COMPARISON OF BODY COMPOSITION MEASUREMENTS ACROSS PHASE OF
THE MENSTRUAL CYCLE: HYDROSTATIC WEIGHING, AIR-DISPLACEMENT
PLETHYSMOGRAPHY VS THREE COMPARTMENT MODEL

By

Kate A. Reynolds

A Thesis Presented to
The Faculty of Humboldt State University
In Partial Fulfillment of the Requirements for the Degree
Master of Science in Kinesiology: Exercise Science

Committee Membership
Rock Braithwaite, Ed.D., Graduate Coordinator, Committee Chair
Tina Manos, Ed.D., Committee Member
Young Sub Kwon, Ph.D., Committee Member
Patrick Hyland, M.S., Committee Member

May 2015
Abstract

COMPARISON OF BODY COMPOSITION MEASUREMENTS ACROSS PHASE OF THE MENSTRUAL CYCLE: HYDROSTATIC WEIGHING, AIR-DISPLACEMENT PLETHYSMOGRAPHY VS THREE COMPARTMENT MODEL

Kate Alise Reynolds

Purpose: The purpose of this study was compare %BF values measured by BODPOD, HW to the Siri 3-Compartment Model equation, and to determine if the fluctuations of body weight as a result of fluid retention during the luteal phase of the menstrual cycle will affect the results of body composition measurements. Methods: Twenty subjects, 18-26 yrs old, participated in this study. There were 9 female, no birth control (FNBC), 6 female, birth control (FBC), and 5 Control (male) subjects. Each subject was assessed three times over the course of one month or one menstrual cycle. Four values of %BF were obtained, BODPOD, HW, Siri3C_{BODPOD}, and Siri3C_{HW}. Results: Three by three Repeated Measures ANOVA revealed significance difference (p<0.05) between HW and Siri3C_{BODPOD} for FNBC and FBC (p<0.000) and the Control (p=0.001). There was significance between HW and Siri3C_{HW} for FNBC (p<0.000), FBC (p=0.006), and Control (p=0.001). There was no significance between times of assessment for all groups. Significant changes between TBW and weight were not detected. Conclusion: The "sex bias" still needs further investigation. Further research needs to investigate changes in TBW and weight across the menstrual cycle, and if potential changes influence %BF.
Acknowledgements

In addition to thanking my committee, Dr. Rock Braithwaite, Dr. Tina Manos, Dr. Young Sub Kwon, and Patrick Hyland for supporting and encouraging me to pursue my thesis topic, I want to give a special thanks to my research assistants, Jessica Lorge and Sara Benson for your help and tolerating me throughout this process. Thank you to friend Madison Kirkpatrick for your support and peer reviewing my paper countless times; to my friends Cassandra Winczura and Katlyn Mannatt for all of your support; and to the love of my life Taylor Swain for all of your love, comfort, and support.

Most importantly to my family who if it wasn't for them, this would not have been possible. And greatest thanks of all, to my mother Sherrie Reynolds, for keeping me level headed throughout this process and raising me to be ambitious, strong young woman.
# Table of Contents

Abstract ............................................................................................................................... ii  
Acknowledgements ............................................................................................................ iii  
List of Tables ..................................................................................................................... vi  
List of Figures ................................................................................................................... vii  
Introduction ......................................................................................................................... 1  
  Methods of Body Composition Measurements .............................................................. 2  
  Air Displacement Plethysmography ............................................................................... 3  
    Factors that Influence Reliability and Validity of ADP .................................................. 5  
  Gender Bias ..................................................................................................................... 8  
  Menstrual Cycle ............................................................................................................. 10  
  Luteal Phase .................................................................................................................. 11  
  Measuring TBW Fluctuations During the Menstrual Cycle ............................................ 12  
  Ovulation Tests ............................................................................................................. 14  
Method .............................................................................................................................. 17  
  Participants .................................................................................................................... 17  
  Experimental Procedures .............................................................................................. 18  
Body Composition Assessment Tests .................................................................................. 19  
  Bioelectrical Impedance Analysis ............................................................................. 19  
  Air Displacement Plethysmography ........................................................................... 20  
  Hydrostatic Weighing ................................................................................................. 22  
  Three Compartment Model ....................................................................................... 23
Statistical Analysis ........................................................................................................ 23
Results ............................................................................................................................ 25
Discussion ....................................................................................................................... 32
References ....................................................................................................................... 39
Appendix A: Recruitment Flyers .................................................................................. 45
Appendix B: Permission to Recruit Letter ..................................................................... 47
Appendix C: Informed Consent ...................................................................................... 49
Appendix D: Questionnaire ......................................................................................... 56
Appendix E: Testing Order Chart .................................................................................. 60
Appendix F: FNBC Testing Chart ................................................................................ 61
Appendix G: FBC Testing Chart-Example ..................................................................... 62
List of Tables

Table 1: Subject Characteristics ................................................................. 27

Table 2: Mean Weight, TBW, %BF, and %BF Siri3C for FNBC, FBC, Control Groups 28
List of Figures

Figure 1: *Siri 3C Equation* ................................................................. 23

Figure 2: *Mean %BF for FNBC per Session for HW, BODPOD, Siri3C HW and Siri3C BODPOD* ................................................................. 29

Figure 3: *Mean %BF for FBC per Session for HW, BODPOD, Siri3C HW and Siri3C BODPOD* ................................................................. 29

Figure 4: *Mean %BF for FBC per Session for HW, BODPOD, Siri3C HW and Siri3C BODPOD* ................................................................. 30

Figure 5: *Mean TBW per Session for FNBC, FBC, and Control* ............ 31

Figure 6: *Mean Weight per Session for FNBC, FBC, and Control* ........... 31
Introduction

Body composition assessment is a popular method of obtaining information to build training and weight loss programs for endurance athletes and those who want to change their body fat percentage. Endurance athletes strive to reach an optimal body composition to maximize their performance abilities. A lower body fat percentage (%BF) has been found to be correlated with enhanced performance (Fahey, Akka, & Rolph, 1975; Loucks, 2004; McLester, Green, Wickwire, & Crews, 2008). Body composition assessments should be easily accessible, valid and reliable. However, it has been suggested that there is “gender bias” when using certain methods of assessing body composition (Fields et al., 2001; Vescovi, Hildenbrandt, Miller, Hammer, and Spiller, 2002). Many studies regarding body composition testing indicate that some methods overestimate for some populations and underestimate for others (Bentzur, Kravitz, and Lockner 2008; Biaggi et al., 1999; Levenhagen et al., 1999; Vescovi et al., 2002) which may result in clients receiving inaccurate results to build training and weight loss programs. Therefore, there is a need to determine consistency of results within individuals. Many factors, both physiological and technical, can influence percent body fat (%BF) results from various methods of assessment.

Variation of hydration status may be especially influential on estimates of body composition results. During the female menstrual cycle, increases in the sodium to potassium ratio occurs due to the increases in progesterone concentrations during the luteal phase (Frankovich and Lebrun, 2000). Progesterone may be the cause of water
retention, a premenstrual symptom, which consequently may result in fluctuations in body density measurements upon which body fat percentage is determined (O’Brien, Selby, & Symonds, 1980). Body composition assessments over different phases of the menstrual cycle could influence estimations of %BF, thus suggesting that timing of body composition measurements in female athletes during the menstrual cycles is critical for reliability and repeatability. Technical error, such as not calibrating the equipment prior to testing, and the environmental conditions for some methods, such as room temperature and pressure, can also result in incorrect estimations of %BF. Several methods of body composition assessment are available and are discussed below.

**Methods of Body Composition Measurements**

Body composition can be assessed in a variety of ways, including skinfolds (SF), hydrostatic weighing (HW), bioelectrical impedance analysis (BIA), Dual Energy X-Ray Absorptiometry (DEXA), and air displacement plethysmography (ADP). SF are measured by pinching the skin and underlying subcutaneous adipose tissue at various anatomical sites with calipers. HW, or hydrodensitometry, measures body volume based on the use of Archimedes' Principle, which states that the volume of an object is equal to the object's loss of weight in water, with corrections that are appropriate for the density of water (Dempster & Aitkens, 1995). Estimates of body fat through a resistance of flow from an electrical current through the body tissues are determined through BIA. Lean tissue consists primarily water, which is a good conductor of electricity. Fatty tissue, which is very low in water, is a poor conductor, and will provide resistance. Thus, BIA estimates
resistance to calculate total body water (TBW), fat-free mass (FFM), and %BF. DEXA
generates low dose X-rays, and estimates %BF through an interface with a computer
system for the imaging of the scanned areas (Ghoch et al., 2012). The DEXA measures
body fat mass (FM), FFM, and bone mineral density. ADP is a methodology to estimate
%BF by measuring body volume of air displaced by an individual when placed in an
empty chamber. The previously stated gender bias is seen when using ADP via the
BODPOD as the method of body composition assessment and thus will be the primary
focus for this research.

