ABSTRACT

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The potential influence of the menstrual cycle on percent body fat (%BF) measurements from bioimpedance analysis (BIA) was investigated on 36 female Ss (19-40 yr). Subjects were divided into 2 groups based on oral contraceptive agent (OCA) use. Those women not using an OCA were placed into the OFF group (n = 20) and those using an OCA were placed into the ON group (n = 16). The menstrual cycle was divided into 4 phases: premenstrual, menstrual, postmenstrual, and intermenstrual. All subjects were weighed and tested for %BF using BIA a minimum of 12 times, at least 3 times within each cycle phase. Subjects were also underwater weighed a single time, approximately at the middle of their respective cycles. Repeated measures ANOVAs were used to test for significance between and within groups for weight, resistance, and %BF across the 12 measurements and across the 4 phases. No significance was found for any variable tested with the exception of weight within each group across the 12 measurements ($p < .05$). Percent body fat data from hydrostatic weighing (HW) were compared to BIA values from each of the 4 phases using a repeated measures ANOVA and PPMC. No significant differences were found ($p > .05$) between HW and BIA for any phase. The average correlation between HW and BIA was .78, SEE = 3.3%. Overall, neither OCA use nor the menstrual cycle itself appeared to influence %BF measurements from BIA.

# THE EFFECT OF THE MENSTRUAL CYCLE ON BIOIMPEDANCE RELIABILITY

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# CHAPTER I

# INTRODUCTION

## Background

Obesity is defined by the National Institutes of Health (NIH) (1985), as "an excess of body fat frequently resulting in a significant impairment of health." According to the second National Health and Nutrition Examination Survey (NHANES II), which was conducted by the NIH, a strong association exists between the prevalence of obesity and cardiovascular disease risk factors. These include hypertension, hypercholesterolemia, and noninsulin dependent diabetes mellitus, all of which have been shown to be potentially reversible with weight loss. Obesity predisposes individuals to other disorders such as degenerative joint disease and gallstones. Some forms of cancer have also been linked to obesity (Bray, 1985).

Traditional methods of determining overweightness such as height/weight tables and body mass index do not take body composition into consideration, and therefore may not be accurately assessing body fatness. A more significant measurement from a health or a fitness standpoint is percent body fat (%BF) (Francis, 1990).

There are a number of methods available for assessing %BF, such as skinfold measurements, total body potassium,

dual-photon absorptiometry, and densitometry. Densitometry, otherwise known as underwater weighing, is generally considered the gold standard method for the measurement of %BF. However, this procedure requires expensive, nonportable equipment, experienced technicians, and considerable time. Often a more convenient method of %BF estimation is desired. One such method is bioelectrical impedance analysis (BIA).

Bioelectrical impedance analysis is based upon the principle that lean tissue or fat-free mass (FFM) is a much better electrical conductor, because of its high water and electrolyte content, than is adipose tissue, which tends to impede an electrical current (Pethig, 1979). Assuming that 1) the height of an individual is equivalent to conductor length, and 2) FFM has a constant hydration factor of 73%, equations have been derived to produce estimates of total body water (TBW), FFM, and %BF (Martin & Drinkwater, 1991; Van Loan, 1990).

Since impedance measurements are based upon standard proportions of intra- and extracellular water, it logically follows that any factor which alters the body's normal hydration status may also alter the reliability of impedance measurements. Of particular concern with regard to women is the water retention often reported during certain phases of the menstrual cycle.

## Statement of the Problem

Little research has been done concerning the influence of the menstrual cycle on BIA variability. If water retention and the weight gain associated were found to influence the %BF measurement, recommendations could be made accordingly. Therefore, this is an area which could benefit from additional research.

## Purpose of the Study

The purpose of this study was to investigate the possible influence of the menstrual cycle on the BIA method of estimating %BF, comparing women who used an oral contraceptive agent (OCA) with those who did not.

## Hypotheses

The hypotheses of this study were:

1. No significant difference will be found in the mean weight measurements between the premenstrual, menstrual, postmenstrual, and intermenstrual phases among the group using an OCA (the ON group), and the group not using an OCA (the OFF group).
2. No significant difference will be found in the mean impedance measurements between the premenstrual, menstrual, postmenstrual, and intermenstrual phases among the ON and OFF groups.
3. No significant difference will be found in the mean %BF measurements between the premenstrual, menstrual,

postmenstrual, and intermenstrual phases among the ON and OFF groups.

4. No significant difference will be found in the %BF measurements between hydrostatic weighing and BIA analysis for any phase among the ON and OFF groups.

## Assumptions

The following assumptions were made for this study:

1. The true %BF of subjects did not change over the testing period.
2. The days on which measurements were performed for each cycle phase were representative of that phase.
3. No unreported menstrual abnormalities or irregularities existed.
4. Monophasic and triphasic OCAs produced similar water retention effects, allowing those subjects on these agents to be grouped together.
5. The menstrual cycles observed were typical for those subjects.
6. Subjects maintained a consistent level of sodium intake throughout the study as per initial instructions.
7. Subjects followed the protocol for BIA testing.

## Delimitations

This study had the following delimitations:

1. The subjects were between 19 and 40 years of age.
2. The subjects did not have any unresolved menstrual cycle abnormalities or irregularities.

3. The subjects had a typical cycle length of between 21 and 35 days.
4. The subjects were not greater than 130% of ideal body weight.
5. The subjects were not on any medications, such as diuretics or steroidal drugs, which could have altered normal hydration.
6. The subjects did not alter their diet or exercise patterns to any significant degree.

## Limitations

This study had the following limitations:

1. The subjects were not selected randomly which could have affected the external validity of the study.
2. The subjects were limited to those who were available for all 12 testing days at a specified time and place.
3. No enforcement of the testing protocol was possible which may have influenced the subjects' compliance.
4. Due to time limitations, subjects were tested over one menstrual cycle only.

## Definition of Terms

Bioelectrical Impedance Analysis (BIA) - A method of estimating %BF using whole body impedance to a low level current. It is based on the fact that FFM in the body contains virtually all of the water and electrolytes, and hence will conduct the current, while the fat mass (FM) will not.

Densitometry (HW)- Otherwise known as hydrostatic or underwater weighing, it is considered the gold standard for %BF measurement. By determining the subject's weight when submerged in water after a complete exhalation, total body density (TBD) is calculated. Assuming constant values for the densities of FFM and FM, a number of equations have been derived to estimate %BF from TBD.

Ideal Body Weight (IBW) - Based upon height, the reference weight desired for an individual. In this study, the following equation for females, known as the Divine Equation (J. Rye, personal communication, January 28, 1989), was used to determine IBW:

(Ht in inches - 60) x 5 lbs per inch + 100 lbs = IBW

Intermenstrual Phase (Phase 4) - All remaining days of the menstrual cycle between the post- and premenstrual phases.

