THE EFFECT OF MENSTRUAL CYCLE AND SUBMAXIMAL EXERCISE ON ACUTE
BODY COMPOSITION ESTIMATES FROM BIOELECTRICAL IMPEDANCE

Shoshanna Danielle Moody

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Approved by:

Dr. Robert McMurray
Dr. Bonita Marks
Mrs. Lauren Mangili
ABSTRACT

SHOSHANNA MOODY: The Effect of Menstrual Cycle and Submaximal Exercise on Acute Body Composition Estimates From Bioelectrical Impedance (Under the Direction of Dr. Robert McMurray)

The purpose of this study was to investigate changes in percent body fat (BF%) estimates through bioelectrical impedance analysis (BIA) before and after moderate intensity exercise during the follicular and luteal phases of the menstrual cycle. Ten healthy eumenorrheic female subjects performed 45 min of exercise during the follicular phase and during the luteal phase of their menstrual cycle. BIA estimates of BF% and total body water were examined immediately before exercise and 30 minutes after the end of exercise, without fluid replacement. The results indicated no significant effects of exercise (p = 0.170) or menstrual phase (p = 0.688) on BF%. These results suggest that neither menstrual cycle nor acute exercise needs to be considered when using BIA to estimate BF% in moderately exercising women.
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LIST OF ABBREVIATIONS

BF%: Body Fat Percentage

BIA: Bioelectrical Impedance Analysis

DEXA: Dual-energy x-ray absorptiometry

FFM: Fat-Free Mass (kg)

HR: Heart Rate (beats/min)

OC: Oral Contraceptives

TBW: Total Body Water (kg)

THR: Target Heart Rate (beats/min)

R: Resistance (Ω) to the current used in BIA

SKF: Skinfold caliper testing for body composition
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Chapter I: Introduction

Bioelectrical Impedance Analysis (BIA) has become an extremely popular technique to estimate body fat percentage (BF%) because of its simplicity, portability, and low cost. BIA estimates total body water (TBW), which then is used to predict fat-free mass (FFM) (Kyle et al., 2004; NIH, 1996; Van Loan, 1990). Body fat mass is calculated from the subject’s total body mass minus the estimated FFM. Thus, any factor that could influence body water content could influence the ability of BIA to accurately assess body fat. Normal fluctuations in hydration status can be amplified by the effects of both exercise and menstrual cycle phase on body water content and these factors may need to be taken into consideration when using BIA to estimate body fat. (Bickers and Woods, 1951; Demura et al., 2002; Deurenberg et al., 1988; Gleichauf and Roe, 1989; O'brien et al., 1980; Stump et al., 1988). However, little conclusive data exists from which to determine the exact effects on BIA caused by moderate exercise and menstrual phase. This study proposed to examine these effects and aid in the application of guidelines for the use of BIA.

BIA and Hydration

BIA passes multifrequency currents through the skin and tissues. These currents travel through the electrolyte-rich water supply and meet with resistance (R, measured in ohms; Ω). The amount of R is determined by the amount of water in the body. There will be more resistance to the flow of current with less total body water or increased fat mass and less R when TBW is higher or fat mass is low. TBW measurements from BIA are directly
correlated to amount of fat-free mass, which is approximately 73% water (Kyle et al., 2004). Therefore, BIA can be used to estimate both FFM and fat mass by interpreting TBW levels.

Higher TBW is interpreted by BIA as a lower body fat percentage (Pialoux et al., 2004; Stump et al., 1988). Not all of the literature agrees, however, as some research has demonstrated lower BF% estimates from BIA after dehydration or exercise (Demura et al., 2002; Thompson et al., 1991). This lack of agreement suggests that there are more factors influencing BIA measurements than total body water. One of those factors may be water exchange between muscles and other tissues that could occur during exercise. Another factor influencing BIA measurements may be body electrolyte balance. Research has shown that extracellular hyperosmolality, caused by an increase in sodium concentration, increases electrical conductivity and thereby decreases impedance—even when total body water remains constant (Berneis and Keller, 2000). Any change in electrolyte balance may affect BIA, apart from TBW content. Exercise and menstrual cycle phase are two circumstances in which TBW and electrolyte balance might differ from normal homeostatic conditions.

**Exercise and Hydration**

Exercise without fluid replacement tends to cause a dehydrating effect on the body. During exercise, sweat loss, plasma volume shifts, and water exchange between muscles and other tissue compartments contribute to changes both in TBW and electrolyte content. Dehydrating exercise results in a decrease in TBW which causes an underestimation of FFM and overestimation of BF% when using BIA.

Recreationally active women typically perform short exercise bouts of 30-60 min/day at a moderate intensity, which may not cause excessive sweat loss. However, few studies have been done to determine the effects of this short term exercise on BIA estimates of BF%.
One study demonstrated an increase in BF% estimates using BIA 1-hr after a 30 min moderate intensity exercise bout (Stump et al., 1988). Conversely, another study showed decreased BF% estimates using BIA immediately following a 60 min moderate intensity exercise bout (Demura et al., 2002). The current study hopes to clarify this relationship between BIA and hydration shifts following moderate short term exercise.

**Menstrual Cycle and Hydration**

The menstrual cycle affects a number of physiological processes. Progesterone levels increase during the luteal phase of the cycle, which is thought to affect renal output (O’Brien et al., 1980) and lead to water retention and an increase in TBW (Bickers and Woods, 1951; Deurenberg et al., 1988). Increases in TBW during the luteal phase may cause a decrease in R, an overestimation of FFM, and subsequent decrease in BF% estimates. In addition to water changes, studies have shown that changes in extracellular sodium during the menstrual cycle, which may confound the R measured through BIA (Bickers and Woods, 1951; Deurenberg et al., 1988; Gleichauf and Roe, 1989; Thompson et al., 1991). However, it is not known exactly how these changes affect BIA measurements. This study will test BF% estimates from BIA in both the follicular and luteal phases to add to the current data concerning bioelectrical impedance and body composition variations throughout the menstrual cycle.

**Purpose**

The purpose of the current study is to clarify the effects of exercise and menstrual cycle on estimates of body fat using BIA.
**Hypotheses**

1) There will be a lower BF% estimated by BIA before exercise and higher relative BF% after 45 min of exercise.

2) There will be a relatively higher BF% estimated by BIA in the follicular phase than in the luteal phase of the menstrual cycle.

**Significance**

The relationship between moderate exercise, menstrual cycle, and BIA estimates of BF% is presently inconsistent and needs clarification. The significance of this study lays in the applicability of the results to the general population. Both clinicians and fitness consumers will benefit from increased knowledge on the appropriate physiological conditions under which to use BIA.
Chapter II: Review of Literature

A review of previous research is needed to understand the principles behind BIA and the need for the current study. This review of literature first discusses the theory of Bioelectrical Impedance Analysis (BIA) and examines its validity and reliability. Second, the effects of hydration and electrolyte changes on BIA estimates are explained. Finally, studies showing changes in hydration and BIA after exercise and during the menstrual cycle are reviewed. The need for the current study will be evident after a review of the variability and disagreement among the existing literature.

*What is Bioelectrical Impedance Analysis?*

Bioelectrical Impedance Analysis (BIA) is a method of estimating body composition, which assumes there are three major body compartments: water, fat free mass (organs, muscle, bone), and fat mass. The assumption of three compartments is important because BIA measures FFM and back computes BF% from estimates of total body water and total body mass. BIA introduces a current of a known frequency into skin surface electrodes (Kyle et al., 2004). The current flows from a source electrode to a detector electrode, passing through all of the conducting material in-between (National Institutes of Health, 1996; Van Loan, 1990). In the human body, charged ions, which are directly related to tissue hydration levels, constitute the carriers of this current (National Institutes of Health, 1996).

Kyle et al. (2004), writing for the European Society for Clinical Nutrition and Metabolism, Van Loan (1990), and the NIH (1996) have each reviewed the principles and methods behind BIA. Theoretically, the resistance (R) to the flow of a current across a
section of a uniform cylinder is proportional to its length and inversely proportional to its cross sectional area (Figure 1). Knowing this, the volume of the cylinder can be determined by dividing the length squared by R. When the human body is exposed to electrical current, electrolyte-rich body water conducts this current. The current will meet with resistance based on the volume of water in the body. Therefore, a relationship exists between R and the volume of fat-free mass, which is typically 73% water (Kyle et al., 2004).

**Figure 1.** Principles of BIA illustrated through the relationship between impedance, cross-sectional area, and length. Resistance to the flow of current at a known frequency can be used to estimate volume of the container. Adapted from Kyle et al. (2004)

The human body is not uniformly cylindrical, nor is its conductivity constant, so the assumption that FFM and R are so simply related is misleading for two reasons. First, the human body is not a homogenous conductor, so equations are adapted to relate body height and mass to a perfect cylinder. These equations take into consideration differences in conductivity through the arms, legs, and torso. Second, the relationship between FFM and resistance is confounded by the body exhibiting two different types of R when exposed to an electrical current (Kyle, et al., 2004). Capacitative R (reactance) is caused by impedance of the body cell membranes and resistive R (resistance) originates in the fluid from both intra- and extra-cellular space. A very low frequency current would only pass through extracellular space, and would not yield values for the resistance of cell membranes or intracellular water,
whereas an infinite or very high frequency current would reveal a perfect R measurement for intra- and extracellular fluid (Kyle et al., 2004). For this reason, most modern research-grade BIA machines use multiple frequencies of current.

