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THE EFFECT OF POLYCYSTIC OVARIAN SYNDROME (PCOS) ON THE HUMAN SKELETON

By

Sarah Bruckler

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The Effect of Polycystic Ovarian Syndrome (PCOS) on the Human Skeleton

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Abstract

The purpose of this pilot study is to determine the systemic effects of Polycystic Ovary Syndrome (PCOS) on bone mineral density (BMD). Excessive male sex hormones, excessive insulin levels, and weight gain characterize PCOS, and are correlated to increased BMD. PCOS is also associated with symptoms and comorbid conditions, like chronic vitamin D deficiency, menstrual dysfunction, and hypothyroidism, which are all correlated with decreased BMD. Existing research on this topic reports conflicting results; some studies show a significant correlation between PCOS and increased BMD, while others suggest that no significant correlation exists. These previous studies focused on load-bearing areas of the skeleton, which may obscure results because of a strong correlation between PCOS and high body mass indices (BMI). The current study uses full-body, dual-energy X-ray absorptiometry (DEXA) scans to compare the cranial bone and total BMD of 18 to 45 year old, non-smoking subjects with PCOS with healthy, age- and BMI-matched controls. The present study found that cranial bone BMD is a region of the skeleton that is not affected by mechanical loading, and is a useful metric for assessing systemic changes to BMD. Secondly, a significant, positive correlation between age and cranial BMD was observed in the PCOS sample that was not observed in the control sample. Finally, no correlation was observed between cranial BMD and total BMD in the PCOS sample, but was observed in the control sample. These results suggest that PCOS has a systemic effect on BMD independent of weight.

Introduction

Polycystic Ovary Syndrome (PCOS) affects 6.5 to 8.0% of women, making it the most common endocrine disorder in women.[1] The syndrome is characterized by a variety of common symptoms, like excessive male sex hormones, insulin resistance, menstrual and ovulatory dysfunction, and cystic ovaries.[1] Other common symptoms include imbalanced female sex hormones, infertility, hirsutism (unwanted hair growth on the face, chest, and back), increased lean muscle mass, and high body mass indices (BMI).[1,2,3] In addition to the symptoms of PCOS, patients with the syndrome often suffer from comorbid conditions such as hypothyroidism, temporomandibular joint disorder, systemic inflammation and osteoarthritis, and nutrient deficiencies such as vitamin D deficiency.[4,5,6,7,8] Increased risk of metabolic syndrome, certain reproductive cancers, high cholesterol, non-alcoholic fatty liver disease, and other complications have been observed in people with PCOS.[9,10,11] Together, the symptomology, comorbidities, and complications associated with PCOS make this syndrome a serious public health concern. Despite this, little is known about what causes PCOS, and scientific knowledge about the immediate and long-term consequences of the syndrome is incomplete.

One area of knowledge that is particularly confounding is whether PCOS has a positive, negative, or no effect on bone mineral density (BMD). Much of the research reports a statistically significant positive correlation between PCOS and increased BMD (e.g. patients with the disease have higher BMD than healthy controls).[3,12,13,14] Multiple theories exist about what aspect of PCOS may cause the observed gains in BMD. One hypothesis is that the excessive endogenous male sex hormones characteristic of PCOS cause patients with the syndrome to have bone growth similar to that of males because of

the important role these hormones play in bone growth and development.[15] Another theory is that excessive male sex hormones make it easier for PCOS patients to develop lean muscle mass, which is positively correlated with high BMD.[2,13,16,17] Conversely, other research suggests that the BMD of individuals with PCOS is not significantly greater than that of healthy individuals.[2,13,18] It is possible that symptoms like menstrual dysfunction, hypothyroidism, and chronic vitamin D deficiency may counteract any effect of excessive male sex hormones on bone growth and lead to low BMD.

The existing research about the effect of PCOS on BMD focuses on load-bearing areas of the skeleton, like the head of the femur and the lumbar spine. However, mechanical loading increases BMD locally, which potentially masks the systemic effects of PCOS on BMD. It is important to note that a study by Douchi and colleagues on the effect of PCOS on BMD uses the left arm, which these researchers argue is a non-load-bearing area of the skeleton.[16] While this may be true in people with healthy BMI, it is not the case in people who are overweight or obese. This is because individuals who are overweight or obese require the use of their arms to support their weight during sit-to-stand movement. In a study by Hills and colleagues (2002), 70% of obese children required assistance when asked to stand without using their upper bodies. Other studies show that morbidly obese adults also require the use of their upper limbs to stand and to avoid injury during activity.[19,20,21] Patients with PCOS are more often overweight or obese than lean, so the radius should not be assumed to be a non-load-bearing region of the skeleton when studying the effect of PCOS on BMD. Load-bearing areas of the skeleton become denser as weight increases, and because patients with PCOS commonly have high BMI compared to healthy individuals, these patients will most likely have

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higher BMD localized to load-bearing areas of the skeleton. Studies that focus on load-bearing areas will have a difficult time differentiating whether a patient's BMD is high because of increased weight or because of some other aspect of PCOS. Furthermore, studies that focus on load-bearing areas cannot discriminate whether the effect of PCOS on BMD is localized to these regions of the skeleton, or if the effect is systemic.

These gaps in the existing research inspired this undergraduate Honors thesis. In the current study, the goal is to determine whether PCOS has a statistically significant effect on BMD, and if so, whether that effect is systemic. To answer these questions, the investigators use total body dual-energy x-ray absorptiometry (DEXA) scans to gather data about body composition and BMD. This data can be used to compare total body, head, and vault BMD (head excluding teeth) to determine whether there is a significant difference in BMD of these regions between test and control group members. This research also allows the investigators to confirm whether vault BMD is affected by load-bearing or not. If cranial vault BMD is not affected by load-bearing, but does exhibit a significant difference in BMD when comparing the test and control group subjects, it would suggest that the effect of PCOS on the skeleton is systemic rather than localized to load-bearing areas of the skeleton.

While the study presented in this paper is a pilot study with a small sample size, it is an important step toward a better understanding of the effects of PCOS on the body. A richer understanding of the effect of PCOS on BMD can help clinicians choose treatment options for patients that are directed toward improving BMD and preventing BMD loss leading to osteoporosis (i.e., increased fracture risk). Given that PCOS is a public health concern, this research will benefit patients with PCOS, and society as a whole, by

increasing the general knowledge about this remarkably common disease. With each new piece of information about PCOS, clinicians can develop better treatment plans. Patients with PCOS, in turn, can expect better clinical outcomes, improving their health and well-being.

This paper begins with a comprehensive literature review in which PCOS is more clearly defined and the diagnostic criteria, various symptoms, comorbid conditions, and complications of the syndrome are explained.