Menstrual Phase (Phase 2) - The days of the menstrual cycle during which menses occurs.

Percent Body Fat (%BF) - The percentage of body weight which is composed of adipose tissue.

Postmenstrual Phase (Phase 3) - The 7 days following the cessation of menses.

Premenstrual Phase (Phase 1) - The 7 days prior to the onset of menses.

## CHAPTER II

## REVIEW OF RELATED LITERATURE

### Introduction

The advantages of using BIA as a method of assessing body composition are obvious. It is a fast, noninvasive, convenient, and comparatively inexpensive technique, requiring little skill or experience to perform. However, a debate exists among researchers as to the validity of BIA measurements. Some limitations to this method are well-documented, while other influencing or potentially influencing factors have yet to be researched. Special regard was given in this study to the menstrual cycle and the possible effect it may have on bioimpedance measurements.

### Bioelectrical Impedance

#### History

The first connection between body impedance and the measurement of physiological parameters was made by Nyboer in 1943, when he used electrical impedance plethysmography to demonstrate that biological volumes were inversely related to impedance. He was followed by Thomasset who in 1962, conducted the initial studies using bipolar electrical impedance to measure total body water (TBW) (Lukaski, Johnson, Bolonchuk, & Lykken, 1985).

In 1969, as cited in Lukaski, et al. (1985), Hoeffer made a further advancement in the use of bioimpedance when he reported a high level of correlation (r = .92) between whole body impedance and TBW. This was the first time the tetrapolar impedance technique, measured from wrist to ankle, was employed. According to Lukaski (1987), the primary advantage of this technique is the minimization of contact impedance or skin and electrode interactions. This has since become the standard method for BIA measurement (Lukaski et al., 1985). From these early observations came the development of the use of BIA for the purpose of estimating %BF.

Physical Principle

The method of using BIA for the measurement of %BF is based upon the conduction of an applied electrical current through the body. In a living organism, water and electrolytes behave as electrical conductors while cell membranes act as electrical condensors. At low frequencies (approximately 1 kHz), the current passes primarily through extracellular fluid, while at higher frequencies (500 to 800 kHz), it passes through both extra- and intracellular fluids (Lukaski et al., 1985).

As cited by Kushner and Schoeller (1986), the measurement of TBW by BIA is based upon the principle that impedance is related to the conductor length and configuration, its cross-sectional area, and the signal

frequency. The flow of the current can be related to the volume of the conductor when the signal frequency and conductor configuration are constant:

$$Z = pL/A$$

where Z is resistance in ohms, p is lean tissue resistivity in ohm-cm, L is conductor length in cm, and A is conductor cross-sectional area in $cm^2$. This equation can be rearranged to:

$$Z = pL^2/AL$$

where AL is equal to volume (V). Substituting gives:

$$Z = pL^2/V$$

Impedance has two geometrical components, resistance (R) and reactance (Xc). Resistance is the sum of in-phase vectors or the pure opposition to the flow of a current, while reactance is the sum of out-of-phase vectors or the dielectric component which results from the additional opposition to current flow due to the presence of capacitance or storage of voltage by a condensor (Chumlea & Baumgartner, 1990). However, Lukaski et al. (1985) found the magnitude of Xc to be small compared to R, and R to be a better predictor of impedance than Xc; therefore, Xc can be ignored and R can be substituted for Z in the above equation to give:

$$R = pL^2/V$$

where R is measured in ohms, and L is standing height in cm. This can be rearranged to:

$$V = pL^2/R$$

If p is assumed to be constant, then $L^2/R$ will be directly proportional to, and hence a measure of, the lean compartment volume. Although the human body is not perfectly cylindrical in shape, this relationship has been used to derive models for the prediction of human body composition (Lukaski et al., 1985).

Validity of the Measurement

Bioimpedance analysis has not as yet been accepted by the scientific community as a valid method of determining %BF. Research performed in this area has not produced conclusive results. Most research attempting to validate or discredit BIA has compared bioimpedance results to those obtained by HW.

Lukaski et al. (1985) conducted such a study on 37 men. A high correlation ($r = .98$) was found when $ht^2/R$ was regressed against FFM obtained by HW.

Another study by Lukaski, Bolonchuk, Hall, and Siders (1986) compared BIA, skinfold measurements, and HW in 114 subjects, including both men and women. For women a correlation coefficient of .954 was found between BIA and

HW, while men demonstrated an even higher correlation (r = .979). Overall, the BIA method had a lower standard error of the estimate (SEE) than skinfolds (2.7 vs. 3.9%).

The results of a second study were not in agreement with those found by Lukaski et al. (1986). Jackson, Pollock, Graves, and Mahar (1988) compared skinfolds, BIA, and HW, looking at both day-to-day fluctuations and intertester error. A correlation of .792 was found for BIA and HW in female subjects, with an SEE of 6.4%. The correlation for skinfolds and HW, measured on the same subjects, was higher (r = .88, SEE = 3.6%), indicating that the skinfold method was a better predictor of %BF than BIA. An interesting note to this study was that more than 60% of the measurement error for BIA came from day-to-day changes within the subject. This was in contrast to the skinfold method, where the greatest source of measurement error came from intertester error.

A four-site cross-validation study by Segal, Van Loan, Fitzgerald, Hodgdon, and Van Itallie (1988) compared BIA and densitometrically determined LBM in 1,567 men and women who varied widely in age and body fat content. Bioimpedance was found to be a valid predictor of %BF when data were pooled and fatness-specific equations for predicting LBM were derived. The results of this study indicate that BIA results are reproducible in independent laboratories.

In general, BIA has not yet been established as a valid method of determining %BF. In studies where the manufacturer's equation was used, rather than an equation developed by regression analysis of collected data, correlations have been much lower. Predictive accuracy and generalizability of BIA can only be established by additional cross-validation studies performed by different investigators using large, heterogenous samples (Jackson et al., 1988).

Limitations of Bioimpedance Analysis

Obesity. One of the most frequent problems noted with bioimpedance is the tendency to overpredict FFM in obese individuals and to underpredict FFM in extremely lean subjects (Chumlea, Roche, Guo, & Woynarowska, 1987; Hodgdon & Fitzgerald, 1987; Keim, Barbieri, & Van Loan, 1990; Khaled, McCutcheon, Reddy, Pearman, Hunter, & Weinsier, 1988; Nash, 1985). In a study by Segal, Gutin, Presta, Wang, and Van Itallie (1985) the relationship between LBM as determined by HW and as predicted by BIA was determined. The residual scores, which were the observed minus the predicted LBM values, were significantly related to percent body fat ($r = .796$), such that with increasing fatness LBM was overpredicted by BIA.

