.14 0.28	-0.12 0.34	-0.14 0.28
Triglycerides, mg/dl	-0.03 0.75	0.03 0.73	-0.03 0.77	-0.02 0.88	-0.02 0.88	-0.02 0.88
Fasting Glucose, mg/dl	-0.01 0.93	0.12 0.22	0.02 0.87	0.11 0.39	0.13 0.28	0.11 0.39
Insulin, µU/ml	0.11 0.27	0.16 0.11	0.13 0.19	-0.07 0.58	-0.07 0.57	-0.07 0.58
Estradiol pg/ml	0.23 0.06	0.15 0.23	0.26 **0.03***	0.01 0.94	0.00 0.97	0.01 0.94
Total Testosterone nmol/L	0.15 0.13	0.23 **0.02***	0.17 0.08	-0.10 0.41	-0.09 0.46	-0.10 0.41
SHBG nmol/L	-0.13 0.20	-0.16 0.11	-0.11 0.26	-0.13 0.31	-0.14 0.27	-0.13 0.31
LH µU/ml	-0.27 **0.01***	-0.06 0.58	-0.24 **0.01***	0.05 0.67	0.07 0.57	0.05 0.67
FSH µU/ml	-0.39 **0.00***	-0.13 0.18	-0.39 **0.00***	-0.08 0.51	-0.06 0.65	-0.08 0.51
Progesterone pg/ml	0.27 **0.01***	0.16 0.11	0.27 **0.00***	0.04 0.73	0.01 0.91	0.04 0.74

Values are Correlation Coefficient, p = value
* Significant at α = 0.05

4.3 ASSESSMENTS OF PCOS STATUS A PREDICTOR OF BMD AFTER CONTROLLING FOR UNIVARIATE CORRELATES

The final analysis considered the univariate correlates of lumbar BMD investigated in the previous step, and adjusted for their effects on BMD in order to determine if PCOS case-control had a significant effect BMD after their effects were removed. First, a univariate regression model with PCOS status as a predictor of lumbar BMD was performed. It did not show a significant effect of PCOS case-controls status on lumbar BMD ($p = 0.142$); See Table 16, Model 1. A multivariate model added the effects of the strata determined from the literature and investigated in the previous step: age, BMI, and menopausal status (peri- or post-menopausal was defined as not having a menstrual periods in the last 12 months. This model reduced the effect of PCOS case-control status on lumbar BMD (PCOS status $p = 0.70$); however, the effects of age and menopausal status were significant predictors of lumbar BMD in this model (both $p <0.01$); See Table 16, Model 2. The addition of ethnicity (black or white) further reduced the significance of the effects of PCOS status on lumbar BMD (PCOS status $p = 0.891$), though the effects of age, menopausal status and ethnicity were all significant predictors of lumbar BMD in this model ($p <0.001$ for all). See Table 16, Model 3.

Lastly, a multivariate regression was performed to assess the effect of PCOS case-control status on lumbar BMD independent of the effects of other significant predicator factors as determined from a step-wise linear regression. This model also considered the effects of hormones. As previously described, estradiol was significantly correlated with BMD in controls, but was not measured for all women. As a result, two separate stepwise regressions were run, one with estradiol and one without. When estradiol was not included, menopausal status ($p = 0.002$), age ($p = <0.001$), ethnicity ($p = 0.008$) and FSH ($p = 0.033$) were significant predictors of

when adjusted for the effects of these factors ($p = 0.668$); See Table 16, Model 4. When estradiol was included, menopausal status ($p = 0.001$), age ($p < 0.001$), ethnicity ($p = 0.009$), estradiol ($p = 0.034$) and glucose ($p = 0.047$) were significant predictors of lumbar BMD and case/control status was not a significant predictor of lumbar BMD when adjusted for the effects of these factors ($p = 0.880$); See Table 16, Model 5.lumbar BMD, PCOS and case/control status was not a significant predictor of lumbar BMD

