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Comparison of various methods of measuring body composition to underwater weighing in adult men and women

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COMPARISON OF VARIOUS METHODS OF MEASURING BODY COMPOSITION TO UNDERWATER WEIGHING IN ADULT MEN AND WOMEN

by

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ABSTRACT

Comparison of Various Methods of measuring Body Composition to Underwater Weighing in Adult Men and Women

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Nine different methods of measuring body composition were compared to underwater weighing in average Caucasian adult men and women, to determine which method correlated highest with UWW. Fifty participants were tested on underwater weighing, air displacement plethysmoghraphy, Dual Energy X-Ray Absorptiometry, bioelectrical impedance analysis, ultrasound, near infrared reactance, and skinfolds – sum of 7, 4, and 3 sites.

All correlations for both genders were high at the .05 level. For men the highest correlation was between UWW and the sum of 4 skinfolds at .971 and the lowest between UWW and BIA of .748. For women the highest correlation was between UWW and the sum of 4 skinfolds at .962 and the lowest between UWW and ultrasound of .778.

This study concluded that accuracy of different techniques would depend on population specific limitations. For the present sample accuracy of methods differed slightly between genders, but did not differ significantly from UWW.

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CHAPTER 1

INTRODUCTION

The accurate measurement of adipose tissue in the human body is of great physiological and medical importance. Body fatness, in addition to fat distribution, may greatly influence mortality and morbidity. Accurate measurement of total body fat is important since excess fat can increase the risk for several metabolic disorders, cardiovascular disease, as well as the effect of various drug therapies on these disorders. As interest in measuring body composition increased, various methods to measure body composition were developed. A common assumption in the assessment of body composition was that the body consists of two main compartments: fat and lean body mass (LBM), which consists of muscles, bones, organs, blood, skin, and the brain. The methods using the two-compartment models included underwater weighing (UWW); air-displacement plethysmography (ADP) Bod Pod[1]; skinfold measurements, and bioelectrical impedance analysis (BIA). There are also body composition methods that use a three-compartment model, which measures bone mineral composition in addition to fat and lean body mass. An example of the three-compartment is Dual-energy X-ray Absorptiometry (DEXA)[2]. In addition, Magnetic Resonance Imaging (MRI) scanner is

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[1] Bod Pod Body Composition System (Life Measuremen Instruments, Concord, CA, USA)

[2] Lunar DPX Madison, WI, software version 3.6y

also trying to determine body composition assessment, by computing both subcutaneous and internal fat content. Near Infrared Reactance (NIR) and ultrasound are also becoming popular in body composition studies. The number and diversity of body composition methods used raises the question concerning the reliability and validity of the various methods, especially when measuring different populations. For example, differences in gender (males versus females), the degree of obesity between individuals, the differences between ethic groups and difference in old and young populations.

Purpose

The purpose of this study was to determine which method of body composition assessment is closest to underwater weighing and to likewise determine the rank order of seven body composition techniques to underwater weighing. The nine techniques used in this study were: underwater weighing, air displacement plethysmography (Bod Pod), sum of seven skinfold measurements, sum of four skinfold measurements, sum of three skinfold measurements, bioelectrical impedance (BIA), infrared, ultrasound, dual energy X- ray absorptiometry (DEXA), and magnetic resonance imaging (MRI).

Background

For decades underwater weighting, has been considered the "gold standard" in body composition assessment. It has been validated in several human cadaver and animal studies. Many studies use the underwater weighing method to validate their body composition data. Underwater weighting is minimally affected by hydration status, where as methods such as BIA are sensitive to hydration status. Body dimensions do not affect underwater weighing, as long as the underwater weighing tank is large enough to accommodate individuals of different proportions. In addition, underwater weighing is not affected by fat distribution, where as techniques such as skinfolds are affected by size and distribution. Underwater weighing measures body density, using the accepted formula of body mass divided by body volume. The densities of fat and lean tissue have been determined as 0.9001grams/milliliter for fat tissue and 1.100g/mL for lean tissues. The error for fat estimated from underwater weighing ranges from 2 to 3 percent.

Another two-compartment method is air-displacement plethysmography (ADP). It uses the changes of air pressure to determine individual's volume. The volume of the person is measured indirectly by measuring the volume of air he/she displaces inside an enclosed chamber. The Bod Pod is a commercially available version of air-displacement plethysmography that is currently being used in clinical and research settings. The Bod Pod has been studied in order to evaluate its validity and reliability in assessing body composition in a large variety of populations, such as athletes, apparently healthy adults, special populations, and children. These studies have shown good validity and reliability when compared other with well-established two and three compartments model-methods, such as underwater weighing and DEXA. However, no study has directly compared the Bod Pod's validity and reliability when measuring fat in lean, normal weight, or obese populations.

Skinfolds are a widely used anthropometric method for estimating body fat content. This method uses two-compartment model for measuring body composition. It is widely used in field, laboratory and clinical setting, because of the minimal, inexpensive equipment and time it requires for testing. There are several different skinfold sites used, depending on the equation selected to obtain percent body fat. The commonly used sites are pectoral, abdominal, suprailiac, subscapular, triceps, axilla, and thigh measurements. These measurements are taken on the right side of the body in millimeters of skinfold thickness. The disadvantages, which this method presents, are associated with the number of population specific equations, and high level of technical skill required. In addition, the validity and reliability of the test may be affected by edema and the compressibility of fat tissue. The error for skinfold equations is minimal ranging from 3 to 4 percent.

Bioelectrical impedance analysis (BIA) is a two-compartment model for measuring body composition. This method measures fat free mass (FFM), total body water (TBW), percent fat, intracellular water (ICW), extracellular water (ECW), and body cell mass. BIA measures the resistance of different body tissues to an electrical current. There are factors that can influence this measurement including the concentration of salts in the bodily fluids, the motility and the strength of the ions in body fluids, as well as the geometric form of the fluid. The BIA method was validated next to other more accurate methods for measuring body composition. From this validation a prediction equation was developed for the BIA to allow it to make these measurements. There are various BIA instruments diverse in the frequency of their electrical current. The most commonly used 50kHz model is not as accurate as the higher frequency models or deuterium dilution, which is considered to be the "gold standard" for measuring total body water. The reason for this lack of this accuracy is that such a low frequency electrical wave is unable to penetrate into the cells, and therefore is unable to measure intracellular water, but instead measures only extracellular water. Further research showed that even though the 50 kHz

BIA is failing to measure intracellular water it is still of value for measuring total body water. It was found that extracellular water and total body water or lean body mass are highly correlated. The only limitation factor for these relationships to be held stable is that 50 kHz BIA should be used only on healthy individuals.

Near infrared reactance (NIR) method estimates body fat percentage from the reflectance of near infrared light off the underlying tissue. The Futrex NIR analyzers estimate body fat percentage from optical density (OD) measurements at only one site: biceps brachii. The less NIR light reflected (i.e., more light absorbed), the greater the amount of subcutaneous fat. Futrex 6100 is designed for adults only, and uses body weight, height, OD, gender, and age for predictors in its equations. And NIR method for measuring percent body fat requires little technician skills compared to other methods such as skinfolds. There is little difference in biceps OD when two different testers measure the same person. Limited information is present on how hydration status affects the NIR results, including eating, drinking, exercise, and menstrual cycle stages. In addition, skin tone and color account for 12 to 16 percent in the variability in OD measurements at the biceps site.

Ultrasound is another method used for measuring body composition. Ultrasound measures subcutaneous fat tissue, using the same sites used in skinfold measurements, as well as commonly used formulae such as Jackson and Pollack. Ultrasound is proposed as method of measuring subcutaneous fat that reduces the limitations of the skinfold method. For example, one of the disadvantages to skinfolds is that they compress the fat tissue. In addition, skinfolds may be very hard to measure on obese individuals, but the ultrasound method does not require as high technician skills as the skinfold method. The Ultrasound method is suggested to eliminate the disadvantages of skinfold.

Dual- Energy X-ray Absorptiometry (DEXA) is a commonly used three-compartment model for measuring body composition. It is used to measure bone mineral density, bone mass, as well as body composition. The DEXA uses the attenuation of a collimate x-ray beam passing through the body. The scan is used in body composition studies to measure not only bone mineral density but also fat content, lean body mass, and fat mass distribution. The accuracy of the DEXA scan depends on two major factors- the software and hardware the systems uses, and hydration level of the individual. When using newer software versions, DEXA will estimate body fat content within 1 to 3 percent accuracy. Furthermore, the hydration level of the subject or more specifically the water content of lean body mass may also alternate the end results of the test. Another source of error in measuring body composition by DEXA may occur when the sum of the weight of bodily parts measured by DEXA does not correspond to the whole body weight measured by a beam weight scale (they should be within 1kg/2.2lbs difference).

Magnetic Resonance Imaging (MRI) can be used to measuring body composition. The MRI is able to measure both the subcutaneous fat and the internal fat of a human. MRI has been validated in animals, human cadavers and manufactured phantoms to compare the MRI measurements of body fat compared to fat obtained by chemical analysis. From several body composition studies using MRI to measure body fat content it was found that multiple-slice acquisition data set gives more accurate measurements than single-slice acquisitions, but the ability to obtain full data sets depends on factors such as cost, time for testing and data analysis available. Studies show that obtaining 10 millimeters thick transverse images of the body with 30 millimeters gaps between them in the arms and legs, and 10 millimeters gaps in the torso provides the researcher with enough information to accurately measure the fat content of a body. The scanner differentiate the fat tissue from all other tissues in the body by viewing the fat tissue as a high frequency signal compared to muted background from all other tissues. The MRI is equipped with software, which measures the voxels in each picture taken. A voxel is a volume element (volumetric pixel) representing a value in three- dimensional space, corresponding to a pixel for a given slice thickness. This is analogous to a pixel, which represents two- dimensional image data. Voxels are frequently used in the visualization and analysis of medical data. The MRI pixel intensity is proportional to the signal intensity of the appropriate voxel. Voxels with noise lower than the background noise were not taken for measurements since they were corresponding to lean tissue. Voxels with higher frequency noise, above the threshold, were accounted as fat tissue voxels. Additional manual work on the images may also be required for more accurate measurement of fat content.

CHAPTER 2

LITERATURE REVIEW

Introduction

Body composition assessments are of great interest in the clinical setting; in exercise research; in the practical fields of health and fitness and athletics and even in education situations. For body composition to be of value, the assessment must be reliable and valid. There are several different methods for assessing body composition and there are also several equations, which estimate or predict body composition. Many of the methods are only valid for certain populations based on sex, age and ethnicity. Models for measuring body composition are subdivided into groups based on how they compartmentalize the body. For example, there are two compartment models in that divide the body into two compartments: fat and lean body mass. There are other methods that use three and four compartment models. The three-compartment model divides the body into fat, protein and mineral, and water. The four-compartment model divides the body into fat, protein, mineral and water.

Two-compartment models are determined by measuring body density (Db), which is calculated from dividing body mass (weight in kilograms) by body volume (in liters). The main factor affecting body density is the amount of excess fat. For two-compartment models the density of lean body mass is assumed to be constant for all individuals.

Brozek and Keys, and Siri developed equations to convert body density into body fat. These equations were based on the following assumptions about the density of lean body mass: the density of lean body mass components (water, mineral, and protein) are the same of all individuals; the proportions of water, mineral, and protein in the lean body mass are constant within and between individuals; and that the individual being measured differ from a reference body only by the amount of body fat. Siri developed his equation by estimating the errors associated with using two-compartment models to estimate body fat from body density. These errors were associated with biological variations of the percent body water, protein-to-mineral ratio in the lean body mass. Siri estimated that a 2 percent variation in body water leads to 2.7 percent variation in body fat percent; also that an error estimated from variation of protein-to-mineral ratio leads to 2.1 percent variation in percent body fat. Two-compartment model equations give a good estimation of percent body fat as long as the assumptions are met. Also, conversion formulas for converting body density to percent body fat are developed for specific populations, based on measurements of total body water and/or bone mineral density to estimate lean body mass for each age group, sex, and ethnic group. Table 1 presents the formulas for percent body fat as well as the density of lean mass tissue for Caucasian adult men and women. Table 2 shows the assumed values for components of the fat-free body mass.

Table 1 Percent Body Fat Formulas for Caucasian Men and Women

Ethnicity	Age	Gender	Body Fat (%)	Lean Body Mass(g/cc)
Caucasian	18-59	Male	(4.95/Db) – 4.50	1.100
	18-59	Female	(4.96/Db) – 4.51	1.101

Heyward et. al. (2004) Applied Body Composition Assessment

Table 2 Assumed Values for Components of the Fat-Free Body and Reference Body

Component	Density (g/cc)	Fat-free body (%)	Reference Body (%)
Water	0.9937	73.8	
Mineral	3.038	6.8	
Protein	1.34	19.4	
Fat-free body	1.1000	100.0	84.7
Fat	0.9007		15.3
Reference body	1.064		100.0

Heyward et. al. (2004) Applied Body Composition Assessment
Data from Brozek et. al. 1963

Thee-compartment models were developed to account for interindividual variability in total body water. Three-compartment models assess fat, water, and solids as three building compartments of the body, making an assumption about the protein-to-mineral ratio. Siri developed a three-compartment equation, which may be a better prediction of percent body fat in people with a wide range of variation in their hydration status and obese individuals. Dual Energy X-ray Absorptiometry (DEXA) is also a three-compartment model that measures fat, mineral, and protein + water with assumed constant density for protein+ mineral of 1.0486 g/ml. DEXA scanner is used to measure body density and estimate total body mineral from bone. Table 3 presents body composition equations for three-compartment models. DEXA uses two separate sets of two-compartment equations to measure percent body fat. The first set of equations is used to separate the bone and soft tissue mass, and the second set of equations is used to separate soft tissue mass into lean mass and fat mass.

Table 3 Body Composition Equations for Three- Compartment Models

Model	Equation	Reference
Body Weight= fat+water+(mineral and protein combined)	%BF=[(2.118/Db)-0.78BW-1.354]x100	Siri 1961
Body Weight= fat+mineral+(water and protein combined)	%BF=[(6.386/Db)+3.961BW-6.090]x100	Lohman 1986

Heyward et. al. (2004) Applied Body Composition Assessment

Nine different methods for measuring body composition were used in this study: Underwater weighing (UWW), Skinfold measurements (SF) – sum of seven skinfolds, sum of four skinfolds, and sum of three skinfolds, Bioelectrical Impedance Analysis (BIA), Air Displacement plethysmography (ADP), Near Infrared Reactance (NIR), Ultrasound, Dual Energy X-ray Absorptiometry (DEXA), and Magnetic Resonance Imaging (MRI).

Underwater Weighing

Underwater weighing (UWW) is a two-compartment model for estimating body fat from body density. For many years UWW has been accepted as the "gold standard" in measuring body composition. Many body composition studies use UWW as the reference method for estimating body density and body fat. It needs to be emphasized that the UWW must be done well. There are facilities that use UWW but estimate residual volume and other facilities that measure residual volume "out of the water" and use that value as residual volume, assuming that the value obtained on land is the same as the value in the water. These procedures introduce possible errors. Residual volume must be measured at the time of weighing, especially with non-swimmer subjects. For the purpose of the present study functional residual volume is measured at the time of weighing (see method chapter).

Archimedes' Principle and Body Density

Archimedes' Principle estimates body density (Db) by dividing body mass (land weight) in kilograms to body volume in liters. UWW estimates body volume using Archimedes's principle states that a body immersed in a fluid is buoyed up by a force equal to the weight of the displaced fluid. The loss of weight underwater is the weight of the water displaced. One liter of water weighs one kilogram.

Equation 1

$$Density = \frac{Mass}{Volume}$$

Since the UWW procedure uses scales for measuring body weight under water, the body volume is estimated by the amount of weight lost from the body measured on land and the body measured under water, where the body weight loss under water is directly proportional to the body volume, rather than the initial Archimedes's principle.

Equation 2

$$Density = \frac{Wa}{\frac{Wa - Ww}{Dw}}$$

Were: Wa is the body weight in air

Ww is the body weight under water

Dw is the density of water

The density of the water must also be taken under account. There are charts with measured water density for specific water temperature. The density of distilled water is equal to 1.000g/cc. Non-distilled water has density value slightly under 1.000, which density decreases as the temperature of the water increases.

When body volume estimated from UWW it is corrected for residual volume (RV) and for air in the gastrointestinal tract UWW provides a very good estimation of body density.

Equation 3

$$Density = \frac{Wa}{\frac{(Wa - Ww)}{Dw} - (RV + GIA)}$$

Were: RV is residual volume

GIA is air in the gastrointestinal tract

From previous studies the amount of air in the gastrointestinal tract has been estimated as an average of 100 milliliters for most healthy individuals. Residual volume is the volume of air in the lungs that is left inside the lungs after full forceful exhalation, averaging between 1 and 2 liters of air for healthy individuals. The major limitation to this method is related to the variation of the body's density associated with changes in mineral, protein and water content independent from fat content.

UWW can be very useful method for measuring body fat if specific equations are developed for each of the different populations. After the body density is estimated it is further converted to percent body fat by using Siri's or Brozek's equations:

Equation 4

$$\text{Siri equation: } \%BF = (4.95/Db - 4.50) \times 100$$

Equation 5

$$\text{Brozek equation: } \%BF = (4.570/Db - 4.142) \times 100$$

The average essential fat for men is 5% and for women is 12%. Lean body mass density is less than $1.100 g/cm^3$. Lean body mass contains 2-3% essential fat. Fat free body mass contains no fat at all. The density of fat tissue is $0.9001 g/cm^3$.

Errors of Estimation for Underwater Weighing

There are three major physiological sources of error when estimating body fat percentage using densitometry: water, fat, and mineral content of the lean body mass. The variation of these three factors causes 2-4% error of the estimation of the fat content in the body of a given population. Siri (1956) proposed 4% error in standard deviation for estimating body fat content from body density for all ages, gender, and ethnicity. Siri

(1961) also proposed that there might be 2% error due to variation of body water, which will lead to 2.7% error in estimation of body fat. Also, he found 2.1% error in estimation of body fat content due to variation of body's protein to mineral ratio, as well as 1.9% error due to variation of the composition of the fat tissue itself. This method is not ideal for validation in research studies involving wide age range of participants, or the method used is estimated to have more than 5% error. For participant over the age of 55 the density should be previously adjusted for changes in body water and mineral content. Lohman (1981) found that if the sample for a research is drawn from a population with same gender, ethnicity and not a wide variation in age, the changes of lean body mass density for this specific population would have 2.77% error. This error of estimation of body fat content for a specific population can be caused by a biological variation. In young adult population the standard error ranger from 2.0% to 2.8% (0.0059g/ml) in fat estimation and this error increases for young and elderly populations.

Reliability and Validity of Underwater Weighing

The reliability of UWW is obtained though test- retest measurements of the same individuals after weeks or months to assess the changes in body density between measurements. Durnin and Taylor (1960) used 10 men to test the changes in body density. All participants were asked to maintain their caloric intake for two weeks in order to maintain body weight and composition. The standard error for single observation of body density was found to be $\pm$ 0.0023g/ml. In ninety percent of the cases the error of a single measurement was $\pm$ 0.004g/ml. Buskirk (1961) summarized the conclusions from several other studies to conclude that the variability of body density between measurements ranged from 0.0004 to 0.0043 g/ml.

Keys and Brozek (1953) tested 35 young men. All participants were under calorie balance to sustain their body weight and composition. Multiple density measurements were taken to conclude a replicate standard deviation for density of ± 0.0015g/ml. In this study was reported that researchers who took measurements 30 minutes apart gave even better results with smaller replicate standard error of ± 0.0004g/ml. Keys and Brozek (1953) used male schizophrenic patients, to conclude that the mean of the absolute differences between the two measurements taken one week apart was ± 0.0024g/ml. The standard deviation of replication for this study was ± 0.0026 g/ml. In this study the errors related with obtaining body weight in water and in air, measurements of RV and hydration levels were taken into account.

Durnin and Taylor (1960) concluded that the duplication and reliability of underwater weighing will depend on personal expertise, subject training, reliability of laboratory techniques, accuracy of equipments, and procedure, therefore the reliabilities can not be generalized.