Background

Defining PCOS

PCOS is a cluster of consistently comorbid symptoms disrupting the endocrine systems of affected patients. PCOS is characterized by hyperandrogenemia (excessive male sex hormones). There exist other hyperandrogenic conditions (characterized by excessive male sex hormones), like Cushing's Disease.[1] Additional symptoms are noted and the occurrence of these symptoms tends to be highly varied, leading researchers to regard PCOS as a mostly heterogeneous condition.[1] Aside from hyperandrogenemia, the most common symptoms are oligomenorrhea (infrequent menstruation) or amenorrhea (absent menstruation) and ovarian cysts. These two symptoms are included in the diagnostic criteria of the syndrome.[1] Patients with PCOS may also present with symptoms such as: Insulin Resistance (IR) or hyperinsulinemia, hirsutism (excessive hair growth, particularly on the face, chest, and back), weight gain and obesity, infertility, acne, alopecia (hair loss, particularly on the scalp), vitamin D deficiency, osteoarthritis, and temporomandibular joint disorders.[1,6,7,8]

Additional, long-term effects of PCOS exist. Patients with PCOS are at greater risk of developing metabolic syndrome, which are obesity related conditions that increase a person's risk of diabetes, cardiovascular disease, and stroke.[9] PCOS increases the risk of reproductive cancers, like endometrial cancer, due to menstrual and ovulatory dysfunction.[9,10] Patients with PCOS are also at increased risk of developing other chronic diseases as they age, including non-alcoholic fatty liver disease, dyslipidemia, and hypertension.[9,11] However, many of these long-term risks can be mitigated or prevented with appropriate lifestyle changes and treatment.[9]

Diagnosing PCOS

The high variability of PCOS has made it difficult for clinicians and researchers to determine a singular diagnostic criterion. According to Ricardo Azziz in *Androgen Excess Disorders in Women* (2006), PCOS should not be diagnosed until other hyperandrogenic disorders like Cushing's Disease have been ruled out.[1] Once other possible disorders are excluded, clinicians can use one of two possible diagnostic criteria to determine if a patient has PCOS. The first criteria was set by the National Institutes of Health (NIH) in 1990 and defines PCOS as the presence of hyperandrogenemia and chronic anovulation (the absence of ovulation), and the exclusion of other possible disorders.[1] The second criteria, called the Rotterdam Criteria, was developed in 2003 to broaden the definition of PCOS to include additional phenotypes of the syndrome. The Rotterdam Criterion defines PCOS as the presence of at least two out of three specific symptoms: hyperandrogenism, menstrual dysfunction, and/or the presence of ovarian cysts.[1]

Clinicians use a combination of techniques to determine whether patients have the

characteristic symptoms of either the NIH or Rotterdam criteria. First, doctors may observe phenotypical (visually observable) characteristics of PCOS in their patients. These characteristics include hirsutism, masculine features, male-pattern balding, obesity, and acne.[1,22] Second, doctors rely on patient reports of their symptoms. Patients may report menstrual irregularities, infertility, unwanted hair growth on their face, chest, or back, unwanted hair loss on their scalp, weight gain, or infertility. Doctors also use clinical tests to confirm diagnosis. These tests include blood tests to determine hormone levels, pelvic exams and ultrasound to determine if a patient has ovarian cysts, and menstrual cycle tracking to diagnose menstrual irregularities and oligo- or anovulation.[1,22]

The NIH and Rotterdam criteria are both useful for the diagnosis of PCOS, but approach diagnosis differently. The NIH criteria is most useful for diagnosing patients who are at high risk of metabolic dysfunction, while the Rotterdam criteria is useful for further defining phenotypes of PCOS where patients are more affected by reproductive dysfunction.[23] However, both the NIH and Rotterdam criteria are controversial. The NIH criteria was developed through an informal poll of experts who diagnose and treat PCOS, inquiring what the recognized features of the syndrome were.[23] The NIH criteria excluded polycystic ovaries because, despite its name, cysts are not always present in patients with the other characteristic symptoms of PCOS.[23] It was this exclusion that led to the development of the Rotterdam criteria.[23] However, rather than clarifying how to diagnose PCOS, the Rotterdam criteria only served to demonstrate that there are multiple types of PCOS. While this is useful knowledge to have, it has led to confusion in research and clinical outcomes because the effects and complications of PCOS vary by

phenotype.[23] Most studies on PCOS do not constrain selection criteria to a single phenotype, so it can be difficult to differentiate how the syndrome is affecting patients differently, or how PCOS is responding differently to various treatments.

PCOS and Bone Density

Due to its extreme variability, PCOS is a difficult syndrome to understand and much of the research available about its effect on BMD appear contradictory. Most research suggests that certain symptoms of PCOS, such as hyperandrogenemia, hyperinsulinemia (Insulin Resistance), and obesity, may positively affect bone growth and mineral accumulation.[3] For instance, weight gain and obesity may cause patients with PCOS to have higher BMD in load-bearing regions of the skeleton, like the femoral head or lumbar spine, since load-bearing bone density increases as weight increases. The visceral fat associated with PCOS weight gain can cause excessive free androgen production, which may be associated with increased BMD in non-load-bearing areas of the skeleton.[16] However, existing research does not show whether the effect of PCOS-related obesity on bone density is a localized effect, increasing BMD only in specific areas of the body, or a systemic one.

Contrary studies exist that suggest that patients with PCOS do not have higher BMDs than healthy controls.[2] This may be due to symptoms associated with PCOS that have been shown to have a deleterious effect on BMD. Menstrual and ovulatory dysfunction are significantly correlated to decreased BMD.[24] Chronic vitamin D deficiency, hypothyroidism, and hypoprogesteronemia (low progesterone) may counteract any beneficial effect of PCOS on BMD.[4,8,25] Additionally, there is some research that suggests that common treatments for PCOS, like insulin sensitizing agents

and androgen blockers, may reverse the beneficial effect of the symptoms those medicines treat.[2,3,26]

Hyperandrogenemia

The primary concern of most researchers who study the skeletal effects of PCOS is how hyperandrogenemia might cause patients with the syndrome to have different BMD than healthy patients. Male and female sex hormones regulate the growth of bone, accumulation of bone mineral, and signal epiphyseal plate fusion.[15,27] When these hormones are imbalanced, bone development in both men and women becomes abnormal.[27] It is possible that excessive androgen levels in patients with PCOS may stimulate bone growth and mineralization at a faster or greater rate, causing patients with PCOS to have a different body morphology than people without the syndrome. Indeed, many do. A study in 2015 determined that lean patients with PCOS were more likely to have a mesomorphic endomorph body type than healthy controls.[17] Mesomorphy refers to one's muscle mass, while endomorph refers to one's body fat.[17] Other hormones, like insulin, also affect how bone develops, and androgen seems to regulate how insulin is received by bone tissue.[27]

High testosterone levels have also been linked to greater lean muscle mass in both lean and obese patients with PCOS. In a study by Douchi and colleagues (2001), free androgens associated with visceral fat increased lean muscle mass in arms, legs, and the trunk, and that increased lean muscle mass was significantly associated with increased regional BMD, regardless of load-bearing.[13] However, the same study showed that serum androgen levels were not associated with increased BMD.[13] A later study by the same authors determined that patients with PCOS who are lean, or whose fat is distributed