Similar results were reported by Gray, Bray, Gemayel, and Kaplan (1989). They found that when FFM, as determined by HW, was compared with predicted FFM from BIA, correlation

coefficients were high at all levels of body fatness ($r = .94 - .99$). However, impedance equations tended to overestimate FFM in subjects with greater than 42% body fat.

A study involving 827 subjects was performed by Deurenberg, Van Der Kooy, Leinen, Westerstrate, and Seidell (1990), comparing HW to BIA. Researchers concluded that the %BF values in obese subjects were underestimated by BIA, and they hypothesized that this might have been due to altered water distribution between intra- and extracellular spaces in these subjects.

Results of these studies indicate that equations developed for the general population may not produce reliable results in the obese. Until specific equations have been developed and cross-validated for this subgroup, obese individuals should seek a different method of estimating %BF.

Skin temperature. Since BIA is determined by the water and electrolyte content of the body, it is possible that any factor which alters the distribution of body water relative to the impedance electrodes could alter resistance and therefore the %BF measurement. One such factor is altered skin blood flow, measured by ambient skin temperature.

Caton, Molé, Adams, and Heustis (1988) tested the possible effects that changes in skin temperature could have on bioimpedance. They found that when skin temperature increased, resistance decreased. This resulted in a

significant increase in predicted TBW, and therefore a lower predicted %BF. The opposite was true for cool skin. Decreased skin temperature was found to increase resistance and predicted %BF. Lukaski (1990) reported the same results with skin temperature fluctuation.

Hydration. The primary assumptions made with BIA are that the percentage of FFM composed of water is 73.2%, and that this water is distributed throughout the body uniformly and symmetrically (Cohn, 1985). Subjects are assumed to be in a normal state of hydration; therefore, any condition which alters hydration will also invalidate the measurement (Deurenberg, Westerstrate, Hautvast, & Van Der Kooy, 1991).

A number of conditions could cause hydration levels to fluctuate. Some of these include strenuous exercise, excessive alcohol consumption, use of diuretics or steroidal drugs, and ingestion of a meal or large quantity of fluids prior to testing (Brodie, Eston, Coxon, Kreitzman, Stockdale, & Howard, 1991; Lukaski, Bolonchuk, Siders, & Hall, 1990; Nash, 1985).

One possible factor influencing normal hydration which has yet to be fully explored is the effect of the menstrual cycle on BIA measurements. Premenstrual bloating is a common complaint associated with the menstrual cycle (Watson & Robertson, 1965). If water retention is significant enough to alter normal hydration status and corresponding weight, recommendations can be made to avoid testing during

this time. If significant differences are not found between phases of the menstrual cycle, there is no need to limit BIA use according to menstrual cycle phase.

## The Menstrual Cycle

In order to discuss the possible effects the menstrual cycle may have on BIA measurements, it is first necessary to review the changes that occur within the female over the course of the menstrual cycle. Hormonal fluctuations, both normally and in the woman using oral contraceptives are of specific interest. The following information regarding the ovarian cycle is taken from Marieb (1992).

### The Ovarian Cycle

The ovarian cycle can be described in terms of three consecutive phases: the follicular, the ovulatory, and the luteal phase. The follicular phase occurs from day 1 to day 10 of the ovarian cycle and is characterized by follicular growth; the ovulatory phase extends from day 11 to day 14 and results in ovulation; and the luteal phase, the period of corpus luteum activity, encompasses days 14 to 28.

Typical cycle length is 28 days; however, somewhat longer or shorter cycles are not uncommon. In these situations, the length of the follicular phase may vary, as may the timing of ovulation. Only the luteal phase remains constant, since the end of the ovarian cycle will always occur 14 days after ovulation.

The follicular phase of the ovarian cycle involves the maturation of a primordial follicle from which the female sex cell, called the oocyte or ovum, is released during ovulation. Follicle growth and oocyte maturation are controlled by the interaction of three hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen. The first two hormones are produced and released from the anterior pituitary, while estrogen is generated by the ovaries.

The exact mechanism of hormonal control of the ovarian cycle is not known; however, the most current hypothesis is described here. In the following discussion a 28-day cycle is assumed. It is also assumed that impregnation does not occur.

1. On day 1 of the cycle, rising levels of a hormone called gonadotropin-releasing hormone (GnRH), released from the hypothalamus, stimulate the production and release of FSH and LH in pulse-like spurts by the anterior pituitary.

2. The presence of FSH and LH stimulates the growth and maturation of immature ovarian follicles. As the follicles enlarge, they begin to secrete increasing quantities of estrogen.

3. As plasma estrogen levels rise, a negative feedback mechanism works on the anterior pituitary. This causes an increase in production of FSH and LH, at the same time preventing their release. The result is an accumulation of

these hormones in the anterior pituitary. Within the ovary, estrogen intensifies the effect of FSH on follicle growth and maturation, resulting in further estrogen production.

4. Once estrogen reaches a critical blood level, it exerts positive feedback on the brain and anterior pituitary. The stimulatory effects of high estrogen levels cause a series of events to be set in motion, the first of which is a sudden burstlike release of accumulated LH from the anterior pituitary.

5. The sudden flush of LH stimulates the complete maturation of the ovum. The follicle ruptures and ovulation occurs at approximately day 14 of the cycle. The role, if any, of FSH in this process is unknown. Soon after ovulation, estrogen levels begin to decline.

6. The ruptured follicle is transformed into a corpus luteum. It is stimulated by LH to produce another hormone, progesterone, and smaller amounts of estrogen.

7. Rising blood levels of progesterone and estrogen exert a powerful negative feedback effect on the release of FSH and LH from the anterior pituitary. As these gonadotropins decline, development of new follicles is inhibited.

8. As LH blood levels slowly decrease, the stimulus for corpus luteum activity ends, and the corpus luteum

begins to degenerate. Consequently, the plasma levels of estrogen and progesterone drop sharply.

9. The decline of ovarian hormones at the end of the cycle (days 26-28) ends their blockade of FSH and LH secretion, and a new cycle begins.

The peak levels for FSH and LH occur just prior to ovulation, as does the peak concentration of estrogen. Progesterone reaches its highest level after ovulation, when the corpus luteum is actively secreting hormones. Estrogen concentration increases during this time as well, but does not reach the level seen prior to ovulation.

These hormone fluctuations are typical for the normal female. However, taking exogenous hormones in the form of an OCA alters the usual cycle of hormone production and secretion.