The validity of UWW is difficult to be determined since this method is usually used as the reference method in body composition studies. Regardless, this method still has sources of errors. In underwater weighing, a major error, which may occur is associated with the measurement of residual lung volume. With much smaller contribution to the error in estimating body density from underwater weighing are the body weight measurement, water temperature measurement, and the body weight measurement under water. If all three measurements are within 0.02 kilograms, 0.0005 degrees, and 0.02 kilograms respectively, when added together they contribute with error of 0.0006g/ml. If these three errors are combined with the error from the estimation of residual lung volume by oxygen dilution method they all contribute to 0.0015 to 0.0020 g/ml or approximately 0.7% of fat mass. These changes in the measurement of body density are present and normal to the underwater weighing as a method of measurement. If the error exceeds the 0.0020g/ml (less than 1 percent body fat) for any given participant this shows that there is a higher error in any of the possible sources, or in more than one of them, and the problem should be repaired to be more accurate. To validate the precision of the underwater weighing system it should be tested on within participant variations using more than one person. Another technical error may occur when the same participant is tested repeatedly over the course of several days. This technical error can contribute with 0.0003g/ml variation in the density measurement, which translates to 1.1 percent of body fat error for men and 1.2 percent for women when using underwater weighing. Moreover, variation of the water mount in lean tissues for a give participant from one day to another can cause an error more than a technical error during the measurement. Also, the amount of gas in the gastrointestinal track of the participant may be variable even that it is set at 100ml for all persons. Overall the technical error while using underwater weighing for obtaining body density is minimal if the residual volume is measured with oxygen dilution method, and all other possible sources of variation are also well controlled and accurately measured. If variables in this method are managed properly the technical error of estimation can be less than 1%. Still the precision of the measurement depends on the proper calibration and use of all equipment used in the underwater weighing procedure. The difference in the results of one participant measured by underwater weighing should not vary with more than 0.0015g/ml from one laboratory to another if tested within reasonable time frame. Body fat percentage estimation by this method can be altered by the equation used to convert body density to percent body fat. This error is related to the variation of lean body mass composition between individuals. Siri (1956) estimated this error of 3.9 percent body fat, and Lohman (1992) estimated this error to be 2.8 percent body fat. Studies using four-compartment models compared values to UWW and concluded that four-compartment models overestimate percent body fat with average of 0.6 percent compared to UWW, ranging from 0.1 percent and 1.2 percent body fat. More recent studies showed that the conversion formulas used in UWW average an error of -2.8 percent to 1.8 percent when compared to four-compartment models. Clasey et.al. (1999) showed ranges from 8.1 to 12.0 percent body fat from Siri (1961) when used in two-compartment models compared to four-compartment models for different ages and genders. Wang et. al. (1998) compared UWW to six-compartment chemical model and estimated standard error of 2.2 kilograms when using Siri (1961), which would correspond to 3.1 percent body fat for 70 kilogram men.

Body Density and Water Content

Siri (1956, 1961) developed one of the first multi-component equations where he established a relationship between body density, body water and percent body fat:

Equation 6

$$\text{Percent Body Fat} = (2.118/Db - 0.78\,w - 1.354) \times 100$$

which was derived from the equation below:

$$1/Db = f/df + w/dw + p/dp + m/dm$$

Where:

Db = density of the body

df = density of fat	f = fraction of body weight as fat
dw = density of water	w = fraction of body weight as water
dp = density of protein	p = fraction of body weight as protein
dm = density of mineral	m = fraction of body weight as mineral

Siri made an assumption for the ratio of mineral and protein to be 5 to 12 in lean body mass and a constant density of the protein and mineral (1.565g/ml). Bunt et al. (1989) measured body density of seven women with regular menstrual cycles twice. The first measurement was done when their body weight was lowest, and the second measurement was done when their body weight was at its highest point or during their cycle.

Participant's Factors

For some individuals it may be difficult to exhale all the air while submerged in water, which will make the individual more buoyant, resulting in lower underwater weight, lower body density, and higher percent body fat. This is not a significant problem in the method used in this study because all air in the lungs is measured - functional residual volume (see method chapter).

Individuals often have difficulty in staying under water when being weighed especially individuals with higher body fat content because they are more buoyant than leaner individuals. This can be overcome by using a weighted jacket or belt. The weight added can be weighed underwater to be subtracted from their underwater body weight.

For female participants who experience large weight fluctuations during their menstrual cycle due to water retention may obtain significantly different estimates of their body density if measured during this time. Bunt et. al. (1989) showed that water retention in females can have partial effect on their body density measurements. These findings concluded that females should be measured at the end of their menstrual cycle to obtain the most accurate density measurements. Percent body fat values can fluctuate 3 to 4 percent due to water retention. Because women have different lengths of their menstrual cycle, the same time can not be used for all females. The easiest method for observing body weight fluctuations is by measuring land weight every day for one full cycle to determine when this individual is at her lowest body weight.

Equipment and Technician Skill

When underwater weighing is performed using scale attached to load cells, it must be acknowledged that these cells are very sensitive to movement. When the participant is measured underwater, he or she must snorkel under water until the scale reading is as stable as possible before recording a measurement. This will provide more accurate underwater weight than if the scale is moving due to the submersion of the participant. Also, the participant can be previously instructed to submerge slowly and carefully to minimize water movement. When using load cell systems this problem is minimal, because the load cells sent electrical output, which generates into an analog recorder with a digital display.

Conversion Formulas

For accurate measurement of percent body fat with two-compartment models, it is very important to select the appropriate conversion formula to change body density to percent body fat. The appropriate formula is chosen based on the age, gender, and ethnicity of the sample measured.

Anthropometry – Skinfolds

During the 1990's anthropometry was the only available method for measuring the human body composition. Skinfolds are good measure of subcutaneous fat and it measures two layers of skin and the underlying fat tissue. Orphanidou et. al. (1994) concluded that the estimation of subcutaneous fat from skinfolds at specific sites is significantly less than the same measurements obtained from Magnetic Resonance Imaging. There are two possible explanations for this: one is associated with the distortion of MRI images on the posterior side of the body since the participant is lying in supine position during the scan; the other reason may be explained by the amount of subcutaneous fat tissue that is picked up during the measurement, which depends on the technician skill. The assumption made when using skinfolds is that the distribution of subcutaneous and internal fat is similar between all individuals. This assumption is questionable because older individuals from the same gender may have less subcutaneous body fat than younger individuals from the same gender (Orphanidou et. al. (1994)). In addition, the total body fat affects the amount of internal fat. Lohman (1981) estimated negative correlation between total body fatness and the amount of internal fat; when the total body fatness decreases, the amount of the internal fat increases. Another assumption of the skinfold method is that there is a relationship between the equation using the sum of seven skinfold sites and total body fat content, therefore the total body fat content can be estimated by the sum of seven skinfold's equation. Lohman (1981) also estimated that 50 to 70 percent of the total body fat is located under the skin. There is a considerable variation in the fat content of different tissues of the body: fat in the bone marrow, the central nervous system, the muscle tissue, and in the fat around some organs. Fat distribution is also affected by age, gender, ethnicity, and total body fatness. Another assumption when using the skinfold method is that the sum of the thickness of the sites is related with body density. This relationship is linear for ethnicity using population specific equations, and non-linear for sex. In addition, age is an independent predictor of body density estimated from skinfold thickness for both sexes.

Skinfold Equations

Equations were developed for estimating body composition from skinfold thickness. There are several different population specific equations, which are developed for single population of individuals with similar characteristics in regard to age, sex, ethnicity, and physical activity level. Studies show that at any given age women have higher percentage of body fatness compared to men. The highest value for skinfold thickness in both genders is observed in middle-aged adults. Black female adults have higher values for skinfold thickness in five of the commonly measured sites (thigh, back, hip, abdomen, and triceps) compared to white and Asian. The same results are observed for male populations as well. Another observation made showed that white male and female adults show the highest increase in abdominal skinfold thickness as they age.

Equation 8

Jackson & Pollack Σ 7 sites (Lange caliper) for male:

$$Db(g/cc)= 1.112- 0.00043499(\Sigma 7SF)+ 0.00000055(\Sigma 7SF)^{2}- 0.00028826(Age)$$

Siri (men):

$$\%\ BF= [(4.95/Db) - 4.50] \times 100$$

Equation 9

Jackson & Pollack Σ 7 sites (Lange caliper) for female:

$$Db(g/cc)= 1.097- 0.00046971(\Sigma 7SF)+ 0.00000056(\Sigma 7SF)^{2}- 0.00012828(Age)$$

Siri (women):

$$\%\ BF= [(4.96/Db) - 4.51] \times 100$$

White females: Jackson and Pollack underestimates body fat compared to UWW, DEXA. Durnin (Heyward et. al. (2004)) overestimates body fat compared to Pollack, (Heyward et. al. (2004)) underestimates BF compared to DEXA, and overestimates BF compared to UWW for female ≤ 60 years old and underestimates for females ≥60 years old compared to UWW. DEXA overestimates women under the age of 75 compared to UWW and underestimates women over the age of 75 compared to UWW.

White males: Durnin (Heyward et. al. (2004)) is less than Pollack in all ages. Pollack is less than DEXA in all ages. DEXA is less than UWW in all ages.

Population specific equations are developed based on a linear relationship between skinfolds and body density, but there is a curvilinear relationship between skinfold measurements and body density for a large range of body fat content, which explains why population specific equations will underestimate total body fat for overweight individuals and overestimates it for leaner individuals.

Accuracy of Skinfold Measurements

Skinfold equations use multiple sites for measuring subcutaneous fat thickness from upper and lower parts of the body. This technique is widely used because it is inexpensive, portable, requires minimal equipment, and it can be performed in a laboratory and clinical settings as well as in field studies. In general, the accuracy of the skinfold measurement for estimating body density is 0.0075g/ml, which is equivalent to 3.3 percent body fat due to biological variability of subcutaneous tissue, as well as interindividual differences in the relationship between subcutaneous and total body fat. Prediction errors of no more than ± 3.5 percent body fat or ±0.0080g/ml for skinfold equations are considered acceptable. Pollack (1984) noted that errors in estimating body fat from skinfold methods can come from several sources: the skill level of the person taking the measurements, the type of skinfold caliper used, the equation used for obtaining body density, and specific characteristics of the person being measured. If the tester is experienced with good technique and uses accurately calibrated skinfold calipers the estimation of body fatness will be very accurate. The technician skill level is the major source of error account for 3 to 9 percent variability in skinfold measurements (Pollack 1984). The thigh and abdomen are the two sites with the largest errors of measurement, these are of 7.1 and 8.8 percent respectively. Furthermore, the reliability of the trunk measurements are higher compared to limb measurements. It is very important that the tester is well trained and practiced with proper technique of the use of calipers. This is achieved by repeated training until the numbers obtained are consistent. The tester should be knowledgeable of the position of all skinfold sites being measured, the position from which they are taken, the proper technique for hand grasp and the direction of the fold, as well as the position of the caliper in relation to the hand holding the skinfold. The tester should also be aware of the pressure developed between the calipers while using them and how long they should be kept at the skinfold site for accurate measurement before they start to compress the fold and give a smaller reading. Finally the tester should correlate the body composition derived from the skinfold measurements with that developed from underwater weighing. It is possible to be reliable but not valid.

The literature uses four different skinfold calipers the Lange, Harpenden, Adipometer, and Holtain. The Lange is the most commonly used caliper. Of the four calipers, the Adipometer is the least expensive. The Adipometer's scale measure 80 millimeters. The Lange caliper's scale measures to 60 millimeters and its design allows it to be used on lean, normal and obese individuals as well. It is also found to give higher readings compared to the other calipers (see Table 4).

Table 4 Lange Caliper

Type	Avg. Pressure (g/mm^2)	Range (mm)	Scale precision (mm)	Accuracy	Durability	Cost
Lange	8.4	0-60	0.5	Lange>Harpenden	Excellent	$180

Heyward et. al. (2004) Applied Body Composition Assessment

Over fifty different equations are developed for calculating body fat from skinfold thickness with standard error of estimate that ranges form 3-7%. Durnin and Womersley's and Jackson and Pollack's (Heyward et. al. (2004)) equations specific to age and gender are the two most commonly used equations in the literature. Both equations were compared to underwater weighting and dual energy x-ray absorptiometry and show to underestimate the percent fat in both cases. The differences increased even more for females as the age increases. The most common sites used for the development of these equations as well as in research are triceps, subscapular, abdominal and iliac crest, thigh, biceps and calf, chest, and abdominal, presented from the most frequently used to the least frequently used.

Skinfold measurements are taken on the right side of the body. Skinfold thickness differs very little between the left and right side of the body (1-2mm).

Skinfold thickness may be difficult to obtain on extremely obese or very muscular individuals because it may be hard to separate subcutaneous fat from the underlying muscle tissue. When measuring extremely obese individuals their subcutaneous fat thickness may exceed the maximum capacity of the caliper scale.

The advantages to the skinfold method for measuring body composition are that it is simple, portable, and inexpensive. The disadvantages behind this method are related to the absence of standard methodologies and that it requires very well trained testers in order to obtain accurate measurements. It is very cheap and practical way of estimating body fatness especially in adult populations. Jackson and Pollack equations for measuring body fatness use estimation of body density from three, four or seven skinfold sites (see equations 8 and 9). These equations are primarily done with Lange calipers and are widely tested on different populations, genders, and age groups for validations. They show to be accurate for individuals ranging from 10-40% body fat using Lange calipers. Individuals who are over 40% body fat may be underestimated. Also, Jackson and Pollack developed easy to use charts and tables for conversion of the sum of the skinfolds into body fat percentage depending on age. Based on their equations for skinfolds Jackson and Pollack found small standard error of estimation compared to BMI.

There are two problems that can occur when using skinfold method. One is related to the skinfold equation's specificity to a particular population for which they are developed. Second, inaccuracy may occur due to error in taking the skinfold measurements, which may be related to poor technique, inability to locate the measuring site accurately, inexperience of the tester, improper use of the skinfold calipers, compressibility of the subcutaneous fat tissue. Furthermore, the number of participants in the study as well as their demographics such as age, gender, and ethnicity should be listed. The skinfold caliper type should also be described, since different calipers will not give the same mean for fat percentage in a specific population when used with different equations. Finally, there is also a difference between the same skinfold measurement by two different testers and the use of different calipers as well. The sites with biggest variation between testers are abdomen, suprailiac, and thigh, and smaller variations occur when measurements are taken at the triceps site and subscapular site.

Teran et al. in 1991 conducted a study with 221 obese females to show that the use of Jackson and Pollack or Durnin and Womersley equations in women with more than 35% body fat showed limitations causing underestimation of body fat for these individuals. Both equations were developed on the two-compartment model principle and they were assuming constant density of lean body mass with aging. Also, if using Jackson and Pollack's three site equation for women (abdomen, suprailiac, and triceps) to measure young adult and compared the results to the results of an older adult female, the older adult will have higher body fat percentage than the younger individual. This might be due to different fat distribution over the years.

Most researchers agree that skinfolds are better than body mass index. In 1977 Durnin and Womersley made improvements in their anthropometric research for men but not for women. In 1985 Jackson and Pollack and in 1988 Jackson et al. advanced the skinfold method as being better than BMI. The major reason why BMI is popular is that the body mass index is easier to use and requires no technician skill.

One of the major concerns why the skinfold measurements can not be completely accurate as a method of measuring body fat is because only the subcutaneous fat is measured. The body contains more fat than just what's subcutaneous; there is visceral fat located in the trunk area, for example the thoracic and abdominal cavities, as well as the around the various organs of the trunk. In addition, there is inter-muscular fat that is not considered with subcutaneous fat.

Limb fat is one of the four major compartments of body fat and it is well measured by skinfold measurements. In 1988 Hawthrone et al. claimed that if four skinfolds from limb and trunk locations are taken their sum would be highly correlated with total fat (subscapular, triceps, abdomen, and iliac). Fat patterning is highly associated with genetics, as well as age, gender, ethnicity, and skinfold thickness. In 1986 Baumgartner et al., found that if the total body fatness is controlled, then half of the variation of fat patterning will be fifty percent due to trunk and extremity dimensions. Boileau et al. in 1987 compared the sum of five skinfolds measurements of obese and non-obese adults, where the body fatness was also removed by dividing each skinfold by the sum of all skinfolds to compare central and peripheral fatness. The conclusions from these studies were that obese men and women have more fat in their trunk area compared to non-obese individuals and that the obese adult females have more adipose tissue in the upper trunk part and the obese adult males have more of their trunk fat stored at the lower trunk regions.

Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) is easy, fast, and a relatively inexpensive method of measuring body composition and can be used in clinical, laboratory, and field settings. BIA is used to estimate total body water from water and electrolytes in the body tissues because they are very good conductor of electrical current. BIA sends low-level electrical current with previously set frequency throughout the body tissues. Each of the different tissues has their own conduction properties. The resistance in ohms measured from all the tissues is used to estimate the amount of lean tissue and fat tissue in the body. Higher resistance to the electrical current indicates higher amounts of fat tissue in the body, since fat tissue contains significantly less amounts of water and electrolytes (approximately 2 percent water) compared to lean muscle mass (approximately 73 percent water). Therefore, individuals with large amounts of lean mass will have less resistance to the electrical current. The BIA method may be preferable in certain settings because it does not require high technician skill level, it does not present any discomfort to the participant, and it is easy to use on obese individuals. BIA is recommended for use only in healthy individuals with normal hydration status and normal fluid distribution.

Bioelectrical Impedance Analysis Assumptions

There are two main assumptions that the BIA method uses. One assumption is that the body is shaped as perfect cylinder with a uniform length and cross-sectional area. This assumption is not completely true because the human body represents five different cylinders: one for the trunk area, two for the arms, and two for the legs. Also, because body parts are not with uniform lengths or cross-sectional area the resistance to the flow of the current will be different for each part of the body. Because of this the segmental BIA uses the sum of the resistances from the segments of the body to estimate total body volume. Another assumption in the BIA method is that if the body is shaped as perfect uniform cylinder, at the fixed frequency signal (50 kHz), the impedance (resistance) Z to the current flow through the body is directly related to the length (L) of the body (height) and inversely related to the body cross-sectional area (A).

Equation 10

$$(A): Z = \rho(L/A),$$

where ρ is the specific gravity of tissues and it is assumed to be constant

L is the length of the body (height)

A is the cross-sectional area of the body

Z is the impedance (resistance) of the body.

Two principles are associated with the use of BIA method. One is that biological tissues act as conductor or isolator, and the flow of the current in the body will follow the path with least resistance. Following this principle the flow of the current will pass through lean body tissues, because they contain approximately 73 percent water and electrolytes, which makes these tissues better conductor of electrical current. BIA using 50 kHz frequency is only able to measure extracellular water rather than total body water because this low frequency is unable to penetrate the cell membrane and measure intracellular water. Another principle use in BIA is that the impedance is a function of resistance (R) and reactance (Xc). Kushner (1992) explained that the resistance (R) is measured by the resistance of the current flow by different tissues in the body, and reactance (Xc) is the opposition to the current flow caused by capacitance (voltage storage) produced by the cell membrane.

BIA also uses prediction equations that are age, sex, and ethnicity specific. Fitness level and physical activity was also added to generate population-specific equations. Alternatively, generalized equations have been developed to measure more diverse populations varying in their age, sex, and percent body fat. The use of the BIA, regardless of the equation used, requires body weight and height as well. These measurements are included into the BIA equations because the assumptions that human body is an uniform cylinder and the specific resistivity if tissues is constant are not true, therefore including body weight may account for the more complex geometrical shape of the body. Most BIA equations use either $Height^2/\mathrm{Re}sis\tan ce$ or either of the two factors separately to predict lean body mass. Xc is typically not included in the BIA equations. Xc reflects changes in the distribution of fluids and the water content of fat and lean tissues associated with increase in percent body fat.

Large changes of the fluid content in the trunk will still have little to no effect on the wrist to ankle impedance. Studies examining the ability of the BIA, and particularly the accuracy of the algorithm to estimate total body water (TBW) in individuals undergoing dialysis showed that the BIA significantly overestimates the volume of fluid loss for these individuals. Also, other studies have shown that using a wrist to ankle BIA in individuals with cystic fibrosis is not a good method for accurate estimation of body composition. Researchers used a segmental technique to measure TBW via BIA by obtaining trunk measurements as well as arm and leg measurements separately from each other and comparing them to numbers obtained by water dilution isotope. It was assumed that the body is built by five interconnected cylinders representing the trunk area and both arms and legs. Their volumes are measured separately by measuring the impedance and the length of each segment. At the end all of the measurements are combined to present the whole body volume. Wotton et. al. (2000) study showed that using multiple regression on the segmental method of measuring total body water by BIA gives same or better prediction compared to whole body measurement, but this may apply only to healthy individuals to keep the assumption that the distribution of water is constant between subjects. Furthermore, a consistency can be assumed in the distribution of intracellular and extracellular water in healthy individuals. Wotton et. al. (2000) concluded that the whole body measurement by BIA is adequate for estimating total body water. In a different study with similar design was found that there is no difference in the numbers obtained between the whole body measurement and the segmental measurements. This technique did not work for both genders Organ et. al. (1994). Another important note that can be made from these trials is that the fluid distribution in the legs and arms may be relatively constant when comparing healthy individuals but this may not be the case for non- healthy persons.

A study done by Ward et. al. (2000), showing the relationship between ethnicity, body mass index and bioelectrical impedance pointed that there is a need to have a group specific data. Another conclusion drawn from this study suggests that the differences in impedance between the groups may be related to differences in body type as shown by body mass index. This study also concluded that for each specific population the body resistance would show different characteristics. If there is no independent body composition data present, it will be very hard to determine if the differences are due to the shape of the body or its electrical conductivity. Also, this study found that there is a gender difference even if the participants are all in a similar age range and ethnicity is excluded. Furthermore, there is a difference between ethnic groups within the same gender.