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more peripherally, did not have the same regionally increased BMDs as patients with viscerally distributed fat.[16] It was determined that the cause of this anomaly was that peripheral fat does not produce free androgens the way visceral fat does, and the authors determined that this non-load-bearing effect of visceral fat was more significantly associated with increased BMD than its load-bearing effects.[16]

Menstrual and Ovulatory Dysfunction

According to a 1990 study by Davies, Hall, and Jacobs, there is a significant correlation between amenorrhea and vertebral bone mineral density loss in premenopausal women, except in women with PCOS.[24] The authors of the study hypothesize that the cause of bone mineral density loss in most cases of amenorrhea is due to estrogen deficiency.[24] This hypothesis is based on the purpose that estrogen serves in bone growth and maintenance, which is to signal epiphyseal fusion, support the retention of calcium in bone, and strengthen the bone matrix.[15] However, amenorrhea in patients with PCOS occurs without an estrogen deficiency because many women with the syndrome have excessive estrogen secretion.[12] Individuals with a known history of amenorrhea related to PCOS may not have vertebral bone density loss, or may have a decreased rate of vertebral bone density loss than either healthy individuals or those with amenorrhea unrelated to PCOS. Unfortunately, research comparing vertebral bone mineral density of women with PCOS-related amenorrhea and healthy controls is lacking.

Insulin Resistance, Obesity, and Lean Muscle Mass

Another concern of researchers is the effect of hyperinsulinemia and obesity on the skeletons of patients with PCOS. Insulin Resistance (IR) is a condition where the

body's insulin receptors do not work properly, and this causes the body to overproduce insulin, a state referred to as hyperinsulinemia.[26] While IR is not considered a part of the diagnostic criteria of PCOS, it is a symptom that is as common in patients with the syndrome as hyperandrogenemia, and the importance of IR in diagnosing and treating PCOS is well-recognized by researchers and clinicians.[26]

Obesity is also common in patients with PCOS, and while lean women may develop the syndrome, obese and overweight body types are the most common.[26] According to a study by Carmina and colleagues, PCOS was correlated with increased lean muscle mass, and the factors most associated with increased lean muscle mass are obesity and hyperinsulinemia.[2] According to a meta study of research done on lean muscle mass and femoral neck BMD by Ho-Pham and colleagues in 2013, lean muscle mass is a statistically significant indicator of higher BMD in healthy men and women.[28] Whether the associations between hyperinsulinemia, obesity, and increased lean muscle mass correlate to higher BMD in patients with PCOS is not yet known. The meta-study which suggests greater lean muscle mass correlates to greater BMD was conducted by reviewing studies that looked at healthy individuals and focused on a load-bearing area of the body. Given the difference between these populations and the population of patients with PCOS, it is not possible to assume or infer that the results can be accurately applied to the PCOS population. Carmina and colleagues (2009) found that the BMD of patients with PCOS was similar to that of healthy controls.[2] This contradicts the results of other studies and suggests that hyperinsulinemia, obesity, and the resulting increased muscle mass may not have a significant impact on BMD.[2]

It is also useful to note that insulin, like male and female sex hormones, is an

important hormone for bone development, signaling bone tissue growth, mineralization, remodeling, and epiphyseal plate fusion.[15] There are currently three recognized phenotypes of IR which are characterized by defects of insulin receptors: autoimmune disruption of insulin receptors (or the combination of hyperandrogenemia), IR, and acanthosis nigricans (dark, thick patches of skin).[29] Defects of insulin receptors and autoimmune disruption of receptors are less common, but the third phenotype, called HAIR-AN syndrome, affects 3% of patients with hyperandrogenic conditions, including PCOS.[29] It is possible that the hyperinsulinemic state caused by IR could heighten the stimulation of bone mineralization to a degree that would explain the increased BMD associated with PCOS. Hyperinsulinemia has also been shown to increase the production of androgens in visceral fat, and may play a role in the correlation between hyperandrogenemia and increased BMD.[16] Unfortunately, the effect of IR and hyperinsulinemia on BMD is not well known because there is a lack of studies on this possible avenue of skeletal change in PCOS. More research is needed to understand what effect, if any, hyperinsulinemia and obesity have on the skeletons of patients with PCOS.

Comorbid Conditions with Possibly Deleterious Effect on BMD

At first glance, it would seem that the symptoms of PCOS create a net-benefit to BMD, but there are specific comorbid conditions and treatment options that may have a deleterious effect on bone mineralization. First, chronic and severe vitamin D deficiency is common in patients with PCOS, and is associated with osteomalacia, which is a demineralization in adult bone.[8] There is little existing research on the incidence rate of osteomalacia in PCOS patients, and it is not known whether chronic vitamin D deficiency affects patients with PCOS in a similar fashion to the way it affects other patients.

Secondly, a study by Armanini and colleagues (2013) found that many patients with PCOS also had chronic, autoimmune thyroiditis (AIT) with hypothyroidism.[4] In that study, 27% of patients with PCOS had AIT, with 47% of those patients having subclinical hypothyroidism, compared to 8% of healthy controls.[4] Autoimmune hypothyroidism is caused by a deficiency in thyroxine, and both this deficiency and its most common treatment, thyroxine replacement, have been indicated as risk factors of bone density loss.[4,5] AIT and hypothyroidism-associated BMD loss may occur through thyroxine deficiency, while BMD loss associated with thyroxine replacement therapy may be caused by thyroxine toxicity, though thyroxine toxicity is uncommon.[4,5] The existing research on the effect of thyroid hormone replacement therapy (HRT) on BMD conflicts, but this may be due to the fact that thyroid HRT is heavily regulated to prevent thyroxine toxicity.

In addition, a statistically significant difference in progesterone was observed during the luteal phase (after ovulation and before menstruation) of the menstrual cycle in patients with PCOS compared to healthy controls.[25] It seems that the effect of low progesterone in PCOS is primarily that it increases the risk factor of patients with PCOS for pregnancy-related disorders and loss of pregnancy.[25] However, because progesterone plays a role in bone growth and development, like other sex hormones, low progesterone levels may have an impact on BMD.[25] However, very little research exists concerning the possible effect of low progesterone and BMD in patients with PCOS.

Common Treatments and BMD

Treatment plans for PCOS are necessarily varied in response to the syndrome's heterogeneous nature. Lifestyle changes and exercise are included in all treatment plans

because these options have been shown to be useful across all phenotypes of PCOS.[9] In addition to lifestyle changes, patients with PCOS are commonly treated with one or more of the following medications: androgen blocking medications, insulin sensitizing agents, or hormonal birth control. These treatments may affect the way in which PCOS affects BMD.