Oral Contraceptives

Oral contraceptive use involves the administration of estrogen and progesterone given in the appropriate quantities during the first half of the menstrual cycle. Ovulation is prevented by suppression of the surge of LH secretion which is necessary for ovulation to occur. Since any natural decrease in estrogen secretion would not be detected if exogenous estrogen was taken, OCAs may work by masking the normal estrogen depression which signals the beginning of ovulation.

According to Walker and Bancroft (1990), two primary types of OCAs are currently prescribed. Monophasic pills provide a low dose of combined estrogen and progesterone given in constant amounts. Triphasic pills provide a constant level of estrogen, but the progesterone level varies to imitate normal fluctuations of this hormone.

With either type of OCA women are placed on a 4-week cycle, with hormone-containing pills taken daily for 3 weeks. In order to induce menstruation, a placebo containing no hormones is taken on the fourth week of each menstrual cycle. Therefore, in contrast to normal hormone fluctuations, individuals on an OCA experience a drop in estrogen only when the placebo is being taken. Progesterone levels may or may not vary, depending on the type of OCA prescribed. Since the release of LH and FSH is inhibited by high levels of ovarian hormones, these hormone concentrations remain low until placebos are taken.

## BIA and the Menstrual Cycle

As mentioned previously, water retention is a common problem reported during certain phases of the menstrual cycle, most typically premenstrually (Sloane, 1985). Although no established physiological basis has been determined for these problems, hormone fluctuation of some kind has been proposed as a causative factor since the 1930's (Laessle, Tuschl, Schweiger, & Pirke, 1990).

Research is still being done in an attempt to learn more about premenstrual symptoms and their etiology.

In 1979, Abplanalp, Donnelly, and Robert measured levels of estrogen and progesterone in women, comparing these results to subjective complaints of symptoms. They found no systematic variation in symptoms across endocrinologically defined cycle phases. Nor did they find any significant relationship between levels of ovarian hormones and complaints of symptoms. This indicated that the physical problems did not appear to be connected to any particular hormonal change.

Walker and Bancroft (1990) compared 122 women who used either a monophasic OCA, triphasic OCA, or who used a different form of birth control, in regard to menstrual cycle symptoms. Subjects kept records of symptoms for two to three cycles. One finding was that "body swelling" was the most prevalent complaint for all three groups. However, there was a tendency for the monophasic group to report this symptom more often during menstruation, while the triphasic group reported feeling bloated both premenstrually and during menstruation. Those women not using an OCA reported the most water retention premenstrually. The conclusion reached by the researchers was that pill cycles and normal ovarian cycles produced very similar results, again suggesting that symptoms had little to do with direct hormone effects.

In 1990, a study by Laessle et al. found that specific physical complaints occurred during the luteal, premenstrual, and menstrual phases. Researchers speculated that premenstrual problems could be due to decreased levels of progesterone in these phases. However, symptoms were not prevalent during the follicular phase, when progesterone was also low, suggesting that there was no correlation between absolute hormone level and presence of symptoms. Also, this study did not include bloating or water retention as a variable to be monitored.

Another study by Magos (1989) tested 150 women, rating symptoms on a daily basis for one full menstrual cycle. He found that 85.3% of subjects reported premenstrual water retention. An interesting note to these findings is that while "feeling swollen" was the most frequently reported symptom, actual weight gain was only the fifth most prevalent problem. This would seem to indicate that perception of bloating was not always accompanied by physical evidence of water retention.

Food cravings are also typically associated with either the premenstrual or menstrual phase. A study was performed by Dalvit (1982) on eight women to determine whether their food intake was affected by menstrual cycle phase. His results indicated that caloric intake was significantly higher during the 10 days postovulatory than during the 10 days preovulatory. Based on this, he hypothesized that

progesterone could be acting as an appetite stimulant. However, the data collected on dietary intake were determined by subject recall, and therefore were not objective. Two studies were found which directly addressed the issue of bioimpedance validity over the course of the menstrual cycle. Chumlea, et al. (1987) investigated the influence of oral contraceptive use on BIA. Twenty-nine women, 21 to 38 years of age, were subjects in this study. Eleven of these women were taking an OCA, while the remaining 18 were not. Weight and bioimpedance measurements were taken on these subjects for 35 consecutive days. To test the ability of a single BIA measurement to predict the true value of resistance throughout the menstrual cycle, correlations were determined between BIA on a random day and the mean value for BIA for all 35 days tested. The correlation coefficients were .89 ($p < .05$) for women taking oral contraceptives and .97 ($p < .05$) for women not on an OCA. These results indicated that any single BIA measurement would accurately predict BIA, with no effect of menstrual cycle day on resistance measurements. Nor did researchers find that OCA use had any significant effect on bioimpedance.

A second study examining the effect of the menstrual cycle on bioimpedance was performed by Gleichauf and Roe (1989). Weight and bioimpedance were measured daily on 25 women for a full cycle. Sodium intake was also assessed.

The menstrual cycle was divided into four phases: menses, follicular, postovulatory, and premenstrual. While significant differences were found between some phases for weight or resistance, no significant differences were found for %BF, indicating that BIA was consistent throughout the menstrual cycle. However, these researchers did suggest that women who experience large weight fluctuations may want to take the average of several bioimpedance measurements during the cycle rather than a single measurement, in order to better estimate %BF.

In general, the studies which directly addressed effects of the menstrual cycle on BIA did not find any significant relationship between cycle phase and alteration in resistance or %BF measurement. Even those studies which were concerned only with symptoms of the menstrual cycle could not identify any particular pattern of symptoms, indicating that individuals do not react in the same way to specific changes in the different hormone concentrations.

# CHAPTER III

## METHODS AND PROCEDURES

### Introduction

The methodology of this study consisted of subject selection, testing procedures, and statistical analysis. Subjects were selected with the goal of obtaining individuals who represented a normal, healthy population of premenopausal women. Testing methods were devised which would provide adequate data for interpretation yet be realistic to complete within the testing schedule.

### Subject Selection

Recruitment of subjects was done primarily via word-of-mouth, with most volunteers coming from a local hospital, university, and fitness center. Potential subjects were contacted by telephone and were informed of what the study would entail. If the individual indicated interest in participating, arrangements were made for her to complete a brief health history form (see Appendix A) to screen for delimiting factors.

Subjects who fit the necessary criteria were then placed into one of two groups based on their current use of oral contraceptives. Those currently using oral contraceptives were placed in the ON group while those not on an OCA were categorized as the OFF group. Each subject

was given a testing time and location based on her schedule. Every effort was made to minimize inconvenience for the subjects in order to encourage compliance.

