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The Association Between Measures of Adiposity and Anovulation in Women With Regular Menstural Cycles

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THE ASSOCIATION BETWEEN MEASURES OF ADIPOSITY AND ANOVULATION IN WOMEN WITH REGULAR MENSTRUAL CYCLES

A Thesis Presented

by

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ABSTRACT

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Anovulation accounts for approximately 12 percent of all female infertility in the United States. Prior studies suggest women with high body mass index (BMI) have an increased risk of infertility, particularly obese women with abnormal cycle lengths. To date no studies have examined the relationship between measures of adiposity, including BMI and percent body fat measured by DXA scan (%BF), and anovulation among women with regular menstrual cycles assessed with biomarkers. We evaluated this association using data from the BioCycle study, a prospective cohort of 259 women with regular menstrual cycles. All measures of adiposity and covariates were collected at baseline. Anovulation was assessed via luteinizing hormone and progesterone levels in urine samples collected 16 times throughout two menstrual cycles.

A total of 34 women had at least one anovulatory cycle during the study. Unadjusted models for BMI show a significant decrease in risk comparing highest BMI quartile to the lowest, (OR: 0.29; 95% CI .090-.968). Once multivariable logistic

regression was used to adjust for age no significant associations were found in any BMI quartile, but point estimates did not change significantly. Similar trends were found using other measures of adiposity. Results show that there is a non-significant inverse trend between adiposity and anovulation in healthy women with regular menstrual cycles. This relationship can possibly be explained by age due to the influence of time since menarche (TSM). Further research is needed to examine this relationship.

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CHAPTER I

INTRODUCTION

According to the clinical definition, infertility occurs when a couple, not using contraception, is unable to become pregnant after twelve or more months of trying (1). In 2002, the National Survey of Family Growth estimated that 7.4% or 2.1 million women in the US are infertile (1). Infertility can be divided into four categories: female infertility, male infertility, female and male combined infertility, and unknown cause. It is estimated that somewhere between 50-60% of infertility cases are due to female infertility and 30-40% are due to both male and female infertility problems (2).

Female infertility can be further subdivided into 8 categories: hypothalamic-pituitary factors, ovarian factors, tubal/peritoneal factors, uterine factors, cervical factors, vaginal factors, genetic factors, and unknown etiology. It is estimated that approximately 12% of all female infertility can be attributed to ovarian dysfunction; either the inadequate formation of the corpus luteum or inability to produce an oocyte (2). Women with regular menstrual cycles may have infertility and not be aware of the fact until they try to become pregnant because anovulatory cycles are asymptomatic.

Increased adiposity may contribute to anovulation through disturbances in the hypothalamic-pituitary-ovarian (HPO) axis, which regulates reproductive hormones. There is research supporting the association between obesity and the disruption in ovulation via two mechanisms: 1) hyperandrogenism and 2) insulin resistance (3, 4).

To date no epidemiologic studies have evaluated the association between adiposity and anovulation in a population of women with regular menstrual cycles using

biomarkers as a measure for anovulation. However, studies using other measures of infertility found an increase in risk between body mass index (BMI) and infertility (5-21). In addition BMI has been found to affect fertility and response to fertility treatments among women with reproductive disorders known to have anovulation as a symptom (22-25). There also have been no studies that look at the association between multiple measures of adiposity and anovulation using a better predictor of adiposity other than BMI.

The identification of a modifiable factor such as adiposity, which can affect fertility in all women, would give physicians other options prior to using infertility treatment. Because anovulation is not easily detected without a biomarker it is important that studies incorporate this measure. It is important to look at the normal menstrual cycle in a healthy population in an attempt to identify possible changes that are present before a woman needs to seek medical treatment for fertility problems. Therefore, we propose to assess the relationship between BMI and anovulation using data from the BioCycle Study, a prospective study of women who reported regular menstrual cycles and did not have any other medical conditions that could affect ovulation. The participants had 16 visits during which both blood and urine were collected to measure multiple biomarkers of menstrual cycle status and ovulation.

CHAPTER II

PHYSIOLOGY OF ADIPOSITY AND ANOVULATION

There are a several biological mechanisms through which obesity may increase the risk for anovulation. These mechanisms center on the HPO axis, which regulates both the menstrual cycle and ovulatory function through a complex hormonal regulation system. The two main disturbances to the HPO axis occur through either hyperandrogenism or insulin resistance (4).

Hyperandrogenism is a biological condition where there is an excess production or secretion of androgens, which include sex hormones (4). Adipose tissue has been shown to have the potential to alter the secretion of sex hormones, given its essential role in both androgen production and in the conversion of androgens into sex hormones (3). Both androgen production and conversion affects the carrier protein sex hormone-binding globulin (SHBG), an important hormone in the HPO axis (3, 4). Therefore, increased adiposity can lead to an excess of adipose tissue, and in turn a disturbance in the production of SHBG and other sex hormones, disrupting normal ovulatory function (3, 4). Therefore, it is plausible that adiposity contributes to ovulatory disorder.

In terms of a second mechanism, insulin resistance can lead to disturbance in ovulatory function. The ovary is a target organ for insulin, requiring insulin to stimulate the production of sex hormones (3). Adipose tissue affects insulin by producing metabolites used in insulin secretion and metabolism. An increased amount of adipose tissue may lead to an increased amount of insulin secreted and altered metabolism, in turn causing insulin resistance throughout the body, thereby increasing the amount of

circulating insulin (4). This increased insulin level will negatively affect the ovary and its sex hormone production, affecting the HPO axis and altering ovarian function. Therefore, it is plausible that increased adiposity contributes to altered ovarian function.

In summary, there is biological evidence supporting the hypothesis that obesity increases the risk for anovulation through the disruption of HPO axis by hyperandrogenism, and/or insulin resistance.

CHAPTER III

EPIDEMIOLOGY OF ADIPOSITY AND ANOVULATION

Approximately 21 studies have examined the association between obesity and anovulation. Epidemiologic studies looking at BMI and anovulation measured by biomarkers are sparse (9, 20, 23, 24). Previous studies have examined obesity and fecundability, which is the probability for a woman to conceive during a given menstrual cycle, (5, 6, 8, 17-19), and/or measuring time to pregnancy (TTP), which is a measure of length of time it takes to become pregnant (7, 10-16, 21). These studies were conducted among women diagnosed as subfertile (5-19), or women with polycystic ovarian syndrome (PCOS) (22-25). To our knowledge no previous studies have looked at women with regular menstrual cycles using the design we are interested in investigating.

Studies looking at BMI and fecundability or TTP have consistently observed inverse associations (5-8, 14-16, 18, 21, 26). Other studies focusing on infertility, in a case-control setting, found that those who had increased obesity measured by BMI were at greater risk for infertility when compared to those who were fertile (10-13, 19). Finally, studies conducted among women who have PCOS, a reproductive syndrome with decreased ovulation as one of its main symptoms, found that there was an inverse association between obesity and fertility (22-25). None of these studies were conducted among women with regular menstrual cycles and none have measured ovulation using biomarkers.

To our knowledge no studies to date have used multiple measures of adiposity in an attempt to examine the amount of misclassification that can be introduced by only

using BMI. Previous studies have been done looking at measures other than the standard BMI and anovulation (27) and have found a similar increased risk of anovulation with increased adiposity. There have been studies that have looked at multiple measures of adiposity as predictors for all cause mortality (28) and cardiovascular disease (29) and were used to identify potential exposure variables reflecting adiposity.