First, women with PCOS may be treated with exogenous sex hormones in the form of oral, implantable, or injectable contraceptives. Hormonal oral contraceptives are a common treatment for oligo- and amenorrhea in premenopausal women with PCOS.[30] In women with oligo- or amenorrhea that is not associated with PCOS, these contraceptives may prevent the BMD loss that is associated with menstrual and ovulatory dysfunction.[24] However, as mentioned earlier, patients with PCOS-related amenorrhea have increased estrogen secretion, which may have a positive effect on BMD. This may mean that any positive impact on BMD associated with hormonal oral contraceptives is absent, or that oral contraceptives do not have a similar effect in PCOS patients as the effect observed in non-PCOS patients. The effect of implantable and injectable contraceptives on bone density seems to be the subject of some debate among medical professionals. In one study, depo medroxyprogesterone acetate injectable contraceptive may have induced estrogen deficiency in the subjects studied, causing a statistically significant decrease in bone density in those subjects compared to control subjects who did not receive the injection.[31] However, this study has been criticized for poorly matching women in the test and control groups for other risk factors of low BMD like cigarette smoking.[32] Critics also pointed out that medroxyprogesterone has been shown to be useful in the treatment of osteoporosis.[32] Treatment of PCOS-associated oligo- and

amenorrhea with injectable or implantable forms of contraceptives may have an effect on the BMD of patients. However, little research has been done in this area, and conflicting evidence of the effect of implantable and injectable contraceptives on BMD makes it difficult to determine if these medications will induce estrogen deficiencies in patients with PCOS to a degree that would have any effect on their BMD.

Secondly, insulin sensitizing medications like metformin hydrochloride are useful in treating PCOS, making them a common addition to many patient treatment plans. The medications are meant to staunch excessive insulin production as a response to Insulin Resistance (IR), and mitigate the production of excessive male sex hormones and follicle stimulating hormone.[26] Therefore, insulin sensitizing agents may counteract the effect of IR and hyperandrogenemia on bone growth and development. Similarly, androgen suppression medications are used to prevent excessive secretion of male sex hormones, and it is within the realm of possibility that these medications could lessen the effect of hyperandrogenemia on BMD in patients with PCOS. Vitamin D supplementation, which is used to resolve osteomalacia and other symptoms of vitamin D deficiency, may stop or correct any demineralization that occurs in response to the chronic vitamin D deficiency associated with PCOS. Finally, as mentioned in an earlier section, thyroid replacement therapy to treat hypothyroidism may resolve any effect that hypothyroid conditions have on BMD loss in patients with PCOS, or, in the event of over-medication, may cause BMD loss.

Rationale

The literature about the skeletal effects of PCOS is incomplete, lacking in both longitudinal studies, and those which compare full-body DEXA scans to more thoroughly

correct for load-bearing bone density. Understanding PCOS and its impact on the skeleton is crucial because it affects some 6.5-8.0% of the female population according to projections made using the NIH Diagnostic Criterion.[1] This literature review highlights a deficit of understanding of the effects of PCOS on BMD, a deficit that could be mended through a study comparing the BMD of lean and obese patients with PCOS to that of healthy controls. The goal of the current study is to determine whether there is a systemic effect of PCOS on BMD and the direction of that effect. The secondary goal of the present study is to determine whether the cranial vault is a useful region to study when determining if changes to BMD in response to PCOS occur independently of mechanical loading.

Further justification for a comparative study is that individuals with PCOS are significantly more likely to suffer psychological distress than healthy women.[33] One of the reasons for this psychological stress is that patients with the disease feel they have different, and unfeminine, bodies from healthy women.[33] A more comprehensive picture of how this syndrome impacts the lives of patients could help doctors better treat and inform patients to increase their overall quality of life. Effective treatments like diet, exercise, supplements, hormone therapies, and insulin sensitizing medications have been shown to improve the quality of life of patients.[33]

Along with the burden on the physical and emotional well-being of patients, the diagnosis and treatment of PCOS presents a massive economic burden on the healthcare system. In 2006, the estimated cost of PCOS in the United States was $4.37 billion.[34] Early detection of PCOS is a vitally important, cost effective way to reduce the economic burden of the syndrome.[34] However, early detection requires a more thorough

understanding of the syndrome and its effects on the body. For these reasons, it is prudent for researchers to bolster existing knowledge by creating studies that fill in gaps and more rigorously attend to confounding issues.

Materials

This study was approved by the Eastern Michigan University Human Subjects Review Committee, protocol number 896868-1. Subjects were required to confirm that they were not pregnant before a DEXA scan could be completed. Over-the-counter pregnancy tests purchased from a local pharmacy were used to ensure that no subjects were pregnant. Female subjects were recruited from the Eastern Michigan University student, faculty, and staff population, and from around Ypsilanti, Michigan. The selection criteria for participation in the study included non-smoking, non-pregnant subjects aged 18 to 45 years who did or did not have PCOS. Subjects were separated into two groups based on their health status. PCOS group subjects self-reported that they had been diagnosed with PCOS and control group subjects self-reported that they had not been diagnosed with PCOS. Study participants were recruited at EMU via email and posted signs. Two subjects were recruited by word of mouth from other participants. The PCOS group consisted of 10 subjects with PCOS, and the control group consisted of 5 healthy, age- and weight- matched subjects, none of whom were pregnant or at risk of being pregnant.

The subject's height and weight were recorded for the purposes of finding age and weight matched controls. Subjects were also given a given a full-body dual-energy x-ray absorptiometry (DEXA) scan to measure total body BMD, as described below. The

scanner was operated by a trained member of the investigative team, thesis advisor Dr.

Megan Moore. The DEXA device used was a GE Healthcare Lunar Prodigy Pro housed

in the Eastern Michigan University Office of Nutrition Services. Printouts of each body

composition scan provided the data for this study, and statistical analysis of the data was

completed using IBM SPSS™ Statistics 21.0 and Microsoft Excel™.

Methods

At the beginning of each scanning session, the study protocol was explained and

informed consent was obtained via a consent form that explained the purpose,

procedures, requirements, and details of the study, including any risks and policies in

place to mitigate risk. Subjects were then escorted to a restroom and provided an over-

the-counter pregnancy test. Pregnancy tests were self-administered, and one of the

investigators verified the results of each test. Pregnancy waiver forms stating that the

subject was not pregnant were signed by each subject after a negative pregnancy test

result was obtained, and signed by the investigator, who acted as witness to the test result.

Subjects were then assigned a numerical code to ensure anonymity of their data. A health

status questionnaire was administered to determine if the subjects had conditions known

to be comorbid with PCOS and to record whether they were taking any medicines to treat

PCOS, insulin, or if they used hormonal implantable or hormonal injectable

contraceptives, which are known to affect BMD (Appendix A). Subjects were then asked

to weigh themselves so that the DEXA device could be calibrated for their body size.