**Air Displacement Plethysmography**

The BODPOD (COSMED, Concord, CA) is an easily accessible method of
measuring body composition for many laboratories. The BODPOD uses ADP as a
method of measuring body composition. ADP is a method of determining body volume
(BV) based on the pressure-volume relationship. Isothermal and adiabatic conditions can
have significant effects on the accurate measurement of BV determined by
plethysmography (Dempster & Aitkens, 1995). ADP relies on the understanding and
application of Boyle’s Law, and Poisson’s Law. Boyle’s law states the product of
pressure and volume will remain constant when the amount of air compressed under
isothermal conditions decrease in volume proportionally to an increase in pressure,
\[ P_1/P_2 = V_2/V_1 \] (Dempster & Aitkens, 1995). However, under adiabatic conditions,
temperature of air does not remain constant as its volume changes, which is described by
Poisson’s Law. Poisson's Law describes the relationship between the pressure and
volume of air under adiabatic conditions. Poisson's Law states that \( \frac{P_1}{P_2} = \frac{V_2}{V_1} \gamma \), where \( \gamma \) is described as the ratio of the specific heat of a gas at a constant pressure to that of constant volume (Dempster & Aitkens, 1995).

Dempster and Aitkens (1995) were the first to report a detailed description of the BODPOD. The BODPOD contains two chambers in a single structure. Subjects sit in the front chamber of the BODPOD, where a molded fiberglass seat forms a wall that separates the front chamber from the rear chamber. The door is sealed by electromagnets during data collection, and has a large acrylic window, which allows the subject a comfortable environment that minimizes any potential events of claustrophobia. The rear chamber, which is the reference chamber, contains pressure transducers, electronics, breathing circuit, valves and an air circulation system. There is a mounted diaphragm between the 450 L front chamber and the 300 L rear chamber. During body composition assessment, this diaphragm oscillates back and forth to create sinusoidal volume perturbations in the two chambers. The volume perturbations are exactly equal in magnitude but opposite in sign, and lead to small and complementary pressure fluctuations in the two chambers. Along with the sinusoidal volume perturbations, Fourier coefficients are used to calculate pressure amplitude at the frequency of oscillation, which rejects noise at other frequencies and higher harmonies. These two variables, sinusoidal perturbations and Fourier coefficients, effectively eliminate the deleterious effects of temperature change during the measurement (Dempster & Aitkens, 1995).
Factors that Influence Reliability and Validity of ADP

There are two different air conditions that influence the pressure-volume relationship previously mentioned, isothermal conditions and adiabatic conditions. A gas under isothermal conditions will maintain a constant temperature, whereas adiabatic conditions exist when a gas is under constant changing temperatures (Fields, Higgins, & Radley, 2005). Air under isothermal conditions is 40% more compressible than air under adiabatic conditions. Isothermal air will also, for a given body volume, produce a lower pressure output signal. This will result in underestimation of body volume. Isothermal, as well as adiabatic conditions are minimized in the BODPOD through the oscillating diaphragm that was previously described, as well as a mechanism that circulates the air within the chamber (Dempster & Aitkens, 1995). The BODPOD controls for the air conditions that may occur during body composition assessments.

Body hair may also have an effect on ADP results. Higgins, Fields, Hunter, and Gower (2001) investigated the potential for these effects. The authors tested 25 men under four conditions: 1) beard shaved and swim cap worn, 2) facial hair present and swim cap worn, 3) facial hair present and no swim cap, and 4) no facial hair and no scalp hair. Body hair, facial hair, beards, and scalp hair was found to cause an underestimation of %BF (Higgins et al., 2001).

Vescovi, Zimmerman, Miller and Fernhall (2002) studied the effects of clothing on the reliability and accuracy of ADP. It had been previously determined that the isothermal conditions of cloth are easily compressed and can cause a negative volume during ADP measurements (Dempster and Aitkens, 1995). The authors theorized that
clothing as minimal as a swimsuit would affect measurements in ADP, and that nude conditions may provide more accurate results (Vescovi et al., 2002). The primary finding of Vescovi et al. (2002) was that there was a significant underestimation of body fat percentage when the subjects wore a hospital gown during ADP measurements when compared with swimsuit or nude conditions. Nude conditions did not result in significantly different body fatness when compared to the swimsuit condition. From this study, it was determined that ADP results are effected by excess clothing due to isothermal conditions; and although there was a high sensitivity to clothing, nude vs. swimsuit conditions did not result in a significant difference in the accuracy and reliability of the %BF measures (Vescovi et al., 2002).

Isothermal conditions may not only be a result of body hair or clothing, but also air from the lungs (Dempster & Aitkens, 1995). When a subject enters the BODPOD, the air that is close to the skin of the subject, hair, and clothing will be isothermal, meaning the temperature will change. However, the large amount of air in the lungs will remain in isothermal conditions. Since there are many factors, such as lung volume and surface area artifact (i.e. skin, hair and clothing) that will alter the accuracy of the body volume measurements, it is necessary to eliminate or account for those factors. One such way to account for the isothermal conditions of the air in the lungs is by measuring thoracic gas volume ($V_{TG}$). The $V_{TG}$ measures the lung air volume and air that could be trapped in the thorax.

$V_{TG}$ air is under isothermal conditions and the air inside the front chamber is under adiabatic conditions. This results in the volume of air in the chamber seeming to be
greater than what it actually is by 40%. The BODPOD corrects for this overestimation, otherwise there would be a 40% underestimation of %BF if this correction was failed to be made (Demptster & Aitkens, 1995; McCrory, Mole, Gomez, Dewey, & Bernauer, 1998). There is an alternative to the measured VTG, which is a predicted thoracic gas volume (VTGpredicted). The VTGpredicted is beneficial when trying to save time and test a large group of subjects, or one subject repeatedly. McCrory et al. (1998) found that VTGpredicted and measured VTG did not differ significantly in a sample of 50 subjects, comprised of 36 women and 14 men.

Environment is also a major factor that can influence the reliability and validity of the BODPOD. ADP relies on pressure measurements, and pressure is affected by volume and temperature. If the room temperature or the chamber temperature changes, then the pressure will change; if the pressure in the room is changed (e.g. the door to the testing room is left open) then the volume measurements will be affected. It is important to ensure reliability among different BODPOD units. Ball (2005) investigated the interdevice variability in %BF between two BODPOD units. Both units were placed into the same room, under the same room conditions and operated by one technician. Two tests were performed in immediate succession for 50 subjects. The author found an agreement between both group and individual measurements of %BF. It was also suggested that any errors or variability were due to technological and biological factors rather than variations in manufacturing (Ball, 2005). Collins et al. (2004) investigated within and between laboratory accuracy of measuring %BF using ADP. The between measurements, or reproducibility, showed that sex did not cause any significant
differences in %BF. The within laboratory assessment, or repeatability, showed greater imprecision due to the inconsistencies, although minimal, with biological factors such as skin and lung temperature, clothing, movement in the chamber. The authors determined that the change in temperature was significant between laboratories. However, only the bias from the first laboratory was significant. Thus the changes in temperature between the initial body composition assessment and the return assessment were significantly related to the differences in body volume measurements.

**Gender Bias**

The accuracy of body composition assessment between and within methods has continuously been investigated. Bentzur, Kravitz, and Lockner (2008) evaluated ADP estimated %BF with other methods such as HW, DEXA and SF. However, there were noticeable variations in estimations of %BF among different methods of measuring body fat percentage, most particularly in females. Researchers that have tested female subjects find that ADP overestimates %BF compared to multiple methods such as HW (Bentzur et al., 2008; Levenhagen et al., 1999; Vescovi et al., 2002). Studies that look at male subjects, however, find an underestimation of %BF using ADP (Biaggi et al., 1999; Levenhagen et al., 1999). Fields et al. (2001) found an underestimation in body fat percentage in females when comparing the ADP to the Four Compartment Model (4-C Model). The authors suggested that there is a possible limitation in measuring lean subjects, as well as a possible gender bias by the BODPOD (Bentzur et al., 2008). Ball
(2005) found that differences between two BODPOD units produced a higher variability for female subjects than male subjects.