BIA equipment uses either single frequency or multi-frequency techniques. Single-frequency BIA sends a current of 800µA, typically at a frequency of 50 kHz, between two skin electrodes, arranged in one of three locations: bipolar foot-to-foot, bipolar hand-to-hand or tetrapolar hand-to-foot (Kyle et al., 2004; NIH, 1996; Van Loan, 1990). The low frequency of 50 kHz used in single-frequency BIA can estimate FFM, but according to Kyle et al. (2004), it has limited use in appropriately estimating FFM in non-normally hydrated subjects. Multi-frequency BIA uses the body’s resistance to frequencies from 1-500 kHz to estimate FFM and TBW. The multi-frequency technique has the advantage of being able to penetrate cell membranes and estimate intra- and extra-cellular fluid, yielding a better estimate of TBW than single-frequency BIA would allow.

Of the three types of BIA, foot-to-foot and hand-to-hand placement of electrodes typically use single-frequency currents, whereas tetrapolar hand-to-foot placement uses multi-frequency currents. Dittmar (2004) examined 146 subjects of the same ethnicity to determine if the three techniques were different in their estimation of BF%. On one single day within a 0.5 hour period, subjects were analyzed for BF% using all three techniques: hand-to-foot, hand-to-hand, and foot-to-foot. Mean BF% estimates were similar across all 3 techniques. Mean percentages were 22.23, 22.58, and 22.58 for hand-to-foot, foot-to-foot, and hand-to-hand techniques, respectively. The standard deviation for tetrapolar hand-to-foot was slightly smaller (8.00%) than for the other two techniques (8.37 and 8.27 for foot-to-foot and hand-to-hand, respectively). The author states that compared to the tetrapolar technique,
hand-to-hand methods tended to overestimate BF% in males and underestimate BF% in females. The lower variability of hand-to-foot techniques, as demonstrated by smaller standard deviations, may indicate better reliability for this method.

BIA has been utilized without adverse event for over two decades, however, when a current is sent through the body the obvious question raised is: how safe is this procedure for use on human subjects? The National Institutes of Health (NIH) Consensus Statement (1996) claims that the BIA method is considered safe for two reasons: first, the frequencies used to detect bioelectrical impedance do not excite the nerves or cardiac tissue, and second, the human body does not perceive the low current magnitude of less than 1 miliampere (mA) used in BIA.

In the present study, a hand-to-foot tetrapolar technique will be used. The NIH (1996) has come to a consensus about proper use and placement of hand-to-foot BIA electrodes. The skin must first be cleaned with alcohol and spot or foil electrodes used for proper electrical conduction. Two source electrodes are placed on the dorsal surfaces of the hand and foot next to the metacarpal and metatarsal phalangeal joints. Two detector electrodes are placed at the wrist and ankle. Proper placement of electrodes is critical because as little as a 1-cm shift in electrode site can lead to a 2-percent shift in resistance (NIH, 2006).

**Bioelectrical Impedance: Validity and Reliability**

Despite its obvious appeals, the validity and reliability of BIA as a proper tool for body composition estimation has been questioned. Other techniques such as skinfold measurement (SKF), hydrodensitometry (body density determined by under-water weighing), and dual-energy x-ray absorptiometry (DEXA), are commonly used and accepted methods
for determining body composition, but at its inception BIA had yet to gain such acceptance. Over the past two decades, numerous studies have investigated the issues of BIA validity and reliability. (Heyward, 1996; Jackson et al., 1998; Kushner and Haas, 1988; Segal et al., 1985; Segal et al., 1988; Sun et al., 2005)

Early work comparing BIA to standard reference methods of body fat estimation was performed by Segal, Gutin, Presta, Wang, and Van Itallie (1985). They compared hydrodensitometry, total body water analysis, and anthropometry/SKF, to BIA. Seventy-five adults (41 females and 34 males) aged 17-59 years took part in this study; their body fat percentages ranged from 4.9 to 54.9%. Hydrodensitometry was performed using standard procedures and the Goldman and Buskirk formula for body density calculation. Percent body fat and fat-free mass was calculated from body density measurements. Total body water (TBW) was also measured by giving a dose of oral $^3$H$_2$O. FFM and TBW measures were then combined using a formula to determine percent fat. Percent body fat was also determined through 4-site and 8-site SKF testing (Segal et al., 1985). FFM was then back calculated from the SKF estimated body fat (FFM = weight $\text{kg} - \text{fat mass}\ $kg). BIA was performed to determine body fat and FFM.

Pearson product moment correlations were calculated to compare FFM determined by hydrodensitometry, SKF, BIA, and the FFM/TBW combined compartments (Segal et al., 1985). FFM determined from hydrodensitometry, most strongly correlated with estimates of FFM from BIA; $r = 0.935$. The two combined body compartments of FFM/TBW also strongly correlated with BIA values; $r = 0.934$. BIA estimates of FFM also correlated strongly with calculations of FFM through 4-site and 8-site SKF, with $r$ values of 0.856 and 0.859, respectively. These results suggest promising data towards validating BIA against the
reference methods of hydrodensitometry and SKF for FFM estimates. However, this study compared FFM, and not BF% estimates. Further, while the authors provided correlation coefficients, these high r values only signify that the methods are highly related. Correlation coefficients do not provide any information regarding the accuracy of BIA against the other reference methods.

In another study, Segal, Van Loan, Fitzgerald, Hodgdon, and Van Itallie (1988) determined the validity of BIA compared to hydrodensitometry in 1567 subjects aged 17-62, with body fat ranging from 3-56 percent. Subjects underwent hydrodensitometry to determine FFM and BF%. Total body resistance was captured using a tetrapolar electrode bioelectrical impedance technique. FFM and BF% was estimated from the BIA measures of resistance. Stepwise multiple regression analyses, with hydrodensitometrically determined FFM as the dependent variable, found that resistance and height$^2$ were separately better predictors of FFM than when used in the ratio of height$^2$ to resistance. Correlation coefficients comparing hydrodensitometry BF% and BIA-predicted BF% were $r = 0.809$ and $r = 0.852$ for men and women, respectively. From these data, Segal et al. (1988) determined BIA is valid if using fatness-specific and gender-specific prediction equations for estimating FFM and body fat. Best-fit regression lines follow better trends among males, among females, and among the lean and obese separately than when the populations are combined. Therefore, the authors warn that it is not appropriate to apply one single prediction equation for BIA to people of any size or gender.

As demonstrated by the studies discussed above, BIA has been shown to be comparable to hyrodensitometry, a long-used standard reference technique for determining body composition (Segal et al., 1985; Segal et al., 1988). There are two other commonly
performed methods for body composition estimation, skinfold caliper testing and dual-energy x-ray absorptiometry. Kushner and Haas (1988) attempted to validate BIA against SKF. Eighty healthy subjects (50 female, 30 male) aged 21-60 years were placed into one of four groups based on their body mass index (BMI). BMI is a ratio of body weight in kg divided by height in meters squared. The classifications of BMI for this study were: lean BMI < 12.0, normal BMI 17.0-26.9, obese 27.0-39.9, and superobese BMI ≥ 40.

The fasted subjects were measured for height, body mass, SKF, body circumferences, and BIA (right sided tetrapolar model). SKF measurements were used to compute BF% and FFM and the average of the three FFM estimates by BIA was also calculated. Regression analysis between FFM predicted by SKF and BIA was performed. Correlation coefficients in all four groups (lean, normal, obese, superobese) were significant with r values > 0.924 (p < 0.001), demonstrating evidence for FFM determined by BIA as a valid predictor of FFM determined by SKF caliper testing. However, as in the Segal et al. (1985) study, a high correlation between SKF and BIA measurements suggests a relationship between methods, but does not imply the accuracy of the BIA method.

A recent 2005 study by Sun et al. (2005) compared DEXA and BIA in the assessment of body fat percentage in healthy populations. This study examined 591 healthy non-diseased subjects aged 19-60 years with BMI ranging from 16.98-54.27. After a 12 hour fast, subjects were measured for height, mass, DEXA whole-body scan, and BIA using the same multifrequency tetrapolar devise used in the current study, QuadScan 4000 (Bodystat, Douglas, UK). Paired t-tests were used to compare the predicted BF% outcomes of BIA and DEXA and Pearson product moment correlations were performed between BF% estimated by BIA and measured by DEXA. The correlation between the two methods for the entire
study group was \( r = 0.88 \), with correlation coefficients for men and women equaling 0.78 and 0.85, respectively. However, when placed into groups of low (<20%), moderate (20-30%), or high (>30%) body fat, BIA overestimated BF% by approximately 3.9% for lean subjects while underestimating BF% by nearly 3% for high body fat subjects. Sun et al. concluded that BIA and DEXA compare favorably for subjects falling within a normal body fat range, but that caution should be used when applying BIA to very lean or obese individuals.

The validity of BIA has been established for healthy, non-diseased individuals, and can be applied in these populations as a comparable method for determining BF% and FFM in place of SKF, hydrodensitometry, and DEXA (Heward, 1996; Kushner and Haas, 1988; Segal et al., 1985; Segal et al., 1988; Sun et al., 2005). Reliability is different than validity. Reliability is a measure of how accurately an apparatus can replicate measurements.