In a large retrospective study among 10,903 Danish women who attended antenatal care for their first planned and successful pregnancy, Jensen *et al.* assessed the relationship between BMI and the fecundability odds ratio (FR) (5). Around the twentieth week of gestation, women were asked the number of months they were trying to conceive, if they had normal menstrual cycles, and their height and pre-pregnancy weight. This information was then used to calculate TTP and BMI. After adjusting for potential confounders, the fecundability for an "overweight" woman (defined as a BMI >25 kg/m^2) was decreased when compared to that of a "normal weight" woman (defined as a BMI 20-25 kg/m^2) (FR: 0.77; 95% confidence interval (CI) 0.70-0.84). Those with a BMI below normal (<20 kg/m^2) also had decreased fecundability as compared to normal weight woman, although this was not statistically significant (FR: 0.95; 95% CI 0.90-1.01).

Due to the exclusion of non-pregnant women, those at highest risk were likely not included. In addition, the measure of fecundability relied on each woman's definition of "trying" to become pregnant and is therefore subject to "wantedness bias" (30). This is not an accurate measure of true ovulatory function, which is instead more accurately measured through biomarkers that can assess the level of hormones and the point of

ovulation in a woman's cycle. This information would tell the researcher whether each particular cycle was even ovulatory.

Rich-Edwards *et al.* were the first to examine the relationship between BMI and infertility. In a nested case-control study using data from the Nurses' Health Study II, the authors defined cases as married nulliparous women who self reported inability to become pregnant for at least one year because of an ovulatory disorder (n=2,527). Controls were defined as married parous women who had no history of infertility (n=46,718) (11). Self reported height and weight at age 18 was used to calculate BMI for all study participants. After adjustment for potential confounders those in the highest BMI category of ≥ 32 kg/m^2 were at an almost three-fold increased risk for infertility when compared to normal weight women (BMI category 20-21.9 kg/m^2) (OR 2.7; 95% CI 2.0-3.7). Women with a BMI above 23.9 kg/m^2 also had a statistically significant increase in risk.

Both exposure and outcome in this study could be misclassified due to data collection technique. The self reported value of BMI makes nondifferential misclassification of the exposure probable and this was not validated by the study. The use of self reported ovulatory infertility has its limitations but was validated via a secondary questionnaire and medical records. Among 40 out of 75 medical records collected, a 95% confirmation rate was found.

Finally, Al-azemi *et al.* conducted a prospective study of 270 women who were diagnosed with PCOS and seeking infertility treatment. The authors measured the association between BMI and ovulation after medical induction (22). Women in the highest BMI category (BMI ≥ 35 kg/m^2) had a lower percentage of ovulation at 12

months when compared to women in the BMI category of 18-24 kg/m^2 (38.3% vs. 91.9% $p < 0.0001$). The authors also found statistically significant differences in the 6 month ovulation percentages for the highest BMI category and for the BMI category of 30-34 kg/m^2 as compared to those in the BMI category of 18-24 kg/m^2.

This study has limited generalizability because it was limited to a group of women with a reproductive disorder that affects ovulation. The authors of the study did not specify if they adjusted their findings for age. It is possible that the results they found are confounded if they did not adjust for this important variable. It is important to know the effect of BMI in healthy women because it can be used as a potential risk factor for ovulatory problems.

In summary, no previous studies have prospectively assessed the association of BMI and anovulation using biomarkers among a population of women with regular menstrual cycles. However, prior epidemiological studies have found an inverse association between BMI and fecundability and TTP (5-9, 12-21). Researchers have also looked at women diagnosed with infertility and found this association (10, 11, 22-25).

CHAPTER IV

SUMMARY

Infertility is estimated to affect 7.4% of the US population and 50-60% of cases can be attributed to female infertility. Anovulation is an asymptomatic event that is difficult to measure without biological measures (1, 2). BMI may lead to ovulation disruption via hormonal disruptions of the HPO axis due to hyperandrogenism and insulin resistance (3, 4).

Epidemiologic evidence suggests that a positive relationship between BMI and decreased fecundity, increased TTP, and increased infertility (5-9, 12-21). Other studies limited to women with reproductive disorders also found a relationship between BMI and fertility (10, 11, 22-25). However, none of these studies have prospectively examined this association among women with regular menstrual cycles using biomarkers to assess ovulation.

Therefore, we examined the relationship between measures of adiposity and anovulation using biomarkers in a population of women with regular menstrual cycles and no history of other infertility problems.

CHAPTER V

HYPOTHESIS AND SPECIFIC AIMS

Using a prospective cohort design, we evaluated the relationship between multiple measures of adiposity and anovulation among women with regular menstrual cycles. The following aim was addressed:

Specific Aim 1: To evaluate the association between BMI and anovulation in women with regular menstrual cycles.

Hypothesis 1: Among adult females with regular menstrual cycles, those who have higher BMI will have an increased risk for anovulation as compared to those with normal BMI.

CHAPTER VI

METHODS

Study Design and Population

Using a prospective cohort study design we evaluated the association between BMI and anovulation. We also evaluated other measures of adiposity as possible predictors of anovulation. We used data collected as part of the BioCycle Study conducted at University of Buffalo in Buffalo, New York between 2005 and 2007.

A detailed description of the study population and design has previously been published (26, 31-34). Briefly, healthy, regularly menstruating premenopausal women were recruited from clinical practices, University of Buffalo health services, flyers, radio & television ads, and local newspapers. The study was designed to include a total of 16 cycle visits over two menstrual cycles with visits usually scheduled to match key biologic processes in menstrual cycle function. The initial schedule was based on a normal 28 day cycle and had women come into the clinic on day 2, 7, 12, 13, 14, 18, 22, and 27. Fertility monitors were used to modify the schedule of clinic visits based on the individual woman's cycle. At these visits, blood and urine samples were obtained and in-person interviews were conducted.

Interested participants were scheduled for a screening visit where all exclusion factors were measured. At the end of this exclusion visit a baseline/enrollment visit was scheduled 1-2 weeks prior to the subjects' next menstrual period. At this visit, physical and anthropometric measures were taken, blood and urine samples were obtained, and questionnaires were completed. The participants were given a daily diary and home fertility monitor to take home with them and detailed instructions were provided for use

of both tools. The participants were then required to call the center on the first day of their next period to schedule an appointment for the following day. All following visit days were scheduled based on an algorithm using cycle length in attempt to time visits on the correct days of the menstrual cycle (35). The participants began using the fertility monitor on day 6 of their cycle and were instructed to come in if the monitor indicated "peak fertility" on a day without a scheduled visit. If there was no positive indication on the monitor by day 14, a visit was scheduled and the subject was instructed to continue to monitor for 10 additional days. The fertility monitor was only used as a tool to schedule visits close to ovulation. The measures from the monitor were not used in this study. A total of 259 women with regular menstrual cycles participated in the study.