There are possible explanations for the variations in body fat percentages among female subjects when using the BODPOD. However, no studies have attempted to determine the causes of variations. One possible explanation for the discrepancies in estimations of %BF is that changes over the menstrual cycle may influence factors that determine %BF from the BODPOD. The menstrual cycle has three phases: the follicular, ovulatory, and luteal phases. During the luteal phase, there is an increase in progesterone which may cause extra water retention. Water retention may lead to increased weight that could cause variations in body composition measurement.

Previous researchers have shown that there were no significant differences in body weight and %BF between the follicular and luteal phase (Lebrun, McKenzie, Prior & Taunton, 1995) when body fatness was assessed with SF and HW. In these studies the phase of menstrual cycle was determined by the amount of premenstrual symptoms and basal body temperature, confirmed with serum hormone levels. However, ADP was not studied. It is therefore not known if changes in body water over the menstrual cycle would result in significant differences in estimated %BF when using ADP.

Moon et al. (2009) investigated the validity of various laboratory methods for estimating %BF in female NCAA Division I athletes and found that ADP overestimated %BF by as much as 5.38% compared to the five compartment (5-C) model and underestimated the leaner subjects by as much as 5.19%. The authors suggested that due to changes in peak body mass during the menstrual cycle, using 2 compartment (2-C)
methods of body composition assessment would lead to inaccurate estimations of %BF. However, these researchers did not control for the menstrual cycle, but suggested using a three compartment (3-C) equation that would account for total body water (TBW) to consider the weight fluctuations due to water retention during the menstrual cycle (Moon et al., 2009).

Menstrual Cycle

A "normal" menstrual cycle lasts 28 days, but young females' menstrual cycle can vary from 25-34 days (Mihm, Gangooly, & Muttukrishna, 2011). Controlled by hypothalamic, hypophyseal, and ovarian hormones, the menstrual cycle promotes many changes in various parts of the body (Constantini, Dubnov, & Lebrun, 2005). The menstrual cycle is often divided up into three phases, follicular, ovulatory, and luteal phase. The follicular phase of the menstrual cycle starts on the first day of the menses, and on average lasts 9 days, but can last from 10-23 days (Constantini et al., 2005; Mihm et al., 2011). During this phase, the follicles are grown due to the influence of hypophyseal follicle-stimulating hormone (FSH). Around the follicle, cells secrete estrogen and thus increases the hormone levels. This in turn will stimulate the secretion of the hypophyseal luteinizing hormone (LH) (Constantini et al, 2005).

There is a surge of LH after the increase in estrogen and, one day later, ovulation occurs. This marks the beginning of the ovulatory phase which, on average, lasts approximately 5 days. The ovulatory phase prepares the uterus for receiving an embryo by thickening of the endometrium. The follicle then releases the ovum, and a few days
later it becomes the corpus luteum, which secretes progesterone and initiates the luteal phase. The luteal phase lasts about 14 days, but can vary between 7 and 19 days in some women. At the end of this phase the secretion of progesterone from the corpus luteum stops, the endometrium is then no longer supported, and the endometrium releases as menstrual bleeding (Constantini et al., 2005; Mihm et al., 2011). When the estrogen and progesterone levels decrease during the menses, FSH starts to secrete again and starts the cycle all over again.

**Luteal Phase**

When looking at exercise body composition in female athletes, the most important phase of the menstrual cycle is the luteal phase. There are impairments in physical performance during the late luteal phase that are associated with premenstrual symptoms. These symptoms can include fatigue, fluid retention, weight gains, fluctuating moods, increase in basal body temperature, and dysmenorrhoea (Giacomoni, Bernard, Gavarry, Altare & Falgairette, 2000). Whether or not symptoms are minor, Constantini et al. (2005) stated that premenstrual symptoms can prevent athletes from reaching full performance capabilities. Increased estrogen levels help signal the LH surge, and is followed by the increase of progesterone which is responsible for increased water retention (Frankovich and Lebrun, 2000). The primary physiological indicators of predicting ovulation are the LH surge and basal body temperature that occur during the luteal phase, both of which can be determined through ovulation tests.
Measuring TBW Fluctuations During the Menstrual Cycle

Hydrostatic Weighing (HW) has been considered the "gold standard" for body composition assessment in most laboratory settings when DEXA is not available. The methods of HW are derived from Archimedes' Principle, which states that the upward buoyant force that is exerted on a body immersed in a fluid is equal to the weight of the fluid that the body displaces. HW uses three values: weight of the dry body, weight when completely immersed under water, and the density of water. FFM is denser than FM and thus FFM contribute to the density of the body immersed in water while FM floats. Body density is then calculated based on the weight under water. Further, body density (Db) is used to calculate %BF using a given equation for the respected population, the most commonly used equation is the Siri equation:

$$\%BF = \left( \frac{495}{\text{BodyDensity}} \right) - 450.$$

Byrd and Thomas (1983) investigated weight fluctuations during the menstrual cycle and if those fluctuations were significant enough to affect body density and %BF measurements through HW. The authors did not find significant weight differences between the two phases of the menstrual cycle in non-obese young females. However, Bunt, Lohman and Boileau (1989) determined that Byrd and Thomas (1983) did not accurately measure each stage of the menstrual cycle. Therefore, Bunt et al., (1989) calculated TBW to measure water weight changes and body density through HW. The authors found significant changes in Db in relation to changes in TBW. It was noted by
the authors, however, that TBW fluctuations could not account for the changes in Db alone (Bunt et al., 1989).

The most common form used to measure changes in body composition during the menstrual cycle is with the use of Bioelectrical Impedance Analysis (BIA). Resistance and reactance is measured through BIA. Resistance is a measure of the amount of electrical current that a substance, in this case, fat, will stop. Reactance is a measure of a material's ability to slow a current. These two values are able to be used in an equation that will calculate %BF, TBW, extracellular water (ECW), and intracellular water (ICW). Extracellular water accounts for 20-30% of body weight, and ICW accounts for 35-45% of body weight, therefore TBW accounts for 55-65% of body weight. (Shanholtzer & Patterson, 2003). Gleinchauf and Roe (1989) investigated the reliability of estimating body composition from BIA during a single menstrual cycle. The researchers measured mean ± SD resistance, reactance, TBW (L), FFM, FM, and %BF in 26 regularly menstruating women during four predetermined phases of the menstrual cycle. Ovulation tests that predict the luteinizing hormone were used to determine menstrual cycle phase. The authors did not find any significant changes during different phases of the menstrual cycle however, changes were only monitored for one menstrual cycle, and hydration status and skin temperature was not monitored. The authors did not believe that the small changes were due to the water retention that presumably occurs during the menstrual cycle. There was also no additional method of body composition to compare changes in a menstrual cycle (Gleinchauf and Roe, 1989).
McKee and Cameron (1997) attempted to determine changes in body weight and BIA during one menstrual cycle in women and a control group of men. The researchers also found that there were significant body weight changes during the cycle, though it was not of a large enough magnitude to influence FFM and TBW. They determined that body weight changes are not fluid related and BIA is not sensitive enough to determine if body weight fluctuations are due to water retention. However, this study did not involve the use of ovulation tests to predict phase of menstrual cycle during assessment and there were not measures for multiple menstrual cycles (McKee & Cameron, 1997). Shanholtzer & Patterson, (2003) investigated using BIA as a hydration status assessment required female subjects to be scheduled during the follicular phase of their menstrual cycle to control for water retention that occurs in the luteal phase. It has been discussed previously that water retention or body weight changes are seen in the luteal phase. Accurate determination of menstrual cycle phase is important for accurately determining body composition changes, and especially with BIA as it can determine TBW changes. Thus, estimating phase of menstrual cycle over the course of two to three cycles can provide the most accuracy of estimated body composition changes for various methods of body composition assessment.