Jackson, Pollock, Graves, and Mahar (1998) examined the reliability of BIA and compared its precision to hydrostatic weighting and SKF testing. Twenty-four men and 44 women were recruited to participate in this study on two different days within a 7-day range. Subjects reported to the lab and 2 separate technicians, in random order, performed SKF, hydrostatic weighing, and BIA (Jackson et al., 2005). All measurements for each method were repeated four times each by the two technicians. Test-retest reliability coefficients were high, \( r \sim 0.957 \) for BIA. Proportions of the total variance attributable to inter-day and inter-technician error were both very small, but of these two sources of error in BIA, the day to day variability was the largest, accounting for approximately 3% of the variance. The authors suggested that day to day variations in body water content may contribute to the error found in BIA measurements.
Bioelectrical Impedance: Effect of Water and Electrolyte Balance on Body Composition Estimates

BIA estimates BF% from resistance measures that are dependent on TBW (Kyle, 2004; NIH, 1994; Van Loan 1990). Thus, it is important to know exactly how fluctuations in water and electrolyte balance effect BIA measurements. Thompson et al. (1991) conducted research to determine the effects of hydration and dehydration on both BIA and hydrodensitometry measurements. Ten healthy white males aged 18-44 years were randomly assigned to undergo either tetrapolar BIA or hydrodensitometry. Each subject went through two trials: hydration and dehydration. The subjects’ BF% through BIA or hydrodensitometry was determined. In the hydration trial, subjects drank 0.5 L distilled water, waited 30 minutes, and again underwent BIA or hydrostatic weighing. In the dehydration intervention, subjects were asked to dehydrate through both exercise and steam room exposure to a level corresponding to 2-4% of their body mass, then they again underwent BIA or hydrodensitometry. While there was an increase in estimated BF% through BIA between the baseline and hydrated states, the increase did not reach statistical significance. However, there was a significant decrease in BF% estimates by both BIA (-1.7%) and hydrostatic weighing (-1.5%) in the dehydration intervention.

These data are opposite to what was expected: dehydration causes higher resistance leading to increased BF% estimates. The authors concluded that the change in BF% estimates through hydrostatic weighing may have been more due to the significant body mass difference caused by dehydration. The finding that acute dehydration significantly affects BF% estimations through BIA reinforces the concept that water balance is a crucial factor in the use of bioelectrical impedance. The water balance shifts induced in this study were minimal and Thompson et al. (1991) acknowledged that the literature provides conflicting
results on the effect of hydration state on body composition analysis. Furthermore, the dehydration trial in this study used both exercise and heat to accomplish loss of body mass. The effects of dehydration on BIA may have been confounded by the effects of exercise.

Pialoux et al. (2004) recently conducted a study on body composition following dehydrating endurance exercise and after recovery to pre-exercise hydration conditions. Twelve healthy males, either recreationally active or endurance trained, were recruited. Subjects first went through a 1-week control trial in which body composition (through BIA) and TBW (through deuterium oxide dilution) were measured. They were then examined using BIA after four consecutive days of 5 hrs of exercise, broken into 50 minute trials. Next, the subjects rehydrated and were examined for body composition and TBW from the first to the eighth day of recovery, during which time subjects were to refrain from heavy exercise. TBW and FFM estimates increased between the control day and recovery day 1 in both deuterium oxide dilution and BIA. This reinforces the assertion that an increase in total body water leads to a subsequent increase in estimated FFM by BIA (Kyle, 2004; NIH, 1996; Pialoux, 2004; Van Loan, 1990).

Some research has shown that BF% estimated by BIA decreases when TBW increases (Pialoux, 2004); yet contradictory research shows a decrease in BF% after dehydration (Thompson, 1991). These studies may have been confounded by the affect of exercise on BIA measurements. Apart from both exercise and TBW influences, it is also important to know if BIA measurements change when concentration of an important electrolyte, sodium (Na\(^+\)), changes in the body. Berneis and Keller (2000) examined this question by following bioelectrical impedance changes during acute changes of extracellular osmolality. Eight healthy adults aged 24-26 years were recruited for this three part study. In
part one, hyper-osmolality, subjects received 80 ml/h of a 2%-sodium, saline solution for 12 hrs overnight. Subjects then received 200 ml/h of 5% NaCl solution. In part two, hypo-osmolality, subjects received two injections of 4 µg desmopressin (an antidiuretic), a hypotonic saline solution injection, and drank 2-2.5 L tap water. In part three, iso-osmolality, subjects drank water ad libitum. BIA was measured at baseline and after osmolality changes in each trial.

In the Berneis and Keller study (2000), TBW measured through urinary output, remained constant during each trial. The authors concluded that extracellular hyper-osmolality (increase in Na⁺ concentration) increases electrical conductivity and thereby decreases resistance in BIA, even when total body water remained constant. Resistance values in BIA were similar between baseline and final measurement in the iso-osmolality and hypo-osmolality trials. This indicates that increased electrolyte concentration, independent of TBW changes, is an important source of variation in BIA.

**The Menstrual Cycle: Fluctuations in Water and Electrolyte Balance**

Past literature has noted the importance of fluid homeostasis when using BIA (Kyle, 2004), and has shown changes in BIA estimates of body composition under abnormal hydration and electrolyte states (Berneis and Keller, 2000; Pialoux et al., 2004; Thompson et al., 1991). Electrolyte shifts, water retention, and hormonal fluctuations at certain phases of the menstrual cycle have been observed. As early as 1951, researchers Bickers and Woods investigated the relationship between premenstrual tension and abnormal water storage. The researchers examined 22 female subjects with symptoms of pain, bloating, thirst, and pelvic fullness during the approximately 7 days before menstruation. These women were treated with a dehydrating drug, pyrilamine-8-bromo-theophyllinate. During the cycles in which
they received the drug, average premenstrual weight gain was only 1.5 lbs. Yet when
followed during another cycle without receiving the drug, average premenstrual weight gain
was 6.9 lbs. Bickers and Woods (1951) note that most women normally store more water
during the premenstrual phase than during other phases of the cycle, and those women
retaining large quantities of water may be successfully treated with dehydrating drugs.

The cause of this fluid retention in the premenstrual, or luteal, phase may be due to
increases in progesterone concentration. To observe these increases, O’Brien, Selby, and
Symonds (1980) conducted a study comparing 18 women with symptoms of premenstrual
syndrome to 10 women without noticeable symptoms. These women were examined for
changes in mood, 24-hour urinary volume, total sodium, sodium:potassium ratio, and plasma
progesterone concentration. Mean serum progesterone levels in women with premenstrual
symptoms were significantly higher than the control group during the early luteal phase (22.4
versus 8.7 nmol/l, respectively). Both groups also had marked rises in progesterone
concentration in the late luteal phase. Increases in sodium:potassium ratio and urinary
sodium followed the rise in progesterone levels in both groups of women during the early
luteal phase. O’Brien et al. (1980) noted that increased progesterone in the early luteal phase
has a natriuretic effect, causing marked water loss. They suggested that the large swings in
water balance are the cause of premenstrual symptoms: a noticeable diuresis in early luteal
phase followed by a return to baseline hydration, and then large increases in aldosterone in
the late luteal phase which cause sodium and water retention. How these physiological
changes during the menstrual cycle impact BIA estimates of body composition in women has
not been fully substantiated.
Deurenberg, Weststrate, Paymans, and van der Kooy (1988) followed eight women throughout their menstrual cycle and noticed an average increase in resistance through BIA of 8 ohms between the 1 week prior to menstruation (luteal phase) and the 1 week after menstruation (follicular phase). While the authors only reported resistance values, not BF% estimates, lower resistance would yield higher estimated FFM and lower BF% during the luteal than during the follicular phase. While water retention would most likely lead to increased body mass, the authors claim that body weight changes did not confound changes in resistance from BIA in this study. They concluded that the decrease in resistance was due to water and electrolyte retention. However, their data to support this statement is not published.

Gleichauf and Roe (1989) conducted daily BIA estimates of TBW, FFM, and BF% on twenty-six eumenorrheic women aged 20-41 years throughout an entire menstrual cycle. The days of the cycle were divided into four phases: 1) menses, 2) follicular, 3) postovulatory/early luteal and 4) premenstrual/late luteal. Resistance values were significantly higher in phase 2 than phase 1, and significantly lower in phase 4 (late luteal) than in phase 2 (follicular). These resistance values translated to 0.12 kg lower fat free mass (FFM) estimates in phase 2 than in phase 1 and 0.04 kg higher FFM in phase 4 (late luteal) than in phase 2 (follicular). These changes in resistance and FFM were significant, yet so small the authors concluded that BIA use at any time of the cycle is appropriate in the general population of women.

Water retention and electrolyte changes occur during the menstrual cycle (Bickers and Woods, 1951; O’Brien et al., 1980). These changes may cause fluctuations in BIA measurements (Deurenberg et al., 1988; Gleichauf and Roe, 1989). However, not all
research demonstrates changes in BIA during the menstrual cycle. McKee and Cameron (1997) followed 36 non-oral contraceptive (OC) users throughout one menstrual cycle. BIA and body weight were measured several times per week. The researchers found significant increases in body mass before menstruation. However, this luteal phase increase in body mass was not correlated to changes in BIA. Using ANOVA, McKee and Cameron determined no significant changes in BIA throughout the menstrual cycle. The researchers concluded that the weight gain was either not fluid related or BIA was not as sensitive an indicator of changes in body water.

Water retention and body weight gain has been shown to occur in women with normal menstrual cycles not using oral contraceptives (OC). A question that arises is then: do users of OC demonstrate significantly different body water changes throughout the menstrual cycle than do non-OC users? Stachenfeld, Silva, Keefe, Kokoszka, and Nadel (1999) examined the hypothesis that oral contraceptives effect body water balance. Nine healthy women each underwent 6 testing stages: follicular (1) and luteal (2) phases without OC, after 4 weeks of OC (3), during the follicular (4) and luteal (5) phases after a 4 week washout period, and after 4 weeks on a different OC (6). The two OCs administered were estradiol + progestin and progestin only.