Table 16: Multiple Linear Regression With the Dependent Variable Lumbar Bone Mineral Density Measured by Quantitated Computed Tomography

Model (Standardized β values (confidence interval) and p-value)					
	1	**2**	**3**	**4**	**5**
PCOS	-0.104 (-18.19, 2.630) 0.142	-0.23 (-10.33, 6.954) 0.70	-0.008 (-9.076, 7.896) 0.891	0.026 (-7.001, 10.89) 0.668	-0.11 (-11.01, 9.438) 0.880
Menopausal (No period in last 12 months)		-0.318 (-36.34, -15.38) <0.001*	-0.324 (-36.64, -16.13) <0.001*	-0.240 (-30.85, -7.131) 0.002*	-0.287 (-35.46, -9.951) 0.001*
BMI		0.038 (-0.371, 0.735) 0.518	0.004 (-0.537, 0.578) 0.943		
Age		-0.383 (-3.239, -1.613) <0.001*	-0.349 (-3.020, -1.408) <0.001*	-0.319 (-2.937, -1.155) <0.001*	-0.373 (-3.419, -1.373) <0.001*
Ethnicity (African American)			0.189 (6.451, 26.03) 0.001*	0.159 (3.632, 23.78) 0.008*	0.185 (4.096, 28.40) 0.009*
FSH				-2.145 (-0.445, -0.018) 0.033*	
Estradiol					0.151 (0.004, 0.091) 0.034*
Glucose					0.137 (0.003, 0.371) 0.047*
Adjusted R^2	0.006	0.0365	0.396	0.393	0.438
N	200	200	200	181	124

* Significant at $\alpha = 0.05$

5.0 DISCUSSION

5.1 DESCRIPTIVE STATISTICS IN PCOS CASES AND CONTROLS

PCOS cases and controls in the entire CHARM III Visit One cohort and those in the sub-set for QCT analysis displayed expected descriptive characteristics based upon the PCOS symptoms and background studies reviewed in the "Background" section. PCOS cases had higher total testosterone in both groups. This is expected since this is an indicator of elevated androgen levels, one of the diagnostic criteria for PCOS. PCOS cases also had higher BMI measures and waist/hip ratios, indicators of elevated weight and central adiposity previously described in PCOS women (Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R, 1998). This may explain why cases in both cohorts had elevated HDL compared to controls, and why in the entire CHARM III Visit One cohort cases had increased triglycerides compared to controls since these factors are highly correlated to weight. In the QCT analyzed sub-group, cases had significantly higher diastolic blood pressure than controls. This may also result from the increased weight of cases since weight has a significant impact on cardiovascular health. Cases also had significantly elevated levels of insulin compared to controls in both groups, as well as more women with Type-2 Diabetes. As previously discussed, diabetes is more prevalent in women with PCOS than the general population (Stankiewicz and Norman, 2006), (Zborowski J, Talbott E, Cauley J, 2001).

Cases had lower levels of specific hormones related to ovulation and menstrual cycles. In the entire CHARM III Visit One cohort cases had significantly lower SHBG, LH, FSH and progesterone compared to controls. Other studies have indicated lower levels of SHBG and FSH in PCOS cases compared to controls (Kirchengast and Huber, 2001), (Noyan et al, 2004) (Yüksel et al 2001) (To and Wong 2005). SHBG is inversely related to insulin; as cases had higher insulin levels than controls, it is logical that they would have lower SHBG levels. FSH is known to be lowered in women with PCOS and is related to ovulation; since women with PCOS are, by definition, less like to have normal ovulation, it is also expected that they would have lower FSH levels than controls.