Lohman et al. in 1987 and Segal et al. in 1988 concluded that bioelectrical impedance was as good as the skinfold measurements in body composition research and much better compared to body mass index alone. The issue occurring when bioelectrical impedance is used to measure lean body mass or total body water is the equations used to convert the resistance from ohms into percent fat. The same resistance in ohms can lead to two very different fat percentages if put into two different formulas. In 1990 Mazess proposed the idea that the resistance measured by the bioelectrical impedance may be directly related to body composition without the need for height and weight measurement. Also, Mazess proposed that the resistance by itself should be very highly correlated to lean mass. One year later Deurenberg et al. compared bioelectrical impedance in both men and women. The first trial the length was included and the correlations found were 0.85 and 0.77. The second time the length factor was excluded and the correlations were -0.48 and -0.56. In the majority of studies done to measure lean body mass or total body water with bioelectrical impedance the researchers prefer the relationship L^2/R rather than body weight because of the higher regression coefficient of body weight.

From measuring whole body resistance the estimated error for men was 1.4% fat and for women was 1.5% fat. For skinfold thickness the error for both men and women was 1.0% fat. Hydration level is very important when using BIA, which can be the major cause of variance when participants are tested repeatedly over the course of few days. This variance is set at 1.8% for men and 2.4% for women.

In 1986 Lukaski presented the relationship between the lean body mass and bioelectric resistance in ohms (L^2/R, where L is the length of the body and R is the impedance in ohms). In 1987 after reviewing this relationship, Lukaski predicted that the resistance of the body depends on its volume and the conductive properties of its fluids. For the human body, muscle or lean body tissue is more conductive compared to fat mass, which is more resistive to an electrical current. This is due to the different amount of water content of the different tissues: muscle contain more water than fat, therefore muscle is more conductive, or less resistant to electrical current. Furthermore, the amount or volume of lean body tissue can be predicted from the following equation:

Equation 11

$$V = p\,(L^2/R)$$

where:

p is the conductive properties of the lean boy tissue, or more specifically its resistivity to a current,

L represents the length of the body or conductor, and

R represents the resistance of the body.

In 1986 after testing both: men and women, Lukaski et al. found a standard error of estimation for men to be 2.5 kilograms and 2.0 kilograms for women. In 1987 Lohman et al., found the standard error of estimation for men to be 2.8 kilograms and 2.1 kilograms for women. In 1988 Segal et al. found standard errors of estimation for men: 2.9, 3.3, 3.6, and 3.5 kilograms, and for women 2.1, 2.3, 2.4, and 2.5 kilograms. The standard error of estimation for bioelectrical impedance ranges from 2.0 low from the research made by Lukaski et al. in 1986, to high 5.1 kilograms estimated by Jackson et al. in 1988. Lohman (1987) concluded that if using bioelectrical impedance for estimating lean body mass and it is assumed that the standard error of estimation is between 1.5 and 2.1 kilograms and the water content in lean body mass is 73% than the bioelectrical impedance error in estimation of lean body mass will range from 2.1 kilograms to 2.9 kilograms.

Pateyjohns et. al. (2006) used 43 healthy but overweight and obese men in a comparison of estimation of body fat percentage between bioelectrical impedance analysis and dual energy X-ray absorptiometry (DEXA). The results of this study showed that single-frequency bioelectrical impedance analysis have small bias in the absolute differences between both methods, and bioelectrical impedance analysis underestimates percent body fat with 1.7 percent compared to DEXA.

In another study conducted by Bolanowski et. al. (2001) comparing bioelectrical impedance analysis and DEXA 59 adult women and 41 adult men were tested. This study showed highly statistically significant correlations between DEXA and BIA measurements in lean body mass, fat mass, and percent body fat for both genders. There was no influence of age and body mass index observed on the relationship between DEXA and BIA results. In this study if BIA was used as the reference method it would be concluded that DEXA measurements underestimate lean body mass and overestimates body fat in both genders.

Sun et. al. (2005) used total of 591 adult men and women in a wide range of body fat percentages to compare the estimation of body fat percentage between multi-frequency bioelectrical impedance analysis and DEXA. This study showed that the mean body fat percentage obtained by BIA in all participants was significantly lower than that measured by DEXA: 32.89 ± 8.00 percent compared with 34.72 ± 8.66 percent, with similar results for both genders. Furthermore, BIA overestimated percent body fat by 3.56 percent in lean participants (below 20 percent body fat), and underestimated percent body fat with 2.65 percent in obese participants (above 30 percent body fat). For participants with body fat percentages between 20 and 30 percent body fat both methods were very similar in their body fat percentage estimates. When numbers were separate for men and women BIA measurements for men overestimated percent body fat by 3.03 percent for lean men (under 15 percent body fat), underestimated percent body fat by 4.32 percent for obese men (above 25 percent body fat), and for men participant with body fat percentage between 15 and 25 percent both methods had little difference in percent fat estimates, which was considered normal body fat percent range for male participants. For women, BIA overestimated percent body fat by 4.40 percent for lean women (below 25 percent body fat), underestimated percent body fat by 2.71 percent for obese women (above 33 percent body fat), and had little difference with DEXA when percent body fat was between 25 and 33 percent, which was considered normal body fat percent range for female participants.

Evans et. al. (2001) found significant agreement between total body water measured by BIA and deuterium oxide (D_2O) dilution. Using the estimated total body water by BIA and deuterium oxide (D_2O) dilution in a four-compartment model produces very good mean values for percent body fat, but there are high individual differences ranging from -5.6 to 5.5 percent body fat.

Bioelectrical Impedance Analysis Guidelines

Specific pretesting guidelines are used when BIA is used to measure body composition. For accurate estimations of total body water all individuals being tested should follow these guidelines:

- No eating or drinking within 4 hours prior to the test.
- No exercise within 12 hours prior to the test.
- Participant should urinate 30 minutes prior to the test.
- No alcohol consumption within 48 hours prior to the test.
- No testing of females who perceived they are retaining water during that stage of their menstrual cycle.

Table 5 Summary of Factors Affecting Bioelectrical Impedance Measures

Factor	Effect on resistance (Ω)	Effect on fat-free mass (kg)
Eating or drinking within 4 hr	↓ 13-17	↑1.5
Dehydration	↑ 40	↓5.0
Aerobic exercise (low intensity)	No change	No change
Aerobic exercise (moderate-high intensity)	↓50-70	↑12.0
Menstrual cycle (follicular vs. premenstrual)	↓5-8 follicular state	No change
Menstrual cycle (menses vs. follicular)	↑7 menses	No change
Electrode placement	↑10	No change
	↑70	↓11.0
Electrode configuration (right side vs. left side)	No change	No change
Room temperature (14°C vs. 35°C)	↑35 for 14°C	↓2.2

Heyward et. al. (2004) Applied Body Composition Assessment

Air Displacement Plethysmography – Bod Pod

The Bod Pod is an instrument, which estimates body composition using air displacement plethysmography (ADP). The Bod Pod is the commercially available version of ADP that is currently being used in clinical and research settings. The Bod Pod measures body volume, by displacing air rather than water, from which body density and percent fat are then estimated. More specifically, the volume of an object is measure indirectly by measuring the volume of air it displaces inside an enclosed chamber.

Over the last 12 years, the Bod Pod has been researched in order to evaluate its validity and reliability in assessing body composition in many different populations. Studies that have been done have evaluated the validity and reliability of the Bod Pod to accurately measure %fat in athletic populations, apparently healthy adults, special populations, children, and among others. Research has shown positive results of the Bod Pod's validity / reliability in measuring percent fat amongst different populations, when compared with other well established two and three-compartment model-methods, such as underwater weighing and dual energy X-ray absorptiometry.

Using air displacement plethysmography the volume of the participant is measured indirectly by measuring the amount of air the participant displaces as he or she enters an enclosed chamber. Very similar to underwater weighing, the participants body volume is measured as the person enters a chamber, with a previously measured volume while empty, and displaces amount of air equal to his or hers body volume, similar to the underwater weighing procedure where the body volume equals the amount of water that body displaces while fully submerged under water. The Bod Pod uses a relationship between pressure and volume, and tries to control for air temperature and pressure that occurs when the participant enters the chamber. The Bod Pod's chamber is egg shaped fiberglass chamber in which the person is enclosed during the measurement procedure, tightly closed with strong magnets located along the door of the chamber. Air displacement plethysmography also relies on Boyle's and Poisson's gas laws, which describes the pressure – to - volume relationships of gases under isothermal and adiabatic conditions. Boyle's law states that a quantity of air compressed under isothermal conditions will decrease its volume in proportion to the increasing pressure $P_1/P_2 = (V_2/V_1)$ (where P_1 and V_1 represent one pair of conditions when the chamber is empty, and P_2 and V_2 represent a second condition when the participant is inside the enclosed chamber); Poisson's law states that under adiabatic conditions, the temperature of air does not remain constant as its volume changes $P_1/P_2 = (V_2/V_1)^{\lambda}$, where λ is the ratio of the specific heat of the gas at constant pressure to that of constant volume, equal to 1.4 for air representing 40 percent difference between isothermic and adiabatic conditions. Differences in the behavior of gases is very important to the way that the Bod Pod instrument is designed, e.g. the air under isothermal conditions is easier to compress creating negative volume. For this reason participants measured by Bod Pod can not wear bulky clothing during the test because this is going to result in invalid reduction of body volume. A major assumption of Bod Pod is that isothermal effects of clothing, hair, thoracic gas volume, and body surface area can alter the body volume and therefore they have to be controlled. Each individual measured by Pod Pod wears minimal clothing specified by the Bod Pod methodology (swimwear, or compression shorts without padding and compression bra without padding). Also, participants are asked to wear a swim cap that covers all their hair. Body surface area is calculated by measuring the participant's height and weight, which is also used to correct for the isothermal effects at the body's surface. Body's surface area is measured by a formula from DuBois and DuBois (1916), which is inputted into the Bod Pod's software from the manufacturer. Thoracic gas volume is directly measured or estimated by the Bod Pod to account for isothermal conditions in the lungs. The Bod Pod recommends measuring thoracic gas volume over its prediction. Asking the participant to breath into and out of a hose during the thoracic gas volume measurement obtains this measurement. The participant is taking normal tidal volume breaths following a cadence on the screen of the Bod Pod's computer. After 50 seconds of normal breathing the airway closes and the participant has to perform 3 light puffs, alternately contracting and relaxing the diaphragm muscle. This small pressure changes in the lungs and external volume that this puffing creates is used to measure the thoracic gas volume of the individual. A formula input into the Bod Pod software from Dempster and Aitkens (1995) is used to obtain body volume: BV(L)= BV_{raw} - surface area artifact +40%TGV. Body density is calculated by dividing body mass over body volume, and Siri equation is used to convert body density into percent body fat.

The Bod Pod machine consists of two chambers: rear chamber and front chamber where the participant sits during the test. A moving diaphragm is located on the back of fiberglass seat on the wall of the two chambers, which oscillates during the test. The motion of the diaphragm crates small volume changes, equal in magnitude but opposite in sign between the two chambers. Poisson's law for pressure and volume relationship is applied to measure the volume of the front chamber. The volume of the front chamber is measured twice; once empty and once with the individual inside. Body volume is calculated by subtracting the volume of the chamber with the person inside from the volume of the chamber when empty. Further, this raw volume is corrected for body surface area and thoracic gas volume.

Dempster and Aitkens (1995) validated the Bod Pod by using cubes and cylinders of volumes between 25 and 150 liters. The error was estimated to be less than 1 percent and standard error was 0.004 liters.

McCrory et. al. (1995) first tested the Bod Pod on human participants and compared the results to body fat percentage obtained from underwater weighing. This study showed that the Bod Pod underestimated body fat by 0.3 percent compared to underwater weighing.

Vescovi et. al (2001) used lean, average weight, and obese adult men and women to compare the estimation of percent body fat using the Bod Pod and underwater weighing. From this study was concluded that there are no significant differences in the mean values for body density or percent fat measured by the Bod Pod and underwater weighing in either gender. On the other hand, this study also presented a significant underestimation of body density and corresponding overestimation of percent body fat when using the Bod Pod to measure lean individuals, while no differences were observed in percent body fat for the average or overweight individuals. Gender bias may be present when lean individuals are measured by the Bod Pod, while it appears that there was no gender bias for average or overweight individuals. Furthermore, the mean differences for the lean sample used in this study was over 30 times greater and approximately 5 times greater than the average weight participants and the entire sample, respectively. From this study it was concluded that the Bod Pod can not be considered as accurate measure of percent body fat for lean individuals compared to underwater weighing. It appears that estimating percent fat using the Bod Pod is accurate compared to underwater weighing for all individuals except the lean.

Another study done by Ginde et. al. (2005) compared the body density measured by the Bod Pod and body density obtained by underwater weighing in participants ranging form normal weight to severely obese. Total of 123 adult men and women were measured with underwater weighing and Bod Pod using Siri equation. The study concluded that there are no significant difference in body density measured by the Bod Pod and underwater weighing in either normal weight, overweight, obese, or severely obese individuals. Also, there was no significant difference between both methods for measurement in the group mean percent fat estimate. This study showed high validity of the Bod Pod in measuring body density in overweight and obese individuals.

In an overview of Bod Pod studies, the Bod Pod was mainly compared to underwater weighing and Dual Energy X-ray Absorptiometry. When compared to UWW and or DEXA, Bod Pod percent body fat estimates range from -4.0 percent to +1.9 percent with standard error ranging from 2.2 percent to 3.7 percent.

When compared to four-compartment models the Bod Pod significantly underestimated body fat with 1.8 to 2.8 percent body fat, Collins et. al. (1999). Millard-Stanfford et. al. (2001) used body densities estimated by Bod Pod and UWW and input them in the same four-compartment formula to find that the Bod Pod percent body fat is significantly different from the percent body fat estimated by UWW (17.8 percent and 19.3 percent respectively). Fields et. al (2001) concluded that UWW and Bod Pod are very similar in predicting percent body fat when compared to four-compartment models.

Sources of Measurement Error for Air Displacement Plethysmography (ADP)

Dempster and Aitkens (1995) used 50.039 liters cylinder to validate the Bod Pod and found that two measurements made in separate days differ with 3 milliliters. Vescovi et. al. (2001) measured same participants twice and found average difference between the two testing's of 1.7 to 3.4 percent. Nunez et. al. (1999) estimated this range between 2.0 and 2.3 percent between two days.

Viscovi et. al. (2001) measured technical error of the Bod Pod contributes with less than 0.0020 g/cc, and McCrory estimated that technical error contributed with 0.4 percent to the body fat percentage.

For participants with long facial hair, the body fat percentage can be underestimated with approximately 1 percent, and for participants who do not wear swim cap to cover hair the percent fat can be underestimated with 2.3 percent.

Ultrasound

Ultrasound, like skinfolds, measures subcutaneous fat tissue. Skinfold measurements are often difficult to obtain on very fat individuals, and fat compressibility can also be sometimes a problem. Ultrasonic depth measurements can be applied at any site on the body without the disadvantages of skinfolds.

Bullen et. al. (1965) investigated the possibility of using ultrasonic technique for measuring the fat thickness in humans. The ultrasound estimation of subcutaneous fat thickness at three sites was compared to the estimations made with the skinfold calipers. A total of 100 men and women were tested. The three sites measured were: triceps, sub-scapular, and abdomen, and the Lange calipers were used. Thirteen of the participants were also examined with direct needle puncture at the abdominal site for direct measurement of subcutaneous fat thickness. In this study, a high correlation was found between the ultrasound and skinfold calipers measurements at the abdominal and triceps sites for both men and women. The uncompressed ultrasound measurements were compared to one-half of the thickness of the double- folds skinfold measurements. Skinfold thickness and ultrasound depths at the triceps and abdominal sites were compared separately for the men and women groups. Correlations obtained at the triceps site were 0.80 and 0.80 for men and women respectively. At the abdominal site these measurements were 0.90 and 0.85 for men and women respectively. For both men and women, at the abdominal site one-half of the skinfold caliper measurement was 66 percent of the uncompressed ultrasound measurements. At the triceps site the values for men and women were 61 and 67 percent respectively. In this study, no significant difference was observed between the skinfold and ultrasound measurements. There was a high agreement between both methods, reliability coefficients at the triceps, sub-scapular, and abdominal sites were 0.98, 0.98, and 0.99 respectively. The correlation coefficient between the needle puncture and ultrasound measurements at the abdominal site on a subsample of people was 0.98.

Kuczmarski et. al., (1987) compared the ultrasound measurements to skinfold measurements in obese adults. The purpose of the study was to see if ultrasound measurements overcame some of the shortcomings of the skinfold method. The study used 13 men and 31 women. More than half of the subjects were over 40 percent body fat measured by underwater weighing. Body densities estimated by underwater weighing ranged between 0.96 to 1.04g/mL with mean value of 1.01g/mL. The mean value for body fat obtained from underwater weighing calculated by Siri's equation was 41.7 percent for both genders, 36.2 percent mean body fat for men, and 44.0 percent for women. Pearson correlation coefficients between skinfold and ultrasound measurements were significant at all sites. Compared to other body sites, the correlation between skinfold and ultrasound measurements were high for the biceps, triceps, and thigh sites. Ultrasound measurements of subcutaneous fat at the subscapula, abdominal, thigh, and biceps had higher coefficients of correlation with body density than did the same measurements obtained through skinfold measurements. The correlation of the biceps, triceps, and thigh sites with body density were negative and highly significant, regardless of the measurement instrument used. For the skinfold caliper the triceps site was the strongest single correlate of body density, and for the ultrasound the biceps and thigh sites each had the greatest correlation coefficient.

Voltz et.al. (1983) used 66 college age (18- 26 years) females to determine the validity of using ultrasound for the field assessment of body composition. At least three skinfold measurements were also taken on all participants using Lange calipers following procedure described by Brozek and Keys (sites included: abdominal, axilla, ilium, sub-scapular, triceps, biceps, thigh, and calf). One-half of the skinfold thickness was compared to the corresponding ultrasound depth measurement. It was concluded that the one-half of skinfold thickness measurement was significantly different from the ultrasound measurement at all sites with the exception of the biceps and the subscapular sites. All seven sites were correlated to body density obtained from underwater weighing. The iliac site for both ultrasound and skinfold measurements showed the highest correlation to body density: $r=-0.73$, and $r=-0.69$ respectively. The calf measurements demonstrated the lowest single correlation, r=-0.38 for ultrasound, and r=-0.45 for the skinfold value.

Borkan et. al. (1982) measured 39 males between 41 and 76 years of age to compare subcutaneous fat thickness measured by skinfold calipers and ultrasound (Body Composition Meter). A Lange skinfold caliper was used to measure skinfold thickness at 15 sites located at the levels of the chest (anterior, midanterionr, lateral (axilla), and posterior (sub-scapular)), arm (anterior (bicepts), lateral, posterior (triceps), and medial), umbilicus (anterior (umbilicus) lateral (suprailiac), posterior (dorsal iliac)), and leg (anterior, medial, lateral, and posterior). For all skinfold to ultrasound comparisons, the skinfold thickness was divided into half, because skinfolds represents a double fold of fat. The results showed that the correlations for skinfolds were higher compared to the ultrasound for every site except anterior arm. Ultrasound did not achieve any reliability higher than 0.81 and four sites had reliability less than 0.5. The only sites where reliability of ultrasound compared favorably with skinfolds were on the leg. The correlations between ultrasound and skinfolds were strongly positive at many sites, indicating that relative rankings of individuals were similar, even if measurement scales varied. Triceps, subscapular, and lateral leg had correlations greater than 0.80 (i.e., 64 percent of the variance is explained). At other sites the correlations were much lower. To determine if both methods are equally effective in measuring overall body fatness, skinfolds and ultrasound sites were individually compared with total fat weight. In all but the biceps measurement the skinfolds had higher correlations with fat weight than did ultrasound. Subscapular skinfold was the only site that had a correlation with total fat higher than 0.60. The average correlation of skinfold sites with total fat was 0.51, and for ultrasound the correlation was 0.35. Skinfold sites had higher correlations with body weight than ultrasound for most sites. At the majority of skinfold and ultrasound sites, the correlation with fat weight were greater than those for total fat. The results of this study showed that skinfolds measurements done with Lange calipers are a more effective way of measuring subcutaneous body fat than ultrasound using Body Composition Meter.

Fanelli et. al. (1984) used 124 lean white males between the ages of 18 and 30 years to investigate the validity of ultrasound compared to skinfold measurements and density from underwater weighing. Mean body density was calculated 1.07g/ml, from underwater weighing and Siri's formula was used to convert body density to percent fat. Mean percent fat for this sample was 12.7 percent, but more than half of the participants had more than 17 percent body fat measured by underwater weighing. Measurement of subcutaneous body fat by skinfolds and ultrasound were significantly correlated at all seven sites, with measurements taken on the triceps and thigh correlating higher than those taken over the other sites. Compression ranged between 10 and 40 percent for fat thickness values recorded from the skinfold measurements for each body site. Thigh, triceps, and abdominal measurement sites had the least amount of variance in percentage compression. To determine which measurement sites were the most accurate in predicting total body fat, the subcutaneous fat thicknesses obtained from the caliper and ultrasound at each site were individually compared with body density measured by underwater weighing. All correlations were negative and highly significant. The skinfold measurements of subcutaneous fat for most sites had slightly higher correlation with body density than with ultrasound. From the seven sites the abdominal, triceps, and thigh measurements were with higher correlation with body density compared to other sites, regardless of the measurement technique. For the caliper measurements the triceps site were the best single prediction factor of body density (r=0.749), and for ultrasound that best single predictor site was the abdominal site (r=0.736). Subcutaneous fat thickness measured by skinfold calipers, in this study, had slightly higher correlation with body density when compared to ultrasound for five of the seven sites. In the present study, ultrasound measurements showed good agreement with the skinfold measurements. The average subcutaneous fat thicknesses measured by ultrasound were greater than one-half of the average values obtained by skinfold calipers, indicating a compression effect. In this study for the sample of lean men the compression over the abdominal, triceps, and thigh sites did not have significant effect on the accuracy in the prediction of body density. The results of this study point that the skinfold caliper (Lange) is not significantly better and more effective method for measuring subcutaneous body fat than ultrasound.