In each of the 6 stages, plasma volume and osmolality was tested before and after dehydrating exercise (Stachenfeld et al., 1999). Subjects in both the OC and non-OC stages showed an increase in plasma osmolality and decrease in plasma volume after dehydrating exercise. Resting plasma osmolality was significantly lower (- 4 mosmol/kg H$_2$O) in the luteal phase without OC and in the two OC stages than in the follicular phase without OC. Loss of Na$^+$ to sweat was greatest from exercise in the follicular phase without OC than at
other stages of testing. The effect of the dehydrating exercise on plasma volume and osmolality was similar both in the control (non OC) and OC stages. Use of OC did not stimulate an increase in sodium retention over the non-OC trials.

Machado, Tachotti, Cavenague, and Maia (2005) also examined OC use and bioelectrical impedance measures throughout a menstrual cycle. Eighty women were randomly divided into 3 groups: 1) OC containing ethinylestradiol/gestodene, 2) OC containing ethinylestradiol/drospirenone, or 3) male condom/no OC (control group). BIA was used to examine TBW, FFM, and fat mass on the first, 10th, and 21st days of the cycle. There were no significant differences in TBW, FFM, or FM estimates through BIA between the three groups during the cycle. The limitations to this study are that there was no wash-out or adjustment period, and subjects were only followed for one cycle. The limitations in the Machado et al. (2005) and Stachenfield et al. (1999) studies, and the lack of direct knowledge of OC’s effect on BIA parameters may confound the results in the current study. Therefore, the current study will use only female participants with regular menstrual cycles who are not using oral contraceptives.

Research on menstrual cycle changes has shown wide shifts in TBW levels (Bickers and Woods, 1951) and electrolyte levels (O’brien et al., 1980, Stachenfeld et al., 1999). These changes may be related to higher resistance from BIA (Deurenberg et al., 1988) and higher BF% estimates by BIA (Gleichauf and Roe, 1989) during the follicular phase than during the luteal phase. However, changes in body mass throughout the menstrual cycle may also have no effect on BIA changes (McKee and Cameron, 1997). While dehydrating exercise was used to compare hydration responses of OC and non-OC users (Stachenfeld et al., 1999), no research has been conducted to observe effects of moderate exercise between
the phases of the menstrual cycle on BIA. The present study will examine the effects of moderate exercise on BIA and also explore the effects of menstrual cycle phase on BF% estimated by BIA.

**Exercise: Effect on Water Balance**

Exercise can cause water and electrolyte shifts, and water loss through sweat. Without fluid replacement, this water loss would lead to decreased plasma volume, and a subsequent increase in resistance and BF% measures through BIA. The majority of existing research on BIA and exercise uses long bouts of dehydrating exercise, so it is unknown whether exercise or dehydration causes a change in BIA measures. Some research has observed BIA after shorter bouts of moderate intensity exercise. Stump, Houtkooper, Hewitt, Going, and Lohman (1988) examined 7 men and 7 women during a 30 min treadmill run at 60-70% estimated maximum heart rate on two different days, one day without fluid replacement and one day with fluid replacement. Immediately post exercise, there was no change in R values from BIA for the non-fluid replacement trial compared to pre-trial R values. One hour post exercise without fluid replacement, there was an increase in R of approximately 9 ohms. In the fluid replacement trial, there was no change in R for the 3 hr post exercise. The authors concluded that even moderate intensity short term exercise, without fluid replacement, can increase R and change BF% estimates by BIA.

Demura, Yamaji, Goshi, and Nagasawa (2002) reported results opposite to those of Stump et al (1988). Using 15 females and 15 males, they compared BF% estimated through hand-to-hand, foot-to-foot, and hand-to-foot BIA before and after 60 minutes of exercise at 55% estimated maximal heart rate. Decrease in BF% estimates after exercise were evident for all three BIA methods. It is important to note however, that these measurements were
taken immediately following exercise. When BIA is measured immediately following exercise, increased muscle blood flow and increased skin temperature could cause a change in overall impedance. According to Kyle et al. (2004), this change in blood flow and skin temperature could be a confounding variable in the decreased BF% estimates.

**Summary**

BIA is a valid and reliable method to estimate FFM and BF%. However, changes in body water and electrolyte distribution or concentration may affect BIA measurements. The exact effects of acute hydration and dehydration on BIA are not clear. Some research has demonstrated a decrease in BF% by BIA after acute increases in total body water (Pialoux et al., 2004) while other research has shown a decrease in BF% estimates after dehydration (Thompson et al., 1991).

One factor influencing water balance is the menstrual cycle. Fluctuations in progesterone (O’Brien et al., 1980) and other hormones cause water retention (Bickers and Woods, 1951) and decreased plasma osmolality in the luteal phase (Deurenberg et al., 1988). This premenstrual increase in TBW causes an increase in FFM estimates from BIA.

Exercise is a second factor that influences water balance. Little work has been performed to assess BIA estimates of body composition following moderate exercise of 1 hr or less. The research that has been completed is contradictory: some has shown an increase in R by BIA after 45 min exercise (Stump et al., 1988), which should lead to higher BF% estimates. Other research has shown that 60 minutes of exercise decreases BF% estimates from BIA (Demura et al., 2002). The current study seeks to clear the controversy by examining BIA body composition estimates prior to and following moderate intensity exercise during the follicular and luteal phases of the menstrual cycle.
Chapter III: Methodology

Participants

Ten healthy, eumenorrheic women participated in two exercise trials, once during the follicular and once during the luteal phase of their menstrual cycle. Participants were chosen based on the following inclusion criteria: healthy nonsmoking adult women aged 18-40 years with normal menstrual cycles of 28 ± 7 days, recreationally active at least 3 d/wk at 30 min/d. The following exclusion criteria were applied: currently taking diuretic medications, currently using hormonal birth control, classified as obese (BMI > 30; Brooks, Fahey and Baldwin, 2005), inability to conform to study control conditions of caffeine limitation and water consumption.

Instrumentation

The BIA system used in the current study is the BodyStat QuadScan 4000 (Isle of Man, UK) which employs multifrequency currents through a tetrapolar hand-to-foot impedance model. Bodystat has been validated against a three-component model reference method of determining body composition (Parker et al. 2003) and against other whole-body impedance systems (Smye, Sutcliffe, and Pitt, 1993). The reliability of the Bodystat system for measuring total body water has also been demonstrated (Shanholtzer and Patterson, 2003). Polar Heart Rate Monitors were used to track heart rate (HR). Body mass was measured using a Detecto model 3P7044 scale (Jerico, NY) and height was measured with a stadiometer (Perspective Enterprises, Portage, MI). Urine Specific Gravity was measured
with an American Optometrics refractometer (Keene, NH) to estimate state of hydration. A Monark 818 Ergomedic cycle ergometer was used for each exercise trial.

**Procedures**

This study was approved by University of North Carolina Behavioral Institutional Review Board (Approval # 07-0411). Subjects were informed of the study procedures and provided with an informed consent form. Subjects reported to the laboratory on three different occasions: once for informed consent, an initial physical exam conducted by a trained Ph.D., and submaximal graded exercise test, once during the luteal phase and once during the follicular phase of their menstrual cycle. Menstrual cycle phase was determined through self reported dates of menstruation. Approximate follicular phase was defined as the 1-7 days following the end of menstruation, and luteal phase as the time period approximately 8 days prior to onset of menstruation.

**Figure 2.** Timeline of study procedures.
The protocol for visit 1 began with a reading of the study procedures by the tester, and completion of informed consent form by the participant. Participants completed a medical history form, and submitted to a physical exam. Participants were weighed and then fitted with a heart rate monitor. Participants were asked to lie in the supine position for 5 minutes, after which a resting heart rate was recorded. Participants then completed a submaximal exercise test, modified from the YMCA cycle ergometer test procedures. The modification was to increase the RPM to 60. Participants cycled at 60 rpm with a workload of 0.5 kpm, and continued at 60 rpm with increasing resistance every 3 minutes, according to their HR response. The submaximal test was halted when participants reached their age-predicted 60% of maximum heart rate, based on the Karvonen Formula (Brooks et al., 2005).

\[ THR_{60} = 0.60 \times (HR_{\text{max}} - HR_{\text{rest}}) + HR_{\text{rest}} \]

A best-fit line of heart rate responses to varying workloads was used to determine the cycle ergometer resistance level for the subsequent 2 exercise trials. Participant’s workload was set as the predicted resistance needed to elicit a heart rate response of 50% max.

The protocol for the exercise trials during visits 2 and 3 was identical. Participants reported to the lab 8 hours postprandial having consumed no caffeine or electrolyte-containing drinks for 8 hours. The women were asked to consume 64 oz. water during the 24 hours before each trial and an additional 0.5 liters of water throughout the 2 hours prior to reporting to the laboratory. All procedures were performed by the same tester on each visit.

Subjects were asked to void prior to any measurements and the urine sample was tested for specific gravity. After voiding, height and body mass of subject wearing sports bra and shorts was measured. BIA was then performed. Subjects were asked to remove all metal jewelry. Skin at electrode sites was cleaned with alcohol swabs. Electrodes were placed on
the right wrist—the positive charge proximal to the third metacarpal joint and the negative charge inside the wrist closest to the head of the ulna. Two more electrodes were placed on the right ankle, the positive charge proximal to the third metatarsal joint and the negative charge between the medial and lateral malleoli. Electrodes were attached with the subject in the supine position, and the subject remained in this position while multifrequency BIA was performed.