The following criterion was used as exclusion from the original study: Depo-Provera, Norplant or intrauterine device use in the past 12 months; oral contraceptive or other hormone supplement use in the past 3 months; planning to attempt to conceive in the next 3 months; actively trying to conceive in the past 6 months; pregnancy currently or in past 6 months; breast feeding in the last 6 months; abnormal pap smear in last 6 months with no subsequent normal results; laparoscopy confirmed endometriosis; current uterine fibroids or removal in last 12 months; history of polycystic ovary disease; history of Chlamydia infection or positive IgG at screening; untreated gynecological infection or any infection in past 6 months; gynecological surgery in past year; sought treatment for infertility ever; history or clinical signs of gynecological problems; infectious disease treated by physician in past 6 months; treatment for allergies with chronic medication; liver or kidney disease requiring treatment in past year; younger than 18 or older than 44; psychiatric condition requiring medical therapy in last year; BMI <18 or >35.0 kg/m^2

measured in clinic; plan to consume restricted diet for weight loss or medical reasons in next 3 months; gastrointestinal conditions associated with mal-absorption; unwilling to stop regular intake of vitamin or supplements; chronic use of certain medications; antibiotic use in past 3 months; history of chronic disease such as heart disease, diabetes mellitus, cancer, inflammatory disease, autoimmune, liver or kidney, thyroid disease or any other endocrine dysfunction; current treatment for anemia; history of alcohol abuse; dependency disorder or substance abuse in past 30 days; self report of regular illicit drug use in past 30 days; and diet high in phyto-estrogens.

Exposure Assessment

BMI was calculated using height and weight as measured by trained study personnel. The measures were obtained at eight points in the cycle but only height and weight at baseline visit was used. For our analysis, BMI was broken into quartiles. BMI was also be analyzed continuously to evaluate a dose-response relationship (Table 1). We evaluated the following measures of adiposity as possible predictors of anovulation, all of which were measured at baseline: waist to hip ratio (WHR), percent body fat from Dual-energy X-ray absorptiometry (DXA) scan, percent truncal fat from DXA, truncal to leg fat from DXA, and total skinfold thickness. All measures were evaluated at as quartiles and continuous variables (Table 1).

Validity of Exposure Assessment

To date DXA scan is considered one of the gold standards for measuring the true amount of body fat a person has and the validity of the other measures of adiposity are

often compared to this measure (36). The assessment of whether measured BMI is a true measure of percent body fat has been assessed in previous studies (37). Blew *et al.* measured the reliability between BMI calculated using height and weight measured by the same instrument and percent body fat calculated from a DXA scan, which gives a true measure of body fat. They found that among their population of women ages 40-66 there was a high correlation between BMI and %body fat (r=0.81). Sensitivity for BMI was 25.6% and specificity was 99.3% using the NIH definition of obesity at 30 kg/m^2 (37). Taylor *et al.* measured the reliability between WHR and truncal fat measured by DXA and found that among children 3-19 years old there was a correlation of value of 0.73 (38). Durin *et al.* found that when using total skin fold measurements that there was a correlation value of 0.80 when comparing skinfold thickness to %body fat among young women (39).

Outcome Assessment

Anovulation was identified using the biomarkers of LH and progesterone, measured in urine collected at the 16 visits to the clinic during their cycle. A woman was classified as anovulatory if peak progesterone concentration across the cycle was ≤ 5ng/ML, reflective of corpus luteum failure, and there was no LH peak, reflective of failure of oocyte being released, measured during at least one menstrual cycle. This criterion was previously used in other studies using these data (26). The variable was dichotomized into a "yes/no" variable (Table 1).

Validity of Outcome Assessment

The validity of using LH urinary levels as a marker for ovulation has been shown to have high validity. Guida *et al.* found that among 40 women ages 21-42 there was 100% correlation between urinary LH measured daily and ultrasound diagnosis of ovulation with no difference in the accuracy and precision of LH and ultrasound ($p < 0.05$) (40). For this study, interassay coefficients of variation of LH were less than 4% and for progesterone 14% (41, 42).

Covariate Assessment

Data for all covariates were collected via self report during in-person interviews during the study (Table 1). We used baseline/enrollment measures of age; history of smoking; history of alcohol use; stress using Cohen's Perceived Stress Scale, a scale that is used to measure participants perception of stress with high validity and reliability (43); race; education; use of oral contraceptives; prior pregnancy; time since menarche (TSM) and physical activity measured by the International Physical Activity Questionnaire (IPAQ) score, a tool to quantify physical activity that has been shown to have high validity (44). These factors have been found to be important covariates in prior studies of fertility (5, 6, 9-11, 45).

Data Analysis Plan

Specific Aim: To evaluate the association between BMI and anovulation.

Univariate Analysis

We calculated number and percentages of women in relation to the population characteristics as well as their BMI categories and anovulation status (Table 1).

Bivariate Analysis

Covariates were cross tabulated with BMI quartiles (Table 2b) and with anovulation status (Table 3) to evaluate potential confounders. Chi-square tests were used when cell size is sufficient to assess homogeneity in the distribution. If the cell size was not sufficient, Fishers exact test was used instead. P-values were derived from the Chi-square tests for categorical variables and 2 sample t-tests for continuous variables.

Multivariable Analysis

Logistic regression was used to provide an unadjusted odds ratio and 95% confidence interval for the association between BMI and anovulation (Table 4). Multivariable logistic regression was then be used to model the relationship between BMI and anovulation adjusting for potential confounders (Table 4). Covariates which change the estimates for BMI by 10% or greater when added to the model were considered confounders and retained in the multivariable model. Continuous variables were tested for linearity in the logit and included as a continuous variable only if they met this criterion. Generalized estimating equations (GEE) were used to examine the same relationship by cycle instead of woman (Table 9). These models address the correlation due to the multiple cycles per women. Sensitivity analysis was done to select an age where the changes in the association between BMI and anovulation could be explained. We stratified the data based on age, (Table 7). We also examined detailed information on specific covariates based on the stratification groups (Table 5 & 6).

Finally we examined other measures of adiposity to see if the association found between BMI and anovulation was the same among all other measures. We first examined the correlation between all measures of adiposity (Table 2a). We then chose percent body fat (%BF) from DXA scan, which is a golden standard, to look at bivariate relationships between %BF and covariates (Table 2c). Finally we looked at the unadjusted and adjusted models of each measure of adiposity (Table 4).

CHAPTER VII

SIGNIFICANCE

To date no studies have evaluated the association between adiposity and anovulation in women with regular menstrual cycles. These women are an important population to examine because if there is a difference in their risk for anovulation than they can be identified prior to seeking medical help for fertility problems. Given that adiposity is potentially modifiable risk factor for women who are having trouble conceiving, research to evaluate this relationship is pertinent. Results from this study will further research in this area and may suggest a way to increase conception potential among women with regular menstrual cycles.

CHAPTER VIII

HUMAN SUBJECT PROTECTION

The BioCycle study was approved by the Institutional Review Boards of University of Buffalo, the National Institutes of Health, and University of Massachusetts. All participants were required to sign an informed consent statement indicating that they understood the study requirements, that they were under no obligation to participate, and that they could withdraw at any time.

Every effort is made to ensure that confidential information remains secure. Study personnel were trained in privacy protocols and all study records are kept under lock and key at the original study site. Computer files are kept on a secure server that is password protected, with only study personnel able to get access to these files once permission is granted from the study coordinator.

The known risk to participants is adverse reactions to blood draws during the study visit, which all participants were informed of before signing the consent. There is also a possibility that potentially sensitive information about the participants could be obtained if there were to be a confidentiality breach. Given that all study personnel are trained in privacy procedures, this is unlikely to occur. The only known benefit to the participants in the study was the knowledge that they would be advancing science in the area of women's reproductive health.