Once these steps were completed, subjects were asked to remove their shoes, and all

metal jewelry or clothing with metal components. Subjects were then asked to lie still on

the DEXA device, and an investigator ensured that the subject was positioned correctly. The subject's legs were strapped together to prevent movement, and participants were asked not to speak until an investigator cleared them to do so to minimize movement of the chest and arms. Once subjects were properly positioned and still, a total body bone mineral density scan was performed according the GE Healthcare Lunar Prodigy Pro device manufacturer's guidelines. If one of the subjects' arms and/or legs did not fit on the scanning table, the DEXA scanner automatically estimated the BMD and body composition from the opposite side of the body. The DEXA device was operated by a trained member of the investigative team, thesis advisor Dr. Megan Moore. There were no adverse events reported during this research.

Subject's BMI and BMD_{Total} were calculated during the initial scan and no region of interest was selected to obtain these data. After each initial scan, subjects were provided with a printed report of their scan data, along with a pre-printed informational sheet explaining the results of their scan. In addition, Dr. Megan Moore explained the results of the scan to each subject. A second copy of each subject's initial report was printed for use by the investigative team. The default region of interest selected automatically for the head (BMD_{Head}) was used initially, saved, and printed. The region of interest for the head was then manually moved so that the box around the vault started just above the teeth and that the total volume of the cranial vault (BMD_{Vault}) was set to between 118 cc and 121 cc. The results were saved and printed a third time.

Statistical Analysis

Data obtained at scanning sessions was entered into a spreadsheet format for use with IBM SPSS™ Statistics 21.0 software. Partial correlations were used to assess

correlations between BMI and BMD_{Total}, BMD_{Head}, and $BMDv_{ault}$ controlling for age. These tests were completed for the total, control, and PCOS samples. Nonparametric correlations using Kendall's tau coefficient were used to compare Age and BMD_{Total}, BMD_{Head}, and $BMDv_{ault}$ for each sample. Finally, nonparametric correlations using Kendall's tau coefficient were used to compare $BMDv_{ault}$ and BMD_{Total} for each sample.

Partial correlations were used to determine the strength and direction of the linear relationship between BMI and BMD_{Total}, BMD_{Head}, and $BMDv_{ault}$. As a person ages, BMD should change, so it was necessary to set Age as the covariate (control variable) to see how BMI correlated to BMD independently from the effect of subject's ages. Nonparametric correlations, specifically Kendall's tau, were performed because the sample size was too small to assume normal distribution. Kendall's tau is comparable to other nonparametric statistical models, such as Spearman's R, in terms of statistical power, but differs in magnitude and interpretation.[35]

Results

The average age of subjects was 29.5 years in the total sample, 27.3 years in the control sample, and 30.6 years in the PCOS sample (Table 1). The average BMI of the subjects was 35.6 kg/m^2 in the total sample, 29.8 kg/m^2 in the control sample, and 38.4 kg/m^2 in the PCOS sample (Table 1). The average BMD_{Total} of subjects was 1.280 g/cm^2 (SD = 0.071) in the total sample, 1.236 g/cm^2 (SD = 0.078) in the control sample, and 1.303 g/cm^2 (SD = 0.060) in the PCOS sample.

Table 1. Demographic data for Total, Control, and PCOS Samples

	N	Mean Age	Mean BMI
Total Sample	15	29.5	35.6
Control Sample	5	27.3	29.8
PCOS Sample	10	30.6	38.4

A significant correlation between BMD_{Head} and Age was observed in the PCOS sample ($r = 0.644$; $p = 0.009$), and the total sample ($r = 0.383$; $p = 0.047$) (Table 2). However, while the correlation between BMD_{vault} and Age was significant in the PCOS sample ($r = 0.556$; $p = 0.025$), it was not significant in the total sample (Table 2). Correlations between BMD_{Total} and Age were not significant in either the total or PCOS sample (Table 2). However, the correlation of BMD_{Total} and Age in the PCOS sample was stronger than the correlation observed in the control sample ($r = 0.200$; $p = 0.624$).

Table 2. Nonparametric correlations between Age and BMD_{Total}, BMD_{Head}, and BMD_{vault}

	BMD_{Total}	BMD_{Head}	BMD_{Vault}
Total Sample Age	0.329 (0.091)	0.383 (0.047)*	0.356 (0.066)
PCOS Sample Age	0.405 (0.106)	0.644** (0.009)	0.556* (0.025)

P-values shown in parentheses
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Additionally, significant correlations existed between BMD_{Total} and BMD_{vault} in the total sample ($r = 0.386$; $p = 0.047$) and the control sample ($r = 0.800$; $p = 0.050$), but no significant correlation was observed in the PCOS sample ($r = 0.135$; $p = 0.590$) (Table 3). Furthermore, no significant correlation between BMD_{vault} and BMD_{Total} controlling

for age was observed in the PCOS sample (Table 4).

Table 3. Nonparametric correlations between BMD_{Vault}, and BMD_{Total}

	BMD_{Total}
BMD_{Vault} Total Sample	0.386* (0.047)
BMD_{Vault} Control Sample	0.800* (0.050)
BMD_{Vault} PCOS Sample	0.135 (0.590)

P-values shown in parentheses
* Correlation is significant at the 0.05 level (2-tailed).

Table 4. Partial correlations of BMD_{Vault} and BMD_{Total} controlling age in PCOS sample

	BMD_{Total}
BMD_{Vault} PCOS Sample	-0.143 (0.714)