**Ovulation Tests**

An accurate method of determining when the LH surge will occur is through the use of at-home ovulation tests (Nielsen, Barton, Hataska, and Stanford, 2001). At-home ovulation tests are used on a daily basis starting on a given day after the first day of
menses, as instructed per the manufacturer's suggestions. The tests are used every day until a surge in the LH is detected (Fehring, Raveile and Schneider, 2004). The typical use of ovulation tests require anywhere from 10 to 20 daily urine tests per cycle (Fehring, et al., 2004). Using an at-home ovulation test daily will allow a female to accurately know when the LH surge occurs and thus can predict hormone fluctuations.

**Purpose**

It did not appear that the phase of menstrual cycle was reported or controlled for in previous research while using ADP to assess body composition in female subjects (Bentzur et al., 2008; Fields et al., 2001; Moon et al., 2009; Vescovi et al., 2002). One study focused on the influences that each phase of the menstrual cycle had on assessing body composition, however, the only determination of menstrual cycle phase was based on the self-reports of PMS symptoms completed by the subjects (Francek, 2008). Self-reports are not a reliable source of accurately predicting when the LH surge occurs. Also, the ADP is influenced by body weight and body volume, and if there is noticed weight gain during the luteal phase, then results from ADP may not be accurate. To the knowledge of the author, no studies exist in which the LH surge was accurately determined when using the BODPOD and controlled for fluid retention and increased weight.

The purpose of this study was to compare %BF values measured by BODPOD, HW, and the Siri 3 Compartment Model equation. A secondary purpose to this study was to determine if the fluctuations of body weight as a result of fluid retention during the
luteal phase of the menstrual cycle will affect the results of body composition measurements using air displacement plethysmography.

**Hypotheses**

It was hypothesized that %BF measured by air displacement plethysmography via the BODPOD will be significantly higher in the Luteal Phase than the Follicular and Ovulatory Phase in females taking no birth control. It was also hypothesized that a female group taking hormonal contraceptives and a male control group will show no significant changes in %BF during a one month period. It was hypothesized that the Siri3C equation would yield similar values compared to HW in all groups.
Method

An experimental, prospective case-control design was used to determine differences of body fat percentages during different phases of the menstrual cycle in a female non-birth control sub-group, female birth control sub-group, and a male control group. Permission from the Institutional Review Board at Humboldt State University was obtained prior to collection of data. An informed consent (Appendix C) and Questionnaire (Appendix D) was administered prior to any data collection.

Participants

Twenty subjects, 9 females not using birth control, 6 females using birth control, and 5 males, between the ages of 18-30 years were recruited for this study. The subject sample included 11 Caucasians, 3 Caucasian/Asians, 4 Hispanic/Latinos, 1 Caucasian/Hispanic/Latinos, and 1 African American/Hispanic/Latinos. The subjects were not to be on any weight loss program and were asked to maintain their current lifestyle eating and exercise habits. Female subjects not using any form of birth control had to have a normal menstrual cycle (25-34 days). In order to account for disruptions of the normal hormonal fluctuations during the menstrual cycle, a female, birth control, group will be included. Female subjects using birth control were only included into the study if they were using oral contraceptives, "the Pill", or NuvaRing. For both forms of birth control, there also had to be a placebo week each month. Subjects were also excluded if they had any internal pacemaker, defibrillator, medication pump, or any other
medical devices, metal joint replacement or metal rod/pin implants of any kind since these items greatly affect BIA assessment. A questionnaire was used to determine if subjects met inclusion criteria.

**Experimental Procedures**

Subjects were required to come into Humboldt State University’s Human Performance Lab for three body composition assessments over the course of one month or one menstrual cycle. Subjects were required not to eat 2-3 hours prior to assessment and were not allowed to exercise 2-3 hours prior to coming in for each session. Proper and regular hydration was stressed for accuracy of the procedures. During each session the subjects received three body composition assessments; BIA to determine TBW, followed by ADP, and then HW. See Appendix E: Testing Order Chart. To determine if water retention during the menstrual cycle is a factor in the overestimation of body fat percentage, the Siri-3C equation (Siri, 1961) was used to account for TBW and compared to the %BF determined by ADP and HW (see Figure 1). The Siri-3C equation was found to be an accurate method of calculating %BF while considering TBW in Moon et al., (2007), Moon et al., (2008) and Moon et al., (2009).

Female, non-birth control (FNBC) subjects were asked to monitor their menstrual cycle with daily, morning urine digital ovulation tests from ClearBlue® to determine the peak LH surge. FNBC started using the ovulations tests on day five of their menstrual cycle, during the follicular phase. The subjects also came in for their first session on day five. The second visit, the ovulatory phase, was scheduled as the ovulation tests indicated
that the LH surge was occurring, not when the LH hormone was at its peak. The third visit was three to five days before the onset of the next menses, when progesterone levels were to be peaked in the luteal phase. See Appendix F: FNBC Testing Chart.

Abnormal disruptions were accounted for by including a female birth control group (FBC). Subjects were included in the study if they were either using oral contraceptives, aka "The Pill", or NuvaRing. A typical month of using “The Pill” or NuvaRing include three weeks for either taking a hormone pill or three weeks of wearing a NuvaRing, both are followed by placebo week. The placebo week can include either a sugar pill or iron tablet for those using the pill, or removing the NuvaRing for a week. The placebo week therefore results in a menses each month. FBC subjects were tested at the beginning of Week One of their birth control cycle, the end of Week Two of their cycle, and one or two days following the cessation of taking the pill or removing the NuvaRing. See Appendix G: FBC Testing Chart-Example.

To control for the hormonal fluctuations of a menstrual cycle, a male control group was recruited. The males were tested for three sessions, one assessment per week for three weeks. Environmental conditions, temperature, humidity and barometric pressure, were measured in the BODPOD room.

**Body Composition Assessment Tests**

**Bioelectrical Impedance Analysis**

A bioelectrical impedance analyzer was used to estimate TBW following the procedures recommended by the manufacturer (Quantum X, RJL Systems, Clinton
Township, MI). Subjects were to lay supine during the analysis and four electrodes were placed on landmarks specified by the manufacturer: 1) right wrist 2) on the metacarpophalangeal joints of the second digit 3) on the right ankle on an imaginary line that bisects the medial malleolus, and 4) on the right dorsal surface at base of the metatarsophalangeal joints of second toe. After the electrodes were placed and connected to the analyzer, resistance and reactance was then measured, and then used to calculate TBW. To control for any error, the instructions provided by RJL Systems for the placement of the electrodes were followed for each trial. Electrodes were kept in an air-sealed box so the electrode gel on the electrodes would not dry out. Electrodes were only used once and disposed of after each assessment. Standard error of estimate for BIA is ±1.8% and 6.3%. Validity and reliability was established by Levenhagen et al. (1999) between DEXA and BIA $r=0.93$, and HW and BIA $r=0.91$.

**Air Displacement Plethysmography**

For the assessment using the BODPOD subjects were asked to change into a tight fitting swimsuit (or spandex) and to remove all jewelry. They were required to wear a tight fitting swim cap with a shower cap underneath the swim cap for hygienic purposes. Body mass was measured with a calibrated scale and a two-point calibration was performed to determine a baseline measure for the empty chamber and when using a 50.024 L cylinder. The subject was then asked to step into the device and sit still, breathing normally, for at least two 20s trials. If first two volume measurements were inconsistent, a third volume measurement was taken. The software estimates %BF from
the measured body volume and body density. The Siri equation for the general population was selected for all participants. Predicted $V_{TG}$ was used instead of measured values to standardize the protocol. The BODPOD was calibrated prior to every subject to reduce error. Standard error of the estimate for ADP is 4.0%-1.9% when compared to HW. Validity and reliability was established by Levenhagen et al. (1999) between DEXA and ADP $r=0.94$; HW and ADP $r=0.97$.