CHAPTER IX

PERMISSION TO ACCESS DATA

I, Nicole Ash received permission to use the BioCycle Study database from Brian W. Whitcomb, BioCycle Study investigator. The BioCycle Study was approved by IRBs at the University at Buffalo and the National Institutes of Health. Use of the BioCycle Study database for research at UMass was approved by the School of Public Health and Health Sciences IRB.

CHAPTER X

RESULTS

Study population characteristics can be seen in Table 1. Women in the study were more likely to be white, non-smokers who had at least a college education, were past users of oral contraceptives and nulliparous. The average age of the population was 27.29 (SD ±8.2) years with an average BMI of 24.08kg/m^2 (SD ±3.9).

Table 2a shows the correlation between each of the measures of adiposity using Pearson's product-moment correlation calculation. Each of the correlations was statistically significant, with all measures being highly correlated except for WHR.

Distribution of participants by BMI Quartile and %BF quartile can be seen in Table 2b and Table 2c respectively. The only covariate to differ significantly between the quartiles of BMI was age (p=0.04). The same was true for %BF.

Table 3 shows the distribution of participants by anovulatory status. A total of 34 women or 13% had at least one anovulatory cycle. There was a significant difference in age between the ovulatory women versus the anovulatory women. Ovulatory women had an average age 28.28 (SD ±8.3) years verses the anovulatory 20.79 (SD ±2.9) (p=<0.001). Anovulatory women also differed significantly from ovulatory women in regards to education level, with 20.6% of anovulatory women being college graduates compared to 52% of ovulatory women (p=<0.001). Previous pregnancy also differed between the two groups with 43.4% of ovulatory women having a previous pregnancy compared with 14.3% of anovulatory (p=0.04). Finally, past oral contraceptive also

differed between the two groups. 57.4% of the ovulatory women had used oral contraceptives in the past compared with 37.5% of the anovulatory women (p=0.03).

In unadjusted analysis, BMI quartiles 2 and 3, along with the continuous measure of BMI, were not significantly associated with anovulation (Table 4). Women in BMI quartile 2 had a non-significant 29% decreased risk for anovulation compared with women in BMI quartile 1 (95% CI: .28-1.82). The odds ratio comparing BMI quartile 3 to BMI quartile 1 was the same. When BMI quartile 4 was compared to BMI quartile 1, women in quartile 4 had a significant 71% decreased risk for anovulation (95% CI: .090-0.986). Looking at BMI as a continuous variable the unadjusted odds ratio (OR) was 0.91 (95% CI: 0.82-1.01) for each one unit increase in BMI. Once adjusted for age, the OR for women in the second BMI quartile compared to the first was 0.66 (95% CI: 0.24-1.80), while the OR for women in the third quartile compared to the first was 0.73 (95% CI: 0.26-2.01). The OR for the fourth quartile compared to the first became non-significant 0.38 (95% CI: .106-1.33). We also looked at BMI quintiles and sextiles and found similar non-significant inverse trend (Table 8). The analysis using GEE examining cycles instead of women found the same non-significant trend (Table 9). We found similar results among all measures of adiposity with no significant relationships (Table 4).

We were interested in the impact age had on the BMI-anovulation relationship. Sensitivity analyses were done, and age of 22 years was identified as the point where those above and below had differenced in both their BMI and anovulation status. We stratified the women into those less than 22 years of age (Table 5) and greater than or equal to 22 years (Table 6). This showed that women less than 22 who were anovulatory

were more likely to be in BMI quartile 1 and have a less time since menarche (TSM). An unadjusted model of just TSM and anovulation found a significant 31% decrease in risk of anovulation for every year since menarche (95% CI: 0.51-0.94). Women who were 22 or older and anovulatory also were more likely to be in BMI quartile 1 but had a much longer TSM. TSM in this group was not significantly related to anovulation (OR 0.71; 95% CI: 0.50-1.00). When the multivariable models were examined separating the women into these two groups the non-significant trend was still seen but was more pronounced in those less than 22 years (Table 7).

CHAPTER XI

DISCUSSION

In this study of 259 healthy women with regular menstrual cycles, we found no significant associations between adiposity and anovulation, but did find a non-significant inverse trend in the data. We found that women who were younger than 22 years were the ones who had the most cases of anovulation. These women were also found predominately in the first BMI quartile and to have the shortest time since menarche.

Our non-significant results of BMI and anovulation, among women with regular menstrual cycles, is different than previous findings for fecundability and TTP (5-9, 12, 12-21) and women with diagnosed infertility (10, 11, 22-25). We hypothesized that we would see the same relationship in this population of healthy women with regular menstrual cycles as other studies found. However, we found no significant association between adiposity and anovulation in this population even after adjusting for age. We did have a small sample size, limiting power for the study. We had 80% power to detect an odds ratio of 3.25 so it is possible that if we had a larger sample size we may have been able to detect a smaller difference in the groups.

Based on the findings it could be hypothesized that among young healthy women anovulation is strongly associated with age and time since menarche and is minimally influenced by adiposity. Among women $\geq$ 22 years there were very few anovulatory cycles limiting our ability to evaluate the association with BMI.

The range of BMI in our study was also limited by the exclusion criteria. We had no individuals that were over 35 kg/m^2, which excluded those in the higher BMI ranges

and could have also excluded those who would be most likely to be at risk for anovulation. Some of the previous studies found a U-shape association with BMI and it is possible that since we don't have those in the largest BMI categories we could only be seeing part of this U-shape design and if we include women in with higher BMI's then we may have found this U-shape in our results also.

Further research would need to be done to examine these two hypotheses. A study which included a wider range of BMI, along with increased numbers of women, could be beneficial to look closer at this relationship and have the statistical power to detect small differences that may be present.

It is possible that our results were affected by bias. Nondifferential misclassification of exposure may arise if women were to report their own height and weight used to calculate BMI. This type of misclassification, would underestimate any association seen between body mass index (BMI) and anovulation. Because we used trained study personnel to measured the participant's height and weight at baseline, using the same equipment for everyone, the likelihood that misclassification would occur is minimal.

Nondifferential misclassification of the outcome could occur if we inaccurately identify a woman with ovulation as anovulatory regardless of their BMI. If this did occur it would underestimate our results. Cases of anovulation are identified from lab values obtained during the study period. The laboratory coefficient of variance, a measure of reliability, for both LH and progesterone was small therefore reducing this concern (41, 41, 42, 42). We also are using two measures to confirm the presence of anovulation, both

low progesterone levels and missing or late LH elevations. Therefore we feel that there is little chance for misclassification to occur.

Due to the methodology of a prospective study there is little chance for selection bias to occur, because the exposure of BMI is measured before the outcome of an anovulatory cycle has happened. There is a chance for loss to follow-up to introduce bias into a prospective study. This can occur if women lost to follow-up were more likely to be in the high BMI group and also more likely to be anovulatory. If this were to happen it would cause an underestimation of the true relative risk. Because of the asymptomatic nature of anovulation we believe that the 17 participants who dropped out of the study before one cycle was completed did not systematically differ by exposure or outcome and that, therefore selection bias was unlikely to occur.