Significant differences in LH and progesterone disappeared in the QCT analyzed sub-group. It is expected that LH levels would be higher in women with PCOS compared to controls (To and Wong, 2005), but as BMI increases, the levels of LH can rise. As this is, in general, an over-weight group (cases mean BMI = 31.9, controls mean BMI = 28.34), high BMI levels may explain the lack of a significant difference in LH levels between BMD measured cases and controls. In addition, in the entire CHARM III Visit One cohort there was a significant increase in cases which had not had a period in at least 12 months compared to controls; this difference was not significant in the QCT analyzed sub-group. Regular menstruation can help maintain higher levels LH and progesterone, which may explain the discrepancy in this case/control comparison between the whole cohort and the sub-cohort.

In both cohorts cases also had lower levels of estradiol, though the difference was not significant. The lack of significance may be due to the fact that estradiol levels have naturally variation. These results are similar to other studies, which showed non-significantly lower levels

of estradiol in PCOS cases compared to controls (Kirchengast and Huber, 2001) (Yüksel et al 2001) (To and Wong, 2005).

In the entire CHARM III Visit One cohort, controls were significantly older than cases, though the age difference was not significant in the QCT analyzed sub-group. This is likely a random effect related to sampling methods. Basic measures which may impact analysis of PCOS and its factors on BMD were compared between those who were analyzed for QCT BMD and those who were not to determine the applicability of the findings of the QCT analyzed subgroup to those who were not analyzed, and to determine if there was any bias in the selection of the population for QCT analysis. The group selected for QCT analysis had significantly smaller proportions of women with periods in the last 12 months, indicating that there are less women in this sub-group who are either peri- or post-menopausal or who have oligo-amenorrhea. However, the groups analyzed for QCT also had significantly smaller proportion of women who had a hysterectomy and/or oophorectomy, which could partially account for the lower proportion of women without periods in the last year. Therefore, findings for those analyzed in the QCT measured sub-group may not be generally applicable to the entire CHARM III Visit One cohort. As indicated in the stratified analysis of BMD by the number of periods in the last 12 months (see Results), this factor can significantly affect BMD; women with no periods in 12 months had lower BMD compared to those who did. Differences in BMD in the BMD analyzed sub-group may therefore be under-inflated compared to the entire CHARM III Visit One cohort.

5.2 DIFFERENCES IN DETERMINATES OF BMD BETWEEN PCOS CASES AND CONTROLS

Lumbar BMD measured by QCT was not significantly different between PCOS cases and controls when unadjusted BMD scores, 30-year old matched T-scores and age matched Z-scores were compared, though the differences in T-scores were borderline significant ($p = 0.10$) at an alpha level of 0.05. When stratified to adjust for factors known to affect BMD there were still no significant differences in BMD between cases and controls. ANCOVA analysis did not reveal a main effect of PCOS case-control status on lumbar BMD and did not indicate any significant interaction effect of PCOS case-control status with any of the stratified factors. However, three of the stratification factors – age, BMI and race – did show significant effects on BMD. Women ≤48 years of age had lower BMD compared to those aged >48; women with a BMI ≥30 had high BMD compared to those with a BMI <30 and African-American women had higher BMD than Caucasian women. All of these factors are supported by the literature; BMD decreases significantly with age and increases with weight (Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R, 1998), (Angelopoulous et al., 2006) (Murata, 2006) and African Americans also have higher BMD than Caucasians (Seeman, 2002).

The analysis of the effects of menstrual history in the teenage years, twenties and thirties on BMD also did not reveal any significant differences in BMD between women with oligo-anovulation (≤8 periods per year) compared to those with normal menstrual cycles in cases and in controls. A comparison lumbar BMD of PCOS women with <8 periods per year in their 30s to PCOS women with >8 periods per year in their 30s approached significance ($p = 0.12$), but women with PCOS and chronic oligo-anovulation into their 30s represent a "worst case" scenario of life-long menstrual irregularity. Previous studies focused on the effects of

annovulation on BMD in women with exercise and diet-induced amenorrhea (Warren, 2003); however, the causes of irregular menstruation in the current study group more likely related to the effects of PCOS than to behavior as in previous studies. Certain factors within this study group of PCOS cases and controls may also counter-act effects of irregular menstruation on BMD. In particular, the protective effects higher BMI may mediate differences in BMD between oligo-anovulation and normal menstruation groups. Women in previous studies were mostly thin due to behavior and diet. However, in this study group cases and controls were relatively heavy-set. Cases had a mean BMI of 31.9, obese by WHO criteria, and controls had a mean BMI of 28.34, overweight by WHO criteria. The effects of BMI may antagonize any deleterious effects of irregular menstruation.