## Testing Procedures

Subjects were given an informed consent form to sign (see Appendix B) and a protocol for BIA testing (see Appendix C). Protocol restrictions included avoiding alcohol for a minimum of 24 hours prior to testing and avoiding vigorous exercise and caffeine for at least two hours before testing. Subjects were also to be at least two hours postprandial, and were given guidelines to limit their sodium intake (see Appendix D).

Each subject was tested a minimum of 12 times, with at least three measurements taken during each of four phases of the menstrual cycle. These included the premenstrual (Phase 1), menstrual (Phase 2), postmenstrual (Phase 3), and intermenstrual (Phase 4) phases, as previously defined in Chapter I. All testing was done at a consistent time of day for each individual. Prior to each session, subjects were asked a list of questions regarding their compliance to the protocol. If any of the stated restrictions were not adhered to, the subject was rescheduled to test on the following day.

Actual measurements taken each day included weight and impedance measurements from which %BF was calculated. The BIA apparatus used was a Bioelectrical Sciences BES 200Z

In addition to BIA measurements, each subject was hydrostatically weighed on a day approximately midway through her cycle. This procedure involved obtaining a dry weight, determining residual lung volume, and underwater weighing.

The subject was first asked to change into a swimming suit which would not "pocket" air, preferably a two-piece suit, and to void or eliminate, if necessary. Her weight was then measured to the nearest quarter-pound. Next the subject was asked to shower and then to carefully get into the immersion tank. Residual volume was calculated using the oxygen dilution technique. This involved measuring the amount of nitrogen in the subject's exhaled air before and after a known volume of pure oxygen was mixed with it to the point of equilibrium.

With the chair empty and the water still, the weighing equipment was calibrated. Once this was completed, the subject was instructed to sit on the weighing chair and to put on a nose clip. She was then told to hold on to the chair, take a few deep breaths, and to blow out as much air as possible, bending forward and submerging completely as she continued to blow out.

Once as much air as possible was expelled and the subject was totally submerged, she remained stationary while the computer was activated to record her underwater weight. This process was repeated at least six times, and the

average of the three highest readings was used. Percent body fat was then determined using the following equation (Brozek, Grande, Anderson, & Keys, 1963) for densitometrically determined body fat in women:

$$\text{Percent Body Fat} = [4.570 - D] - 4.142\ (100)$$

where D is equal to body density as determined by HW.

## Statistical Analysis

Data collected from BIA and hydrostatic weighing were statistically analyzed. A two-way ANOVA with repeated measures design was used to detect significant differences within and between the ON and OFF groups for body weight, resistance, and %BF across the 12 individual measurements, across the four phases, and within each phase.

Independent t-tests and Pearson product moment correlations were used to compare %BF measurements from BIA for each of the four phases to that obtained from HW.

# CHAPTER IV

# RESULTS AND DISCUSSION

## Introduction

The data collected in this study were analyzed for significant differences in weight, resistance, and %BF across 12 measurements, across four phases, and within each phase. Hydrostatic weighing results were compared to BIA measurements from each phase. The purpose of this chapter is to present the data collected and to discuss the results of the statistical analysis.

## Results

Descriptive statistics were performed on the subject groups, and a dependent t-test was done to ensure that the two groups were not statistically different in regard to height, initial weight, or age. Percent body fat from HW was later compared to see if this differed significantly between groups. Table 1 provides data on the descriptive characteristics for the groups and for the subjects overall.

Measurements of weight, resistance, and %BF were taken on all subjects a minimum of 12 times or at least three times within each cycle phase. Since phases were defined by menstruation, the true cycle phase was not always known at the time of measurement. The result of this was that extra

Table 1. Physical characteristics of the subjects

| Group | n | Age | Height | Weight | %BF# |
|---|---|---|---|---|---|
| OFF | 20 | 30.6* | 65.4 | 133.4 | 24.4 |
| | | 6.37** | 2.76 | 14.31 | 5.65 |
| ON | 16 | 27.1 | 65.1 | 138.2 | 25.6 |
| | | 3.96 | 2.12 | 14.58 | 4.09 |
| Overall | 36 | 29.0 | 65.3 | 135.5 | 25.0 |
| | | 5.64 | 2.46 | 14.43 | 4.91 |

* = Mean
** = Standard Deviation
# = %BF from underwater weighing

measurements were sometimes taken, particularly in phase 4, which by definition was the longest cycle phase. When more than the three required measurements were taken within a phase, all data were averaged and the mean score was used to represent each of the three data points for that phase.

Table 2 contains the mean weights across the 12 measurements for each group and overall. No significant difference ($p > .05$) was found between the ON and OFF groups for this variable. Although a statistically significant difference ($p < .05$) was found within each group of subjects, the absolute differences between the highest and lowest mean weights across the 12 measurements were only 1.6 and 1.5 pounds in the OFF and ON groups, respectively. Since the actual range of weight fluctuation was so narrow, and did not seem to follow any pattern, this statistical significance was disregarded and no post-hoc test was

Table 2. Comparison of body weight across the twelve measurements

| Measurement | OFF (n = 20) | ON (n = 16) | Overall (N = 36) |
|---|---|---|---|
| 1 | 132.9* | 138.7 | 135.5 |
| | 14.07** | 15.06 | |
| 2 | 133.0 | 138.3 | 135.3 |
| | 13.78 | 14.94 | |
| 3 | 133.3 | 138.2 | 135.5 |
| | 14.48 | 15.04 | |
| 4 | 134.0 | 138.1 | 135.8 |
| | 14.23 | 14.71 | |
| 5 | 133.9 | 138.4 | 135.9 |
| | 14.05 | 14.87 | |
| 6 | 133.7 | 138.0 | 135.6 |
| | 13.88 | 14.75 | |
| 7 | 133.0 | 137.3 | 134.9 |
| | 14.06 | 14.70 | |
| 8 | 133.1 | 137.6 | 135.1 |
| | 13.94 | 14.79 | |
| 9 | 133.0 | 137.2 | 134.8 |
| | 14.15 | 14.73 | |
| 10 | 132.4 | 137.9 | 134.9 |
| | 13.64 | 14.89 | |
| 11 | 132.9 | 137.9 | 135.1 |
| | 13.79 | 14.71 | |
| 12 | 133.0 | 138.0 | 135.2 |
| | 14.15 | 14.96 | |

* = Mean ** = Standard Deviation

performed. Data from the 12 measurements were divided into the four, previously defined, menstrual cycle phases. A repeated measures ANOVA was used to detect significance between phases for each of the variables. Mean weights for each group and overall across the four phases are presented in Table 3. No significant differences ($p > .05$) were found across the phases, either within or between groups.