Prospective studies can face information bias when the diseased group of the study is measured more carefully for exposure, or when the exposed group is questioned differently than the unexposed for outcome information. An example of this is if those with anovulation had more study visits measuring BMI than those ovulatory cycles or if the samples provided by those with high BMI were checked more carefully for anovulation. If this did happen we may find more cases of anovulation among those in the high BMI category and this would bias our results by away from the null. Laboratory workers were blinded to the participant's exposure information making it very unlikely that information bias would be present in our study.

We chose covariates to analyze for confounding based on their presence in previous literature and their effects on statistical models. We are not aware of any key confounding factors that were not measured during the study process. It is possible that

there could be some residual confounding if key factors were not measured accurately. An example of this would be looking at physical activity as a confounder. In our study this measure was based on self report and if this was not reported accurately, even after we adjust for it there would still be some confounding effect in the results. Because physical activity is inversely associated with BMI and positively associated with anovulation, failure to control this completely would result in an underestimation of the true relative risk. We believe that any residual confounding would not affect our results significantly.

Our study was limited to women with regular menstrual cycle length and we believe that the results of our study may be generalized to all women with normal menstrual cycle length. This is true because the biological mechanism through which BMI impacts anovulation may differ among women with abnormal menstrual cycles.

In conclusion, we found no significant relationship between BMI and anovulation among women with regular menstrual cycles. It is possible that some biases may have occurred in this study but we feel that they are minimal and any that do occur would bias our results towards the null and would underestimate the true association. The relationship between BMI and anovulation among women with regular menstrual cycles still remains unclear and there is need for further research. Studies with larger populations that are not so restrictive to the exposure of BMI may have different results, and should be conducted before concluding that BMI has no true association with anovulation among women with regular menstrual cycles.

TABLES

Table 1. Population Characteristics; BioCycle Study 2005-2007.

	Overall N=259
Demographics	
Age (years): mean ± SD	27.29 ± 8.2
Current Smoker: n(%)	
No	200 (78.7)
Yes	54 (21.3)
Missing	5
Current Alcohol use: n(%)	
No	85 (33.0)
Yes	172 (66.9)
Missing	2
Perceived Stress Score: n(%)	
Q1(7-18)	78 (30.2)
Q2(19-22)	64 (24.8)
Q3(23-25)	55 (21.3)
Q4(26-40)	61 (23.6)
Missing	1
Race: n(%)	
White	154 (59.5)
Black	51 (19.7)
Other	54 (20.9)
Education Level: n(%)	
High School Graduate or Less	33 (12.7)
Some College	101 (39)
College Graduate and Above	125 (48.3)
Physical Activity: n(%)	
Low	25 (9.7)
Moderate	92 (35.5)
High	142 (54.8)
Previous Pregnancy: n(%)	
No	111 (58.7)
Yes	78 (41.3)
Missing	70
Past OC use: n(%)	
No	115 (45.1)
Yes	140 (54.9)
Missing	4
Measures of adiposity	
BMI, (kg/m^2): mean ± SD	24.08 ± 3.9
BMI, (kg/m^2) Quartiles: n(%)	
Q1 (16.4-21.0)	65 (25.1)
Q2 (21.1-23.5)	65 (25.1)
Q3 (23.6-26.2)	65 (25.1)
Q4 (26.3-35.0)	64 (24.7)
Waist to hip ratio (cm): mean ± SD	0.75 ± 0.06
% Body Fat: mean ± SD	29.54 ± 5.99
% Truncal Fat: mean ± SD	25.11 ± 7.39
Truncal/Leg Fat: mean ± SD	0.71 ± 0.15
Total skinfold thickness (mm): mean ± SD	81.18 ± 22.14
Measures of Anovulation	
Anovulation: n(%)	
No	225 (86.9)
Yes	34 (13.1)

Table 2a. Correlation coefficients between measures of adiposity; BioCycle Study 2005-2007*.

	BMI	BMI Quartile	% Body Fat	% Truncal Fat	Waist to Hip Ratio	Truncal/Leg Fat	Total Skinfold Thickness
Total Skinfold Thickness	0.75	0.69	0.75	0.77	0.3	0.6	1
Truncal/Leg Fat	0.66	0.66	0.62	0.84	0.49	1	
Waist to Hip Ratio	0.33	0.34	0.22	0.35	1		
% Truncal Fat	0.79	0.75	0.95	1			
% Body Fat	0.75	0.7	1				
BMI Quartile	0.92	1					
BMI	1						

*All p values were <.0001

Table 2b. Distribution of covariates according to BMI kg/m^2 quartiles; BioCycle Study 2005-2007.

	BMI Q1 (16.1-21.0)	BMI Q2 (21.0-23.5)	BMI Q3 (23.6-26.2)	BMI Q4 (26.3-35.0)	P Value[1]
Demographics					
Age: mean ± SD	25.9 ± 7.3	26.2 ± 8.00	27.5 ± 8.6	29.6 ± 8.7	0.04
Current Smoker: n(%)					0.57
No	54 (84.4)	50 (79.4)	48 (76.2)	48 (75.0)	
Yes	10 (15.6)	13 (20.6)	15 (23.8)	16 (25.0)	
Missing (5)					
Current Alcohol use: n(%)					0.85
No	24 (36.9)	22 (33.9)	19 (30.2)	20 (31.3)	
Yes	41 (63.1)	43 (66.1)	44 (69.8)	44 (68.8)	
Missing (2)					
Perceived Stress Score: n(%)					0.98
Q1(7-18)	22 (33.9)	19 (29.7)	18 (27.7)	19 (29.7)	
Q2(19-22)	16 (24.6)	16 (25.0)	19 (29.2)	13 (20.3)	
Q3(23-25)	11 (16.9)	14 (21.9)	14 (21.5)	16 (25.0)	
Q4(26-40)	16 (24.6)	15 (23.4)	14 (21.5)	16 (25.0)	
Missing (1)					
Race: n(%)					0.25
White	36 (55.4)	43 (66.2)	37 (56.9)	38 (59.4)	
Black	12 (18.46)	7 (10.8)	15 (23.1)	17 (26.6)	
Other	17 (26.15)	15 (23.1)	13 (20.0)	9 (14.1)	
Education Level: n(%)					0.35
High School Graduate or Less	8 (12.3)	7 (10.8)	6 (9.2)	12 (18.8)	
Some College	21 (32.3)	28 (43.1)	31 (47.7)	21 (32.8)	
College Graduate and Above	36 (55.4)	30 (46.2)	28 (43.1)	31 (48.4)	
Physical Activity: n(%)					0.84
Low	8 (12.3)	4 (6.2)	8 (12.3)	5 (7.8)	
Moderate	24 (36.9)	24 (36.9)	23 (35.4)	21 (32.8)	
High	33 (50.8)	37 (56.9)	34 (52.3)	38 (59.4)	
Previous Pregnancy: n(%)					0.33
No	27 (64.3)	30 (63.8)	30 (60.0)	24 (48.0)	
Yes	15 (35.7)	17 (36.2)	20 (40.0)	26 (52.0)	
Missing (70)					
Past OC use: n(%)					0.72
No	31 (50.8)	29 (44.6)	29 (44.6)	26 (40.6)	
Yes	30 (49.2)	36 (55.4)	36 (55.4)	38 (59.4)	
Missing (4)					
Measures of adiposity					
Waist to hip ratio (cm): mean ± SD	0.78 ± 0.04	0.74 ± 0.05	0.77 ± 0.07	0.77 ± 0.05	<.0001
% Body Fat: mean ± SD	24.9 ± 4.0	26.3± 4.5	31.5 ± 3.8	35.6 ± 4.5	<.0001
% Truncal Fat: mean ± SD	18.9 ± 4.5	21.0 ± 4.9	27.5 ± 4.9	33.0 ± 5.1	<.0001
Truncal/Leg Fat: mean ± SD	0.59 ± 0.10	0.64± 0.10	0.76 ± 0.13	0.85± 0.12	<.0001
Total skinfold thickness (mm): mean ± SD	63.2 ± 12.8	72.3 ± 14.2	86.1 ± 13.4	104.6 ± 21.9	<.0001
Measures of Anovulation					
Anovulation: n(%)					0.23
No	53 (81.5)	56 (86.2)	56 (86.2)	60 (93.8)	
Yes	12 (18.5)	9 (13.9)	9 (13.9)	4 (6.3)	