P-values shown in parentheses

The significant correlation between BMD_{Vault} and Age in PCOS is a positive correlation, meaning that BMD_{Vault} increased with Age in the PCOS sample (Figure 1). The correlation of BMD_{Total} and BMD_{Vault} in the control sample is strongly positive (Figure 2), but this is not so in the PCOS sample (Figure 3). A slightly stronger positive correlation between BMD_{Total} and Age was observed in the PCOS sample ($r = 0.405$; $p = 0.106$) (Figure 4) than in the control sample ($r = 0.200$; $p = 0.624$). However, neither of these correlations is statistically significant ($p = 0.050$).
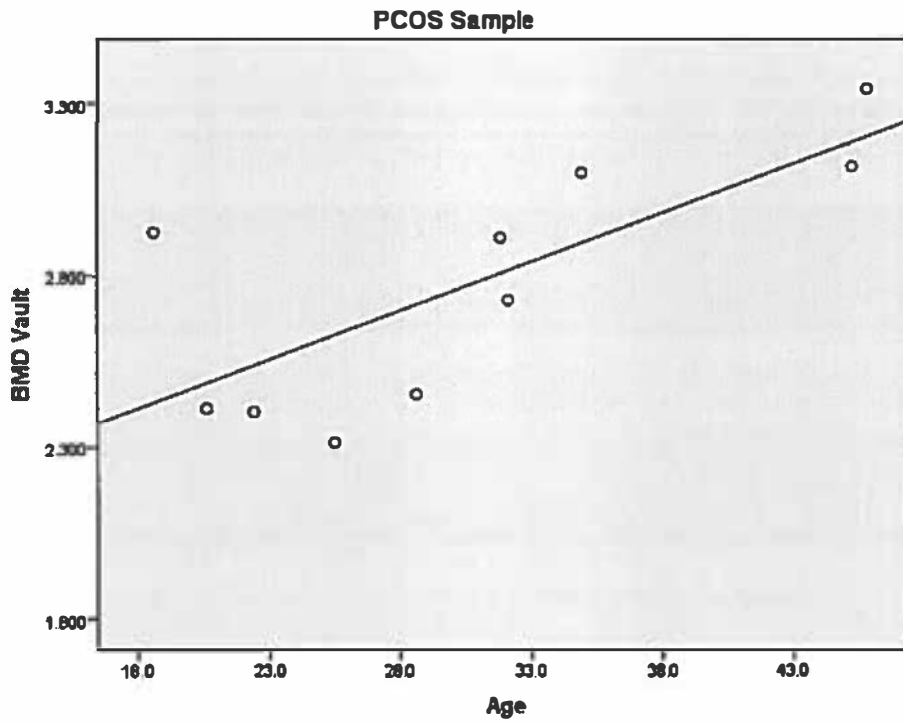
Figure 1. Correlation of BMDv$_{ault}$ and Age in the PCOS Sample (r = 0.556)
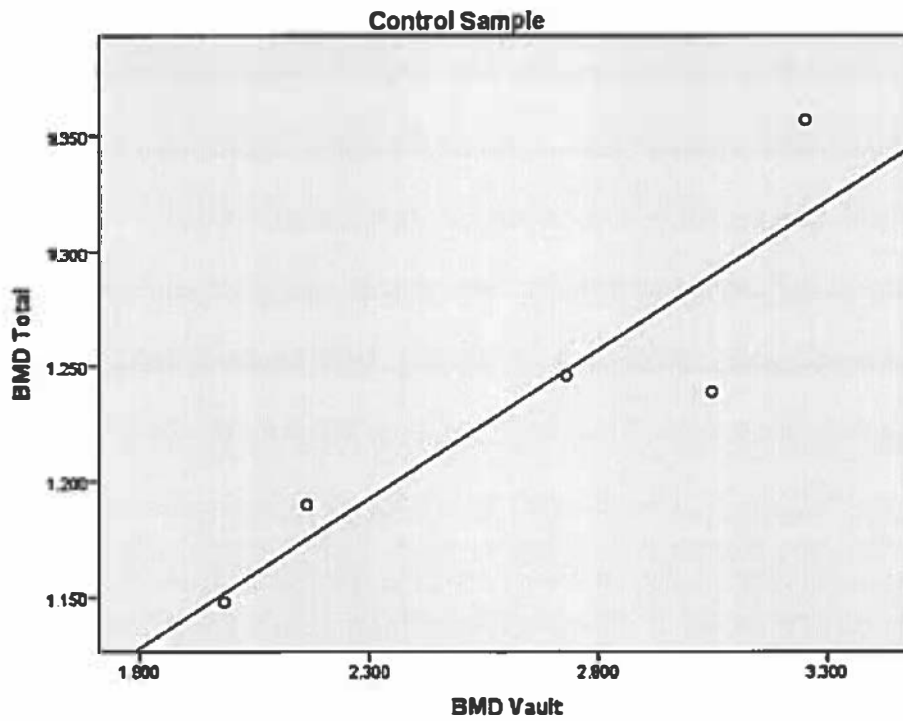
Figure 2. Correlation of BMD$_{Total}$ and BMDv$_{ault}$ in the Control Sample (r = 0.800)

Figure 3. Correlation of BMD_{Total} and BMD_{Vault} in the PCOS Sample ($r = 0.135$)

Figure 4. Correlation between BMD_{Total} and Age in the PCOS Sample ($r = 0.405$)

Discussion

The first purpose of the present study is to determine whether PCOS has an effect on BMD. While much of the existing research on this topic suggests a significant positive correlation between PCOS and BMD, other research suggests no correlation exists. This study found a positive relationship between BMD_{Total} and Age in both th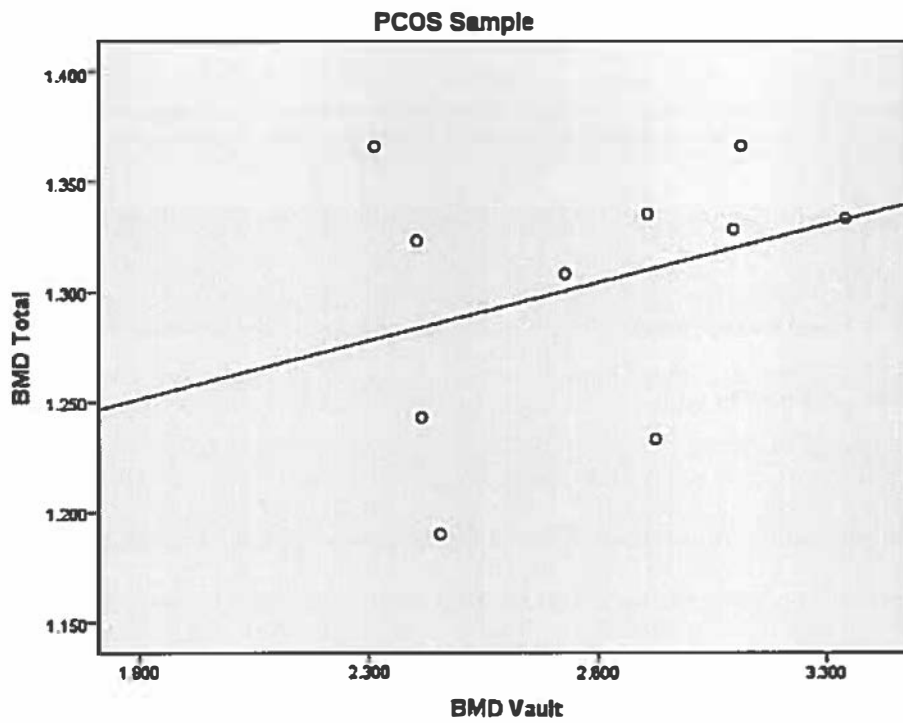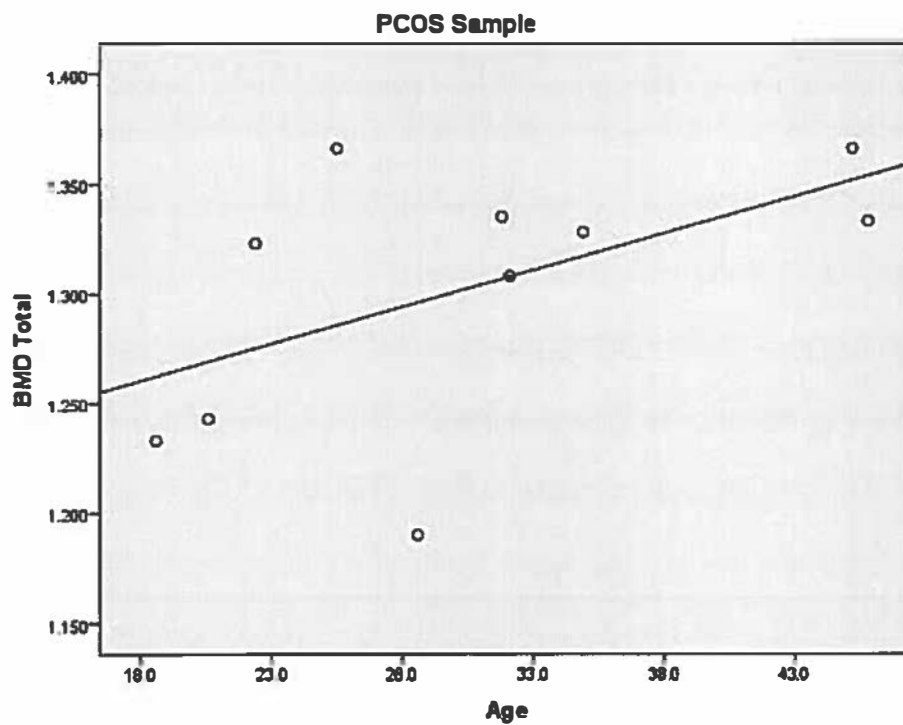e PCOS and control samples, but neither relationship was statistically significant. However, the relationship between BMD_{Total} and Age in the PCOS sample ($r = 0.405$) was slightly stronger than the relationship between BMD_{Total} and Age in the control sample ($r = 0.200$). These results are similar to the results of existing research; increased BMD is observed in subjects with PCOS, but this increase is not significantly different than what is observed in healthy subjects.