Near Infrared Reactance

Infrared Reactance method for measuring body composition has not been studied very much compared to other methods. Conway et. al. (1984), used 53 adults to measure body fatness, by comparing skinfold thickness, infrared reactance, and ultrasound to body water estimation by deuterium dilution. It was found that the standard error of estimate for infrared reactance was 3.0% fat. This percent was larger for ultrasound and the sum of five skinfolds. Infrared reactance uses spectral analysis of the interactance signal, which tests the optical distribution properties of the tissues. Different tissues will have different disperse properties which will affect the shape of the spectrum differently depending on the tissue. Other researcher who did not have the equipment Conway had and were not able to replicated her study but instead used less sophisticated instruments to measure body composition did not agree with Conway that infrared reactance is better in measuring body fatness than the skinfold method.

Israel et. al. (1989) measured 80 white, athletic men to determine the validity of the Futrex-5000 (Near Infrared Reactance (NIR)). Underwater weighing was the criterion method for this study. Siri's formula for converting body density obtained for underwater weighing to body fat percentage was used. Seven sites and three sites skinfold measurements were also performed. Jackson and Pollack equation was used to calculated body density from the skinfold measurements, taken with Harpenden calipers. Body density obtained from skinfold measurements was converted to body fat percentage using Siri's formula. The results of this study showed a significant difference among the methods used to predict body density and percent body fat. The Futrex-5000 significantly overestimated body density and underestimated percent body fat when compared to underwater weighing, three and seven sites skinfolds. In addition, there was no significant difference between underwater weighing and three and seven sites skinfolds. The results of this study suggested that the Futrex-5000 was not accurate estimating body density and percent body fat compared to underwater weighing.

Polito et. al. (1994) used 169 adult men and women, ages 18 to 48 years, with diverse body compositions to investigate the validity of Futrex 5000. The optical density (OD) was measured at six sites: biceps, triceps, subscapulaar, suprailiac, thigh, and calf. Also, subcutaneous body fat thickness was measured on the same sites with Harpenden caliper.

The body density was predicted from sum of four skinfolds using the equation proposed by Durnin and Womersley. A good correlation between OD and skinfold thickness was obtained only from one site- biceps (r=0.81 for men and r=0.84 for women). Underwater weighing used Durning and Rahaman equation to convert body density to percent body fat. The correlation between percent body fat obtained from underwater weighing and percent body fat obtained by the Futrex manufacturer's equation gave correlation of r=0.88. The Futrex underestimated body fat percentage with 2.1 percent for men and 3.9 percent in women, especially in fatter individuals, compared to underwater weighing. The Futrex manufacturer's equation requires the device to be used on the biceps site. In this study was found that this equation predicts body fat percentage with correlation of r=0.88 for men and r=0.72 for women. Also, the standard error of estimation was large: 4 percent for men and 4.7 percent for women, and there was systematic underestimation of total body fat for both genders. From this study was concluded that the Futrex measures accurately the thickness of subcutaneous fat only on biceps site. Also, the accuracy of this device measurement ability is limited only to people with average body composition. Large fat mass in obese participant and the large muscle mass in body builders type individuals is likely to be a reason for attenuation observed in this study in the capacity of OD measures to predict the thickness of fat at the other five sites used. In addition, no improvement was observed when the sites' measurements were added or averaged (calf, thigh, suprailiac, subscapular, and biceps). The conclusion from this study was that NIR is not a valid method for measurement of body composition for individuals who are not in the average normal range. When used on obese individuals or individuals with large muscle mass the NIR could produce biases and obtain unreliable results compared to other more valid instruments such as underwater weighing.

Brooke-Wavell et. al. (1995) used 54 young adults (27 men and 27 women) and 63 middle age men to examine the relationship of the Futrex-5000 with subcutaneous fat and muscle thickness and total body fat content. Sixty-three sedentary middle-aged men were used for cross-validation of the body composition techniques. Ultrasound (Ekoline 20A Ultrasound) was used to measure muscle mass thickness. Skinfold measurements were done using Holtain[3] skinfold calipers. Skinfold measurements were taken at four sites: biceps, triceps, subscapular, and suprailiac; Durnin and Womersley equation was used to obtain body density and Siri equation was used to convert body density to percent body fat. All measurements were made on the left side of the body. Measurements with the Futrex-5000, skinfolds, and ultrasound were made at five sites: biceps, triceps, subscapular, suprailiac, and anterior thigh. Underwater weighing was also performed using Siri equation to calculated percent body fat from body density. The Futrex-5000 measurements were significantly greater in men than women at the biceps, triceps, and thigh measurements. The Futrex-5000 measurements at the biceps were highly correlated with ultrasound in the 54 young men and women, but it was not significant. The contribution of muscle mass thickness was only significant at the biceps site in women, where only 5 percent of the variance was explained by muscle thickness. Skinfold thicknesses were better correlated with ultrasound than with optical density (OD) measured by the Futrex-5000. The differences in correlation coefficients were significant at most sites except biceps. OD values were negatively correlated with body fatness in

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[3] Holtain Ltd, Crymych

middle-aged men. Biceps skinfold measurements agreed with the Futrex-5000 in the measurement of subcutaneous fat tissue significantly better than at other sites, therefore the Futrex-5000 does not provide better measurement of subcutaneous fat than skinfolds. Mean body fat content measured by the Futrex-5000 and skinfolds was significantly higher than that from underwater weighing. Skinfolds were showed to be better predictor of body fatness than the Futrex-5000 in middle-age men. The Futrex-5000 estimates of body fat were not as good as skinfold measurements in the group of middle-ages men; compared to underwater weighing showed that skinfolds have higher correlations and lower standard error than NIR. Also, it was observed that both the Futrex-5000 and skinfolds tended to overestimate fatness of leaner participants, and underestimate fatness in fatter subjects. These trends were considerably higher for the Futrex-5000 than they were for skinfolds. The agreement between percent body fat between the Futrex-5000 and underwater weighing in middle-aged men was not as good as in young men. The conclusions from this study were that the Futrex-5000 related to subcutaneous fat measured by ultrasound is better in young men, and the strength of the relationship depended on the site being measured. Muscle thickness did not have big contribution to the Futrex-5000 measurement. Biceps skinfold thickness was better correlated to body fatness than the Futrex-5000 in middle-aged men. Also, skinfolds were better predictor of total body fatness than the Futrex-5000. In younger populations skinfolds performed equally or better in estimating body fatness than the Futrex-5000.

Dual Energy X-ray Absorptiometry (DEXA)

DEXA is three-compartment model for measuring body composition assessing bone mineral content, fat mass, lean tissue mass, and regional fat distribution. DEXA is a preferred method for measuring whole body mineral content. DEXA requires little effort from the participant, and it does not depend on technician skill. This device is also used to measure body fat content as well as fat free mass. The principle that DEXA uses is that the attenuation of X-rays with high and low photon frequencies is measurable and depends on the thickness, density, and chemical composition of the body tissues. The attenuation, or weakening, of the X-ray frequencies is due to the chemical composition and densities of different tissues like bone, fat, and lean mass. These attenuations of X-ray frequencies of different tissues are assumed to be constant for all individuals. DEXA uses two X-ray beams and a narrow fan-beam mode, and as they pass through the body they are attenuated due to partial absorption of photons. A detector measures this reduction for each of the pixels of the body. Compared to the gold standard for measuring body composition DEXA is able to accurately measure body fat for young and healthy individuals.

One assumption that the DEXA scanner uses is that the amount of fat over the bone is the same as the amount of fat over bone free tissue. In DEXA scan images the amount of soft tissue is calculated from image pixels that do not contain any bone. Lohman (1996) found that approximately, 40 to 45 percent of the DEXA images have pixels with bone in them, therefore the lean mass and fat mass estimated by DEXA are measured from approximately 60 percent of the total pixels obtained from all images. In addition, in body parts such as the arms, the obtained pixels containing bone are much more than the pixels with sift tissue only, therefore fewer pixels are used to estimate fat and lean tissue, which can lead to not as accurate measurements of sort tissue as in other regions of the body.

The manufacturers of DEXA calibrated the scanner using phantoms with known density and quality of bone, lean tissue and fat. The purpose of this calibration was to correct for DEXA's limitation associated with the anterior-posterior thickness of the body. This variation of body thickness may change the attenuation of given tissue- fat, bone, and lean mass- even that it is assumed to be constant for all individuals.

Another assumption of DEXA when measuring whole body composition is associated with the water and electrolyte content of lean tissues. Going et. al (1993) estimated that changes in the water content of lean mass with 1 kilogram will not effect the accuracy of DEXA measurement. Lohman (2000) estimated that 5 percent changes in water content of lean mass would lead to DEXA error of estimation of 1 to 2.5 percent of body fat. Newer software versions improve the ability of DEXA to measure body composition and the hydration level of the participant have little effect on the estimation of percent body fat.

DEXA scanner compared to other methods for measuring body composition presents no discomfort to the participant. DEXA does not require thoracic gas volume or residual volume measurements. DEXA does not require any additional testing or collection of bodily fluids. The manufacturer recommends that no calcium supplements are taken on the day of the test. DEXA scan does not require any nutritional, exercise, or drinking restrictions prior to the test. In addition, DEXA scan is safe method for measuring body composition. The radiation from DEXA scan is estimated to be 3.5mrad, which is similar to the radiation absorbed by the skin for one week (Lang et. al. 1991).

The accuracy of body fat measurement for this device is strongly dependent on the type of DEXA scan (Hologic, Lunar or Norland), as well as software used. This makes the validation of DEXA scan very difficult. Another limitation for accurate measurement of body composition using DEXA can occur when measuring obese individuals because they often do not fit in the scanning area. Genton et. al, (2005) concluded that it is impossible to fit obese women (BMI over $30kg/m^2$) on a Prodigy scanning table, for which he suggested half-body scans. Furthermore, in his study Genton et. al, discuss another limitation to the measurement of body fat by this instrument that can occur when the meat thickness increases from 20.5 to 26 centimeters, which leads to underestimation of the measurement. Genton et. al, (2005) in his research showed that DEXA underestimates body weight in participants over 75 kilograms of body weigh compared to scale weight, but this underestimation was not observed in Lunar Prodigy (+0.5±0.8kg). High power mode software has been developed to correct for this underestimation when measuring obese populations. Deurenberg and Deurenberg-Yap (2001) stated that additional source of error can come from regional fat distribution between different ethnic populations. Lohman et. al. (2000) estimated that when percent fat obtained from DEXA is compared to multi-component models DEXA estimates percent body fat within 1 to 3 percent. Wang et. al. (1998) compared DEXA with six-compartment chemical model and concluded standard error of estimate of 1.7 kilograms of fat mass for the DEXA. In general, the error for DEXA scan in measuring body fat, lean muscle mass, and percent body fat is 1.0 kilogram, 0.8 kilogram, and 1.4 percent respectively. Lohman (1996) estimated that the general error of DEXA when measuring percent body fat is approximately 1 percent.

Pateyjohns et. al, (2006) compared DEXA to single frequency bioelectrical impedance analysis in measuring overweight and obese adult men. It was concluded that both devices have good relative agreement for all measures of body composition and DEXA measures higher body fat percentage with 1.74 percent and underestimated fat free mass with 2.5 kilograms compared to bioelectrical impedance analysis.

In another study comparing DEXA to bioelectrical impedance analysis in measuring body composition in adult men and women, Bolanowski et. al, (2001), found that men had significantly more lean body mass, and women had significantly more body fat when assessed with both DEXA and BIA, where DEXA measured more body fat tissue than BIA in both genders. Also, no significant influence of age and BMI was observed on the relationship of DEXA and BIA results. In this study, if BIA was used for the reference method it can be concluded that DEXA measurements underestimate lean body mass and overestimate body fat in both genders.

Lohman et. al, (2000) argued that the accuracy of the DEXA system for measuring body composition is highly correlated with the software and hardware systems used. Another major source of error concluded from this study was the hydration level of the participant, and more importantly the water content of fat free mass. Also, technical errors in assessing body composition from DEXA are introduced when the weight from the sum of the parts does not agree closely with scale weight (within 1 kilogram), leading to inaccurate estimates of changes in body composition. Snead et. al. (1993) compared DEXA (Hologic 1000, 5.50 software version) to underwater weighing to conclude that

DEXA underestimates percent body fat. He also performed an experimental test placing packages of lard over the abdomen of the participants. This study also showed that the percent fat was underestimated by the DEXA. Later Milliken et. al. (1996) used DEXA (Lunar DEXA-L, 1.3) to test if the device will accurately measure packets of lard placed over the abdominal region of the participant and found good measurements of the lard packages. Kohrt et. al. (1998) reanalyzed Snead's study using Hologic 5.64 software version and showed that the packages of lard are accurately measured compared to underwater weighing. He found that the difference in the mean body fat percentage measurements between both methods was within one percent, and percent body fat for men was underestimated and percent body fat for women was overestimated by DEXA compared to underwater weighing. In general, agreement is found in DEXA estimates of percent body fat and those of the multi-component models when recent software versions are used for both Lunar and Hologic systems- correspondence is found for lean and obese individuals and for all ages. Estimates of body composition from DEXA using recently developed software systems are within 1 to 3 percent of the fat from multi-component models.

Lohman et. al. (2000) also argues that hydration status of adults changes with age, which has an important effect on estimation of body fat percentage by multi-component model. Variation of water content in fat free mass for adults has been estimated to be 2%, Withers et. al. (1998) and Modlesky et. al. (1996) using mass spectrometry. Roubenoff et. al. (1993) hypothesized that abnormal levels of hydration alter the attenuation coefficient for lean tissue using DEXA, which leads to an error in estimating lean tissue for such individuals. On the other hand, Going et. al., (1993) found that acute changes in hydration status have little effect on the estimation of percent body fat by DEXA. Evans et. al., (1999) correlated variations of the hydration level of fat free mass with differences in percent fat between DEXA and four-compartment models, and estimated that 5 percent change in the water content in fat free mass affects DEXA estimates of percent body fat between 1 to 2.5 percent. Theoretical and empirical analysis showed that the level and variability in hydration status of fat free mass is not a major factor affecting the agreement of DEXA with multi-component methods.

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging uses a computer-generated image from radio frequency signals emitted by hydrogen nuclei - hydrogen molecules behave like tiny magnets. If external magnetic field is applied a pulsed radio frequency across the body part makes the hydrogen molecules to line up and absorb energy. When the radio wave is stopped the nuclei give off the absorbed radio signal, and the released signal is used to create an image. MRI is able to measure whole body and regional fat distribution as well as measuring visceral fat. The application of MRI includes whole-body measurement and regional fat tissue distribution, and measurement of visceral fat. The main objective is to obtain MRI images with good contrast between adipose and non-adipose tissue while minimizing the inconvenience to the participant. A typical body composition study using MRI requires the positioning of the subject to be within the center of the magnet in prone or supine position. Typically a 320mm region of the body is captured in a single acquisition, with multiple images (e.g.,7) anywhere within this region obtained in the same time required to obtain a single image. The abdominal region requires around 26 seconds for the imaging sequence to complete, which is short enough time for the participants to be able to hold their breath and eliminate the artifact of respiratory motion on the image quality. After obtaining all imaging acquisition from the body a sophisticated software analysis is required of the given tissue area (cm^2)- Ross et. al. (2000).

Quantitative Measurement of Tissues- The use of MRI in body composition is used to characterize the quality and distribution of fat and skeletal muscle. MRI has been used to measure fat and lean tissue in fetuses, children, normal weight males and females, obese males and females and diabetic and elderly populations. Most of the studies obtained their data from single image, but it is possible to obtain whole body data, which will require approximately 30 minutes. There are many advantages to using whole body acquisitions compared to single image data. Using whole body MRI is giving better on the distribution and quality of regional and total body fat, as well as skeletal muscle distribution – Ross et. al. (2000).

Qualitative Measurement of Tissues- Recent evidence suggests that MRI can be used to measure the quality of various lean tissues in vivo, in particular, skeletal muscle. Proton MRI does not separate the signals from different protons within image voxel, which makes the MRI not useful for determining the fat or water content of particular tissue for example skeletal muscle. Because of this a "chemical- shift" imaging techniques are used which enables the MRI to separate the water and fat signals from other signals in the region of interest- Ross et. al. (2000).

Thomas et. al. (1998) measured total of 67 women to assess different MRI scanning regimes and examine some of the assumptions commonly made when MRI is used to measure body fat content. Fifty four healthy females volunteers were assign to four groups according to their body mass index (BMI); A group included lean participants, B group included participants in the normal range, C group included overweight participants, and D group included obese participants. In addition 13 non-diabetic women with Prader-Willi syndrome were recruited; they were also distributed to one of the four groups depending on their BMI. All participants were measured by MRI and skinfolds, and 58 of them were measured by bioelectrical impedance analysis (BIA). Whole-body cans were performed on each female. The participants were lying in the center of the magnet in supine position with her arms overhead. They were scanned from their fingers to their toes, acquiring 10 millimeters- thick transverse images with 30 millimeters gaps between slices in the extremities, and 10 millimeters gas between slices in the trunk area. The images obtained from the scanning procedure were analyzed. Fat in the images appeared as a high signal against a muted background of other tissues and noise. The images were analyzed by using a software program that used knowledge-based image processing to label voxels as fat and nonfat components. The image processing procedure used a contour- following algorithm to isolate individual structures from images produced by thresholding. The threshold needed to identify the voxels associated with fat was computed automatically gray- intensity histogram analysis and background noise computation. Each side was also manually reviewed using interactive software program, to delete voxels that are not corresponding to fat tissue. This was useful for deleting pixels associated with the liver and bowel content that appears as bright, high-intensity structure in the images. The calculation of the volume (cm^3) of the total body fat content was made by summing relevant voxels counts and multiplying them by the voxels dimensions in cubic centimeters. The fat content volume for the whole body was than calculated by multiplying the fat tissue volumes of each slice by the sum of the slice thickness (10 millimeters) and interslice distance. In general, 10 or more slices are required at a time, obtained over an area of 40 centimeter or more of body area. This is required to keep the image stable. Fewer images taken at a higher distance from the isocenter of the magnet can cause significant image distortion. Waist and hip measurements, skinfold measurements at the biceps, triceps, subscapular, and suprailiac were taken. There were two assumptions made related to the nature of the content of each voxels identified as fat tissue in the MR image. Model A assumption was that each voxel is reflecting only fat, and Model B assumption was that each voxels presents that adipose tissue itself, composed of triglycerides, water, proteins, and minerals. For Model B, mean triglycerides fracture of 80 percent was used. Depending on the model used the volume of total body fat content can be very different. In this study, the sample body fat content ranged from 23 to 68 percent with model A, and 18.5 to 54.5 percent obtained from model B. In this study was observed that individuals with lower BMI had similar body fat percentage with individuals with higher BMI. An increase in total internal fat was observed with increase of subcutaneous body fat. Also, a significant number of lean participants in this study had the same or higher percent of total internal fat than did some obese individuals. The total internal fat was divided into visceral and non-visceral fat. Visceral fat was measured anywhere in the trunk area between the head of the femur and the top of the liver or the bottom of the lungs. Subcutaneous fat in this area was called abdominal fat and did not contribute to the visceral fat percentage. All other internal fat was labeled as nonvisceral. There was a significant correlation between visceral fat and wais to hip ratio in the normal range BMI group B and the overweight range BMI group C. In group A and D corresponding to lean group and obese group this correlation was not observed. The same relationship was found between total internal fat and waist to hip ratio. Also, even that there was a variation in visceral fat among groups, the nonvisceral fat content was relatively constant. For most participants the amount of nonvisceral fat was found to be similar or higher than their visceral fat content in volume and percentage. In this study a significant correlation was found between the percent fat obtained from MRI and the percent fat obtained from BIA in all four groups, taking into consideration only the healthy participants ($r = 0.93$, $p < 0.01$). This correlation was much stronger for individuals in the overweight (C group: $r = 0.84$, $p < 0.01$) and obese (D group $r = 0.90$, $p < 0.02$) groups, than individuals in the lean (A group: $r = 0.54$, $p < 0.02$). On the other hand the correlation between MRI and anthropometric measurements was stronger for lean individuals than it was for overweight and obese individuals (A group: $r = 0.79$, $p < 0.01$; B group: $r = 0.59$, $p < 0.02$; C group: $r = 0.28$, $p < 0.14$; D group $r = 0.29$, $p < 0.28$). MRI and anthropometric measurements did not correlate as well as MRI and BIA ($r = 0.88$, $p < 0.01$). There are numerous assumptions that need to be adopted when measuring body composition, which makes the comparison of methods hard. These assumptions are related to densities of fat and fat free tissues, the chemical content of fat free tissues (water, protein, and minerals), hydration status of fat-free tissues (approximately 73 percent), and the fat content of adipose tissue (approximately 80 percent). These assumptions may be the reason for the generally poor agreement between MRI and other methods when absolute values are compared, even though methods correlated relatively well. A critical difference between MRI and other methods for measuring body composition is that MRI volume measurement is agreeable to absolute calibration, which leaves only tissue distribution and tissue content as the two possible sources of measurement error for different individuals. MRI measurements are less likely to be affected by individual variations, which can lead to achieving a higher statistical power for a given sample size.