[1]P-values obtained from chi-square tests or Fishers exact for categorical variables and T-Tests for Continuous variables

Table 2c. Distribution of covariates according to % Body fat by DXA scan quartiles; BioCycle Study 2005-2007.

	% Body Fat Q1 (15.10-24.65)	% Body Fat Q2 (24.66-29.81)	% Body Fat Q3 (29.82-33.53)	% Body Fat Q4 (33.54-45.25)	P Value[1]
Demographics					
Age: mean ± SD	26.4 ± 7.7	25.8 ± 7.8	27.5 ± 8.9	30.1 ± 8.0	0.02
Current Smoker: n(%)					0.83
No	46 (75.4)	49 (80.3)	47 (78.3)	50 (82.0)	
Yes	15 (24.6)	12 (19.7)	13 (21.7)	11 (18.0)	
Missing (16)					
Current Alcohol use: n(%)					0.57
No	17 (27.4)	20 (32.3)	23 (37.7)	23 (37.7)	
Yes	45 (72.6)	42 (67.7)	38 (62.3)	38 (62.3)	
Missing (13)					
Perceived Stress Score: n(%)					0.36
Q1(7-18)	16 (26.2)	25 (40.3)	16 (25.8)	16 (25.8)	
Q2(19-22)	20 (32.8)	11 (17.7)	16 (25.8)	15 (24.2)	
Q3(23-25)	10 (16.4)	15 (24.2)	12 (19.4)	17 (27.4)	
Q4(26-40)	15 (24.6)	11 (17.7)	18 (29.0)	14 (22.6)	
Missing (12)					
Race: n(%)					0.79
White	38 (61.3)	39 (62.9)	35 (56.5)	35 (56.5)	
Black	14 (22.6)	10 (16.1)	11 (17.7)	15 (24.2)	
Other	10 (16.1)	13 (21.0)	16 (25.8)	12 (19.4)	
Missing (11)					
Education Level: n(%)					0.33
High School Graduate or Less	7 (11.3)	6 (9.7)	6 (9.7)	13 (21.0)	
Some College	23 (37.1)	28 (45.2)	26 (41.9)	18 (29.0)	
College Graduate and Above	32 (51.6)	28 (45.2)	30 (48.4)	31 (50.0)	
Missing (11)					
Physical Activity: n(%)					0.6
Low	4 (6.5)	8 (12.9)	3 (4.8)	7 (11.3)	
Moderate	20 (32.3)	21 (33.9)	25 (40.3)	24 (38.7)	
High	38 (61.3)	33 (53.2)	34 (54.8)	31 (50.0)	
Missing (11)					
Previous Pregnancy: n(%)					0.06
No	27 (58.7)	32 (72.7)	25 (56.8)	21 (44.7)	
Yes	19 (41.3)	12 (27.3)	19 (43.2)	26 (55.3)	
Missing (78)					
Past OC use: n(%)					0.39
No	27 (44.3)	32 (52.5)	29 (46.8)	23 (37.1)	
Yes	34 (55.7)	29 (47.5)	33 (53.2)	39 (62.9)	
Missing (13)					
Measures of adiposity					
BMI, (kg/m^2): mean ± SD	21.14 ± 1.75	22.11 ± 2.57	24.89 ± 2.75	28.25 ± 3.65	<.0001
BMI, (kg/m^2) Quartiles: n(%)					<.0001
Q1 (16.1-21.0)	30 (48.4)	27 (43.6)	5 (8.1)	1 (1.6)	
Q2 (21.0-23.5)	27 (43.6)	19 (30.7)	13 (21.0)	3 (4.8)	
Q3 (23.6-26.2)	5 (8.1)	10 (16.1)	26 (41.9)	19 (30.7)	
Q4 (26.3-35.0)	0 (0.0)	6 (9.7)	18 (29.0)	39 (62.9)	
Missing (11)					
Waist to hip ratio (cm): mean ± SD	0.74 ± 0.04	0.74 ± 0.04	0.76± 0.06	0.77 ± 0.07	0.0006
% Truncal Fat: mean ± SD	16.3 ± 2.6	22.1 ± 2.7	27.8 ± 3.1	34.2 ± 4.1	<.0001
Truncal/Leg Fat: mean ± SD	0.58 ± 0.09	0.66 ± 0.13	0.77 ± 0.13	0.83 ± 0.12	<.0001
Total skinfold thickness (mm): mean ± SD	60.9 ± 11.2	77.9 ± 15.0	84.5 ± 14.7	103.9 ± 22.5	<.0001
Measures of Anovulation					
Anovulation: n(%)					0.22
No	54 (87.1)	52 (83.9)	56 (90.3)	59 (95.2)	
Yes	8 (12.9)	10 (16.1)	6 (9.7)	3 (4.8)	
Missing (11)					

[1]P-values obtained from chi-square tests or Fishers exact for categorical variables and T-Tests for Continuous variables

Table 3. Distribution of covariates according to anovulation status; BioCycle Study 2005-2007.

	Ovulatory	Anovulatory	P-Value[1]
Demographics	N=225	N=34[b]	
Age: mean ± SD	28.3 ± 8.3	20.8 ± 2.9	< 0.001
Current Smoker: n(%)			0.92
No	173 (78.6)	27 (79.4)	
Yes	47 (21.4)	7 (20.6)	
Missing	5	0	
Current Alcohol use: n(%)			0.78
No	73 (32.7)	12 (35.3)	
Yes	150 (67.3)	22 (64.7)	
Missing	2	0	
Perceived Stress Score: n(%)			0.80
Q1(7-18)	67 (29.9)	11 (32.4)	
Q2(19-22)	54 (24.1)	10 (29.4)	
Q3(23-25)	48 (21.4)	7 (20.6)	
Q4(26-40)	55 (24.6)	6 (17.7)	
Missing	1	0	
Race: n(%)			0.70
White	136 (60.4)	18 (52.9)	
Black	43 (19.11)	8 (23.5)	
Other	46 (20.4)	8 (23.5)	
Education Level: n(%)			< 0.001
High School Graduate or Less	28 (12.4)	5 (14.7)	
Some College	79 (35.1)	22 (64.7)	
College Graduate and Above	118 (52.4)	7 (20.6)	
Physical Activity: n(%)			0.65
Low	23 (10.2)	2 (5.9)	
Moderate	81 (36.0)	11 (32.4)	
High	121 (53.8)	21 (61.8)	
Previous Pregnancy: n(%)			0.04
No	99 (56.6)	12 (85.7)	
Yes	76 (43.4)	2 (14.3)	
Missing	50	20	
Past OC use: n(%)			0.03
No	95 (42.6)	20 (62.5)	
Yes	128 (57.4)	12 (37.5)	
Missing			
Measures of adiposity			
BMI, (kg/m^2): mean ± SD	24.2± 3.9	25.0± 3.4	0.08
BMI, (kg/m^2) Quartiles: n(%)			0.23
Q1 (16.1-21.0)	53 (23.6)	12 (35.3)	
Q2 (21.0-23.5)	56 (24.9)	9(26.5)	
Q3 (23.6-26.2)	56 (24.9)	9 (26.5)	
Q4 (26.3-35.0)	60 (26.7)	4 (11.8)	
Waist to hip ratio (cm): mean ± SD	0.75 ± 0.06	0.75 ± 0.04	0.57
% Body Fat: mean ± SD	29.8 ± 6.1	27.7 ± 4.8	0.08
% Truncal Fat: mean ± SD	25.4 ± 7.5	23.0 ± 6.1	0.12
Truncal/Leg Fat: mean ± SD	0.71 ± 0.15	0.69 ± 0.16	0.67
Total skinfold thickness (mm): mean ± SD	81.9 ± 23.1	76.4 ± 14.2	0.18