The second goal of the present study was to determine if any effect of PCOS observed is systemic. The results of this study show a significant positive correlation between BMD_{vault} and Age in the PCOS sample, which is not observed in the control sample. Additionally, the BMD_{vault} of PCOS subjects is increasing independently of BMI. These results suggest that PCOS has a meaningful, systemic effect on BMD that is not occurring in healthy control subjects. Further evidence for a systemic effect on BMD is observed when the correlations between BMD_{vault} and BMD_{Total} of each sample are compared. In the control sample, this correlation is significant ($p = 0.050$), meaning that BMD_{vault} is changing in tandem with BMD_{Total}. The correlation between BMD_{vault} and BMD_{Total} in the PCOS sample is not significant, suggesting that BMD_{vault} is changing independently of BMD_{Total}.

The final purpose of this research is to determine whether the cranial vault region

of the skeleton is a useful region to study to understand what impact PCOS has on BMD. As mentioned above, no significant correlation between BMD_{Vault} and BMD_{Total} was found in the PCOS sample despite a significant correlation between BMD_{Vault} and Age. These results support the conclusion that PCOS has a systemic effect on BMD, because there is not a significant correlation between BMD_{Vault} and BMD_{Total}, like the one observed in the control sample. These results also support the conclusion that BMD_{Vault} is a useful region to study when trying to determine the effect of PCOS on the skeleton. These conclusions illustrate how research that focuses on load-bearing regions of the skeleton may not be providing a complete view of how PCOS is affecting the skeleton.

Results that show a slight increase in BMD_{Total} were expected by the investigators of the present study, given the results of previous research studying the effect of PCOS on BMD. Male and female sex hormones play a similar role in supporting the retention of calcium in bone and strengthening bone matrix.[15] Additionally, one of the primary symptoms of PCOS is excessive secretion of androgens, often presenting as hirsutism, which is positively correlated with increased bone density in women with oligomenorrhea or amenorrhea, likely due to the effect of androgens on bone growth and calcium retention.[1,15,27] Most research on the effect of PCOS on BMD suggests that the high levels of androgens and follicle stimulating hormones present in women with PCOS have a positive effect on BMD, both by influencing bone growth and development, and possibly preventing bone mineral density loss. This hypothesis is supported by the current study because the BMD_{Vault} (unaffected by mechanical loading) *increased with age* in the PCOS sample. The opposite is expected in healthy females, in which bone mineral density decreases with age.[36]

The lack of significant difference between the correlation of BMD_{Total} and Age in the PCOS and control samples is also expected, because many comorbid conditions of PCOS are linked to lower BMD, and many of its treatments may ameliorate the positive effect of high male sex hormones and hirsutism. For instance, Autoimmune Thyroiditis is a commonly comorbid condition with PCOS, and both this disorder and its most common treatment have been linked to BMD loss.[4,5] Additionally, common treatments for PCOS like insulin sensitizing agents and androgen suppressants mitigate symptoms of PCOS that researchers theorize may be the source of the effect of PCOS on BMD, like helping patients lose visceral weight that causes excessive free testosterone secretion.[16,26] It is important to consider that while neither the correlation of BMD_{Total} and Age in the PCOS sample or the control sample are significant, the relationship between BMD_{Total} and Age is slightly stronger in the PCOS sample, suggesting that the syndrome does have an effect on BMD.

The investigators also expected to confirm that the cranial vault region is a truly non-load-bearing region of the skeleton, as existing research on the usefulness of the cranial vault region exists.[37] A study by Turner et. al. (1997) determined that the cranial vault region is not affected by mechanical loading from body weight or activity, and that assessing BMD using the cranial vault can be useful for determining how patients are responding to treatment.[37] The present study takes this information a step further, suggesting that cranial vault BMD is useful for assessing the systemic effect of disease on BMD.

However, unexpected results were obtained by this study. First, it was surprising to see a strong, positive correlation in BMD_{Vault} and Age in the PCOS sample. This result

is the opposite of what was observed in the control sample, and contrary to existing research which suggests that BMD decreases with age.[36] Also, the lack of correlation between BMD_{Vault} and BMD_{Total} when controlling for age in the PCOS sample is unexpected. In the PCOS sample, BMD_{Vault} is changing independently of BMD_{Total}, but this conflicts with general knowledge about BMD. BMD_{Vault} should increase or decrease in tandem to increases or decreases in BMD_{Total}, as illustrated by the strong correlation between the two in the control sample. The unexpected results obtained by this study raise important questions about the role of PCOS in women's bone health, suggesting additional research is needed to understand the complex effect of PCOS on BMD.

Limitations of this Study

There are issues with this study that need to be addressed in an expanded study. First, subjects self-reported their health status, and it was not possible to confirm these diagnoses, or determine free testosterone or serum hormone levels. Therefore, this study cannot determine if the effect of PCOS on BMD observed is equally applicable to all subphenotypes of PCOS. It may not be possible, with the current lack of data on how PCOS manifests and affects the health status of patients suffering from the various phenotypes, to develop a study that could sufficiently delineate between the phenotypes. This is a known issue in PCOS research. Furthermore, it is not possible to determine what factors of PCOS may be causing the syndrome to affect systemic BMD without confirming such things as serum hormone levels and free testosterone levels, and comparing lean muscle mass of PCOS subjects and healthy controls. However, this study is not designed to pinpoint the cause of the effect of PCOS on BMD. The investigators sought only to determine whether PCOS has an effect on BMD, if the effect of PCOS is

systemic or localized, and whether $BMDv_{ault}$ is a sufficient metric of the effect of PCOS on BMD.