Environmental conditions were controlled for when using the BODPOD according to the manufacturer. For room temperature, the BODPOD is not to be near a heater, air conditioners, or fan, not be placed in direct sunlight. Room temperature was to remain between 20ºC and 27ºC and temperature should not vary more than ±0.5º C over the course of a test. Room humidity remained 20-70% (Relative Humidity (non-condensing)) and it could not vary ±5% Relative Humidity over the course of a test. To control room pressure, the door to the BODPOD room was not opened at any point during each test, there were no windows in the BODPOD room, and fan/heating/cooling ducts were turned off. Room noise also was also controlled for by avoiding low frequency acoustic noise such as doors shutting or floor vibrations, as much as possible. The scale was placed on the most level portion of the room to avoid any wobble during mass measurement. The BODPOD was not touched or moved during any assessment and movement inside and outside of the BODPOD was limited. One technician was allowed in the BODPOD room.
Hydrostatic Weighing

Hydrostatic weighing was considered the criterion method for measuring and comparing body composition assessment. Validity and reliability has been established between the DEXA and HW, \( r = 0.94 \) (Levenhagen et al., 1999). Assessment using HW (Exertech, La Crescent, MN) required subjects to enter in a tank, to get their hair wet, to remove any air pockets in their swimsuits, and to rub their skin to rid of any air bubbles. The test administer waited until the water level steadied and was no longer draining after the subject entered before calibrating the scale. After calibrating, the subject was asked to sit on the scale in the center of the tank. Each subject was instructed to hold onto the handles on the seat, exhale all of the air out of their lungs until there were no more bubbles while pulling him or herself under water, and to hold for a few seconds to receive an accurate reading. The subjects were instructed to come out of the water after a test administer slapped the tank. If the subjects could not hold their breath until signaled by a technician, they were instructed to raise his or her head out of the water when they felt ready. For calculation of %BF, the flattest portion in the graph depicting the subjects’ body density in water was selected at 150 samples per second, which automatically calculated body density and %BF. The Siri equation was used for %BF calculations. Standard error of the estimate for HW is ±2.7%. Residual Volume was predicted, using prediction equation provided in the Exertech Software:

**Males:** \( RV(L) = 0.033 \times \text{Height (in)} + 0.022 \times \text{Age (yrs)} - 1.232 \)

**Females:** \( RV(L) = 0.046 \times \text{Height (in)} + 0.016 \times \text{Age (yrs)} - 2.003 \)
Three Compartment Model

HW and ADP use a 2-C model equation. However, methods that use a three compartment (3C) model have been proven to be accurate in determining %BF (Moon et al., 2007; Moon et al., 2008; Moon et al., 2009). The 3-C model considers the ratio of total body water (TBW) to body mass (BM). Moon et al. (2007) determined that the Siri 3C equation is an accurate method to calculate %BF in comparison to %BF estimated by ADP and HW. BIA will be used to measure TBW in each subject. The following equation was used to calculate %BF, where BD is body density and BM is body mass measured by ADP (Siri3C\textsubscript{BODPOD}). Siri3C was calculated again using BD obtained from HW (Siri3C\textsubscript{HW}). There were two Siri3C calculations, Siri3C\textsubscript{BODPOD} and Siri3C\textsubscript{HW}, for each session in FNBC, FBC and Control groups.

Figure 1: Siri 3C Equation

\[
\%BF = \left[ \frac{(2.118}{BD} - (0.78 \left( \frac{TBW}{BM} \right)) - 1.354 \right] \times 100
\]

Statistical Analysis

Statistical analysis was performed using IBM SPSS 21 (IBM SPSS, Chicago, IL). Descriptive statistics for the subjects mean weight (kg), height (cm), average BMI (kg/m\(^2\)), and age are in Table 1. A 3x3 Repeated Measures ANOVA with a Bonferroni adjustment was performed to determine the differences in %BF (BODPOD, HW, and Siri 3C\textsubscript{BODPOD}) and for each group between each phase of the menstrual cycle or session. A
second 3x3 Repeated Measures ANOVA was performed for %BF (BODPOD, HW, and Siri3C_{HW}), and for each group between each phase of the menstrual cycle or session.
Results

A three by three repeated measures ANOVA was performed for all three subjects groups, FNBC, FBC, and Control. Significance was set at $p<0.05$. For the FNBC group, Mauchly’s test indicated that assumption of sphericity had not been violated for the main effects of menstrual cycle phase or for method of assessment, BODPOD, HW, and Siri3C\textsubscript{BODPOD}. There was a significant main effect of the type of body composition assessment, $F(2, 16) = 45.772$, $p=0.000$. Contrasts revealed that BODPOD $F(1, 8) = 5.57$, $p=0.046$, and HW $F(1, 8) = 85.28$, $p=0.000$, were significantly different than Siri3C. However, the significance between BODPOD and Siri3C\textsubscript{BODPOD} is negligible. There was no significant main effect between phase of menstrual cycle $F(2, 16) = 0.803$, $p=0.465$. Contrasts did not reveal significance between phase of menstrual cycle; FP vs LP $F(1, 8) = 0.343$, $p=0.574$ and OP vs LP $F(1, 8) = 1.246$, $p=0.297$. There was no significant interaction between type of body composition assessment and phase of menstrual cycle $F(4, 32) = 0.644$, $p=0.635$. Contrasts were performed comparing type of assessment with all phases of the menstrual cycle. No significance was revealed with the contrasts at any level. Mauchly’s test indicated that sphericity was not violated in the FNBC group.

Sphericity was violated ($p=0.006$) according to the Mauchly’s test for the interaction of type of body composition assessment and time of assessment in the FBC group. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. The main effects of type of body composition assessment and timing of assessment did not violate sphericity. The significant main effect of type of body
composition assessment was $F(2, 10) = 19.10, p=0.000$. Contrasts revealed that there was no significance between BODPOD and Siri3C\textsubscript{BODPOD} $F(1, 5) = 1.559, p=0.267$, and significance between HW and Siri3C\textsubscript{BODPOD} $F(1, 5) = 85.976, p=0.000$. There was no significant main effects for timing of assessment $F(2, 10) = 0.901, p=0.628$. Contrasts did not reveal any significance between Session 1 and Session 3, $F(1, 5) = 1.327, p=0.301$, or between Session 2 and Session 3, $F(1, 5) = 0.875, p=0.393$. There was no main effect for the interaction between type of body composition assessment and time of assessment $F(4, 20) = 0.393, p=0.628$. Contrasts did not reveal any significance at any level.

Mauchly's test indicated that sphericity was not violated for the control group. There was a significant main effect for type of body composition assessment $F(2, 8) = 25.394, p=0.000$. Contrasts further revealed that there was significance between BODPOD and Siri3C\textsubscript{BODPOD}, $F(1, 4) = 10.872, p=0.030$, and a higher significance between HW and Siri3C\textsubscript{BODPOD}, $F(1, 4) = 68.492, p=0.001$. There was no significant main effect for time of assessment, $F(1, 4) = 0.110, p=0.897$. Contrasts did not reveal any significance. There was a significant, although negligible, main effect for the interaction between type of body composition assessment and time of assessment, $F(4, 16) = 3.044, p=0.048$. Contrasts revealed significance between HW and Siri3C\textsubscript{BODPOD} at Session 2 and Session 3, $F(1, 4) = 10.716, p=0.031$, but no significance at any other level.

Another 3x3 Repeated Measures ANOVA was performed for all groups to compare the time of assessment and method (BODPOD, HW, and Siri3C\textsubscript{HW}). There was a significant main effect for type of body composition assessment for FNBC group, $F(2, 16) = 24.273, p<0.000$. Contrasts revealed significance between HW and Siri3C\textsubscript{HW} $F(1,
There was no significant main effect for time of assessment or interaction between type and time of assessment. There was a significant main effect for type of body composition assessment in FBC group, $F(2, 10)=8.723, p=0.006$. Contrasts revealed significance between HW and Siri3CHW, $F(1, 5)=41.377, p=0.001$. There was no significant main effect for time of assessment or interaction between type and time of assessment. There was a significant main effect for type of body composition assessment for the Control group, $F(2, 8)=12.625, p=0.003$. Contrasts revealed significance between HW and Siri3C HW, $F(1, 4)=72.619, p=0.001$. There was no significant main effect for time of assessment or interaction between type and time of assessment.

Table 2 depicts the mean TBW, weight, %BF for HW, BODPOD, Siri3C BODPOD, and Siri3C HW. Figures 2-6 show mean %BF for HW, BODPOD, Siri3C BODPOD, Siri3C HW TBW and Weight per session for each group. There were no significant changes in TBW and Weight between sessions for all subject groups.