Ross et. al. (1993) used 15 healthy obese female participants to establish the MRI as a tool for measuring subcutaneous and visceral adipose tissue distribution in obese women, and to assess the relationship between selected anthropometric values and adipose tissue measured by MRI. Transverse slices with 10 millimeters thickness were taken every 50 millimeters of body area from the fingers to the toes. The participant was lying in supine position with their hands overhead. For each participant a total of 41 images were obtained. The total acquisition time for each participant was approximately 30 minutes, with 8-minute trunk acquisition, and two 4-minutes acquisitions on the arms and legs. The areas (cm^2) adipose tissues in each slice were computed automatically by summing the fat pixels and multiplying them by the pixel surface area. The volume (cm^2) of these regions of adipose tissues was calculated by multiplying the adipose tissue area (cm^2) by the slice thickness (10 millimeters). The total fat volume was calculated by adding by adding the volumes from all 41 slices. The visceral fat volume was calculated by adding the volumes of the seven slice images taken from one slice above the 4th lumbar vertebra to five slices below the 5th lumber vertebra. Skinfold measurements were obtained by Harpenden calipers at: biceps, triceps, chest, subscapular, iliac, calf, thigh, and rib. Circumference measurements were obtained at: biceps, forearm, chest, hip, proximal thigh, calf, and umbilicus. Also, waist to hip ratios were obtained for each of the participants. Even that all participants were android with respect to their fat distribution, there were large differences observed in this study for all MRI measured variables. Subcutaneous fat volume ranged from 26 to 76 liters, and visceral fat volume ranged from 0.9 to 5.5 liters. Subcutaneous fat volume represented 92.3 percent and visceral fat volume represented 6.2 percent of total body fat. The results of this study confirm that MRI can be used to reliably measure body fat distribution, more specifically visceral fat in humans. When the MRI procedures described in this study are used the expected error for measuring subcutaneous body fat is approximately 5 percent and the measurement error for visceral fat is approximately 10 percent.

Summary

Accurate measurement of body composition is important for various health and fitness reasons. There are numerous methods for measuring body composition in clinical, laboratory, and field settings. The wide variety of methods provide opportunities for measuring body composition from tissue to molecular level, dividing the methods from two to six compartment models. Different population-specific equations are developed to calculated body densities from variables measured through these techniques, and further to convert body density to percent body fat.

The literature has indicated that there are inconsistencies in the agreement between different methods of measuring body composition. The results from different studies comparing body composition techniques have been conflicting, showing contradictory comparisons between methods and their correlations with each other. Factors related to age, sex, and ethnicity, as well as the used of different equations for obtaining body density and percent body fat play a major role in the accord between methods. The agreement of different methods when measuring body composition of individuals with wide ranges of body fatness is inconsistent (lean, normal, overweight, and obese). For example, skinfold measurements have been shown to underestimate body fat in obese individuals and overestimate percent body fat in lean individuals when compared to other methods. Similar relationship was found for bioelectrical impedance, and air displacement plethysmography. These results also vary between sex, and ethnicity of the populations being measured. Dual Energy X-ray Absorptiometry is shows to overestimate body fat compared to BIA in obese individuals but to underestimate percent body fat compared to underwater weighing. BIA also correlated very well with Magnetic Resonance Imaging when measuring overweight and obese individuals. Contradictory results were shown in the literature when Near Infrared Reactance method was validated against other methods. NIR presented good correlation with DEXA and underestimated percent fat for normal men and women when compared to underwater weighing. Conflicting results from different studies were shown when NIR was compared to skinfold and ultrasound measurements. Studies comparing ultrasound to skinfold technique also have diverse results in the correlations between body sites of measurement and the validity of ultrasound and skinfold methods compared to underwater weighing. Magnetic Resonance Imaging was better correlated with BIA than skinfolds. MRI correlated well with skinfolds when measuring lean individuals were as MRI and BIA correlated better when measuring obese individuals. MRI is also a good method for measuring internal body fat. In addition, the use of different brands of equipment adds to the different results by showing reverse results when measuring the samples with similar characteristics and comparing them to the same reference methods.

CHAPTER 3

METHODOLOGY

Introduction

This study investigated various methods for measuring body composition in comparison to underwater weighing in Caucasian adult healthy men and women. The purpose was to determine a valid alternative in measuring body composition for each one of the two populations. This chapter describes the methods and procedures used in this study. The study included one testing day for each individual, during which each person completed all testing measurements included.

Participants

The participants were 26 Caucasian adult women and 24 Caucasian adult men, between the ages of 18 and 55 years. All participants were in good general health. Women who were pregnant or breastfeeding were not allow to participate in the study for various reasons involving radiation associated with two of the measurement techniques. Also, participants with metal implants or joint replacements were not included in the study.

Testing Procedures

Each participant was scheduled to complete all tests in one day to eliminate any possible changes in body composition. The completion of the tests took from 1 hour and 30 minutes to 2 hours. In the testing session each participant was measured with Bioelectric Impedance Analysis (BIA), Near Infrared reactance (NIR), skinfolds and equations to convert skinfolds to percent fat, ultrasound, Bod Pod, Dual Energy X-ray absorptiometry (DEXA), and underwater weighing. From the subject pool 5 men and 5 women were scheduled for an additional day of testing to complete Magnetic Resonance testing, and those subjects reported to Nevada Imaging Center to complete a whole body scan.

Informed Consent Forms and Medical Released Forms

All participants were required to read and sign an Informed Consent Form prior to participating in the study. Participants were also required to obtain Dual Energy X-ray absorptiometry (DEXA) prescription from a designated medical specialist. Participant completing Magnetic Resonance Imaging (MRI) scan were required to complete medical questionnaire before the test.

Measurements

The measurements and their descriptions are described below:

1. Body height and weight

Body weight and height were taken at the beginning of each session followed by the seven body composition methods. Body weight was taken to the nearest 0.5 kilogram on a balance beam scale. Participants were measured without shoes and minimal clothing or swimwear. Body height was taken to the nearest centimeter with a wall-mounted stadiometer. Height was measured without shoes.

2. Skinfolds measurements

These measurements of subcutaneous fat were taken on the right side of the body using Lange skinfold calipers. The skinfold sites were: Pectoral, Umbilical, Ilium, Axilla, Triceps, Subscapula and Thigh. The exact locations are presented in Appendix B. Since the reliability and validity of skinfold measurements depends on the tester's expertise, a pilot study was done to determine the tester's reliability. Using 15 participants, the 15 participants were measured on all seven sites on two consecutive days. The author's test-retest reliability coefficients are listed below:

Skinfold Site	Test/retest Reliability
Pectoral	0.98
Umbilical	0.98
Ilium	0.99
Axilla	0.99
Triceps	1.00
Subscapula	0.99
Thight	1.00

Body density from skinfold measurements was calculated using Jackson & Pollack equations for men and women. Siri equation was used to convert body density to percent body fat:

Equation 14. Jackson & Pollack Σ 7 sites (Lange) for male (Heyward et. al. (2004)):

$$Db(g/cc) = 1.112 - 0.00043499(\Sigma 7SF) + 0.00000055(\Sigma 7SF)^2 - 0.00028826(Age)$$

Equation 15. Siri (Density to percent fat -men)(Heyward et. al. (2004)):

$$\%\ BF = [(4.95/Db) - 4.50] \times 100$$

Equation 16. Jackson & Pollack Σ 7 sites (Lange) for female (Heyward et. al. (2004)):

$$Db(g/cc) = 1.097 - 0.00046971(\Sigma 7SF) + 0.00000056(\Sigma 7SF)^2 - 0.00012828(Age)$$

Equation 17. Siri (Density to percent fat -women)(Heyward et. al. (2004)):

$$\%\ BF = [(4.96/Db) - 4.51] \times 100$$

3. Underwater Weighing (Hydrostatic Weighing)

Percent fat from underwater weighing was the criterion methods against which the other body composition methods were compared. Although underwater weighing is considered the most reliable and valid method of determining body composition, it is assumed the procedure was a good procedure. This meant that the air in the lungs at the time of weighing was accurately determined, that the water was still and not moving at the time of weighing, and that all accessory equipment was calibrated and accurate. (Determining oxygen volume in the re-breathing bag, calibration of the gas analyzers, accuracy of the added weights on the subject, and the density and temperature of the water). The underwater measurement consisted of three procedures: calibration of equipment, measurement of body weight under water and residual volume measurements, and gas analysis. Calibration procedure included calibration of the solenoid delivering 5 liters to the re-breathing bag and calibration of the oxygen and carbon dioxide gas analyzers. The Siri equation was used to convert body density from underwater weighing to percent body fat:

$$\%BF = [(4.95 / Db) - 4.5] \times 100$$

A detailed explanation of UWW procedures is presented in Appendix C.

4. Bioelectrical Impedance Analysis (BIA)

By passing a low-grade electrical current (from a 9volt battery) through the body, body water resists (impedes) the current, this is measured in ohms of resistance. Since the amount of water in fat, muscle and bone is known, equations determine from the resistance how much fat is in the body. BIA gives total body water, and then an equation converts total body water to percent fat. The BIA instrument used in this study was by BioAnalogics. There is little technician skill needed making it a viable method for lay workers.

A detailed description of the bioelectrical impedance analysis is presented in Appendix D.

5. Air Displacement Plethysmography (Bod Pod)

The Bod Pod uses the same principle as underwater weighing to get the body's volume except that instead of displacing water air is displaced. Bod Pod is a two-compartment model for measuring body composition that determines body volume from pressure changes. Two gas laws are used in the Bod Pod system. Boyle's law states that volume occupied by a gas at constant temperature is reduced or expended in direct proportion to the pressure placed around it: P1/P2 = (V2/V1) - double the volume, half the pressure. The second law is Poisson's law, which accounts for adiabatic conditions. This law states that gas compresses or expands with temperature changes, which accounts for the heat that the human body will give off.

Sources of error in the Bod Pod (SEE = ±2.2-3.7%) include body hair, testing conditions, and prediction equations used for the conversions of body density to percent body fat. Body hair leads to underestimation of percent body fat, due to smaller body volume measurements. In addition, clothing is another variable that can lead to underestimation of percent body fat, e.g. hospital gown lowers percent body fat by 5 percent. Tight fitting clothing is optimal when Bod Pod technique is used. Furthermore, small variations in percent body fat are found between prediction of thoracic gas volume (TGV) and measurement of thoracic gas volume.

Bod Pod uses volumetric method for determining body volume. It determines the volume of empty chamber, and the volume of the chamber with the person inside. Body volume is calculated as the difference between the two measurements. Furthermore, the volume of the person is corrected for body surface area using DuBois and DuBois (1916) formula, and thoracic gas volume. The body volume is calculated by Dempster and Aitkens (1995) formula where BV (in L) = BVraw – surface area artifact + 40% of TGV. Siri equation is used to convert body density to percent body fat:

$$\%BF = [(4.95 / Db) - 4.5] \times 100.$$

A complete description of the calibration and use of the Bod Pod is presented in Appendix E.

6. Dual energy X-ray absorptiometry (DEXA)

DEXA was not designed for body composition but for bone mineral density. It's the primary test for osteoporosis. However, it also reportedly gives an accurate and reliable percent body fat, and is very user and subject friendly. The DEXA equipment is very expensive, but if available, is could be an excellent instrument for body composition. The subject lies, relaxed on the table and the scanning arm travels the length of the table and body.

DEXA scan sends an invisible beam of low-dose x-ray with two distinct energy peaks throughout the body- one of the peaks is absorbed by the soft tissues and the other one by the bones. The soft tissue amount can be subtracted from the total and what is left is a subject's bone mineral density (BMD). On the other hand the DEXA has special software, which computes and displays the bone density measurements on a computer, are the results can be after print out.

A complete description of the procedure and calibration is presented in Appendix F.

7. Ultrasound

The BodyMetrix BX2000 is a new instrument for determining percent body fat. This ultrasound procedure uses the same seven sites for measuring subcutaneous fat thickness as the skinfold technique. Ultrasound energy produces a low frequency mechanical pressure wave through soft tissue, which is used to measure subcutaneous fat thickness. This technology is relatively inexpensive and portable especially when compared to modalities such as underwater weighing, or DEXA scan. The BodyMetrix BX2000 has number of different formulas in its software used to determine body density, and percent body fat (e.g., Jackson and Pollack and Siri equations). This technique is very easy to use and does not present discomfort to the subject. The printout gives information about percent body fat, BMI, and target weight.

The detailed description of using and measuring with the BodyMetrix is presented in Appendix G.

8. Near Infrared

Near infrared reactance (NIR) method estimates body fat percentage from the reflectance of near infrared light off the underlying tissue. The Futrex NIR analyzers estimate body fat percentage from optical density (OD) measurements at only one site: biceps brachii. The less NIR light reflected (i.e., more light absorbed), the greater the amount of subcutaneous fat. Futrex 6100 is designed for adults only, and uses body weight, height, OD, gender, and age in its equations for predicting percent body fat. NIR method for measuring percent body fat is relatively easy to use technique and it requires little technician skills compared to other methods such as skinfolds. There is little difference in biceps OD when two different testers measure the same person. Limited information is present on how hydration status affects the NIR results, including eating, drinking, exercise, and menstrual cycle stages. In addition, skin tone and color account for 12 to 16 percent in the variability in OD measurements at the biceps site. Using near-infrared spectral analysis the estimation of fat mass and lean mass is based on the light absorption and reflection properties of each tissue. When the light beam meets a certain tissue the light can be transmitted, absorbed, or reflected from that tissue, which gives us information about the chemical composition of the tissues. The test is usually performed on the biceps of the dominant arm of the participant. The printout gives information about body composition, and total body water.

The detailed description of using and measuring with the Futrex 6100 is presented in Appendix H.

9. Magnetic Resonance Imaging (MRI)

Participants were placed in supine position on the MRI table with their arms crossed on top of the abdomen. Velcro belt was placed around their mid- section to assure that the arms will not move during the scanning. Sand bags were placed on the lateral side of the ankles to support the leg, and keep them from external rotation during the test. The participant was than slid inside the magnet with feet first. The scanning procedure started from the head of the participant going down to the feet. The body of each participant was divided into 8 scanning stations along the length of the body. The scanning procedure is set up to take multiple pictures of each of the 8 scanning sessions. Full body scan was performed, with thickness of each image of 6.25mm, without leaving gaps between images. The testing procedure took 30 minutest, and the patient set up took 10 minutes. After all images were obtained they were processed to obtain body fat volume and percent body fat using Osirix software. All of the MRIs will be conducted utilizing Philips MRI scanners at the Nevada Imaging Center (NIC). Additional studies comparing the MR-related assessment will be subjectively and quantitatively reviewed by a full-time physicist from Philips, Tom Perkins, PhD, at NIC.

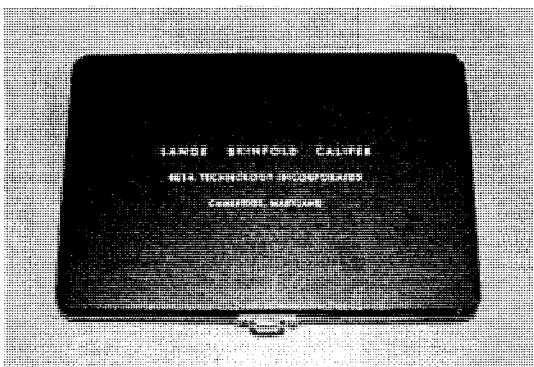
Pretesting Guidelines For Testing Sessions

- No eating or drinking within 4 hours before the test
- No exercise within 12 hours before the test
- Urinate within 30 minutest before the test
- No alcohol consumption within 40 hours before the test
- No diuretic medications within 7 days before the test
- No testing of females who think they may be retaining water during the stage of their menstrual cycle.

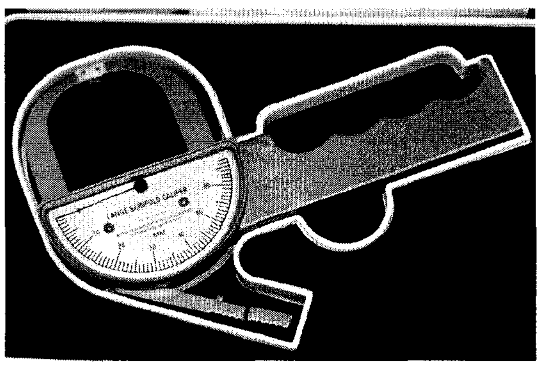
Equipment

The various pieces of equipment used for body composition are pictured below. Company names and addresses are presented in Appendix I.

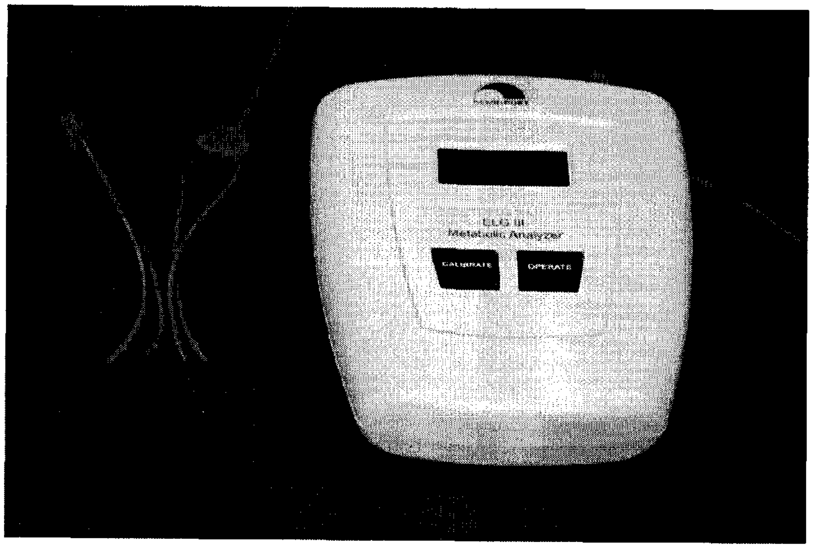
Picture 1 Lange Skinfold Caliper



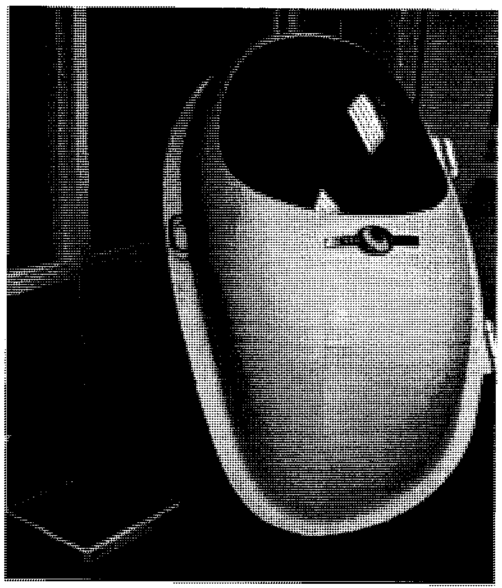
Picture 2 Lange Skinfold Caliper



Picture 3 Bioelectrical Impedance Analysis

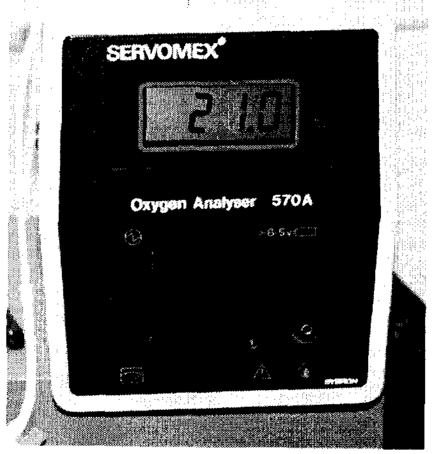


Picture 4 Bod Pod

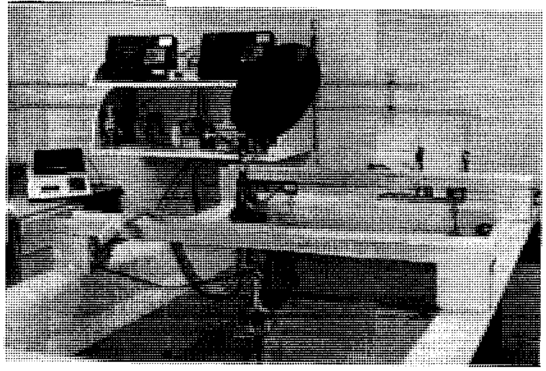


Picture 5
C02 analyzer for UWW:Anard AR 400

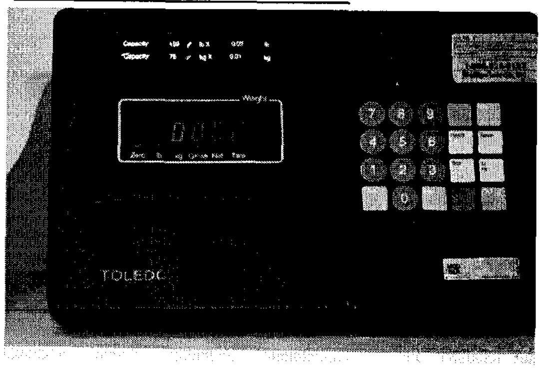
Picture 6
02 Analyzer for UWW Servomex 570A