BIA electrodes were removed. Subject was then fitted with a Polar heart rate monitor. Subject was then asked to do a self-paced warm up for 5 min on a Monark cycle ergometer followed by self-determined stretching. Using the best-fit line prediction of resistance needed to elicit a heart rate response of 50% max, resistance levels were set for each participant. Subject then pedaled on the ergometer for 45 minutes at 60 rpm. Workload was only adjusted if the participant was unable to complete 45 minutes at the initial workload; if workload was adjusted, the same adjustments were made during the second exercise trial. At the end of 45 minutes of exercise, subject exited the cycle ergometer and was allowed to stretch and stand until HR recovered to near resting levels. Subjects were asked to rest or do quiet work for 30 min to allow skin temperature and blood flow to stabilize.

Subjects were then asked to produce a second urine sample to be analyzed for urine specific gravity. After voiding, subjects were re-weighed to account for any exercise-induced weight loss. BIA was again performed using the same procedures as earlier. Subject was allowed to rehydrate, drinking enough water to account for the change in pre- to post-exercise body mass. Subjects remained in the laboratory until all fluids were ingested and HR had returned to steady, resting levels (±10 bt/min).
**Analysis**

BF% estimates were obtained from measurements of resistance taken by the QuadScan 4000. Mean and standard deviation of BF% in each menstrual phase and before and after exercise were calculated. The two hypotheses of this study were: 1) there will be a lower BF% estimated by BIA before exercise and higher relative BF% after 45 min of exercise, and 2) there will be a relatively higher BF% estimated by BIA in the follicular phase than in the luteal phase of the menstrual cycle. To determine if subjects were exercising at the same intensity for both trials, repeated measures ANOVA of rating of perceived exertion and HR responses to exercise was used; the dependent variables were rating of perceived exertion and HR, and the independent variable was time. To determine the effects of exercise (hypothesis 1) and menstrual phase (hypothesis 2) on BF%, two-way within subjects ANOVA (p ≤ 0.05) was used. The independent variables are exercise and menstrual cycle phase, each having 2 levels: pre- and post-exercise, and follicular and luteal phase. The dependent variable is BF% estimated through BIA. To explore factors that may have influenced the effects of menstrual phase and exercise on BF%, body mass, TBW, and USG were analyzed with 2 x 2 within subjects ANOVAS. For these analyses, the dependent variables were body mass, TBW, and USG, and independent variables were exercise and menstrual phase.
Chapter IV: Results

Subject Characteristics

Table 1. Subject characteristics.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age (yrs)</th>
<th>Body Mass (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>Resting Heart Rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>54.5</td>
<td>163.0</td>
<td>20.5</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>54.2</td>
<td>170.5</td>
<td>18.6</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>45.9</td>
<td>158.8</td>
<td>18.2</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>61.0</td>
<td>167.7</td>
<td>21.7</td>
<td>69</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>61.1</td>
<td>165.8</td>
<td>22.2</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>69.2</td>
<td>170.2</td>
<td>23.9</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>63.5</td>
<td>172.5</td>
<td>21.3</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>64.0</td>
<td>169.0</td>
<td>22.4</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>56.6</td>
<td>160.0</td>
<td>22.1</td>
<td>54</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>52.8</td>
<td>162.6</td>
<td>19.9</td>
<td>63</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>24.9 ± 4.8</td>
<td>58.3 ± 6.8</td>
<td>166.0 ± 4.7</td>
<td>21.1 ± 1.8</td>
<td>57.3 ± 6.3</td>
</tr>
</tbody>
</table>

Ten women participated in the study. Their characteristics are shown in Table 1. Participants were eumenorrheic, non-smokers between the ages of 20 – 35 years, falling within the normal BMI range of 18 – 25 kg/m². All participants were recreationally active, with self-reported activity levels of at least 30 minutes of recreational activity 3 days per week. Resting HR values indicate bradycardia, which together with BMI, suggest that the
women were moderately trained. Participants were not using any form of hormonal birth control.

**General Exercise Responses**

The Borg Rating of Perceived Exertion (RPE) scale and heart rate (HR) monitoring were used to track intensity of the two 45-minute exercise trials, as shown in Table 2. Heart rate and rating of perceived exertion data suggest that the exercise was in the moderate-to-high range of intensity. To demonstrate if each participant experienced similar levels of intensity within their two exercise trials, repeated measures ANOVAs were performed on RPE and HR values. There was a significant effect of time on both RPE (p = 0.001) and HR (p ≤ 0.0001), with both increasing as exercise progressed. There was no significant effect of menstrual phase on RPE (p = 0.842) or HR (p = 0.111). There was no significant interaction effect of exercise and phase on RPE (p = 0.717) or HR (p = 0.601).

**Table 2.** Means ± standard deviations for rating of perceived exertion at 10, 20, and 30 minutes during the luteal and follicular exercise trials.

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteal</td>
<td>12.3 ± 1.7</td>
<td>13.2 ± 1.5</td>
<td>13.8 ± 1.3</td>
</tr>
<tr>
<td>Follicular</td>
<td>12.1 ± 1.0</td>
<td>13.2 ± 0.9</td>
<td>13.8 ± 1.1</td>
</tr>
</tbody>
</table>

**Table 3.** Means ± standard deviations for heart rate (bpm) at 5, 15, 25, 35, and 45 minutes during the luteal and follicular exercise trials.

<table>
<thead>
<tr>
<th></th>
<th>5 min</th>
<th>15 min</th>
<th>25 min</th>
<th>35 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteal</td>
<td>138.8 ± 9.9</td>
<td>149.1 ± 14.9</td>
<td>151.6 ± 12.8</td>
<td>152.4 ± 13.1</td>
<td>155.6 ± 12.2</td>
</tr>
<tr>
<td>Follicular</td>
<td>133.9 ± 16.8</td>
<td>144.8 ± 16.7</td>
<td>146.6 ± 14.8</td>
<td>148.8 ± 14.5</td>
<td>148.4 ± 15.6</td>
</tr>
</tbody>
</table>
Urine Specific Gravity (USG) was recorded before and after exercise during both menstrual phases, and mean values are shown in Table 4. To compare USG values, a within-subjects 2x2 ANOVA was run with pre-post exercise as one factor and menstrual phase as another factor. There was no significant effect of exercise (p = 0.129), or interaction effect of menstrual phase and exercise (p = 0.365) on USG. The p-value of 0.055 for menstrual phase demonstrates a trend towards a significant effect of phase on USG.

**Table 4.** Means ± standard deviations for urine specific gravity (g/cc), measured before and after exercise in both menstrual phases.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Exercise</th>
<th>Post-Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteal</td>
<td>1.013 ± .008</td>
<td>1.008 ± .006</td>
</tr>
<tr>
<td>Follicular</td>
<td>1.009 ± .007</td>
<td>1.007 ± .002</td>
</tr>
</tbody>
</table>

All participants had lower body mass values after exercise (p = 0.0001) in both menstrual phase trials. There was a trend towards an effect of menstrual phase on body mass (p = 0.058), with higher body mass in the luteal phase. Mean weight loss from pre- to post-exercise was 0.67 kg in the luteal phase and 0.60 kg in the follicular phase. There was no interaction effect of exercise and phase (p = 0.184).

Total body water content (TBW) was also measured before and after exercise for each menstrual phase. Repeated measures 2x2 within subjects ANOVA was run on TBW values. There was a trend towards an effect of exercise on TBW (p = 0.060), with TBW being lower after exercise. There was no menstrual phase effect (p = 0.378) or interaction of exercise and phase effect (p = 0.262).
Table 5. Means ± standard deviations of total body water (L) and body mass (kg) measured pre- and post-exercise during the luteal and follicular phase trials.

<table>
<thead>
<tr>
<th></th>
<th>Total Body Water</th>
<th>Body Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Ex.</td>
<td>Post-Ex.</td>
</tr>
<tr>
<td>Luteal</td>
<td>34.29 L</td>
<td>33.99 L</td>
</tr>
<tr>
<td>Follicular</td>
<td>34.95 L</td>
<td>33.04 L</td>
</tr>
</tbody>
</table>

**Body Fat Percentage Responses to Exercise**

Body Fat Percentage (BF%) values were recorded before and after exercise during each phase. Means and standard deviations for BF% recordings are illustrated in Figure 2. Repeated measures 2x2 within subjects ANOVA was run on BF% values with pre-post exercise as one factor and menstrual phase as the second factor. There was no exercise effect (p = 0.170), menstrual phase effect (p = 0.688), or interaction of exercise and phase effect (p = 0.334) on BF%.

**Figure 3.** Means and standard deviations of body fat percentage responses to exercise in luteal and follicular phases.

<table>
<thead>
<tr>
<th></th>
<th>PreEx</th>
<th>PostEx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteal</td>
<td>18.31 ± 3.63</td>
<td>18.17 ± 3.68</td>
</tr>
<tr>
<td>Follicular</td>
<td>18.28 ± 3.26</td>
<td>17.83 ± 3.67</td>
</tr>
</tbody>
</table>
Chapter V: Discussion

The purpose of this study was to clarify the effects of exercise and menstrual cycle on estimates of body fat using BIA. Body fat percentage (BF%) was estimated before and after 45 minutes of submaximal exercise during the luteal and follicular phases of the menstrual cycle. This study found no significant effect of menstrual cycle phase or exercise on BF% estimates obtained from BIA. This study found an effect of exercise on body mass and a trend towards an effect of exercise on total body water (TBW). The following discussion will examine these results in relation to past research and explore their practical significance. This discussion will also examine the limitations of the current study and make recommendations for future research.