Table 1: Subject Characteristics

<table>
<thead>
<tr>
<th>Descriptive</th>
<th>FBC (n=6)</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.83</td>
<td>2.64</td>
<td>21.56</td>
<td>2.00</td>
<td>23.40</td>
<td>1.14</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>167.78</td>
<td>8.71</td>
<td>164.12</td>
<td>6.00</td>
<td>174.34</td>
<td>8.32</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td>59.64</td>
<td>1.79</td>
<td>59.19</td>
<td>5.49</td>
<td>73.96</td>
<td>12.75</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>21.32</td>
<td>1.93</td>
<td>21.99</td>
<td>1.80</td>
<td>24.34</td>
<td>3.63</td>
</tr>
</tbody>
</table>

Notes: Weight is average weight from three sessions
Table 2: Mean Weight, TBW, %BF(Hydro and BODPOD), and %BF Siri3C (Hydro and BODPOD), FNBC, FBC, Control Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FNBC</td>
<td>FBC</td>
<td>C</td>
<td>FNBC</td>
<td>FBC</td>
<td>C</td>
<td>FNBC</td>
<td>FBC</td>
<td>C</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>59.25 (5.64)</td>
<td>59.27 (2.01)</td>
<td>74.39 (13.43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>59.07 (5.26)</td>
<td>59.64 (1.51)</td>
<td>74.27 (13.35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>59.25 (5.61)</td>
<td>59.88 (1.86)</td>
<td>74.57 (13.25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBW (L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>29.88 (2.87)</td>
<td>30.33 (1.76)</td>
<td>42.96 (6.46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>29.42 (2.74)</td>
<td>30.82 (1.62)</td>
<td>42.40 (6.70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>29.93 (3.14)</td>
<td>30.93 (1.01)</td>
<td>42.04 (6.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>21.93 (6.10)</td>
<td>23.00 (3.99)</td>
<td>15.02 (8.46)</td>
<td>27.90 (6.43)</td>
<td>27.22 (5.91)</td>
<td>18.16 (8.16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>22.16 (6.43)</td>
<td>22.17 (3.35)</td>
<td>15.10 (8.27)</td>
<td>27.73 (6.57)</td>
<td>27.35 (5.31)</td>
<td>17.70 (8.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>21.84 (5.91)</td>
<td>21.97 (2.35)</td>
<td>14.56 (8.70)</td>
<td>27.64 (6.62)</td>
<td>26.87 (5.39)</td>
<td>18.00 (8.38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat %†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>27.07 (4.88)</td>
<td>26.62 (3.06)</td>
<td>17.94 (8.40)</td>
<td>29.67 (5.05)</td>
<td>28.75 (3.76)</td>
<td>19.46 (8.53)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>27.63 (4.78)</td>
<td>26.32 (3.36)</td>
<td>18.72 (7.92)</td>
<td>30.09 (4.74)</td>
<td>28.60 (3.92)</td>
<td>19.84 (8.12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>26.99 (4.96)</td>
<td>26.23 (1.82)</td>
<td>19.00 (8.06)</td>
<td>29.54 (5.07)</td>
<td>28.25 (3.09)</td>
<td>20.48 (7.91)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Mean (Standard Deviation). †Siri3C equation applied using respective Body Density measurements.
Figure 2: Mean %BF for FNBC per Session for HW, BODPOD, Siri3C HW, and Siri3C BODPOD

Figure 3: Mean %BF for FBC per Session for HW, BODPOD, Siri3C HW, and Siri3C BODPOD
Figure 4: Mean %BF for FBC per Session for HW, BODPOD, Siri3C HW, and Siri3C BODPOD
Figure 5: Mean TBW per Session for FNBC, FBC, and Control

Figure 6: Mean Weight per Session for FNBC, FBC, and Control
Discussion

The primary findings of this study were that %BF determined from Siri3C\textsubscript{BODPOD} was higher than %BF determined via HW for FNBC, FBC, and Control group. Siri3C\textsubscript{BODPOD} produced slightly higher %BF than BODPOD for the FNBC and for the Control, and similar values for FBC. There were no changes in predicted %BF between any phase of the menstrual cycle for FNBC or between sessions for FBC and Control. These results do not follow the original hypothesis that there would be a higher %BF during the luteal phase of the menstrual cycle for the FNBC group. The results support the second hypothesis that there would be no significant changes between sessions. It was believed that the Siri3C equation would result in values similar to values produced by HW, when considering body density and body mass measured via BODPOD. However, Siri3C\textsubscript{BODPOD} significantly overestimated %BF compared to HW, and, in some cases, compared to BODPOD as well.

There was also a significant difference between HW and Siri3C\textsubscript{HW} for FNBC, FBC, and Control groups. Siri3C\textsubscript{HW} produced significantly higher %BF than HW, thus overestimated %BF for all sessions. It should be noted that, in Figures 2 and Figure 3, there was similarity between %BF via BODPOD and %BF calculated via Siri3C\textsubscript{HW}. Although there were noticeable differences in %BF, there are also slight similarities between BODPOD and Siri3C\textsubscript{HW}. Siri3C\textsubscript{BODPOD} overestimated %BF compared to BODPOD, HW and Siri3C\textsubscript{HW} for all subjects.
The results from this study followed the trend of the "gender bias" in literature for female subjects (Ball, 2005; Biaggi et al., 1999; Bentzur et al., 2008; Levenhagen et al., 1999; Vescovi et al., 2002). For the purposes of being politically correct, the "gender bias" has been called the "sex bias". The sex bias is the overestimation of %BF for female subjects measured using BODPOD compared to HW and other methods and the underestimation of %BF for male subjects. The results from the male control group did not follow the underestimation trend in previous research (Biaggi et al., 1999; Levenhagen et al., 1999). Moon et al., (2009) determined that the Siri3C equation is an accurate form of %BF measurement for Caucasian, NCAA Division I female athletes. Moon et al. (2007) and Moon et al. (2008) tested the validity of the Siri3C in college aged women and men and determined that the Siri3C model was a reliable method for predicting %BF. To the knowledge of the authors there are no studies that test the accuracy of the Siri3C equation for the general population.

There were no significant changes in TBW and weight between sessions for any of the groups. It was not expected that there would be changes in the FBC and Control group. The purpose of this study was to determine if %BF would change in females taking no form of birth control as a result of changes in TBW and weight. However there were no significant changes in TBW or weight for the FNBC group. Weight and TBW may not change significantly enough during the menstrual cycle to influence estimates %BF. BIA via RJL Systems Quantum X analyzer may not have been sensitive enough to detect significant changes in TBW; McKee and Cameron (1997) stated that BIA may not be strong enough to detect TBW changes during the menstrual cycle. However, since
there were no significant changes in weight it can be assumed that TBW did not increase at time of assessment. There were also no significant changes in %BF determined by the BODPOD. The results obtained via BODPOD remained consistent over the course of time.

Since the subject sample included those of various ethnicities, the Siri3C may not be an accurate for ethnicities other than Caucasian as investigated by Moon et al. (2009). The Siri equation was used for BODPOD and HW %BF regardless of ethnicity. Using an ethnicity-specific equation for each subject may have yielded different results. Moon et al. (2009) also tested NCAA Division I athletes; athletes and non-athletes were recruited for this study and therefore could have contributed to the results in this study.

Previous BODPOD comparison and validation research had included at least 20 female subjects (Ball, 2005; Biaggi et al., 1999; Bentzur et al., 2008; Ginde et al., 2005; Moon et al., 2009; Vescovi et al., 2002). Nine FNBC and 6 FBC subjects were included in this study, and therefore sample size may have reduced the power to detect significant differences in the current study.

Limitations

Although DEXA is considered the “gold standard” for body composition measurement, it is expensive and not accessible or convenient in most facilities. There has been strong correlation between the DEXA and HW, $r = 0.94$, which make HW a reliable method and was considered the criterion method for this study (Levenhagen et al., 1999). HW requires subjects to exhale maximally, or residual volume, during
assessment. Residual volume is typically measured via nitrogen or helium dilution (Biaggi et al., 1999; Vescovi et al., 2002; Bentzur et al., 2008). However, RV in this study was predicted via equations provided by Exertech software for ease of assessment. Predicting RV rather than measuring could have resulted in the inconsistency of %BF values from HW. If RV is being overestimated there will be an underestimation of %BF, and if RV is being underestimated, there will be an overestimation of %BF.

The accuracy of the Clearblue Advanced Digital Ovulation Tests was a limitation of this study. Subjects were required to take the tests at home and it was assumed by the researchers that the subjects were following instructions correctly. It had to be assumed that subjects were contacting the primary investigator at the beginning of the LH surge, as indicated by the test, and scheduled within 24 hours. Subjects were not always able to make to preferred timing of assessment. Due to subject availability, subjects occasionally had to be scheduled 24-48 hours after the ideal testing date, and the window to test subjects during specified hormone levels could have been missed. Most subject were not able to be tested at the same time of day for all sessions and time of day should be controlled. It is recommended to have sessions in the morning after an 8-12 hour fast to ensure accuracy.