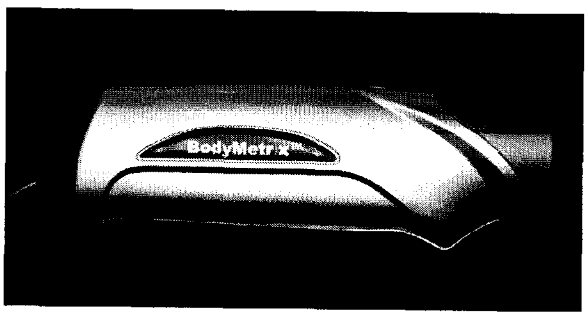
Picture 7
UWW Tank



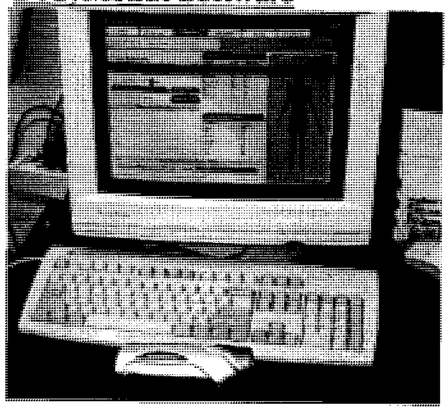
Picture 8
Toledo Scale 8806 Printer



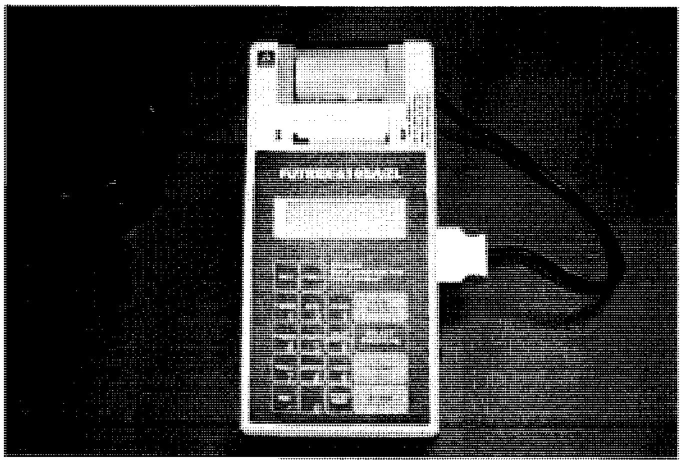
Picture 9
BodyMetrix Ultrasound



Picture 10
BodyMetrix Software



Picture 11 Futrex-6100 A/ZL



Picture 12 DEXA Scan

Statistical Treatment of Data

Two separate Pearson's r correlations were performed for men and women to determined how each of the different body composition measurement methods used in this study correlates with underwater weighing. Regression analysis was also performed to obtain standard error of estimate for each pair of comparisons. Scatter plot charts were created for each of the pair to show graphical illustration of the correlations. Furthermore, Pearson's r correlations were performed to show the correlations between each of the body composition methods used in this study. The purpose of this statistical analysis was to determine how different methods for measuring percent body fat correlate with underwater weighing and the other methods used in the study (sum of seven skinfolds, sum of four skinfolds, sum of three skinfolds, bioelectrical impedance analysis, air displacement plethysmography, dual energy X-ray absorptiometry, near infrared, ultrasound, and magnetic resonance imaging The 0.05 level of probability was selected as the statistical criterion for significance.

CHAPTER 4

RESULTS AND DISCUSSION

Introduction

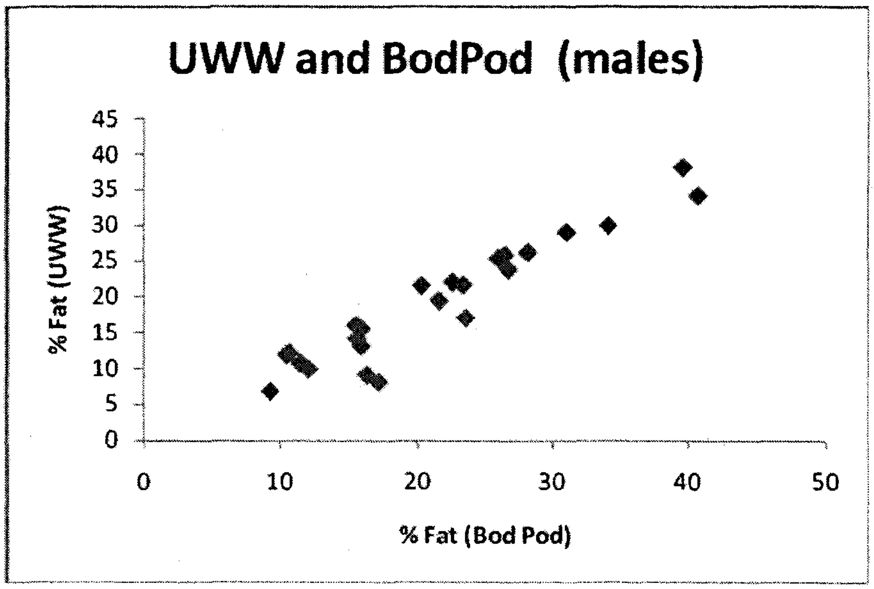
In this study, percent body fat was measured on male (n=24) and female (n=26) participants using nine different methods. The purpose of the study was to measure the degree of relationship between underwater weighing (UWW) and eight additional methods (air displacement plethysmography (Bod Pod), Dual energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), ultrasound, near infrared reactance (NIR), sum of seven skinfolds, sum of four skinfolds, and sum of three skinfolds). Pearson's r and standard error of estimate (SEE) were calculated to determine the degree of linear relationship between UWW and each of the eight methods. Paired t tests ($\alpha = .05$) were used to determine if any of the eight methods over- or underestimated UWW. Scatter plot graphs were created for each pair of variables using Microsoft Excel.

The tester's reliability for underwater weighing was r = 0.976, obtained from a test-retest of 18 females from pilot study data not included in the present study. The tester's reliability for skinfold measurements was obtained from a test-retest of 15 individuals from pilot study data not included in the present study. Reliability for each of the seven skinfold sites ranged from .98 to 1.0.

Underwater Weighing and Air Displacement Plethysmograpy (Bod Pod)

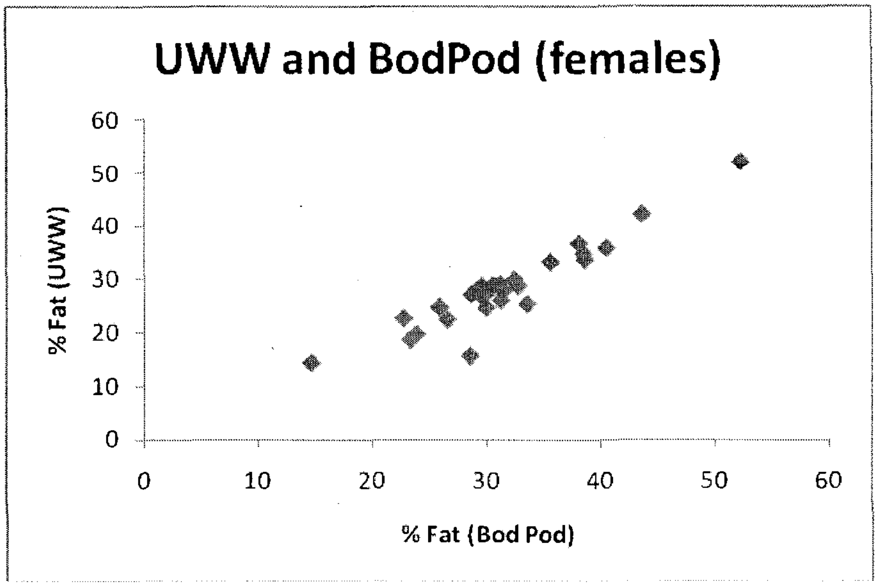
The correlation between UWW and Bod Pod for males in this study was $r = .951$, $p<.001$, SEE = 2.81 % (see Figure 1). The Bod Pod underestimated males' percent body fat by an average of 2.3% ($t_{23} = 4.02$, $p<.001$). A similar result was found by McCrory et. al (1995) with Bod Pod underestimating percent body fat by 0.3 compared to UWW.

Figure 1 UWW and Bod Pod (males)



The correlation between UWW and Bod Pod for females in this study was r = .939, p < .001, SEE = 2.62% (see Figure 2). The Bod Pod underestimated females' percent body fat by an average of 3.3% ($t_{25} = 6.1$, p<.001).

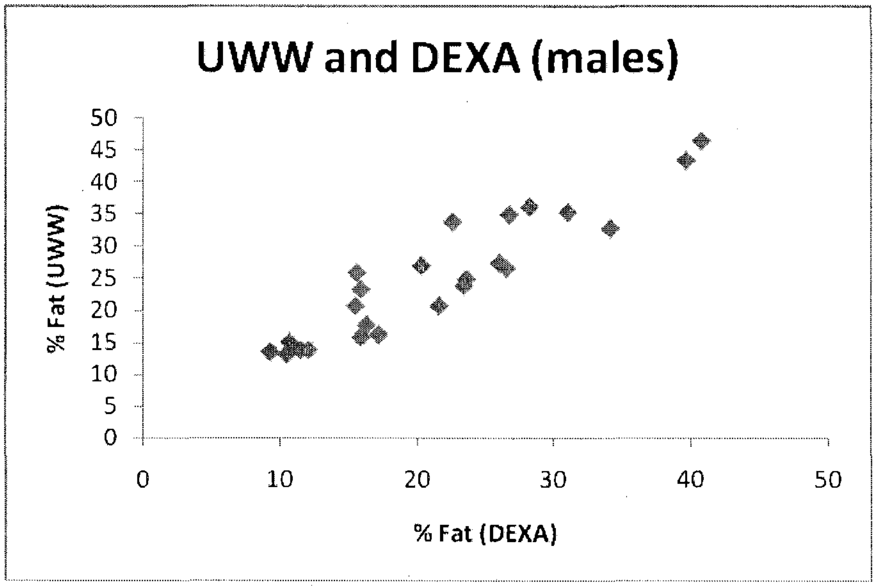
Figure 2 UWW and Bod Pod (females)



Underwater Weighing and Dual Energy X-ray Absorptiometry

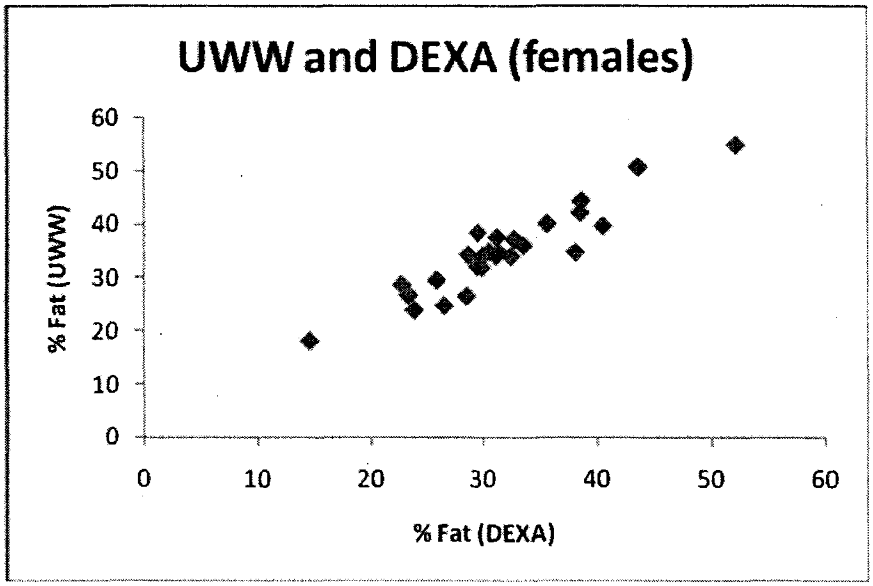
The correlation between UWW and DEXA for males in this study was $r = .929$, $p<.001$, SEE = 3.38% (see Figure 3). The DEXA overestimated males' percent body fat by an average of 3.6% ($t_{23} = -4.89$, $p<.001$).

Figure 3 UWW and DEXA (males)



The correlation between UWW and DEXA for females in this study was r = .932, p < .001, SEE = 2.77% (see Figure 4). The DEXA overestimated females' percent body fat by an average of 3.3% (t_{25} = -5.80, p<.001).

Figure 4 UWW and DEXA (females)



These results agree with studies presented in the literature. The accuracy of the DEXA scan compared to UWW will depend on the type of DEXA scan, and the software version used by the scan. This study agrees that DEXA scans using newer software versions will have an average error of measurement of body fat from 1 to 3%. Lohman (1996) concluded that DEXA scans measure percent body fat with an error of approximately 1%. Snead et. al. (1993) concluded that DEXA (Hologic 1000, 5.50 software version) underestimated percent body fat compared to UWW.

Milliken et. al. (1996) used DEXA (Lunar DEXA-L, 1.3) and showed accurate measurements of packages of lard compared to UWW, which was later repeated by

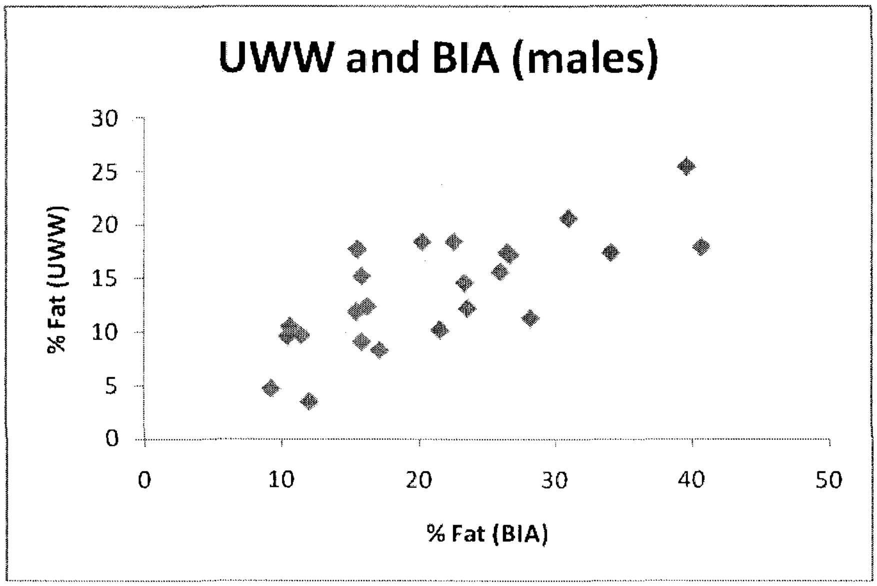
Kohrt et. al. (1998) to show the same agreement between UWW and DEXA (Hologic 5.64 software). He found that there was an average of 1% difference between the two methods and that DEXA underestimated percent fat when compared to UWW for males, which does not agree with the results from this study. On the other hand, Kohrt et. al. (1998) found that DEXA overestimates percent body fat when compared to UWW for females, which corresponds to the findings in this study.

No comparison between the measurement of fat weight and muscle weight by DEXA and UWW was made since DEXA is a three-compartment method and it measures not only fat and muscle weight, but also bone mineral weight, whereas UWW is a two-compartment model and only fat weight and muscle weight are used.

Underwater Weighing and Bioelectrical Impedance Analysis

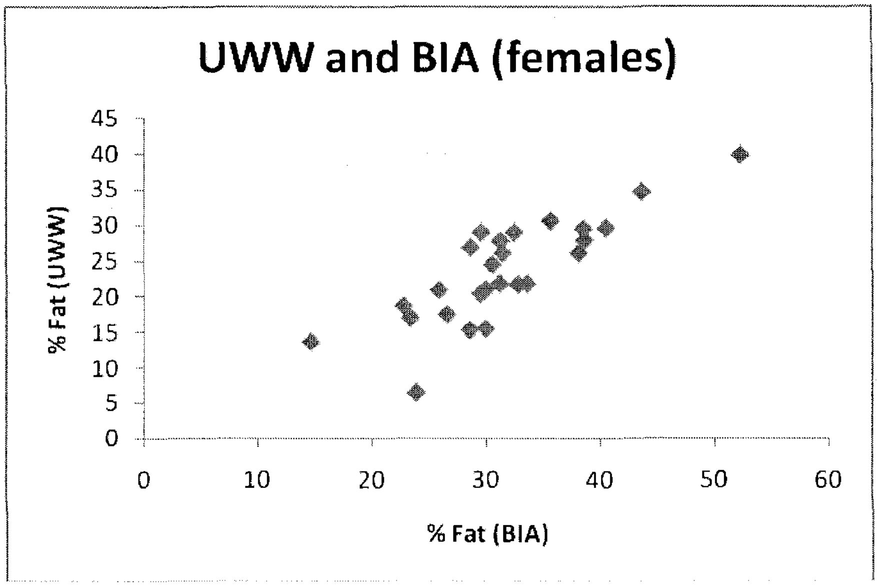
The correlation between UWW and BIA for males in this study was $r = .748$, $p<.001$, SEE = 6.05% (see Figure 5). The BIA underestimated males' percent body fat by an average of 7.7% ($t_{23} = 6.21$, $p<.001$).

Figure 5 UWW and BIA (males)



The correlation between UWW and BIA for females in this study was r = .825, p < .001, SEE = 2.77% (see Figure 6). The BIA underestimated females' percent body fat by an average of 8.1% ($t_{25} = 9.44$, p<.001).

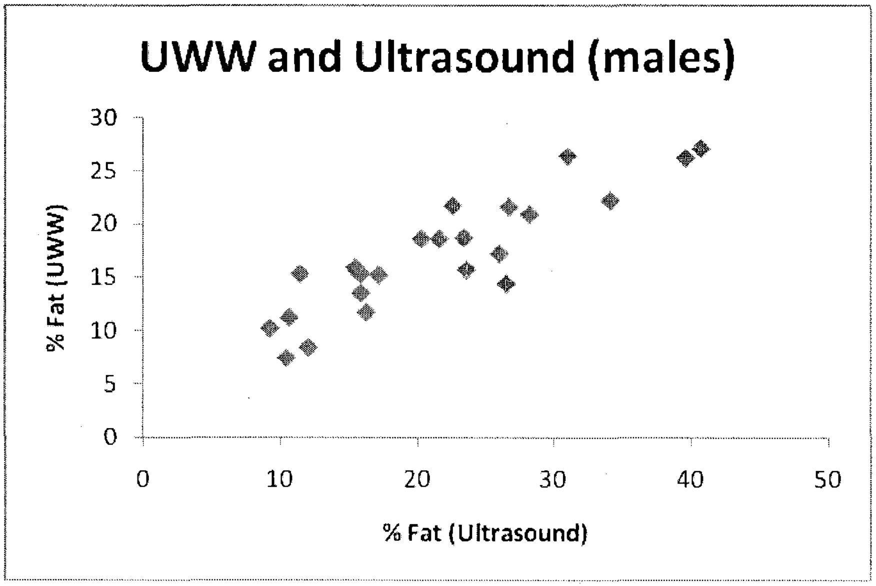
Figure 6 UWW and BIA (females)



Underwater Weighing and Ultrasound

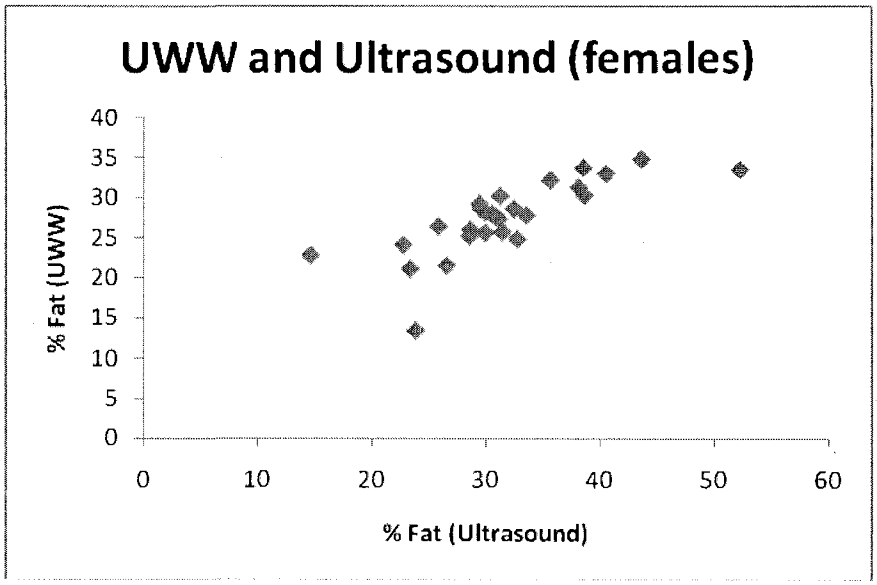
The correlation between UWW and ultrasound for males in this study was r = .887, p<.001, SEE = 4.21% (see Figure 7). The ultrasound underestimated males' percent body fat by an average of 4.4% (t_{23} = 4.49, p<.001).