Exercise Effect on Body Fat Percentage

One of the main findings of the current study was no statistically significant effect of exercise on BF% estimated through BIA (p = 0.170). This finding failed to support the conclusions made by other studies on the effects of exercise and phase on BIA. The initial premise of this study, based on empirical knowledge, was that BF% estimated through BIA would increase after exercise. Dehydration or lower body water after exercise should lead to increased resistance (R) to current in BIA, and a subsequently higher BF% estimation. In addition, Stump et al. (1988) observed an increase in R through BIA one hour after a 30 minute treadmill run. This study was similar to the Stump et al. study in that participants were asked to rest for 30 minutes after exercise before BIA was performed. However,
contrary to the findings of Stump et al., the current study found no significant effect of exercise on BF% estimates.

Demura et al. (2002) concluded that there were significant decreases in BF% estimates immediately after 60 minutes of exercise. One reason for the decreased BF% in the Demura et al. study could have been that BIA was performed directly after the exercise session. Increases in blood flow, skin temperature, and muscle electrical activity immediately after exercise may affect the R of BIA currents, leading to a confounding variable in the BF% estimation. The current study, finding no effect of exercise on BF%, fails to support the Demura et al. findings.

The findings of the two studies examining BF% through BIA before and after short term exercise disagree. Stump et al. shows increased BF% after exercise and Demura et al. shows decreased BF%. The current study used some methods in common to both studies, yet slightly differing in duration of exercise trial and period of rest after exercise, before performing BIA. No consensus between the three studies can be determined. This lack of agreement among the studies might be explained by differences in methodology, number of participants, and duration of the exercise trials. This also suggests that redistribution of water during exercise and in the rest period after exercise may be an important factor in the determination of R when using BIA.

During exercise, water is continually being shifted between cells, interstitial fluid, and plasma. Water is also lost in sweat and shunted from non-vital organs in order to supply working muscles with adequate blood flow. After exercise, body water continues to shift as muscle cell and organ blood flow normalize. This redistribution of water may affect BIA measurements, as BIA works by analyzing body water content. Further, the galvanic skin
response is an occurrence causing changes in electrical activity of the skin due to changes in emotional state, skin temperature, or sweat response (Wikipedia 2007). After exercise, evaporation of sweat on the skin surface leads to a cooling of the skin. This may affect the galvanic skin response, and interfere with the electrical reading of impedance through BIA. These factors, combined with redistribution of water during and after exercise, may contribute to differences among the current literature on the exact effects of exercise on BF% estimated through BIA. There are three possible reasons for the conflicting results of the current literature: 1) there is no change in BIA readings because there is truly no significant water loss, 2) the BIA machine may not have the precision or sensitivity to measure such a small effect, or 3) both weight loss and water loss occur at the same time. Since BIA uses a combination of entered body mass and measured TBW to determine BF% estimations, a proportionally lower body mass and lower TBW reading after exercise may cause BF% to be estimated as the same both before and after an exercise trial.

**Menstrual Phase Effect on Body Fat Percentage**

Another main finding of the current study was no menstrual phase effect on BF% estimated through BIA (p = 0.688). This finding is different from other research that demonstrates higher BIA resistance (higher BF%) in the follicular phase than in the luteal phase (Deureberg et al., 1988; Gleichauf and Roe, 1989). Further, research has shown that women tend to have increases in total body water (TBW) during the luteal phase (Bickers and Woods, 1951). However, the current study showed no significant increase in TBW from the follicular to the luteal phases; hence, no difference in BF%. A reason for the lack of agreement may be the sensitivity of the BIA instruments used, as explained above. Another reason may be the window of time used to categorize luteal and follicular phases in the
current study. Gelichauf and Roe divided the menstrual cycle into four phases: menses, follicular, postovulatory, and premenstrual. The current study combined both postovulatory and premenstrual phases into the luteal phase. Also, the current study uses time frames similar to Deurenberg et al.: 1 week prior to menstruation for luteal phase and 1 week after menstruation for follicular phase.

The current study does support the findings of McKee and Cameron (1997) that demonstrated an increase in body mass in the luteal phase, yet no significant increase in TBW or BF% through BIA. The body weights in the present study were approximately 0.65 kg higher in the luteal phase, whereas TBW changes were marginal. Both the McKee and Cameron study and the current study followed non-oral contraceptive users and found no significant change in BIA measurements based on menstrual phase. While failing to support some prior research on the effects of phase on BIA, the current study does support others. The lack of agreement among the literature may be cause for future research on the subject.

**Body Mass and Body Water: Relationship to Exercise, Phase, and BIA**

Body mass was significantly lower following both 45 minute exercise trials. This result can be partially explained by the loss in total body water from pre- to post-exercise. While there was not significantly lower TBW after exercise, there was a trend towards an effect of exercise on lowering TBW (p = 0.060). Since weight loss over a 45 min period is due to water loss, rather than actual loss of fat mass, you would expect TBW to decline. Since TBW did not change significantly, this suggests that the BIA lacks the sensitivity to determine changes in TBW of less than 0.7 kg. Urine samples were not weighed. However, some of the weight loss demonstrated may be explained by weight loss in the urine sample. Additional weight loss can be explained by water lost in sweat during exercise.
The BIA apparatus uses body mass to interpret resistance and body water readings. With lower overall body mass, and a trend towards lower TBW content after exercise, the finding of no effect of exercise on BF% becomes clearer. If weight and TBW decrease concurrently, BF% estimations through BIA would not change significantly (as explained on page 32).

Urine specific gravity (USG) can be used to estimate hydration status. According to the National Library of Medicine statement by the NIH (2006), normal ranges of USG fall between 1.002 and 1.028. Higher USG values indicate more concentrated urine and a more dehydrated state. The participants’ USG values before exercise for both trials fell within this normal range. In the current study, mean USG values were significantly lower after exercise for both trials. While these lower values after exercise would typically indicate better state of hydration, weight loss from pre- to post-exercise and lower total body water (TBW) values after exercise indicate the opposite.

One reason for this unexpected decrease in USG may be the pre-exercise water intake requirements. Participants were asked to consume 0.5 L of water in the 2 hrs prior to each exercise trial. This water consumption may have caused a false representation of the participant’s true pre-exercise USG. Further, USG samples were taken 30 minutes after exercise. During exercise, kidney blood flow is minimal, and urine output is reduced. Thirty minutes after exercise, blood flow to the kidneys will have resumed, leading to increased water delivery into the urine and a more dilute USG measurement. If the USG would have also been measured directly after exercise, the typical increase in USG seen immediately post exercise would probably have been evident.
There was also a trend towards a significant effect of menstrual phase on USG values (p = 0.055), with luteal USG values higher than follicular. Higher USG values indicate more concentrated urine. Participants followed the same water consumption guidelines prior to exercise in both phase trials. The trend towards an effect of menstrual phase on USG is not associated with a trend towards a phase effect on BF%. Therefore, this trend may be related to water retention during the luteal phase. Water retention would result in less urine output and thus, more concentrated urine. There was a trend towards an effect of menstrual phase on body mass (p = 0.058). This trend is towards a slightly higher body mass during the luteal phase than during the follicular phase; ~0.65 kg. One might assume that the weight gain was due to water retention premenstrually, which is supported by the findings of higher luteal USG in the current study. This assumption would support data from with other studies that have suggested water retention occurs during the luteal phase (Bickers and Woods, 1951; Deurenberg et al. 1988). However, the current study showed no significant effect of phase on TBW. This could be interpreted to mean that body water retention did not occur simultaneously with body mass increases, or that the BIA apparatus is not sensitive to the small changes in TBW. Also, the trend towards an effect of phase on body mass did not occur with any significant effect of phase on BF% or muscle mass (as indicated by BIA). The current study’s finding of higher premenstrual body mass, yet no increase in TBW or decrease in BF% through BIA supports the findings of McKee and Cameron (1997).

**General Exercise Responses**

During exercise, heart rate (HR) increases linearly with time. As muscles tire, rating of perceived exertion (RPE) may also increase. The significant effects of time on both RPE (p = 0.001) and HR (p ≤ 0.0001) in the current study demonstrate that as the time progressed
from rest to 45 minutes, HR and RPE increased significantly. This is a normal effect of exercise, and occurred in all participants. Interestingly, menstrual cycle phase did not significantly affect HR (p = 0.111) or RPE (p = 0.842), which indicates that participants performed trials of similar intensity.

**Strengths and Limitations**

One limitation of the current study was the small sample size. The power of the statistical analyses to determine a menstrual phase or exercise effect on BF% would be increased by a larger sample size. However, since the subject’s responses were quite variable, the addition of numerous subjects would be required to even show a trend. All participants were asked to follow certain pre-exercise guidelines to standardize the testing conditions and adherence to these guidelines was self reported. This self-reporting could be a limitation to the current study if all guidelines were not properly followed. The guideline to consume additional water may have overshadowed normal urine output or body water responses. Also, post-exercise urine samples were taken before post-exercise BIA, which may affect the TBW estimation. Further, participants self reported their dates of menstruation. Participants had nothing to gain from reporting menstruation inaccurately. However, the issue of self-reporting may be a limitation in this case as well.

Luteal phase was defined as the 8 days before onset of menstruation and follicular phase as the 7 days after the end of menstruation. Based on participant’s prediction of their menstrual start date, luteal phase visits were scheduled. This became a limiting factor for retaining subjects. Of the 16 participants enrolled in the study, only 10 completed all trials. The drop out rate was due mostly to inaccurate predictions of menstrual start dates. A more precise method of estimating phase such as oral temperature, ovulation kits, progesterone
measurements, or more lead-in time to track the menstrual cycles of each participant, could have reduced the attrition rate. In addition, a more accurate way to analyze menstrual phase and BIA might be to divide the menstrual cycle into 4 phases: 1) menses, 2) follicular, 3) postovulatory/early luteal and 4) premenstrual/late luteal, similar to the design of Gleichauf and Roe (1989). This method may help to capture more small changes in BIA throughout the menstrual cycle.