The inability of subjects to follow pre-test instructions was another potential limitation to this study. Since subjects were recruited from a college student population and sessions occasionally were first thing in the morning, it had to be assumed that subjects did not ingest coffee or food 2-3 hours prior to assessment. A number of subjects did have either septum or ear piercings that were not able to be removed at the first
assessment. The subjects were then asked to keep the piercings in for all sessions to ensure consistency. Ideally, subjects were to have removed all jewelry prior to assessment.

Subjects were instructed to maintain their normal workout habits, however, they were asked to not exercise prior assessment. Exercising can result in fluid loss and increase in temperature that could have resulted in inaccurate measurements from BIA, BODPOD and HW. Fields, Higgins and Hunter (2004) found that increased heat and moisture in the BODPOD contributed to small but significant underestimation of %BF. It can be assumed that an increase in temperature due to exercise may influence the results of the BODPOD. Another researcher found that %BF in women increased 2 hours post exercise when measured by BODPOD (Harrop, 2013). The author speculated that the increase in %BF is due to an increase in TGV. TGV was predicted in the current study and it is unknown if there were any changes in TGV the three assessments.

**Future Research**

Future research investigating body composition changes during the menstrual cycle should consider measuring rather than predicting RV. This will provide greater accuracy for results. Although the at-home ovulation tests did give a better estimation of phase of menstrual cycle versus predicting via premenstrual symptoms, more accurate methods should be used in future research. Blood sampling or advanced clinical urine hormone tests to measure hormone levels will provide a more accurate representation of peak hormones and phase of menstrual cycle. Direito, Bailly, Mariani, and Ecochard
(2013) collected daily first morning urine samples and assayed for FSH and LH hormone levels when comparing LH surge to other menstrual cycle symptoms. Cole, Ladner, and Byrn (2009) collected daily urine samples and used automated tests to detect highest LH result, or the LH peak. Lenton, Landgren and Sexton (1984) used daily blood samples to predict LH peak when identifying short luteal phases in women with apparently normal ovulatory cycles. Future researchers may also want to consider testing multiple times per phase of menstrual cycle to ensure that female subjects are being tested at peak hormone levels.

It is recommended that future studies counterbalance both female subgroups, randomly selecting a phase of the cycle at the start of testing and testing for at least three menstrual cycles. Time of day for assessment should remain consistent for all subjects. Further research also needs to be done to test the validity of using the Siri 3C equation for the general population. Ethnicity-specific body fat equations should be used to compare to the Siri3C equation used in this study. A larger sample size is recommended for a greater power. A sample size of at least 20 for each subgroup, FNBC, FBC and Control (male) is highly recommended.

Predicted TGV has been shown to be accurate when compared to TGV (McCrory et al., 1998). However, increase in body temperature can occur by simply being active and moving throughout the day, whether due to exercise or not. Fields et al., (2004) saw changes in %BF with as little as a 0.6°C change in temperature. If TGV is influenced by temperature, it is recommended to measure TGV especially if time of day is not controlled for, since activity levels may change throughout the day.
Subjects may have needed two sessions (two different days) to become used to the procedure for HW. The HW procedure is difficult for most and although subjects were given two practice trials on their first session, multiple practice session should be considered prior to data collection as results may differ significantly after familiarization.

A different population is needed for the purposes of this study. Recruiting from a rural, college town population was not ideal for this study. College students have fluctuating schedules with both school and work. Needing to test female students the day when the ovulation tests signaled peaking LH was difficult as students do not have the availability necessary. It had to be assumed that FBC subjects were using their birth control correctly, and it is possible that subjects were inconsistent with taking their birth control as prescribed. An education session or questionnaire is encouraged if a birth control subgroup is included to ensure that subjects are using their birth control correctly.

Conclusion

The "sex bias" still needs further investigation. It is unknown to the investigator of this study the cause of the sex bias with ADP. Further research needs to be conducted on changes in TBW and weight across the menstrual cycle, and if so, are changes enough to influence %BF.
References


air-displacement plethysmography: Influence of body temperature and moisture.

*Dynamic Medicine, 3*(1), 3-10.


several one-step home urinary luteinizing hormone detection test kits to


Volunteers Wanted
Research Opportunity
To qualify:

- Males and Females 18-30yrs
- Not on any weight loss programs
- Females: not on any form of birth control, normal menstrual cycle (25-35 days), not pregnant or planning to become pregnant

Research Requirements:

- Commit to 9 hours (6 sessions, 1.5 hours each) of **FREE body composition assessment**
- 6 body composition assessments with each of the following: BIA, Hydrostatic Weighing, and BODPOD

(Valued at $90-180!)

Contact:
Kate A. Reynolds, ACSM-CPT
951-217-1169 (text friendly)
kar810@humboldt.edu
Volunteers Wanted
Research Opportunity

To qualify:
• Females 18-30yrs
• Not on any weight loss programs
• Using ORAL CONTRACEPTIVES (birth control)
  aka “The PILL”, or Nuva Ring, not pregnant or planning to become pregnant

Research Requirements:
• Commit to 9 hours (6 sessions, 1.5 hours each) of FREE body composition assessment
• 6 body composition assessments with each of the following: BIA, Hydrostatic Weighing, and BODPOD

(Valued at $90-180!)

Contact:
Kate A. Reynolds, B.S., ACSM-CPT
951-217-1169 (text friendly)
kar810@humboldt.edu
Appendix B: Permission to Recruit Letter

Company Name:

Company Address:

Company City, State, Zip:

To Whom It May Concern:

I am collecting data for my thesis research project as a requirement for my Master’s Degree in Kinesiology-Exercise Science. I am requesting permission to post the attached flyer at ________________________________ to recruit potential participants for my study.

The purpose of my study is to monitor body composition changes during the menstrual cycle for females. Males will also have their body composition assessed to test the reliability of our equipment/procedures.

Please advise if this is acceptable, and please inform me of any posting restrictions (such as size of the flyer or any restricted areas, etc.)

If you have any further questions or concerns, please do not hesitate to contact me.
Sincerely,

Kate A. Reynolds
Appendix C: Informed Consent

Evaluation of Body Composition Differences During the Menstrual Cycle: Informed Consent

Principal Investigator:
Kate A. Reynolds, B.S., ACSM-CPT
Humboldt State University
951-217-1169
kar810@humboldt.edu

Faculty Supervisor:
Dr. Rock Braithwaite
Humboldt State University
707-826-4543
reb22@humboldt.edu
Project Description:
This study will assess your body composition changes over the course of your menstrual cycle, for females, and/or the accuracy of equipment in testing your body composition for two months, for male and female participants.

Participation Requirements:
- Male or Female: 18-30 years old
- Male or Female: Not on a weight loss program or planning to lose weight for two months
- Male or Female: Cannot have any internal pacemakers, or defibrillators; and/or implanted metal/rods, joint replacements, magnets that cannot be removed, etc.
- Females: regular menstrual cycle 25-35 days
- Females: are not pregnant, and will not become pregnant during participation
- Females: currently NOT using any form of birth control or stopped taking birth control within the last two months
  OR
- Females: using ORAL CONTRACEPTIVES (aka “The Pill”) or NuvaRing with Placebo Week for the last 3-6 months

Procedures:
If you agree to participate in this study, you will:

1) Complete a questionnaire to ensure qualification for the study. If you do not qualify you will be thanked and will not be able to participate in this research.

2) Be asked to complete a number of tests. These tests will be performed in the Human Performance Lab at Humboldt State University. Testing will take place during 6 visits over 2 months. **Total time required will be 9 hours.** Each session will take up to 1.5 hours.
3) wear a tight fitting swimsuit or spandex

4) have hands and feet bare

5) have your body composition estimated by three methods, Bioelectrical Impedance Analysis (BIA), the BODPOD® and Hydrostatic Weighing (HW).
   a) BIA: have two electrodes placed on your right foot, and two on your right hand. A small unnoticeable amount of electricity is sent through your body while you lay on a table.
   b) BODPOD: height, weight, density and volume of the your body will be measured. You will be required to wear a tight fitting swimsuit or spandex clothing, which will be provided. You will sit in an egg-shaped chamber for several minutes and be assessed for two 40-second trials. The BODPOD is non-invasive, the subjects will only be sitting in the BODPOD and instructed to breathe normally in the chamber.
   c) HW: have to be completely submerged under water and exhale all of the air in your lungs and hold breath for about 10 seconds, however you dictate the time spent under water. This may require as many as 4 to 5 trials.