Correlations and linear regression analysis in cases and controls revealed significant relationships of BMD to various estrogens, age, ethnicity and the number of periods in the last 12 months. However, in controls, all significant correlations disappeared after adjustment for age. This may be because controls were slightly older than cases, and the strong effects of age on BMD may mask any effects of estrogens and other factors on BMD.

5.3 ASSESSMENTS OF PCOS STATUS A PREDICTOR OF BMD AFTER CONTROLLING FOR UNIVARIATE CORRELATES

The final step in this study consisted of controlling for the univariate correlates of BMD examined in the previous steps to determine if PCOS case-control status had a significant effect on BMD. Case-control status approached significance ($p = 0.14$) in a univariate regression of PCOS case-control status and lumbar BMD. However, the addition of other factors determined

from the literature (age, menopausal status, BMI and ethnicity) removed the effects on PCOS case-controls status on lumbar BMD. Finally, step-wise analysis was performed to analyze both the variables from the literature and other factors, such as hormones, to adjust the effects of PCOS case-control status on BMD for all significant predicator factors of lumbar BMD. Since estradiol can affect BMD, but was not measured in all women, two step-wise models were performed: one with estradiol and one without. Age, menopausal status, and ethnicity were significant in both models. In the step-wise regression model without estradiol, levels of FSH also significantly predicted BMD. In the multivariate model with estradiol, FSH dropped from the model and levels of estradiol and glucose were significant. The protective effects of estradiol on BMD are well known (Notelotiz, 2002) (Seeman, 2002). In addition, studies have shown that FSH is associated with increased bone turnover marker activity and is positively correlated with bone loss (Khan AA, Syed Z, 2004). This may explain the significance of FSH in the multivariate model when estradiol was removed; any protective effects of FSH on bone may be masked by more significant effects of estradiol. When PCOS case-control status was added to step-wise models with and without estradiol, its effects were still not significant predictors of lumbar BMD.

These results are inconsistent with the BMD results obtain by Zborowski in the DXA analysis of lumbar BMD in the earlier CHARM II study. CHARM II PCOS cases had a 3.5% higher lumbar BMD compared to controls ($p = 0.049$) and a significant interaction was found for PCOS status and BMI at the lumbar spine. Several factors may account for the lack of difference in BMD between PCOS cases and controls in this particular group. This study population was several years older than the study population analyzed by Zborowski; 74 of the cases and 93 of the controls analyzed by QCT were part of the DXA analysis and would have aged at least 3 to 4

years in between the two studies. The current study population of women was approaching middle age, with an average age of 47.6 for the QCT analyzed sub-group. The impending deleterious effects of age may counteract any protective effects of PCOS case status on BMD. In addition, the age of the women suggests that they are approaching menopause. Menopausal status was found to a be a significant predictor of PCOS, and while the majority of the women in the QCT analyzed group (69.2%) were pre-menopausal (defined as having at least one menstrual period in the last 12 months), it is to be expected from their average age that many are quickly approaching menopausal status. In addition, though cases and controls analyzed for QCT BMD measures had significantly different BMIs, both groups were, as previously described, relatively heavy-set. Therefore, BMI may have been over a protective threshold in both controls and cases. Any expected protective effect of high BMI in PCOS cases would also be present in cases. The high BMI of the current study may also explain differences in results from the current study group to results obtained by Adami et al, Yüksel et al and To and Wong who found a lower lumbar and spinal BMD in PCOS cases compare to various controls groups. The current study group was much heavier than in these reviewed studies (See Tables 1 and 2).