[1]P-values obtained from chi-square tests or Fishers exact for categorical variables and T-Tests for Continuous variables

[b]At least one anovulatory cycle. 24 women had 1 anovulatory cycle and 10 had 2 anovulatory cycles. Total of 44 anovulatory cycles out of 509 in the study.

Table 4. Odds ratios and 95% CI of anovulation by baseline adiposity measures; BioCycle Study

	Cases		Unadjusted		Multivariable[1]	
	N	**%**	**OR**	**95% CI**	**OR**	**95% CI**
BMI Quartiles						
Q1 (16.1-21.0)	12	35.3	1.00	Referent	1.00	Referent
Q2 (21.0-23.5)	9	26.5	0.71	(.28-1.82)	0.66	(.24-1.80)
Q3 (23.6-26.2)	9	26.5	0.71	(.28-1.82)	0.73	(.26-2.01)
Q4 (26.3-35.0)	4	11.8	0.29	(.090-0.99)	0.38	(.106-1.33)
Continuous BMI	34		0.91	(0.82-1.01)	0.93	(0.83-1.04)
%Body Fat Quartiles						
Q1 (15.1-24.7)	8	3.23	1.00	Referent	1.00	Referent
Q2 (24.8-29.8)	10	4.03	1.3	(.475-3.55)	1.05	(.36-3.10)
Q3 (29.9-33.5)	6	2.42	0.72	(.235-2.22)	0.69	(.21-2.29)
Q4 (33.6-44.3)	3	1.21	0.34	(.087-1.36)	0.54	(.13-2.32)
%Body Fat Continuous	27		0.941	(.878-1.01)	0.96	(.89-1.03)
%Truncal Fat Quartiles						
Q1 (10.6-19.0)	10	4.03	1.00	Referent	1.00	Referent
Q2 (19.1-24.8)	6	2.42	0.56	(.189-1.64)	0.38	(.11-1.20)
Q3 (24.9-30.9)	8	3.23	0.77	(.282-2.10)	0.83	(.28-2.49)
Q4 (31.0-45.0)	3	1.21	0.26	(.069-1.01)	0.39	(.09-1.64)
%Truncal Fat Continuous	27		0.956	(.903-1.01)	0.97	(.91-1.04)
Truncal/Leg Fat Quartiles						
Q1 (.364-.590)	8	3.23	1.00	Referent	1.00	Referent
Q2 (.591-.705)	5	2.02	0.592	(.182-1.92)	0.58	(.17-2.02)
Q3 (.706-.801)	7	2.82	0.859	(.291-2.53)	0.77	(.24-2.45)
Q4 (.802-1.14)	7	2.82	0.859	(.291-2.53)	1.34	(.41-4.41)
Truncal/Leg Fat Continuous	27		0.581	(.040-8.37)	1.14	(.06-22.17)
Waist to Hip ratio Quartiles						
Q1 (.60-.717)	6	2.34	1.00	Referent	1.00	Referent
Q2 (.718-.746)	12	4.69	2.15	(.754-6.14)	1.75	(.58-5.22)
Q3 (.747-.781)	9	3.52	1.5	(.501-4.49)	2.01	(.63-6.43)
Q4 (.782-1.16)	7	2.73	1.21	(.382-3.83)	1.84	(.54-6.26)
Waist to Hip ratio Continuous	34		0.127	(<.001-136.09)	5.01	(.003-999.99)
Total Skinfold Thickness Quartiles						
Q1 (32.8-66.0)	9	3.53	1.00	Referent	1.00	Referent
Q2 (66.1-78.0)	11	4.31	1.39	(.533-3.62)	1.19	(.43-3.29)
Q3 (78.1-94.0)	10	3.92	1.22	(.459-3.22)	1.37	(.48-3.92)
Q4 (94.1-156.5)	4	1.57	0.437	(.127-1.50)	0.59	(.16-2.16)
Total Skinfold Thickness Continuous	34		0.988	(.971-1.01)	0.99	(.97-1.02)

[1]Multivariable model adjusted for age.

Table 5. Characteristic of women younger than 22 years; BioCycle Study 2005-2007.

	All	Ovulatory	Anovulatory
BMI			
Q1 (16.1-21.0)	22 (24.18)	14 (21.5)	8 (30.8)
Q2 (21.0-23.5)	27 (29.67)	19 (29.2)	8 (30.8)
Q3 (23.6-26.2)	25 (27.47)	17 (26.2)	8 (30.8)
Q4 (26.3-35.0)	17 (18.68)	15 (23.1)	2 (7.7)
Time since menarche (TSM)			
3	1 (1.11)	0 (0.00)	1 (3.4)
4	2 (2.22)	0 (0.00)	2 (7.7)
5	9 (10.00)	5 (7.8)	4 (15.4)
6	13 (14.44)	9 (14.1)	4 (30.77)
7	20 (22.22)	16 (25.0)	4 (15.4)
8	21 (23.33)	14 (21.9)	7 (26.9)
9	14 (15.56)	11 (17.2)	3 (11.5)
10	10 (11.11)	9 (14.1)	1 (3.9)
Missing	1		
Age			
18	9 (9.89)	5 (7.7)	4 (15.4)
19	30 (32.97)	23 (35.4)	7 (26.9)
20	34 (37.36)	23 (35.4)	11 (42.3)
21	18 (19.78)	14 (21.5)	4 (15.4)
Odds Ratio for anovulation		Unadjusted	
		OR	**95% CI**
TSM Continuous		0.69	(.51-.94)

Table 6. Characteristics of women 22 years and older; BioCycle Study 2005-2007.