Table 3. Comparison of weight across the four phases of the menstrual cycle

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 20 | 133.0* | 133.9 | 133.0 | 132.8 |
| | | 14.10** | 14.04 | 14.03 | 13.83 |
| ON | 16 | 138.4 | 138.2 | 137.4 | 137.9 |
| | | 14.99 | 14.76 | 14.70 | 14.84 |
| Overall | 36 | 135.4 | 135.8 | 135.0 | 135.1 |

* = Mean ** = Standard Deviation

Resistance was the second variable analyzed. The same statistical treatment was performed, first testing for significance across the 12 measurements, then across the four menstrual cycle phases. No significance ($p > .05$) was detected between or within groups in either design. The resistance data from the 12 measurements format is shown in Table 4, while Table 5 contains resistance data across the four phases.

Table 4. Comparison of resistance across the twelve measurements

| Measurement | OFF (n = 20) | ON (n = 16) | Overall (N = 36) |
|---|---|---|---|
| 1 | 594.4* | 558.3 | 578.3 |
| | 60.39** | 40.47 | |
| 2 | 593.1 | 561.8 | 579.2 |
| | 60.75 | 37.11 | |
| 3 | 588.5 | 568.1 | 579.4 |
| | 61.61 | 34.81 | |
| 4 | 588.2 | 562.6 | 576.8 |
| | 68.96 | 37.87 | |
| 5 | 583.8 | 567.8 | 576.7 |
| | 66.37 | 35.31 | |
| 6 | 587.8 | 570.2 | 579.9 |
| | 66.00 | 43.94 | |
| 7 | 599.1 | 573.2 | 587.6 |
| | 64.84 | 45.45 | |
| 8 | 592.8 | 571.0 | 583.1 |
| | 68.04 | 40.76 | |
| 9 | 592.0 | 575.0 | 584.4 |
| | 73.48 | 39.93 | |
| 10 | 596.1 | 570.5 | 584.7 |
| | 64.24 | 37.69 | |
| 11 | 591.8 | 569.1 | 581.7 |
| | 61.36 | 44.53 | |
| 12 | 591.8 | 564.5 | 579.6 |
| | 59.59 | 43.61 | |

* = Mean ** = Standard Deviation

Table 5. Comparison of resistance across the four phases of the menstrual cycle

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 20 | 592.0* | 586.6 | 594.6 | 593.2 |
| | | 60.22** | 66.55 | 67.80 | 60.65 |
| ON | 16 | 562.7 | 566.9 | 573.1 | 568.0 |
| | | 36.55 | 37.77 | 40.69 | 40.54 |
| Overall | 36 | 578.0 | 577.8 | 585.0 | 582.0 |

* = Mean ** = Standard Deviation

The third variable measured was %BF. These results were of the greatest interest, since the purpose of this study was to determine if the menstrual cycle caused BIA estimates of %BF to fluctuate significantly. Again, a repeated measures ANOVA was used to test for significance across the 12 measurements and then across the four phases. There were no significant differences ($p > .05$) in %BF between or within groups for either of the methods tested. The data for %BF across the 12 measurements are given in Table 6, while the values across the four menstrual cycle phases are presented in Table 7.

As stated previously, within the two subject groups the differences between the highest and lowest mean values for weight across the 12 measurements were only 1.5 and 1.6 pounds. However, individual data revealed a considerably larger range of values.

Table 6. Comparison of percent body fat across the twelve measurements

| Measurement | OFF (n = 20) | ON (n = 16) | Overall (N = 36) |
|---|---|---|---|
| 1 | 26.0* | 25.5 | 25.8 |
| | 5.88** | 4.40 | |
| 2 | 26.0 | 25.7 | 25.8 |
| | 6.02 | 4.24 | |
| 3 | 25.6 | 26.3 | 25.9 |
| | 6.19 | 4.60 | |
| 4 | 25.8 | 25.8 | 25.8 |
| | 6.15 | 4.75 | |
| 5 | 25.8 | 26.3 | 26.0 |
| | 5.93 | 4.10 | |
| 6 | 25.7 | 26.4 | 26.0 |
| | 6.09 | 4.18 | |
| 7 | 26.5 | 26.3 | 26.4 |
| | 5.91 | 4.57 | |
| 8 | 26.0 | 26.3 | 26.1 |
| | 6.21 | 4.23 | |
| 9 | 25.7 | 26.5 | 26.1 |
| | 6.30 | 4.51 | |
| 10 | 26.0 | 26.4 | 26.1 |
| | 6.41 | 4.25 | |
| 11 | 25.8 | 26.2 | 26.0 |
| | 6.25 | 4.11 | |
| 12 | 25.9 | 25.8 | 25.8 |
| | 5.85 | 4.40 | |

* = Mean ** = Standard Deviation

Table 7. Comparison of percent body fat across the four phases of the menstrual cycle

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 20 | 25.9* | 25.8 | 26.1 | 25.9 |
| | | 5.98** | 5.95 | 6.06 | 6.10 |
| ON | 16 | 25.8 | 26.2 | 26.4 | 26.1 |
| | | 4.35 | 4.23 | 4.36 | 4.16 |
| Overall | 36 | 25.9 | 26.0 | 26.2 | 26.0 |

* = Mean ** = Standard Deviation

The average individual fluctuation in weight found across all measurements was 3.7 pounds, with a range of 1.75 to 6.5 pounds. In fact, 14 subjects, nine in the OFF group and five in the ON group, displayed a fluctuation in weight of four pounds or greater. It was then hypothesized that perhaps not all subjects had experienced water retention; and, that possibly only those subjects who showed the greater amount of weight fluctuation were susceptible to BIA variability in connection to the menstrual cycle.

In order to test this hypothesis, the data from the 14 subjects who had experienced a four pound or greater weight change were placed in a subgroup. A statistical analysis was then performed on this data, using a repeated measures ANOVA to detect any significant differences across the 12 measurements and the four phases for weight, resistance, and %BF.

The data for mean weights in this subgroup across the four menstrual cycle phases are presented in Table 8. The resistance data are shown in Table 9, and Table 10 gives %BF values across the four phases. No significant differences ($p > .05$) were found within or between groups for any variable.