5.4 STUDY CONSIDERATIONS

The current study had several strengths worth mentioning. The number of women analyzed (156 cases, 171 controls total; 105 cases and 97 controls assessed for BMD), much larger than previous groups analyzed in the literature for associations between PCOS and BMD. The current study also included many factors that can potentially affect BMD, including hormones, lifestyles factors, menstrual history and medical history. This allowed for a robust analysis of the potential

effects of PCOS on BMD and consideration of a variety of confounders and modifiers. While the study considered many covariates that may not commonly be obtained at one time (for example, the combination of cardiovascular measures such as HDL and LDL and hormone measures such as testosterone), the hormone measures that were significant in multivariate modeling (FSH, estradiol) are often analyzed in fertility screening. Glucose, which also figured significantly in the model with estradiol, can also be easily tested. This increases the utility of these models. In addition, cases were drawn from a larger study which initially selected its cases from a random community sample. This minimized selection bias, therefore increasing generalizablity of the cases to the population at large. However, the controls were matched to cases based on age, race and location, and thus may not be generalizable to the general population since, unlike cases, they were not a random sample of the population.

There are several other restrictions one must keep in mind when considering the study results. As reported, the muli-variate regression modeling did not consider all categorical variables. The study showed many significant effects of ethnicity on BMD. However, minorities (primarily African Americans) made up only 17 of the cases (16%) and 12 of the controls (12%) assessed for lumbar BMD. In addition, there is a lack of prospective data for this study (as well as for studies of PCOS and BMD in general) to determine how temporal changes in many of the significant factors may change BMD. Finally, QCT measures of BMD are not as frequently used as DXA measures. There are no DXA or other standardized clinical measures to compare to for accuracy of the measures obtained by QCT in this analysis. Finally, though larger than other studies on BMD in women with PCOS analyzed in the literature, this study group was still relatively small. Zborowski estimated that 150 cases and 150 controls would be needed in order to detect a 1 unit change in BMD measured by DXA with 80% power.

There is a need for large, prospective studies that thoroughly assess changes in PCOS characteristics and risk factors and their effects on BMD. Many of the PCOS characteristics shown in the current analysis, such as hormone levels, have very strong age related components. Thoroughly understanding changes in these risk factors over time can allow for better management of PCOS symptoms in general, and their relationship to BMD in particular. In addition, more studies that investigate the effects of PCOS minorities are needed. The effect of ethnicity on BMD in PCOS women was shown to be significant. Minority status may also affect other effects and health of PCOS which need to be thoroughly understood in order to effectively diagnose and manage PCOS in all ethnicities.

6.0 CONCLUSIONS

In conclusion, the current study had several aims: analyzing descriptive characteristics of CHARM III Visit One and the sub-group of CHARM III Visit One with QCT measures of lumbar BMD; considering the determinants of BMD as measured by QCT from the literature, and then determine if a significant difference exists between PCOS case and control groups analyzed by these determinates; and finally adjusting for these univariate correlates via multivariate logistic regression to determine if PCOS case status is an independent predictor of BMD status after controlling for these factors.

No significant differences were found in lumbar BMD measured by QCT between PCOS cases and controls in any univariate comparisons, nor were any significant differences found in any multivariate adjusted comparisons. The deleterious effects of middle age (mean age was 47.6) and impending menopausal status and the protective effect of heavy BMI in controls as well as cases may mediate some potential protective effects of PCOS case status on BMD.

The study is of public health importance in several ways. Firstly, the factors that affect BMD in women with PCOS can be used to aid in management of PCOS and its long-term effects on BMD. Effects on BMD ascertained from this analysis can also be investigated in other common bone-density related diseases, such as osteoporosis. In addition, though no significant differences were found in BMD using QCT methods, the current study is one of only a handful to use QCT methods to assess BMD in women with PCOS, and the largest study to do so when

compared to the published literature. Results from the study can serve as the basis of comparison for other studies that use QCT methods to assess BMD.

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