6) Females (NO BIRTH CONTROL): be using Clearblue® Ovulation Tests for the first two weeks of each month of participation to know when you are ovulating for accurate scheduling of tests. You will receive addition instruction on how to use this after qualifying for the study.
7) Females (BIRTH CONTROL): Complete one test each week for the three weeks of Pills or usage of NuvaRing. Repeat for a second month. You will not be tested during your Placebo week (during Menses/Period).

Possible Risks and Discomforts:

Due to the nature of the tests, risks that you may experience:

1) have some sensitivity to the electrodes used for BIA
2) feel some discomfort in small spaces in the BODPOD
3) feel some discomfort when submerged under water during HW
4) breathe in or swallow water during the HW test
5) slip while getting in and out of tank/walking through the lab

Risk Management:

- You will be screened for any pacemakers, defibrillators, or other implanted medical electronic devices. If you have any, you cannot participate in the study.
- All metal jewelry, magnetic objects of any form must be removed for BIA. If you have any metal rods/pins/implants that cannot be removed, you cannot participate in the study.
- You may feel discomfort being in small spaces when sitting in the BODPOD and that if you do, there is a blue button by their left leg that will abort the tests and you will be removed from the BODPOD immediately.
- If you cannot hold your breath for HW testing, you may get out of the water at any point. The research team is trained in CPR and AED for very rare, but extreme situations.
- There will be a mat or towel placed at the base of the steps to get into the tank. This will be used to prevent falls.

Benefits:

Participants will be able to determine their body composition through many methods of testing. The value of these tests is about $90-$180.
**Responsibilities:**

You as a participant are responsible filling out the questionnaire to the best of your knowledge as well as letting the research team know if you can no longer participate in the study. If you feel unsafe or any discomfort during any point in the study, it is your responsibility to inform the research team of any issues. It is also your responsibility to follow pretest instructions that are attached to this form.

**Questions:**

Please ask questions at any point during participation in the study. Please use the contact information at the top of this form, or please see below.

**Confidentiality:**

All of your information and results will be kept in a locked cabinet in the Human Performance Lab. Only the research team will have access to your files, but those files will not be removed from the lab or used for any other purposes other than this research. Your information will not be visible to any other participant.

**Freedom of Consent:**

By signing this form you acknowledge that you are voluntarily taking part in this study. You also have the freedom to stop testing at any point during the study. At that point, your information will be destroyed and unusable for research.

**Contacts:**

The Investigator will answer any questions you have about this study. Your participation is voluntary and you may stop at any time.
If you have any concerns with this study, contact the Chair of the Institutional Review Board for the Protection of Human Subjects, Dr. Ethan Gahtan, at eg51@humboldt.edu or (707) 826-4545.

If you have questions about your rights as a participant, report them to the Humboldt State University Dean of Research, Dr. Rhea Williamson, at Rhea.Williamson@humboldt.edu or (707) 826-5169.

**Signature:**

Your signature below shows that you are voluntarily participating in this study.

I, ________________________________, have read and agree to participate in the above study.  (Please PRINT name)

______________________________  _________________
(Participant Signature)      (Date)

______________________________  _________________
(Primary Investigator)       (Date)
Evaluation of Body Composition Differences During the Menstrual Cycle: Pretest Instructions

**General Instructions for all tests:**

- Refrain from ingesting food, alcohol, or caffeine within 3 hours of testing
- Avoid significantly exercise or exertion on the day of testing
- Drink ample fluids over the 24-hour period preceding the exercise test to ensure normal hydration status
- Please use the restroom prior to coming to the lab

**Instructions for Specific Tests:**

**Bioelectrical Impedance Analysis:**

- Do not wear any jewelry or have any metal in contact with the skin.
- Do not participate in this test if you have an internal pacemaker
- Wear clothing that will allow your feet and hands to be easily accessible
- You cannot be pregnant for this test

**Air Displacement Plethysmography (BODPOD):**

- You will be required to wear a tight fitting spandex unitard. If you prefer to bring in your own tight fitting spandex swimsuit, sports bra, spandex shorts, you MUST bring in the same clothing for EACH test day.
- Please do not wear any jewelry, it will have to removed for assessment
- If possible, please remove/shave any body hair on the face, arms or legs, as it affects results

**Hydrostatic Weighing:**

- You will also need to wear a spandex unitard and a towel will be provided to you.
Appendix D: Questionnaire

Evaluation of Body Composition Differences during the Menstrual Cycle:

Subject Questionnaire

Name:___________________ Age:_____ DOB:___/___/_______
Height:_____ ft _____ in  Weight:_______ lbs ________ kg
Sex: Male    Female

Ethnicity (Please Circle):
African America/Black  Hispanic/Latino  Caucasian  Asian
Other:__________________

SECTION I:
YES   NO  1. Are you an athlete?
If so, what sport? _________________ HSU Athlete? Yes   No
Are you currently: In Season/Training?   Yes   No
Type of training? (i.e. strength & conditioning, cardio, etc)
________________________
When is your season? _________________
Training Frequency (x per week and length) ___________ days
_______________ mins

YES  NO  2. Are you trying to lose or gain weight?

YES  NO  3. Will your training program, whether on a sports team or not, result in
INTENTIONAL weight/body composition changes while participating in
this study?

YES  NO  4. Do you have any internal medical devices such as defibrillators,
pacemakers, magnets, metal rods/plates (i.e. knee, hip, etc. replacements)
etc.?

YES  NO  5. Are you allergic to any alcohol swabs, or gel that may cause irritation
when in contact with the skin?

YES  NO  6. Do you have a fear of small, enclosed spaces, AND/OR fear of
submersion in water?

If you have answered YES to question 2, 3, or 4 you can NOT participate in this study.

Thank you for your eagerness to participate.

If you answered YES to question 5 and/or 6, it is advised that you NOT participate in this
study but you will qualify for this study after speaking with the primary
investigator/research team.

SECTION II:

Females:
YES  NO   7. Are you on any form of birth control?

If so, what kind?

a.  The Pill  
b.  Nuvaring  
c.  IUD (Intrauterine Device)  
d.  Depo Provera (The Shot)  
e.  Other:______________________

If you have answered YES, but chose option C, D, or E, you can NOT participate in this study. If you have answered YES and selected option A or B, please answer Question 7a. If you have answered NO, please complete Questions 8, 9, and 10.

YES  NO   7a. If you chose “a. The Pill” or “b. Nuvaring”, do you have a placebo week? i.e. do you have a period each month?

If you have answered NO to Question 7a, you can NOT participate in this study. If you answered NO but start taking birth control or become unexpectedly pregnant during this study, you can no longer participate in the study.

YES  NO   8. Have you stopped using birth control within the last 6 months?

YES  NO   9. Are you pregnant or planning to get pregnant during the course of this study?

YES  NO   10. Do you have an irregular menstrual cycle? Is it shorter or longer than 25-35 days? Does it vary?

If you have answered YES to questions 8, 9 or 10, you can NOT participate in this study.
If you have answered NO to all of the questions, you QUALIFY for this study. By signing below you acknowledge that you answered these questions to the best of your knowledge and understand that if any of these conditions were to change you will be removed from the study and your data will be destroyed.

RESEARCH TEAM: If you sign below, you acknowledge that you reviewed this questionnaire and helped the participant understand the questions to the best of your abilities.

_________________________________________  ____________________
(Signature of Participant)                           (Date)

_________________________________________  ____________________
(Signature of Research Team Member)               (Date)
Appendix E: Testing Order Chart

Bioelectrical Impedance Analysis
- Measure Total Body Water (TBW)

ADP via BODPOD
- Measure Body Mass (BM), Body Density (BD), Body Volume (BV), Body Fat Percentage (%BF)

Hydrostatic Weighing
- Measure Body Density in water (BD), Body Fat Percentage (%BF)
Appendix F: FNBC Testing Chart
Appendix G: FBC Testing Chart-Example

Hormone Pill  Placebo Pill

Session 1  Session 2  Session 3