Table 8. Mean subgroup weights across the four phases

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 9 | 137.6* | 139.0 | 137.5 | 137.0 |
| | | 16.08** | 15.84 | 16.12 | 15.59 |
| ON | 5 | 141.6 | 141.0 | 139.4 | 140.1 |
| | | 12.66 | 13.09 | 12.38 | 11.89 |
| Overall | 14 | 139.0 | 139.7 | 138.2 | 138.2 |

* = Mean ** = Standard Deviation

Table 9. Subgroup resistance values across the four phases

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 9 | 566.5* | 555.0 | 569.3 | 573.7 |
| | | 48.85** | 51.31 | 59.73 | 59.20 |
| ON | 5 | 574.8 | 582.6 | 591.9 | 585.8 |
| | | 39.63 | 49.71 | 48.67 | 39.14 |
| Overall | 14 | 569.5 | 564.9 | 577.4 | 578.0 |

* = Mean ** = Standard Deviation

Table 10. Subgroup percent body fat comparison across the four phases

| Group | n | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| OFF | 9 | 24.0* | 23.8 | 24.2 | 24.4 |
| | | 5.39** | 5.38 | 5.45 | 5.93 |
| ON | 5 | 27.7 | 28.2 | 28.3 | 28.2 |
| | | 3.51 | 3.43 | 3.16 | 3.47 |
| Overall | 14 | 25.3 | 25.4 | 25.7 | 25.8 |

* = Mean ** = Standard Deviation

In order to validate the %BF measurements from BIA, these data were compared to %BF from hydrostatic weighing, which is considered to be the gold standard for %BF measurement. Paired t-tests and Pearson product moment correlations were used to test for significant differences between hydrostatically determined %BF and BIA results from each of the four phases. These values are presented in Table 11.

No significant differences ($p > .05$) were found between hydrostatic %BF and BIA measurements for any of the four phases. It should be noted that HW was performed a single time, and these results were assumed to be constant throughout the menstrual cycle (Byrd & Thomas, 1983). The average correlation between HW and BIA values for all phases was .78 (.75 to .81) while the mean SEE was 3.3% (3.1 to 3.5%).

Table 11. Comparison of percent body fat from hydrostatic weighing and bioimpedance analysis across the four menstrual cycle phases (N = 36)

| Method | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| HW | 25.0* | 25.0 | 25.0 | 25.0 |
| | 4.91** | 4.91 | 4.91 | 4.91 |
| BIA | 25.4 | 25.6 | 25.9 | 25.5 |
| | 5.13 | 5.10 | 5.20 | 5.08 |
| Overall | | | | |
| r | .81 | .77 | .75 | .79 |
| SEE | 3.1 | 3.3 | 3.5 | 3.2 |

* = Mean ** = Standard Deviation

## Discussion

### Validity of BIA

Although the purpose of this study was not to test the validity of BIA as a method of determining %BF, BIA measurements from each of the four menstrual cycle phases were compared to %BF measurements from HW. As was discussed earlier, the average correlation between BIA, across the four phases, and HW data was quite good (r = .78). These results are in agreement with those found by Lukaski, et al. (1985) who found a correlation of .98 when BIA results were compared to %BF from HW in a study involving 37 male subjects.

A larger study by Lukaski, et al. (1986) of 114 subjects, both male and female, also found high correlations

between HW and BIA. For females the correlation was .954, while the correlation for males was .979.

Although the correlations between BIA and HW found in this study were not as high as those cited by other researchers, a correlation of .78 would indicate that the values for %BF from BIA were fairly reliable, assuming that HW most accurately measured %BF in these subjects. Also, since the manufacturer's equation for BIA was used, rather than an equation developed by regression analysis of the collected data, as was done in the studies cited, a somewhat lower correlation is to be expected.

Bioimpedance and the Menstrual Cycle

As was stated previously, mean weights across the 12 measurements did not change significantly for either group of subjects. There was a subgroup of individuals whose weight fluctuated at least four pounds thoughout the cycle tested; however, even in this subgroup there were no significant changes in mean weights. In general, weight showed no true pattern of fluctuation as is illustrated by the data in Tables 2, 3, and 8. Rather, changes in body weight appeared to be random in nature with no apparent correlation to menstrual cycle phase.

A tendency existed for resistance values to decrease when there was an increase in weight, and vice-versa. This would be expected if the weight changes were due to fluctuations in body water. The assumption being that %BF

was held constant in these subjects over the testing time frame, resistance should have changed in relation to weight for BIA to remain valid. However, changes in resistance often seemed to overcompensate for weight fluctuations, resulting in many of the higher %BF measurements actually being found at lower weights.

The results found in this study agree with those of Chumlea et al. (1987) and those of Gleichauf and Roe (1989) who found that the menstrual cycle did not seem to influence BIA measurements to any significant degree. This study also had findings similar to that of Magos (1989) who found that the prevalence of reported feelings of water retention greatly exceeded the actual occurrence of measurable weight gain. In the present study, it was not uncommon for complaints of feeling bloated to be unaccompanied by the weight gain which would be expected if water retention was, in fact, occurring.

One possible explanation for this discrepancy is that body water was redistributed premenstrually, with a greater than normal percentage of TBW found in the abdominal region. Since one of the primary assumptions made with bioimpedance is that body water is distributed uniformly and symmetrically throughout the body (Cohn, 1985), if water redistribution were taking place, it is conceivable that this could have altered BIA measurements in some individuals.

A similar situation occurred with %BF scores as took place with weight. Although no significant differences were found between mean %BF values either between or within groups, individual %BF measurements varied considerably. The average individual %BF fluctuation over the cycle tested was 4.0%, with a range of 1.4 to 6.4%. Because no trend or pattern existed to the fluctuations, the mean scores concealed the degree of variability that was present.

# CHAPTER V

# SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

## Summary

This study examined the possibility that water retention frequently reported by women during certain phases of the menstrual cycle could alter %BF measurements from BIA. Weight, resistance, and %BF were the variables analyzed.

Apart from a small, but statistically significant ($p < .05$) difference in mean weight measurements within groups, no significant differences were found across the 12 measurements or across the four menstrual cycle phases for any of the variables analyzed. This indicated that there was no true pattern of weight change due to water retention; therefore, menstrual cycle phase would not have an effect on bioimpedance measurements in the general female population.

However, despite the lack of significant group findings, individual variability was great. The average weight fluctuation over the cycle tested was 3.7 pounds, with more than one-third of subjects experiencing a weight fluctuation greater than or equal to 4 pounds. Also the average individual %BF fluctuation was 4.0%, considerably more than the mean fluctuations in either of the data arrangements.

This demonstrated that, despite high individual variability in weight, resistance, and %BF, no pattern existed in these fluctuations which could be attributable to body changes related to the menstrual cycle.

## Conclusions

Based on the findings of this study, neither the menstrual cycle nor oral contraceptive use influenced either weight changes or the resulting %BF measurements from BIA, in any predictable fashion. Therefore, most women would not need to take these factors into consideration when deciding to have %BF tested using this method.

However, due to the great deal of individual variablity found in this study, it would be advisable for women who experience large weight fluctuations from day to day, whether due to the menstrual cycle or other influence, to either take the average of several measurements from different days, or to seek another means of %BF estimation.