Figure 7 UWW and Ultrasound (males)



The correlation between UWW and ultrasound for females in this study was r = .778, p < .001, SEE = 4.80% (see Figure 8). The ultrasound underestimated females' percent body fat by an average of 4.2% (t_{25} = 4.42, p<.001).

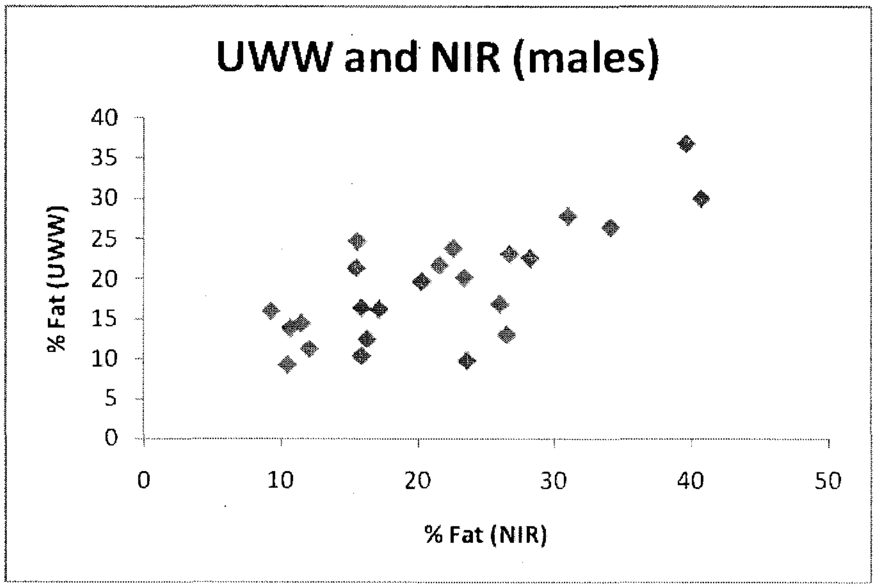
Figure 8 UWW and Ultrasound (females)



Underwater Weighing and Near Infrared Reactance

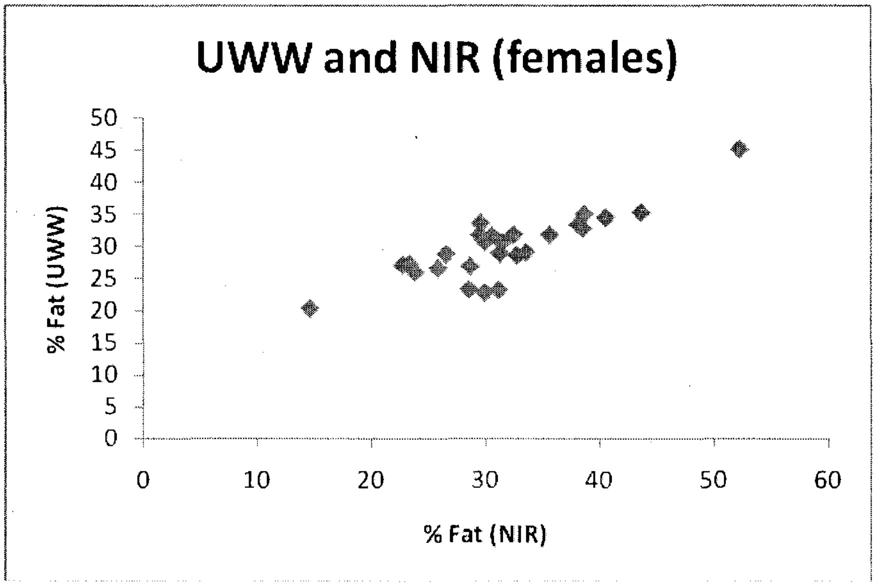
The correlation between UWW and NIR for males in this study was r = .750, $p<.001$, SEE = 6.02% (see Figure 9). The NIR underestimated males' percent body fat by an average of 2.4%, but this difference was not significant ($t_{23} = 1.97$, $p=.060$).

Figure 9 UWW and NIR (males)



The correlation between UWW and NIR for females in this study was r = .842, p < .001, SEE = 4.80% (see Figure 10). The NIR underestimated females' percent body fat by an average of 1.7%, but this difference was not significant ($t_{25} = 2.00$, p = .057).

Figure 10 UWW and NIR (females)

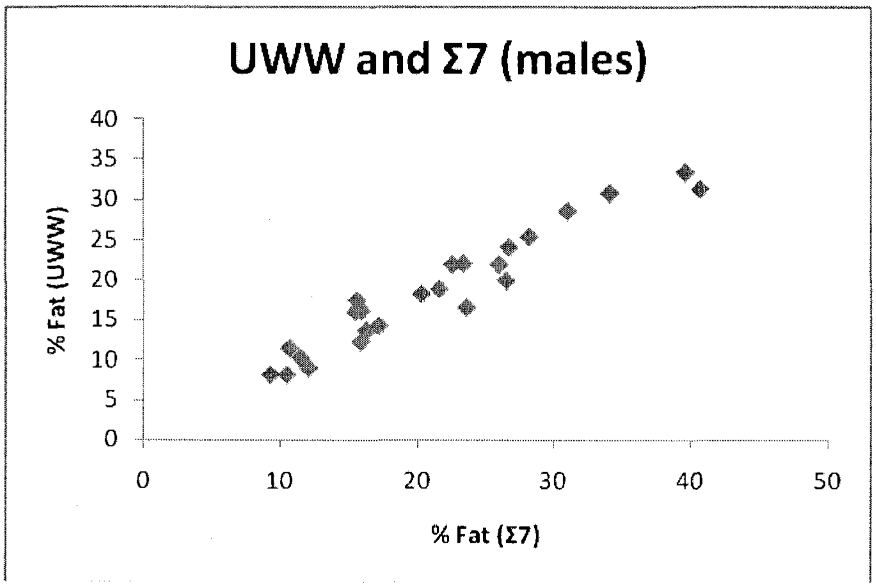


The results disagree with the study conducted by Israel et. al. (1989). Israel tested 80 white male and female participants and found a significant difference between the methods in predicting body density and percent body fat, showing that NIR significantly overestimates percent body fat when compared to UWW. However, Polito et. al. (1994) found that the correlation between UWW and NIR is .88 for men and .72 for women with a standard error of estimate of 4% for men and 4.7% for women; these findings agree with the data from the present study. Polito et. al. (1994) also reported that NIR underestimates percent body fat with 2.1% for men and 3.9% for women, which also agrees with the present study. Brooke- Wavell et. al. (1995) concluded that NIR does not correlate as well as skinfolds to UWW for both men and women, which also agrees with this study's findings.

Underwater Weighing and Sum of 7 Skinfolds

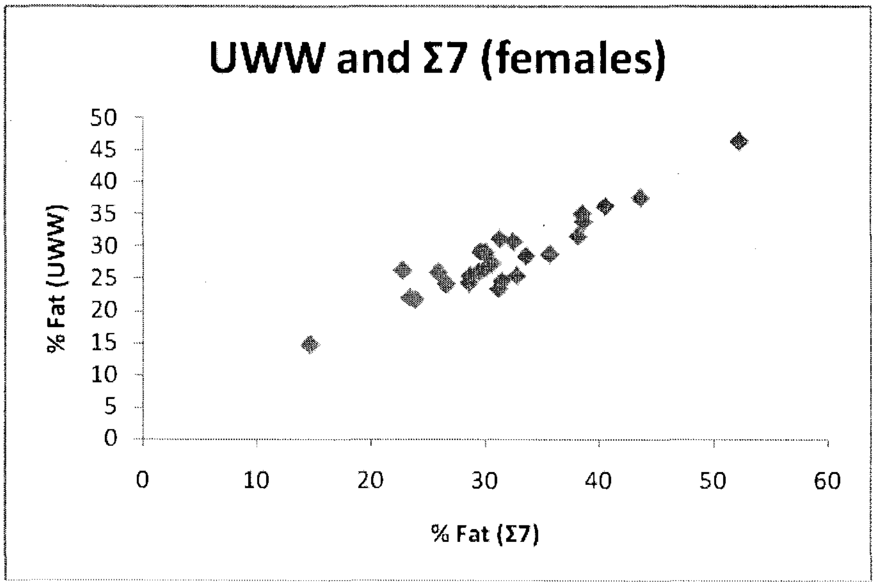
The correlation between UWW and Σ7 for males in this study was r = .965, p<.001, SEE = 2.4% (see Figure 11). The Σ7 underestimated males' percent body fat by an average of 2.7% (t_{23} = 4.98, p< .001).

Figure 11 UWW and Sum of Seven Skinfolds (males)



The correlation between UWW and Σ7 for females in this study was $r = .934$, $p < .001$, SEE = 2.73% (see Figure 12). The Σ7 underestimated females' percent body fat by an average of 3.3% ($t_{25} = 5.96$, $p < .001$).

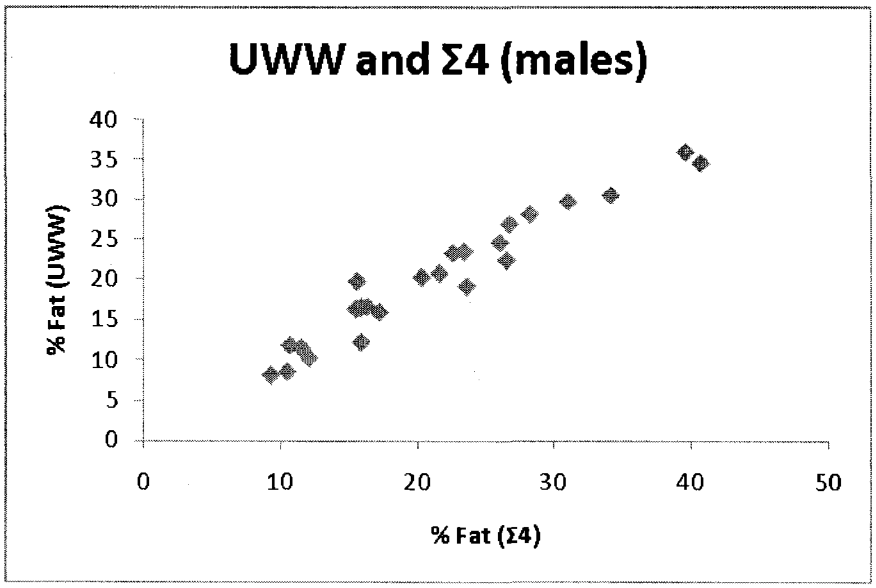
Figure 12 UWW and Sum of Seven Skinfolds (females)



Underwater Weighing and Sum of 4 Skinfolds

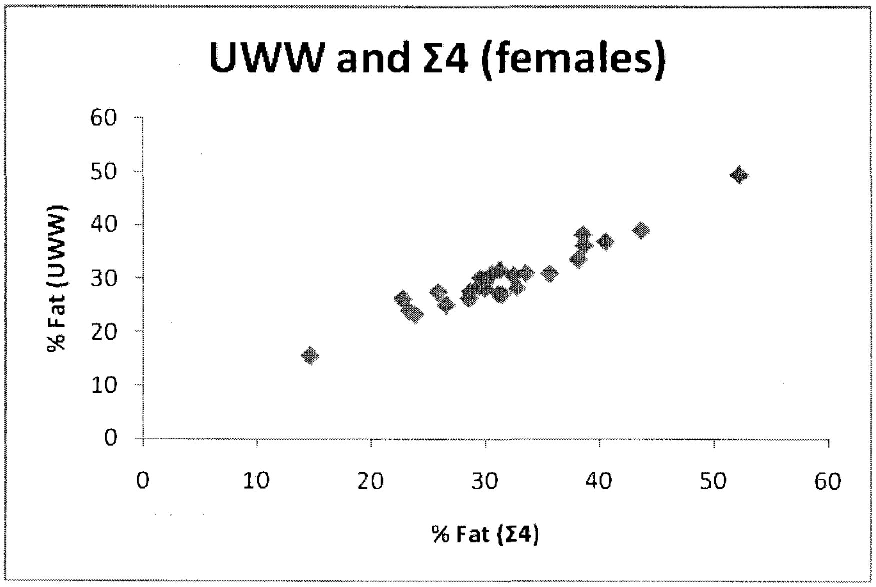
The correlation between UWW and Σ4 for males in this study was r = .971, $p<.001$, SEE = 2.19% (see Figure 13). The correlation between percent fat from UWW and four site skinfolds for males was the highest correlation obtained from all seven methods, although the difference among the correlations was not statistically significant. The Σ4 underestimated males' percent body fat by an average of 1.2% ($t_{23} = 2.39$, p= .025).

Figure 13 UWW and Sum of Four Skinfolds (males)



The correlation between UWW and Σ4 for females in this study was $r = .962$, $p < .001$, SEE = 2.08% (see Figure 14). The correlation between percent fat from UWW and four site skinfolds for females was the highest correlation obtained from all seven methods, although the difference among the correlations was not statistically significant. The Σ4 underestimated females' percent body fat by an average of 1.5% ($t_{25} = 3.35$, $p = .003$).

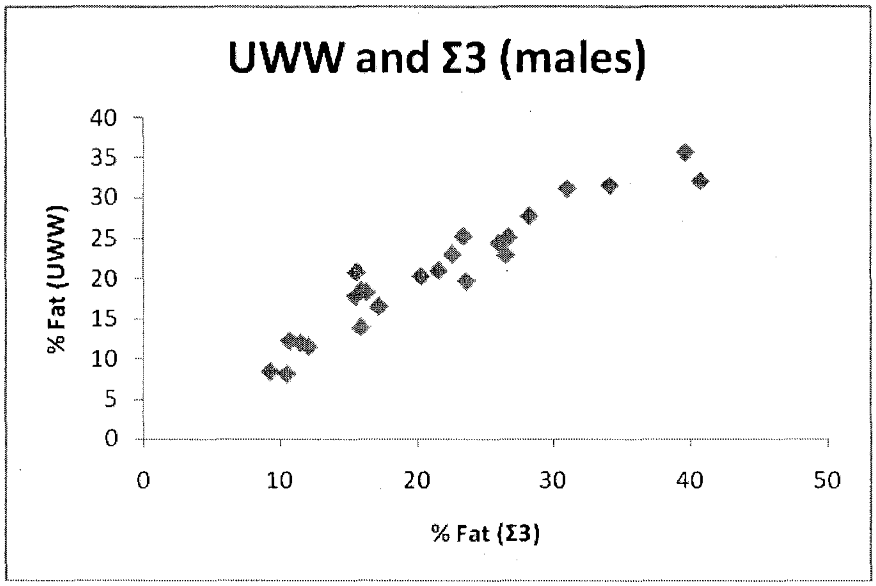
Figure 14 UWW and Sum of Four Skinfolds (females)



Underwater Weighing and Sum of 3 Skinfolds

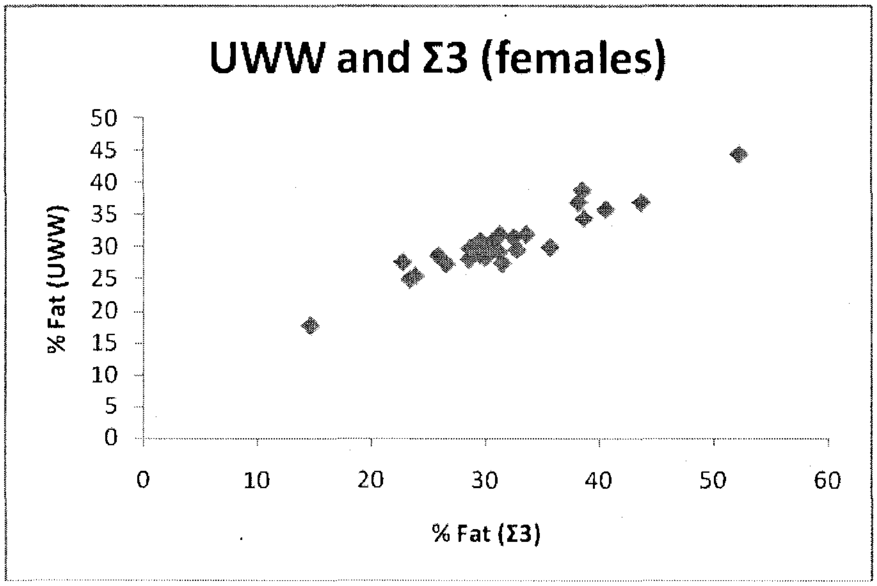
The correlation between UWW and Σ3 for males in this study was $r = .955$, $p < .001$, SEE = 2.69% (see Figure 15). The Σ3 underestimated males' percent body fat by an average of 0.7%, but the difference was not significant ($t_{23} = 1.26$, $p = .220$).

Figure 15 UWW and Sum of Three Skinfolds (males)



The correlation between UWW and Σ3 for females in this study was $r = .946$, $p < .001$, SEE = 2.48% (see Figure 16). The Σ3 underestimated females' percent body fat by an average of 1.0% ($t_{25} = 1.65$, $p = .112$).

Figure 16 UWW and Sum of Three Skinfolds (females)



Magnetic Resonance Imaging

Five male and five female participants were tested on MRI to obtain percent body fat. Total body scans were performed on each individual acquiring 60 images per person. The data was analyzed with software[4]. Each image was transformed so only fat pixels were presented in the images, and tissues different than fat were thresholded to zero and appeared black on the image. For each image the number of pixels corresponding to fat were counted and summed for all 60 images for each individual. Since MR images are three-dimensional, the building blocks of each image are called voxels instead of pixels,

[4] OsiriX v.3.2.1 32 bit

which are the building cells for two- dimensional images. The total sum of the voxels corresponding to fat were converted to volume units by multiplying the total number of voxels to their dimensions in centimeters (x= 0.156cm, y= 0.156cm, z= 0.625 cm). After the volume of the fat voxels was obtained for the whole body it was multiplied by .9001g/ml, which is the density of fat and divided by body weight to obtain percent body fat. The data used showed that the percent fat obtained from MRI can vary depending on how the images are threshold. Since all images were thresholded manually, this showed poor validity in obtaining percent body fat, because a slight change in the threshold of the images led to large differences in percent body fat. Furthermore, objectivity by the researcher when thresholding the images can lead to a large variability of percent body. From using MRIs in this study it was concluded that in order to obtain valid percent body fat measures, automated software is needed for analysis of MR images. If automated software is used to calculate percent body fat from MRI this will eliminate the subjective bias within and between measures and will provide more consistent results for percent body fat. Due to the above concern the data collected from MRI was excluded from the study and the statistical analysis and comparison with other methods for measuring body composition.

Correlations Between Other Methods

Tables 6 and 7 present correlations between the eight methods used to measure percent body fat in this study other than under water weighing. Table 13 presents the correlations for the male participants in this study, and table 14 shows the correlations for the female participants.

Table 6 Male Data – Correlations Between Methods

	DEXA	BIA	Ultrasound	NIR	Σ7	Σ4	Σ3
Bod Pod	.925	.815	.871	.775	.952	.945	.930
DEXA		.817	.913	.829	.947	.957	.929
BIA			.776	.733	.817	.817	.823
Ultrasound				.876	.937	.929	.919
NIR					.853	.835	.825
SF7						.992	.989
SF4							.991

Table 7 Female Data – Correlations Between Methods

	DEXA	BIA	Ultrasound	NIR	Σ7	Σ4	Σ3
Bod Pod	.992	.876	.779	.880	.909	.930	.908
DEXA		.887	.802	.816	.905	.931	.885
BIA			.875	.817	.836	.837	.807
Ultrasound				.660	.779	.782	.771
NIR					.887	.885	.844
SF7						.986	.962
SF4							.978

Summary

Eight different methods for measuring body composition were compared to underwater weighing in adult Caucasian men and women, to determine which method correlated best with UWW. All methods used were ranked against UWW for both genders. Table 8 presents the correlations and standard errors of estimate for each method when compared to UWW for males. Table 9 presents these correlations and standard errors of estimation for the females.

The sum of four skinfolds method had the highest correlation with UWW for males at r = .971 and the lowest correlation was between UWW and BIA at r = .748.

Table 8 Summary of Male Correlations

	SF 4	SF 7	SF3	Bod Pod	DEXA	Ultrasound	NIR	BIA
UWW/R	.971	.965	.955	.951	.929	.887	.750	.748
SEE	2.19	2.40	2.69	2.81	3.38	4.21	6.02	6.05

The sum of four skinfolds method also had the highest correlation with UWW for females at r = .962. The lowest correlation was between UWW and Ultrasound at r = .778.