	All	Ovulatory	Anovulatory
BMI			
Q1 (16.1-21.0)	43 (25.60)	39 (24.4)	4 (50.0)
Q2 (21.0-23.5)	38 (22.62)	37 (23.1)	1 (12.5)
Q3 (23.6-26.2)	40 (23.81)	39 (24.4)	1 (12.5)
Q4 (26.3-35.0)	47 (27.98)	45 (28.1)	2 (25.0)
Time since menarche (TSM)			
3	0 (0.00)	0 (0.00)	0 (0.00)
4	0 (0.00)	0 (0.00)	0 (0.00)
5	0 (0.00)	0 (0.00)	0 (0.00)
6	0 (0.00)	0 (0.00)	0 (0.00)
7	0 (0.00)	0 (0.00)	0 (0.00)
8	1 (.61)	0 (0.00)	1 (16.7)
9	14 (8.48)	11 (6.9)	3 (50.0)
10	8 (4.85)	8 (5.0)	0 (0.00)
11	11 (6.67)	11 (6.9)	0 (0.00)
12	14 (8.48)	13 (8.2)	1 (16.7)
13	9 (5.45)	9 (5.7)	0 (0.00)
14	8 (4.85)	8 (5.0)	0 (0.00)
15	11 (6.67)	11 (6.9)	0 (0.00)
16	3 (1.82)	3 (1.9)	0 (0.00)
17	4 (2.42)	4 (2.5)	0 (0.00)
18	2 (1.21)	1 (0.6)	1 (16.7)
19	4 (2.42)	4 (2.5)	0 (0.00)
20	4 (2.42)	4 (2.5)	0 (0.00)
21	3 (1.82)	3 (1.9)	0 (0.00)
22	7 (4.24)	7 (4.4)	0 (0.00)
23	5 (3.03)	5 (3.1)	0 (0.00)
24	5 (3.03)	5 (3.1)	0 (0.00)
25	9 (5.45)	9 (5.7)	0 (0.00)
26	4 (2.42)	4 (2.5)	0 (0.00)
27	6 (3.64)	6 (3.8)	0 (0.00)
28	8 (4.85)	8 (5.0)	0 (0.00)
29	6 (3.64)	6 (3.8)	0 (0.00)
30	10 (6.06)	10 (6.3)	0 (0.00)
31	4 (2.42)	4 (2.5)	0 (0.00)
32	4 (2.42)	4 (2.5)	0 (0.00)
33	1 (.61)	1 (0.6)	0 (0.00)
Missing	3		

Odds Ratio for anovulation	Unadjusted	
	OR	**95% CI**
TSM Continuous	0.71	(.50-1.00)

Table 7. Age stratified results for association between BMI and anovulation; BioCycle Study 2005-2007.

	Unadjusted		Multivariable[1]		Anovulation
	OR	**95% CI**	**OR**	**95% CI**	Frequency n(%)
Where age < 22					
N	91				26
BMI Quartiles					
Q1 (16.1-21.0)	1.00	Referent	1.00	Referent	8 (30.8)
Q2 (21.0-23.5)	0.74	(.222-2.44)	0.82	(.238-2.79)	8 (30.8)
Q3 (23.6-26.2)	0.82	(.246-2.76)	0.86	(.255-2.91)	8 (30.8)
Q4 (26.3-35.0)	0.23	(.042-1.29)	0.23	(.048-1.40)	2 (7.7)
Continuous BMI	0.89	(.763-1.04)	0.89	(.763-1.04)	

[1]Multivariable model adjusted for age.

	Unadjusted		Multivariable[1]		Anovulation
	OR	**95% CI**	**OR**	**95% CI**	Frequency
Where age ≥ 22					
N	168				8
BMI Quartiles					
Q1 (16.1-21.0)	1.00	Referent	1.00	Referent	4 (50.0)
Q2 (21.0-23.5)	0.26	(.028-2.47)	0.27	(.028-2.65)	1 (12.5)
Q3 (23.6-26.2)	0.25	(.027-2.34)	0.35	(.035-3.39)	1 (12.5)
Q4 (26.3-35.0)	0.43	(.075-2.50)	0.79	(.126-4.91)	2 (25.0)
Continuous BMI	0.94	(.781-1.14)	0.99	(.821-1.91)	

[1]Multivariable model adjusted for age.

Table 8. BMI distributed by quintiles and sextiles; BioCycle Study 2005-2007.

	Ovulatory	Anovulatory
BMI, (kg/m2) Quintiles: n(%)		
Q1 (16.14-20.47)	42 (18.7)	10 (29.4)
Q2 (20.48-22.61)	44 (19.6)	8 (23.5)
Q3 (22.62-24.43)	46 (20.4)	6 (17.7)
Q4 (24.44-27.03)	46 (20.4)	6 (17.7)
Q5 (27.04-34.98)	47 (20.9)	4 (11.8)
BMI, (kg/m2) Sextiles: n(%)		
Q1 (16.14-20.21)	36 (16.0)	8 (23.5)
Q2 (20.22-22.12)	34 (15.1)	9 (26.5)
Q3 (22.13-23.46)	39 (17.3)	4 (11.8)
Q4 (23.47-25.07)	38 (16.9)	5 (14.7)
Q5 (25.08-27.97)	39 (17.3)	4 (11.8)
Q6 (27.98-34.98)	39 (17.3)	4 (11.8)

	Unadjusted		Multivariable[1]	
	OR	**95% CI**	**OR**	**95% CI**
BMI Quintiles				
Q1 (16.14-20.47)	1.00	Referent	1.00	Referent
Q2 (20.48-22.61)	0.76	(.275-2.12)	0.87	(.291-2.60)
Q3 (22.62-24.43)	0.55	(.183-1.64)	0.522	(.163-1.67)
Q4 (24.44-27.03)	0.55	(.183-1.64)	0.621	(.192-2.01)
Q5 (27.04-34.98)	0.36	(.104-1.23)	0.53	(.141-1.99)
BMI Sextiles				
Q1 (16.14-20.21)	1.00	Referent	1.00	Referent
Q2 (20.22-22.12)	1.19	(.412-3.44)	1.204	(.376-3.85)
Q3 (22.13-23.46)	0.46	(.128-1.67)	0.358	(.093-1.38)
Q4 (23.47-25.07)	0.59	(.177-1.98)	0.523	(.143-1.92)
Q5 (25.08-27.97)	0.46	(.128-1.67)	0.616	(.154-2.47)
Q6 (27.98-34.98)	0.46	(.128-1.67)	0.507	(.127-2.02)

Table 9. Odds ratios and 95% CI of anovulation per cycle by adiposity measures: BioCycle Study 2005-2007

	Unadjusted		Multivariable[1]	
	OR	**95% CI**	**OR**	**95% CI**
BMI Quartiles				
Q1 (16.1-21.0)	1.00	Referent	1.00	Referent
Q2 (21.0-23.5)	0.67	(0.256-1.73)	0.65	(0.243-1.71)
Q3 (23.6-26.2)	0.66	(0.254-1.70)	0.67	(0.251-1.77)
Q4 (26.3-35.0)	0.20	(0.063-0.650)	0.26	(0.075-1.15)
Continuous BMI	0.89	(0.805-0.98)	0.90	(0.803-1.01)
%Body Fat Quartiles				
Q1 (15.1-24.7)	1.00	Referent	1.00	Referent
Q2 (24.8-29.8)	1.69	(0.611-4.67)	1.35	(0.466-3.90)
Q3 (29.9-33.5)	0.98	(0.251-2.49)	0.76	(0.246-2.35)
Q4 (33.6-44.3)	0.28	(0.073-1.10)	0.44	(0.103-1.90)
%Body Fat Continuous	0.94	(0.889-0.985)	0.95	(0.895-1.01)

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