While the same tester was used for all trials, tester error may have been introduced in the placement of BIA electrodes. Electrodes were placed on the right wrist and right ankle. Care was taken to place the electrodes in the same position each time. However, there may have been very slight changes in the placement of electrodes from pre- to post-exercise, and among different subjects. To reduce this limiting error, skin could have been measured from the bone markers and marked to guarantee exact placement of electrodes each time. While it is unclear whether small changes in electrode placement affect BIA output, this still may have been a limiting factor in the current study (Evans et al. 1998).

Workload for both trials was determined through a graded exercise test adapted from the YMCA submaximal cycle protocol. Using a best-fit line of heart rate (HR) responses to varying workloads, the workload that elicited 50% of maximum age-predicted HR was chosen as the workload for all trials. The rationale for this decision was that participants would slightly overshoot 50% of max HR and be working at an intensity of approximately 60% max aerobic power throughout the 45 minute trial. However, true HR percentage ranged from 54.3 – 84.5% in the luteal phase and 41.5 – 91.5% in the follicular phase. This wide variation in HR values may have affected the BIA results. Despite this limitation, it
participants, individually, were working at a similar intensity during both menstrual phase trials.

There could be several causes for the wide range of HR values. Participants, while all recreationally active, may have been at varying cardiovascular fitness levels. Those with higher fitness levels would be able to sustain 45 minutes of exercise with a more stable HR. Cardiac drift may have occurred in all trials, leading to an increase in HR. A fan was used to circulate air and aid in reducing the effects of cardiac drift.

A final limitation to this study is the unknown causes of body mass changes in different phases and before and after exercise. This study showed a trend towards and effect of menstrual phase on body mass. Since diet was not strictly controlled, it is not known whether premenstrual weight gain was due to a change in eating habits or hormonally induced water retention. Total body water did not change appreciably with menstrual phase, indicating either that luteal phase weight gain may not have been fluid related, or that BIA was not sensitive to these fluid changes. After exercise in both trials, body mass was lowered. However, there was no change in total body water due to exercise, suggesting that the BIA was at fault. If urine samples were weighed, some of the post-exercise weight loss may also have been accounted for.

Despite these limitations, this study is one of only a few studies to explore the relationship between short term submaximal exercise, menstrual phase, and BF% estimates by BIA. Further research is encouraged to more conclusively define this relationship.

Conclusion

The two hypothesis of this study were: 1) there will be a lower BF% estimated by BIA before exercise and higher relative BF% after 45 min of exercise, and 2) there will be a
relatively higher BF% estimated by BIA in the follicular phase than in the luteal phase of the menstrual cycle. The results of this study fail to support either hypothesis. There were no significant effects of exercise or menstrual phase on BF% estimated by BIA. The results of this study agree with some prior research while failing to support other studies. The lack of agreement among the current available research leads to some recommendations for future research.

**Recommendations**

The major findings of this study were that short term exercise and menstrual phase do not affect BF% estimates using BIA. BIA is a commonly used technique for estimating body fat, and is growing in popularity among personal trainers, laboratory technicians, and fitness consumers. The findings of this study suggest that BIA is an appropriate technique to track BF% in females before or after recreational exercise, regardless of menstrual phase. However, the lack of agreement between this study and others leaves room for future research to elucidate the true effects of menstrual phase and exercise on BIA.

To improve future studies, a larger sample size should be used. Further, studies should examine the effects of varying exercise durations. For example, one study to track BIA before and after 30 min, one after 45 min, and one after 60 min of submaximal exercise. The effect of a post-exercise rest period on BIA readings was also not explored in the current study. Future studies may wish to examine the differences in BIA immediately following, 30 min, and 1 hr after exercise. It is not known exactly how the galvanic skin response or changes in skin temperature, blood flow, and muscle electrical activity following exercise affect BIA measurements.
There is a lot of current research on the topic of BIA changes during the menstrual cycle. However, while this study agrees with some of the research, other studies have shown significant changes in BIA measurements throughout the menstrual cycle. Future research may wish to divide the menstrual cycle into more phases or track the menstrual cycle with more precise methods, such as those suggested in the strengths and limitations section of the discussion. This may account for small changes which may not have been detected by the current study’s design of dividing the menstrual cycle into week-long luteal and follicular phases.

An interesting area for future research may be to tease out the issue in both the current study and by McKee and Cameron (1997); premenstrual weight gain, without total body water gain or lower BF%, is noted in the luteal phase. This weight gain may be due to fluid retention, as the current study demonstrated a trend towards a significant effect of menstrual phase on urine specific gravity (USG) values, with luteal phase USG values slightly higher than follicular phase. The trend towards a menstrual phase effect on USG may be an area for future research to examine together with phase changes in body mass. The current study suggests that BIA use is appropriate for females in different physiological states. However, the exact effects of exercise and menstrual phase on BIA are not clear among current research.
APPENDIX A: Consent Form

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form

IRB Study #
Assent Form Version Date:
Title of Study: The effect of menstrual cycle and submaximal exercise on body composition estimates from bioelectrical impedance

Principal Investigator: Shoshanna D. Moody
UNC-Chapel Hill Department: Exercise and Sports Science
UNC-Chapel Hill Phone number: 962-1371
Email Address: sdmoody@email.unc.edu
Co-Investigators:
Faculty Advisor: Robert G. McMurray, Ph.D.
Funding Source: UNC Chapel Hill EXSS

Study Contact telephone number: (919) 962-1371
Study Contact email: sdmoody@email.unc.edu

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, with the health care provider, or with the University of North Carolina-Chapel Hill.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this permission form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Background: This study is designed to examine changes that may occur in body fat estimations during different phases of the menstrual cycle and following a short exercise bout, using a common method to measure body fat, the bioelectrical impedance analysis.
BIA (Bioelectrical Impedance Analysis) is a popular technique for estimating body fat, used in the research and health club settings. BIA estimates body fat based on how much water it measures in the body. BIA works by placing a very small electrical current, the same as a flashlight battery, and measuring the resistance to that electrical current. We know that both exercise and the menstrual cycle influence body water content. Currently, there is little research that has examined both exercise and menstrual cycle, and how they effect body fat estimates using BIA.

**Purpose:** The purpose of this study is to determine if the body water changes that occur in different phases of the menstrual cycle and with moderate exercise will make a difference in the estimations of body fat as determined by bioelectrical impedance analysis.

**Significance:** Bioelectrical Impedance Analysis (BIA) has become one of the most popular and common forms of body fat percentage estimation techniques. It is portable, inexpensive, and easy to use. The significance of this study lays in the applicability of the results to the general population. Both clinicians and fitness consumers will benefit from increased knowledge on the appropriate physiological conditions under which to use BIA.

**Are there any reasons you should not be in this study?**
- You should not participate in this study if you are a smoker, if you are under the age of 18 or over the age of 40 years.
- You should not participate if you have an irregular menstrual cycle.
- You should not participate in this study if you have a body mass index of greater than 30 (BMI = body weight in kilograms / height in meters$^2$).
- You should not participate in this study if you are currently taking diuretic medications.
- You should not participate in this study if you are physically unable to perform 45 minutes of moderate cycling activity.

**How many people will take part in this study?**
If you decide to be in this study, you will be one of approximately 15 people in this research study.

**How long will your part in this study last?**
Your participation in the study will consist of three sessions. The first session will take about 60 minutes and be used to obtain your consent and complete a basic physical exam to be sure you qualify for the two exercise sessions that follow. During the first session, you will complete a submaximal, graded exercise test to determine the cycle resistance settings for the two exercise trial days. One exercise session will occur during the 7 days following your menstrual period. The other exercise session will occur approximately 8 days before the onset of menstruation. Each session will last approximately 90 minutes, including 5 minutes of rest, 5 minutes of warm up, and 45 minutes of moderate cycling exercise followed by a cool down rest.

**What will happen if you take part in the study?**
The first session will be used to inform you of the study procedures and to obtain your consent to participate in the study. You will then fill out the medical history form. Your
height and weight will then be measured. Finally you will have a standard screening physical examination of your heart, lungs and blood pressure to be sure you qualify for the study. During the initial visit, you will also cycle on a stationary indoor cycle, with gradually increasing resistance, to determine the appropriate resistance setting to reach a target heart rate range during your next 2 visits. Once qualified you will meet with the researcher and set up your two exercise session appointments.

One exercise session will occur in the 1-7 days following the end of menstruation. The other session will occur approximately 8 days before the onset of menstruation. Both of these laboratory exercise sessions will be identical.

Before arriving to the Lab for each exercise session, you will have not eaten or consumed caffeine for 8 hours. In the 24 hours before each session, you will consume 64 ounces of water (about 8 cups), and an additional two cups of water during the 2 hours prior to reporting to the Lab.

When you arrive at the Lab for the first exercise session you will be asked to void and collect your urine for analysis of urine specific gravity. You will then have your weight and height measured. You will then be asked to lie on your back. The skin on your right wrist and right ankle will be cleaned with alcohol swabs. Two electrodes from the bioelectrical impedance machine will be placed on your right wrist and two electrodes will be placed on your right ankle. The analysis of your body fat will be performed with you lying still. The BIA machine passes a very small electrical impulse through you and measures the time it takes for the electrical impulse to flow between your wrist and your ankle.

BIA electrodes will be removed. You will then be fitted with a heart rate monitor, across your chest, just underneath your breastbone (below the bra line). You will be asked to lie in the supine position for 10 minutes, after which a resting heart rate will be recorded. You will then be asked to do a self-paced warm up for 5 min on a Monark cycle ergometer followed by 5 min of stretching.