Secondly, the sample size (N = 15) of this study is small and the selection region is primarily constrained to the Eastern Michigan University campus population. The results of this pilot study are not meant for population-level analysis. Rather, the study was intended to provide justification for an expanded study with a larger sample size.

Finally, the eligibility criteria of this study were too broad. The criteria did not exclude or delineate nonparous subjects from those who had been pregnant and had given birth. Pregnancy can have a negative effect on a person's BMD if the pregnant person does not increase calcium intake to mitigate this effect. Secondly, the broad eligibility criteria made it difficult to match participant's weight. A 1-tailed Mann-Whitney test showed a significant difference ($p = 0.049$) between the BMI of the PCOS sample and control sample. Also, as mentioned above, the eligibility criteria did not restrict subjects to one subphenotype of PCOS, or allow the investigators to group PCOS subjects by subphenotype.

Future Research

Multiple avenues of further research are recommended for this topic. This study is a pilot study, and an expanded study with a larger sample size is needed to both confirm the results and apply this research to population-level generalizations. Such a study is justifiable given the results of this study, and could be developed to account for the issues mentioned in the previous section. Additionally, further research could be done to determine whether PCOS has a protective effect on BMD during and after menopause, and if patients with PCOS are at lower risk of fracture than healthy controls. Research

has been conducted to study the effect of PCOS on BMD during menopause that shows strong, positive correlations between serum sex hormone levels and BMD.[38] A long-term study by Brännström and colleagues (2012) also found a significant positive correlation between PCOS and higher sex hormones during and after menopause, but suggests that peri- and postmenopausal patients with PCOS have a similar fracture risk compared to healthy controls.[38] Another study suggests that male sex hormones have a differential effect on types of bone in postmenopausal women, and further research could be initiated to determine how various hyperandrogenic conditions like PCOS influence types of bone.[39]

Other avenues of research include studying the effect of PCOS on BMD in patients who have had children, or whether pregnant patients with PCOS are able to absorb calcium more easily than healthy controls. It is also possible that patients with PCOS reach peak bone mass at a later age than healthy controls, or that bone growth and development in patients with PCOS differs in some other, relevant way to healthy controls. Another avenue of research is determining which symptoms of PCOS affect BMD, and to what degree. For instance, while this study shows a systemic effect of PCOS on BMD that is unrelated to mechanical loading, the increased BMIs and lean muscle mass associated with PCOS may have specific effects on BMD in load-bearing areas of the skeleton.

Conclusion

PCOS is a complex syndrome affecting an estimated 6.5 to 8.0% of women.[1] Despite its considerable incidence rate, the cause of PCOS is not known, and little is

understood about the immediate or long term impact of the syndrome. Clinically, patients with PCOS present with a wide array of symptoms and comorbid conditions of a varying severity, and the syndrome changes as women age. For these reasons, PCOS is considered by many doctors and researchers to be a serious public health concern. Despite the difficulties in studying PCOS, it is important for clinicians and researchers to study the syndrome closely and add to the general knowledge and understanding of the disease. As general knowledge of PCOS increases, the real scope of the syndrome can be determined. Additionally, new methods of managing the disease will emerge in response to a better understanding of PCOS, leading to better clinical outcomes for patients, and de-stigmatization of the disease among medical professionals and the general populace.

One area in which information about the effect of PCOS is scarce is how the syndrome affects BMD. PCOS is characterized by symptoms which may increase BMD, like hyperandrogenemia, insulin resistance, and obesity. However, little is understood about the lasting effects of PCOS, and the syndrome includes common secondary symptoms and comorbid conditions which may reduce BMD, like chronic vitamin D deficiency, and Hashimoto's Thyroiditis. The literature reviewed in this paper suggests that a correlation between PCOS and BMD does exist, but this literature focuses on load-bearing areas of the skeleton when measuring BMD. Such studies may not adequately illustrate whether the effect of PCOS is systemic or localized because patients with PCOS are likely to have overweight or obese BMIs, and significantly more likely to have increased lean muscle mass. These symptoms of PCOS will increase BMD in load-bearing areas of the body, which are naturally denser to accommodate a person's weight.

With these issues in mind, the current pilot study was developed. The goals of this

study were to determine if PCOS has an effect on BMD, if the effect is systemic, and to provide additional justification for the use of the cranial vault BMD as an appropriate metric for clarifying the first two points. The results of this study suggest that BMD_{vault} increases with age in patients with PCOS, though the opposite result is found in healthy controls. Additionally, this study found that BMD_{vault} does not correlate with BMD_{Total}, and that BMD_{Total} does not correlate with BMI in PCOS subjects. However, in control subjects, BMD_{Total} was significantly correlated to BMI. These results suggests that the effect of PCOS on BMD is systemic, and that BMI affects BMD_{Total} differently in patients with PCOS than in healthy controls. Finally, the present study determined that the effect of PCOS on BMD_{vault} is independent of mechanical loading, providing justification for using the cranial vault BMD as a region for determining whether the effect of PCOS on BMD is systemic.

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Appendix A

Health Status Questionnaire

Please answer the questions below as accurately as you are able. You will be able to verify your answers again before you leave your session.

1. Have you been diagnosed with Polycystic Ovarian Syndrome (PCOS)?

   ☐ YES   ☐ NO   ☐ NOT SURE

2. Have you been diagnosed with hypothyroidism, such as Hashimoto's Thyroiditis?

   ☐ YES   ☐ NO   ☐ NOT SURE

3. Have you been diagnosed with a vitamin D deficiency?

   ☐ YES   ☐ NO   ☐ NOT SURE

4. Have you been diagnosed with Type I or Type II Diabetes?

   ☐ YES   ☐ NO   ☐ NOT SURE

5. Have you been diagnosed with Insulin Resistance or hyperinsulinemia?

   ☐ YES   ☐ NO   ☐ NOT SURE

6. Do you take any of the following medications?

Metformin HCl: (Other names: Glucophage, Glucophage XR, Glumetza, Fortamet, Riomet)	☐ YES ☐ NO
Insulin:	☐ YES ☐ NO
Hormonal Injectable Contraceptive: (Depot medroxyprogesterone acetate, Depo-Provera)	☐ YES ☐ NO
Hormonal Implantable Contraceptives: (Other names: Nexplanon, Implanon)	☐ YES ☐ NO

7. If you use hormonal contraceptives, please write the name of the medication below:

   _____

   _____

By completing this questionnaire, you certify that the answers provided are true and

accurate to the best of your knowledge.

To be completed by the investigator:

CODE: _____