## Recommendations

Bioelectrical impedance is a technique which is influenced by many factors. Although no significant findings were revealed in this study regarding the influence of the menstrual cycle on BIA, obviously this area could benefit from additional research. The high degree of individual variability which existed would indicate that despite every effort to follow the established protocol, some factor or factors influenced these measurements.

Future studies dealing with bioimpedance could further validate the assumption that true %BF did not change by taking girth measurements and by underwater weighing subjects prior to and following completion of the testing. If researchers were able to demonstrate that actual %BF remained constant, then any fluctuations in BIA measurements could, with more confidence, be attributed to other influencing factors.

In general, BIA requires further research in order to be firmly established as a valid method of estimating %BF. Additional studies are needed to examine any of the possible influences on BIA in order to make this technique more consistent and reliable.

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# APPENDIX A

# HEALTH HISTORY FORM

## HEALTH HISTORY FORM

Name: _____ Age: _____

Height (in.): _____ Weight (lbs.) _____

**MEDICAL HISTORY**

1. What was your weight one month ago? _____
   six months ago? _____

2. Have you recently changed your exercise habits? **YES** **NO**
   If yes, in what way? _____
   _____

3. Are you currently trying to lose weight? **YES** **NO**

4. Are you currently taking any medications? **YES** **NO**
   If yes, please list: _____
   _____

5. Do you have any history of menstrual cycle abnormalities or irregularities? **YES** **NO**

   If yes, does this condition still exist? **YES** **NO**
   How long has it been resolved? _____

6. Would you say your menstrual cycle is:

   very regular basically regular irregular

7. Are you currently taking oral contraceptives? **YES** **NO**

   If yes, please list the name brand _____
   How long have you been taking them? _____

8. When was the first day of your last period? _____

9. Are you experiencing any symptoms which may indicate the onset of menopause? **YES** **NO**

This information is true and complete to the best of my knowledge.

Signature_____ Date_____

# APPENDIX B

# INFORMED CONSENT FORM

**INFORMED CONSENT**
**BIOIMPEDANCE AND THE MENSTRUAL CYCLE**

As a participant in this thesis project, your written consent is necessary prior to initiation of testing. Please read this document carefully as your signature indicates that you have understood and agreed to the procedures required of you as a subject.

PURPOSE: This study will examine the reliability of bioimpedance analysis as a means of measuring percent body fat across the menstrual cycle.

PROCEDURES: As a subject, you will be asked to undergo bioimpedance measurements a minimum of twelve times, at different points in your menstrual cycle. This procedure will involve the placement of four electrodes, one each on the top of your right foot, ankle, wrist, and hand. An ohm meter will then be attached to these electrodes via lead wires. A very low level current which cannot be felt will pass between these electrodes, and impedance to this current will be measured by the ohm meter. Your lean tissue will conduct the current while your fat tissue will resist or impede it. You will also be weighed along with each measurement. Each testing procedure should take from seven to ten minutes. You are no more at risk during these testing procedures than you are during other daily activities.

In addition to bioimpedance measurements, you will be asked to undergo one hydrostatic weighing procedure. This is considered to be the gold standard method for percent body fat measurement. At a point approximately midway through your menstrual cycle, you will be scheduled to come to the Human Performance Laboratory at UW-La Crosse. This test will involve determining your residual lung volume, or the amount of air left in your lungs after a full expiration. You will then be asked to sit in an immersion tank and exhale fully as you slowly lower your head beneath the water. When you can blow out no more air, your weight will be recorded. This procedure will be repeated until readings correlate closely. Although this may sound intimidating, the actual amount of time spent beneath the water is a matter of seconds. This entire procedure should take no more than one hour.

**BENEFITS:** Most health and fitness centers which offer percent body fat measurements do so for a fee. As a subject in this study, you will have repeated measurements performed at no cost to you. Participation in this project is entirely voluntary, and you are free to withdraw at any time. All data obtained from you is strictly confidential.

I acknowledge that I have read this document in its entirety and understand the contents within.

I have also read the protocol for bioimpedance testing and agree to comply to the best of my ability. If for some reason I am unable to fully comply, I agree to inform the researcher so that the testing session can be rescheduled.

Signature_____Date_____

Witness_____Date_____

# APPENDIX C

# PROTOCOL FOR BIA TESTING

**PROTOCOL FOR BIOIMPEDANCE ANALYSIS**

Bioelectrical impedance measurements for the determination of percent body fat are affected by your hydration level, or how much water your body contains. Because of this, anything which alters hydration levels should be avoided prior to testing. The following is a list of restrictions which must be adhered to for bioimpedance to be accurate.

1. No alcoholic beverages should be consumed for 24 hours prior to testing.

2. Sodium intake should be limited according to the guidelines given on the attached sheet.

3. Vigorous exercise such as running or aerobics should be avoided for at least 2 hours prior to testing.

4. Testing should be done no sooner than 2 hours after consumption of a meal.

5. Caffeine should be avoided for at least 2 hours prior to testing.

These guidelines should be followed as closely as possible. If, for some reason, you are unable to adhere to one or more of these restrictions, please inform the researcher so arrangements can be made for an alternate testing session.

# APPENDIX D

# GUIDELINES FOR SODIUM INTAKE

## GUIDELINES FOR LIMITING SODIUM INTAKE

The consumption of excess sodium is associated with increased water retention. Therefore, it is important for the purpose of this study to limit sodium in your diet, as well as to maintain a consistent level of sodium intake throughout the testing period.

The most important way to decrease your sodium intake is to avoid the use of salt, both on the table and in cooking. Any type of salt, such as garlic salt, is still salt.

The following is a list of high sodium foods. These foods should be consumed in small amounts, if at all. Also, subjects should avoid eating combinations of high sodium foods in the same day.

### HIGH SODIUM FOODS

* Processed, cured, or smoked meats (e.g., ham, bacon, sausage, bologna, salami, pepperoni, and other processed cold cuts)

* Processed cheeses (e.g., American cheese, Velveeta, cheez whiz)

* Canned or boxed soups, unless labelled "low sodium"

* Canned vegetables, unless labelled "no salt added"

* Dill pickles, olives, sauerkraut, and any other food pickled in a salty brine

* Convenience meals (e.g., boxed potato or pasta mixes, Hamburger Helper, canned entrees, frozen dinners unless labelled "low sodium")

* "Fast food" with certain exceptions such as the salad bar and some sandwiches. Check nutrition information, if available, and look for foods containing less than 500 mg of sodium per serving.

* Salty snack foods (e.g., potato chips, pretzels, salted nuts)