Once warmed up, you will pedal on the cycle until your heart rate (HR) reaches 55-65% of your estimated HR max and continue cycling at that intensity for 45 minutes. The intensity of the cycling will be adjusted by the technician to maintain this HR range throughout the exercise bout. At the end of 45 minutes of exercise, you will exit the cycle and be allowed to stretch and stand or sit until HR has recovered to near resting levels (± 10 beats/minute). You will then be asked to remain in a seated or supine resting position for the remained of the 30 minute recovery period to allow skin temperature and blood flow to stabilize. During this 30 minutes, you will be allowed to read or do quiet work.

At the end of the 30 minutes of rest, you will then be asked to produce a urine sample to be analyzed for urine specific gravity. You will then be re-weighed to account for any exercise-induced weight loss. You will then be asked to lie on your back, and skin will again be swabbed with alcohol wipes at the right ankle and right wrist. BIA will again be performed using the same procedures as earlier.
After the completion of the BIA measurement, you will be asked to drink enough water to account for the change in weight and you will be required to remain in the laboratory until all fluids are ingested.

The second visit to the Lab will be identical to the first visit, but at a different menstrual cycle phase.

**What are the possible benefits from being in this study?**
Upon completion of this study researchers and the general public will have a greater understanding of the appropriate physiological conditions under which to use BIA. You will have the benefit of learning your estimated body fat percentage.

**What are the possible risks or discomforts involved with being in this study?**
There should be no risk of psychological harm or emotional distress from completing the research.

You will be asked to complete moderate exercise on a cycle ergometer for 45 minutes. Your daily lifestyle should be at least recreationally active, and the two exercise sessions should not produce any more muscular discomfort that you get during your normal exercise program. However, you may feel moderate muscular soreness after the exercise sessions.

Although no radioactive materials are being used for this study, the UNC Chapel Hill Applied Physiology Laboratory, where the participants will be performing exercise tests, houses radioactive materials. The amount of radioactive material is very small and should pose no health threat to you. All radioactive material is stored in a contained biochemistry section of the Applied Physiology Laboratory, and is kept in compliance with all UNC Office of Environmental, Health & Safety regulations. If you are pregnant or may become pregnant at any time during this study, please notify the researcher.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

**What if we learn about new findings or information during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will your privacy be protected?**
Upon agreement to participate in the study, each participant will be assigned an ID number. All data will be stored by ID in a computer that will be password protected. Only myself and my advisor will have access to this information. Once you have completed both trials, your name, telephone number, and any email addresses will be destroyed and the data kept by ID number with no other identifiers. All data collection sheets and questionnaires that will be used for data collection will be kept in a locked filing cabinet located Rm 25B Fetzer Gym (Dr. McMurray’s office). After each trial, all data will be transferred to the computer by a research team member. Your information will be given to no one other than the research personnel unless you say so in writing to the principal investigator or her faculty advisor listed on the front of this form.
No subjects will be identified in any report or publication about this study; only group statistics. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety. These are very unlikely occurrences, but if disclosure is ever required, the research team will take every step allowable by law to protect the privacy of your personal information.

**What will happen if you are injured by this research?**

All research involves a chance that some injury might occur. This may include the risk of personal injury. In spite of all safety measures, you might develop an injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

**What if you want to stop before your part in the study is complete?**

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

**Will you receive anything for being in this study?**

You will be allowed to retain a copy of your estimated body fat percentage.

**Will it cost you anything to be in this study?**

It will not cost you anything to take part in this study. If you enroll in this study, you will have to pay for your own transportation. Parking behind Fetzer Gym will be provided for those coming from off campus.

**What if you are a UNC student?**

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

**What if you are a UNC employee?**

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

**Who is sponsoring this study?**

This research is funded by the graduate student researcher.
What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research subject?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Title of Study: The effect of menstrual cycle phase and submaximal exercise on body composition estimates from bioelectrical impedance.

Principal Investigator: Shoshanna Danielle Moody

Subject’s Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_________________________________________   _____________________
Signature of Research Subject     Date

_________________________________________
Printed Name of Research Subject

_________________________________________   _____________________
Signature of Person Obtaining Consent     Date

_________________________________________
Printed Name of Person Obtaining Consent
APPENDIX B: Data Collection Forms

VISIT # 1

Date: ________________
Subject Name _______________________  Subject ID # ________________
Age ________________  Age Estimated HR Max ________________ b/m
Height ________________  Body Mass ________________

Eaten in last 2 hours?  □ YES  □ NO

☐ Consent Form Read and Sign
☐ Medical History Form
☐ Physical Exam

☐ Heart Rate Monitor on
☐ 5 min supine rest  RESTING HEART RATE ________________ b/m

☐ THR: 0.60 [ _______ (max) - _____ (resting) ] + _____ (resting) = __________ b/m

☐ Explain Bike / Process

☐ Graded Exercise Test (see attached)

☐ Stage 1:  HR minute 2: ________________ b/m  Workload________
   HR minute 3: ________________ b/m

☐ Stage 2:  HR minute 2: ________________ b/m  Workload________
   HR minute 3: ________________ b/m

☐ Stage 3:  HR minute 2: ________________ b/m  Workload________
   HR minute 3: ________________ b/m

☐ Stage 4:  HR minute 2: ________________ b/m  Workload________
   HR minute 3: ________________ b/m

☐ Date of Visit 2 ________________
VISIT # 2

Date: ___________________   Phase _____________________
Subject Name _______________________  Subject ID # ________________
Age ________________  Age Estimated HR Max ________________ b/m
Height ________________   Body Mass ________________

Caffiene in the last 8 hours?  □ YES  □ NO
Energy / Sports Drinks in last 8 hours?  □ YES  □ NO
Eaten in last 8 hours?  □ YES  □ NO
64 oz. H₂O in last 24 hrs & 2 cups in last 2 hrs?  □ YES  □ NO

☐ RESTING HEART RATE (VISIT 1) ________________ b/m

☐ THR @ 50% MAX __________ b/m  60% max __________ b/m

☐ Urine Sample #1    URINE SPECIFIC GRAVITY BEFORE ___________

☐ Body Comp # 1:   BODY FAT BEFORE: __________ %
  o  TBW _____% _______ L
  o  ECW ____% _______ L
  o  ICW _____% _______ L
  o  Impedance 5 kHz: _______  Impedance 50 kHz: _______
  o  Impedance 100 kHz: ______
  o  Impedance 200 kHz: _______

☐ HR monitor on

☐ 5 minute warm up

☐ Begin exercise: STARTING WORKLOAD = ______________ (to reach 50%)

☐ HR @ 5 min: ________ b/m

☐ HR @ 15 min ________ b/m  RPE: 10 min ___________

☐ HR @ 25 min ________ b/m  RPE: 20 min ___________

☐ HR @ 35 min ________ b/m  RPE: 30 min ___________

☐ HR @ end/45 min ________ b/m
☐ Rest 30 min / walk around

☐ Urine Sample #2   URINE SPECIFIC GRAVITY AFTER __________

☐ Re-weigh: __________

☐ Body Comp # 2:   BODY FAT AFTER: __________ %
  - TBW _____% _______ L
  - ECW _____% _______ L
  - ICW _____% _______ L
  - Impedance 5 kHz: _________   Impedance 50 kHz: _________
  - Impedance 100 kHz: _________
  - Impedance 200 kHz: _________

☐ Rehydrate to weight lost

☐ Date of Visit 3 _____________

---

**VISIT # 3**

Date: ________________   Phase ________________
Subject Name ___________________   Subject ID # ________________
Age ________________   Age Estimated HR Max ________________ b/m
Height ________________   Body Mass ________________

Caffeine in the last 8 hours?   ☐ YES   ☐ NO
Energy / Sports Drinks in last 8 hours?   ☐ YES   ☐ NO
Eaten in last 8 hours?   ☐ YES   ☐ NO
64 oz. H₂O in last 24 hrs & 2 cups in last 2 hrs?   ☐ YES   ☐ NO

☐ RESTING HEART RATE (VISIT 1) ________________ b/m

☐ THR @ 50\%_{MAX} ____________ b/m   60\% max ____________ b/m

☐ Urine Sample #1   URINE SPECIFIC GRAVITY BEFORE __________

☐ Body Comp # 1:   BODY FAT BEFORE: __________ %
  - TBW _____% _______ L
  - ECW _____% _______ L
  - ICW _____% _______ L
  - Impedance 5 kHz: _________   Impedance 50 kHz: _________
HR monitor on

5 minute warm up

Begin exercise: **STARTING WORKLOAD = _____________** (to reach 50%)

HR @ 5 min: _______ b/m

HR @ 15 min _______ b/m \hspace{1cm} RPE: 10 min __________

HR @ 25 min _______ b/m \hspace{1cm} RPE: 20 min __________

HR @ 35 min _______ b/m \hspace{1cm} RPE: 30 min __________

HR @ end/45 min _______ b/m

Rest 30 min / walk around

Urine Sample #2 \hspace{1cm} **URINE SPECIFIC GRAVITY AFTER ____________**

Re-weigh: ____________

Body Comp # 2: \hspace{1cm} **BODY FAT AFTER: __________ %**

- TBW _____% _____ L
- ECW _____% _____ L
- ICW _____% _____ L

Impedance 5 kHz: ________ \hspace{1cm} Impedance 50 kHz: ________

Impedance 100 kHz: ________

Impedance 200 kHz: ________

Rehydrate to weight lost
REFERENCES