Table 9 Summary of Female Correlations

	SF 4	SF 3	Bod Pod	SF7	DEXA	NIR	BIA	Ultrasound
UWW/R	.962	.946	.939	.934	.932	.842	.825	.778
SEE	2.08	2.48	2.62	2.73	2.77	4.12	4.32	4.80

Although the correlations varied between .971 and .748 in the present study, the differences were not significant.

CHAPTER 5

Summary, Conclusions And Recommendations

Summary

Under the assumption that underwater weighing is the most reliable and valid method of determining body composition, the purpose of this study was to determine which method of body composition assessment is closest to hydrostatic weighing and to determine the rank order of nine body composition techniques to hydrostatic weighing. Twenty-four men and twenty-six adult women volunteered as participants. All participants were apparently healthy Caucasian adults. Percent body fat for each participant was obtained from underwater weighing, Bod Pod, dual energy X ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), near infrared reactance (NIR), ultrasound, and skinfold measurements using Jackson and Pollack formulas for sum of seven skinfolds, sum of four skinfolds, and sum of three skinfolds.

After completing all measurements, the percents body fat for male and female participants obtained from the different methods were compared to percent body fat obtained by UWW. Individual correlations were developed between each of the methods and UWW separately for both sexes. In addition, correlations between density from UWW and density from skinfold measurement, and between density from UWW and density from Bod Pod were computed. In addition, another correlation was determined between skinfold measurements and ultrasound, for each of the seven sites of measurement, to determine which site correlated best for male and female participants. For the purpose of this correlation one-half of this skinfold measurement was correlated with the ultrasound measurement for the same site. The reason for this is that skinfold measurements measure a double skinfold thickness.

Conclusions

The conclusions determined from this study are:

1. The variability in measures of body fatness in these healthy adults supports the concept of population specific limitations in these techniques for assessing body composition.
2. As long as there is expert, trained technician, percent body fat obtained form the sum of four skinfold measurements using Jackson and Pollack equation correlates highest with percent body fat obtained from UWW for both sexes in this specific sample.
3. All methods for measuring body composition used showed high correlations with UWW.
4. The ranking of methods for measuring body composition with UWW were slightly different for men and women.
5. Magnetic resonance imaging should not be used as method for measuring body composition until automated software is developed to replace the manual analysis of the images, in order to eliminate subjective bias and reduce the time needed to analyze the images.

Recommendations

Based on the conclusions from this study, it is recommended that:

1. Considerations should be given to the equations used by the DEXA scan to estimate percent body fat and how much bone marrow has affect over the person's percent body fat.
2. Automated software for obtaining percent body fat from MRI should be developed to make this method easier, more valid and reliable in its use for measuring body composition.
3. If studies are using body composition and UWW is not available, skinfold measurements should give the most valid and reliable data. If trained technician are not available for skinfolds, the Bod Pod or DEXA should be used.

APPENDICES

APPENDIX A

Data Sheets

UNIVERSITY OF NEVADA, LAS VEGAS
EXERCISE PHYSIOLOGY LABORATORY
BODY COMPOSITION STUDY 2007/2008
SCORE SHEET

NAME:	16			**AGE:**	29	**DOB**	8/24/87	**DATE:**	0717/08
WEIGHT:	181	lbs.	82.3	kg.	**HEIGHT:**	73.5	in.	187	cm.
BMI	23.6	**BIA**	391	ohms.	**% Fat**	10.6			
BODPOT:	12.1	%	**LBN**	158.6lbs	**Wt**	180.5lbs		**Gender:**	**M**
DEXA:	**% Fat**	15.0	**LBW**	67.3kg	**Fat Wt**	11.9kg	**BMD**	3.9kg	
UWW	**Density**	1.0744	**% Fat**	10.7	**LBW**	161.2lbs			

SKINFOLDS:	**DATE:**		**DATE:**		**DATE:**		**ULTRASOUND**	
Pectoral	6.5	mm.	5.5	mm.	6	mm.	3.9	mm.
Abdominal	14	mm.	14	mm.	13	mm.	5.9	mm.
Illiac	15	mm.	15	mm.	14	mm.	7.5	mm.
Axilla	9	mm.	10	mm.	10	mm.	5.8	mm.
Scapula	12	mm.	14	mm.	13	mm.	5.6	mm.
Triceps	7	mm.	7	mm.	7	mm.	3.6	mm.
Thigh	14	mm.	13	mm.	13	mm.	6.3	mm.

Walst Girth	31.5	in.	80	cm.	**Waist / Hip Ratio**	0.81
Hip Girth:	39	in.	99.1	cm.		

Percent Fat		
Skinfold JP Σ 7	11.5	%
Skinfold JP Σ 3	12.2	%
Skinfold JP Σ 4	11.9	%

	% Fat
UWW	10.7
Bod Pod	12.1
DEXA	15
BIA	10.5
MRI	11.2
Infrared	13.8

Note:
Bod Pod Db: 1.071kg/L

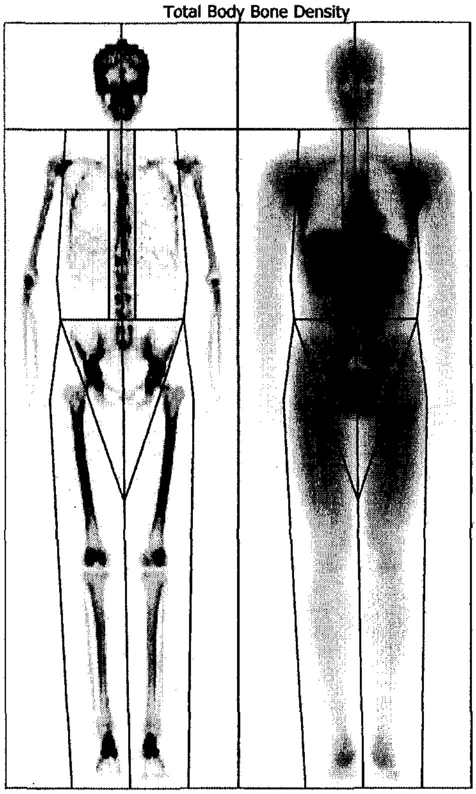
Name			
Date	7/17/2008		
Trial #	1	2	3
Land Wt (lb)	180.50	180.50	180.50
Sinker Wt (lb)	16.77	16.77	16.77
Land Wt (kg)	81.871	81.871	81.871
Sinker Wt (kg)	7.606	7.606	7.606
Water Temp (C)	33	33	33
Water D (g/cm3)	0.99473	0.99473	0.99473
Pre O2 (%)	98.6	98.6	98.6
Pre CO2 (%)	0.0	0.0	0.0
Air in Hose (L)	0.520	0.520	0.520
GIA (L)	0.100	0.100	0.100
Bag #			
Bag Vol (L)	5.2	5.2	5.2
Post O2 (%)	66.0	67.8	64.6
Post CO2 (%)	5.3	5.3	5.4
Wt 1 (lb)	24.99	25.67	24.30
Wt 2 (lb)	24.98	25.67	24.45
Wt 3 (lb)	24.78	26.04	24.45
Wt 1 (kg)	11.335	11.643	11.022
Wt 2 (kg)	11.330	11.643	11.090
Wt 3 (kg)	11.240	11.811	11.090
Ave Wt (lb)	24.917	25.793	24.400
Ave Wt (kg)	11.302	11.699	11.067
Func RV (L)	2.258	1.987	2.466
Body Vol (L)	76.2315	76.1032	76.2588
Body D (g/ml)	1.0740	1.0758	1.0736
% fat (B&K)	**11.32**	**10.61**	**11.48**
% fat (Siri)	**10.91**	**10.13**	**11.07**

UNLV DIVISION OF HEALTH SCIENCES

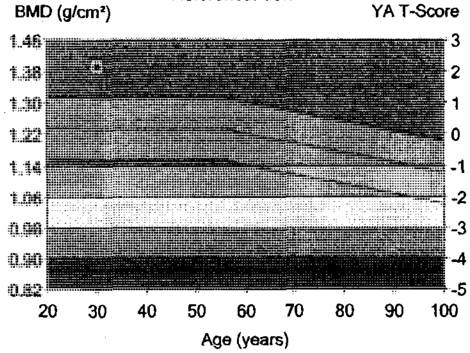
4505 MARYLAND PARKWAY
LAS VEGAS, NV 89154

Patient:		**Facility ID:**	
Birth Date:	8/24/1978 29.8 years	**Referring Physician:**	GOLDING
Height / Weight:	73.5 in. 181.0 lbs.	**Measured:**	7/17/2008 10:16:59 AM (10.50)
Sex / Ethnic:	Male White	**Analyzed:**	7/17/2008 10:17:00 AM (10.50)

Total Body Bone Density



Reference: Total



Region	BMD[1] (g/cm²)	Young-Adult[2] T-Score	Age-Matched[3] Z-Score
Head	2.254	-	-
Arms	1.128	-	-
Legs	1.656	-	-
Trunk	1.090	-	-
Ribs	0.770	-	-
Pelvis	1.375	-	-
Spine	1.222	-	-
Total	1.394	2.2	2.0

COMMENTS:

Image not for diagnosis

Printed: 7/17/2008 10:17:26 AM (10.50)76:0.15:153.85:31.2 0.00:-1.00
4.80x13.00 13.9:%Fat=15.0%
0.00:0.00 0.00:0.00
Filename: seu54kac9.dfb
Scan Mode: Standard 0.4 µGy

1 -Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for Total Body Total)
2 -USA (ages 20-40) Total Body Reference Population (v107)
3 -Matched for Age, Weight (males 25-100 kg), Ethnic

GE Healthcare

Lunar Prodigy
DF+13401

BOD POD® Body Composition Tracking System Analysis

University of Nevada, Las Vegas

Department of Kinesiology
Exercise Physiology Laboratory

SUBJECT INFORMATION	
NAME	
AGE	29
GENDER	Male
HEIGHT	186.7 cm
ID_1	
ID_2	
ETHNICITY	General Population
OPERATOR	mariana
TEST DATE	July 17, 2008
TEST NUMBER	569

BODY COMPOSITION RESULT		
% FAT	12.1	%
% FAT FREE MASS	87.9	%
FAT MASS	9.926	kg
FAT FREE MASS	71.938	kg
BODY MASS	81.864	kg
BODY VOLUME	76.427	L
BODY DENSITY	1.071	kg/L
THORACIC GAS VOLUME	4.193	L

TEST PROFILE	
DENSITY MODEL	Siri
THORACIC GAS VOLUME MODEL	Measured

OPERATOR COMMENTS

ENERGY EXPENDITURE RESULTS

Est. Resting Metabolic Rate (RMR) kcal/day	*Est. Total Energy Expenditure (TEE) kcal/day	Daily Activity Level
1896	2427	Sedentary
	2863	Low Active
	3299	Active
	3944	Very Active
(See RMR Info Sheet for additional info)	*Est. TEE = Est. RMR x Daily Activity Level	

Applies to adults ages 18 and older. Based on information from the Institute of Medicine (2002), Dietary Reference Intakes For Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, And Amino Acids, Part I, pp93-206. Washington, D.C., National Academy of Sciences.

Body Fat: A certain amount of fat is absolutely necessary for good health. Fat plays an important role in protecting internal organs, providing energy, and regulating hormones. The minimal amount of "essential fat" is approximately 3-5% for men, and 12-15% for women. If too much fat accumulates over time, health may be compromised (see table below).

Fat Free Mass: Fat free mass is everything except fat. It includes muscle, water, bone, and internal organs. Muscle is the "metabolic engine" of the body that burns calories (fat) and plays an important role in maintaining strength and energy. Healthy levels of fat-free mass contribute to physical fitness and may prevent conditions such as osteoporosis.

	BODY FAT RATING	MEN	WOMEN	EXPLANATION
☐	Risky (high body fat)	>30%	>40%	Ask your health care professional about how to safely modify your body composition.
☐	Excess Fat	21 – 30%	31 – 40%	Indicates an excess accumulation of fat over time.
☐	Moderately Lean	13 – 20%	23 – 30%	Fat level is generally acceptable for good health.
X	Lean	9 – 12%	19 – 22%	Lower body fat levels than many people. This range is generally excellent for health and longevity.
☐	Ultra Lean	5 – 8%	15 – 18%	Fat levels often found in elite athletes.
☐	Risky (low body fat)	<5%	<15%	Ask your health care professional about how to safely modify your body composition.

Applies to adults ages 18 and older. Based on information from the American College of Sports Medicine, the American Council on Exercise, Exercise Physiology (4th Ed.) by McArdle, Katch, and Katch, and various scientific and epidemiological studies.

Life Measurement, Inc. • 1-800-4 BOD POD • www.lifemeasurement.com

```
****************************
*       Lawrece Goldin     *
*                          *
*      FUTREX 6100A/ZL     *
*      BODY COMPOSITION    *
*          ANALYSIS        *
*                          *
* Name:__________________  *
*    07/17/08   9:35 AM    *
*                          *
* Age:  29                 *
* Sex: MALE                *
* Height:  73 inches       *
* Weight: 181 lb           *
*                          *
*    13.8% Body Fat        *
*                          *
*    Fat weight: 25 lb     *
*                          *
* Your  25 lb of fat have  *
*       three parts:       *
*                          *
* Essential Fat =   9 lb   *
*              -> GOOD <-  *
*                          *
* Reserve Fat   =  16 lb   *
*              -> GOOD <-  *
*                          *
* Excess =    0 lb         *
*              -> GOOD <-  *
*                          *
*   Congratulations, you   *
* have no Excess Fat. You  *
* are at miniumum risk for *
*   the serious diseases   *
*  associated with Excess  *
*        Body Fat.         *
*                          *
*   Lean weight: 156 lb    *
*                          *
*  Estimated Body Water:   *
*  52.4 liters, or 63.8%   *
*                          *
*   Body Mass Index: 24.0  *
****************************
```

BODY COMPOSITION
EXERCISE PHYSIOLOGY LABORATORY
UNIVERSITY OF NEVADA LAS VEGAS
DEPARTMENT OF KINESIOLOGY
LAS VEGAS, NV 89154
702-895-3766
702-873-4449 FAX

Report Generated for:

Report Data for client #00700825

Sex: Male	**Age:** 30	**Date:** November 2, 2008
Height: 6' 1.50"	**Weight:** 181.00 lbs	**Impedance:** 392
BMR: n/a	**ADL:** n/a	**AHR:** n/a

Health Management System
Developed and Marketed by
BIO|ANALOGICS
7909 SW Cirrus Drive
Beaverton, OR 97008

BODY COMPOSITION
ELG Data Report

Client Data:

Impedance: 392
Age: 30
Height: 73.50
Weight: 181.00
Sex: Male

Lean Body Mass:

Weight of Lean Body Mass: 161.84 lbs
Percentage of Lean Body Mass: 89.4%
Lean Body Mass to Fat Ratio: 8.4 to 1
Total Body Water: 55.7 litres

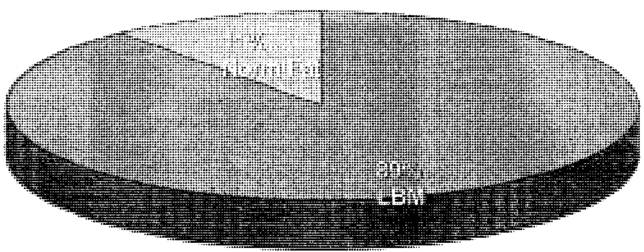
Fat Free Mass is composed of muscles, body fluid, connective tissue and bones. The optimal **Lean to Fat** ratio for you is at least **5.1 to 1**

Body Fat:

Weight of Body Fat: 19.16 lbs
Percentage of Body Fat: 10.6%

Fat is calories stored as energy reserve for your body. The desired range of percent **Body Fat** for you is **8-14%** (or 14-26 lbs). If you consume more calories than your body burns, the excess calories are stored as **Body Fat**. Excess **Body Fat** "frequently results in a significant impairment of health."

Current Status & Goals:



The graphs above show your projected body composition. **"LBM"** represents your **Lean Body Mass** and includes all body components except fat. **"Norm Fat"** represents **Normal Body Fat** which is necessary for proper physical health. **"Excess"** is **Fat** which is in excess of normal limits, and is unhealthy

Your Goal: 181 lbs (10.6% Fat)

Your goal on the **BODY COMPOSITION** will be to **optimize** your body composition. Through proper eating and exercising habits, you will improve your overall body composition, as well as ensuring a healthy lifestyle.

UNLV UNDERWATER WEIGHING PROCEDURES.
RELATIVE DENSITY OF WATER

UNLV Hydrostatic Weighing procedure					
Relative Density of Water					
Temperature			Temperature		
Centigrade	Fahrenheit	Density	Centigrade	Fahrenheit	Density
10	50	0.99730	26	79	0.99681
11	52	0.99360	27	81	0.99654
12	54	0.99520	28	82	0.99626
13	55	0.99400	29	84	0.99597
14	57	0.99270	30	86	0.99567
15	59	0.99910	31	87	0.99537
16	61	0.99897	32	89	0.99505
17	63	0.99880	33	91	0.99473
18	64	0.99862	34	93	0.99440
19	66	0.99843	35	95	0.99406
20	68	0.99823	36	97	0.99371
21	70	0.99802	37	99	0.99336
22	72	0.99780	38	100	0.99299
23	73	0.99756	39	102	0.99262
24	75	0.99732	40	104	0.99224
25	77	0.99707			

APPENDIX B

Skinfolds

The exact sites at which the skinfolds were taken follow:

- Pectoral fold- diagonal fold taken on the pectoral tendon line. For men this measurement was taken halfway between the axillary fold and the nipple. For women this measurement was taken one-third of the way between anterior axillary fold and the nipple.
- Umbilicus fold- vertical fold approximately one inch to the right of the umbilicus.
- Ilium fold- diagonal fold on the midaxillary line and the crest of the Ilium.
- Axilla fold- vertical fold on the midaxillary line at midsternum level.
- Subscapula fold- diagonal fold on the inferior angle of the scapula.
- Triceps fold- vertical fold halfway between the acromion process and olecranon process on the posterior side of the arm with extended and relaxed elbow.
- Thigh fold- vertical fold halfway between the groin line and the top of the patella.

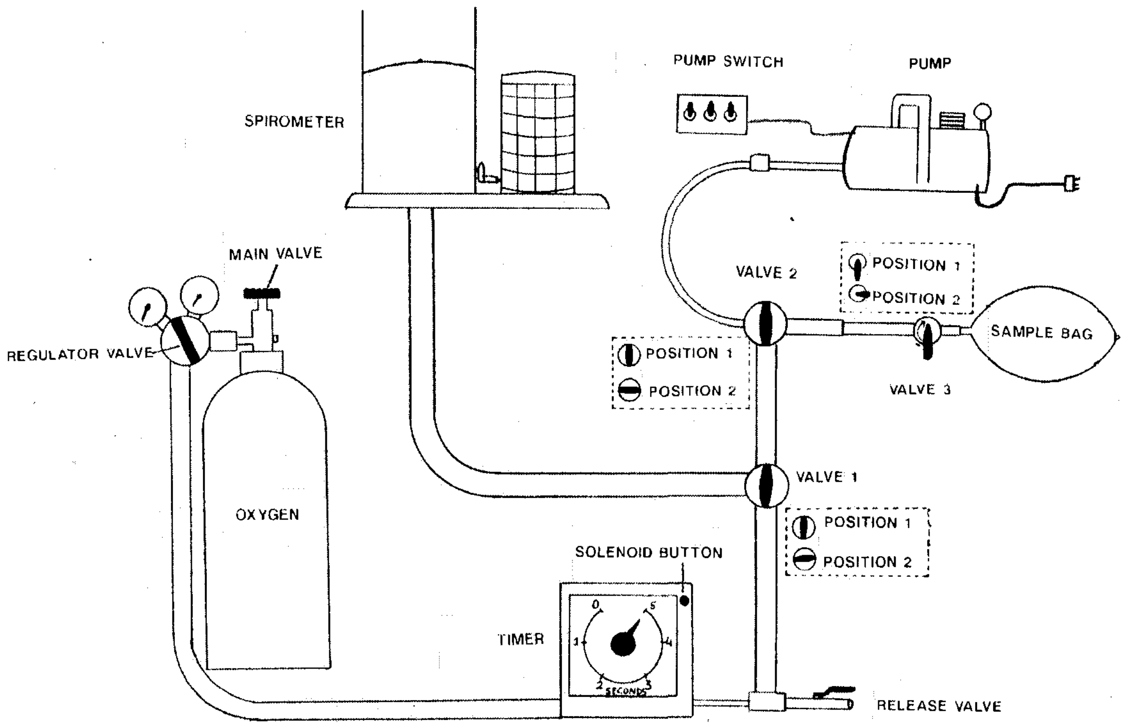
APPENDIX C

Underwater Weighing

Underwater Weighing Procedures:

Calibration of system for filling the re-breathing bag- this system included gas tank filled with pure oxygen (98 percent), timer, spirometer, vacuum pump, release valve for attaching the re-breathing bag, and open valve for releasing air from the spirometer, connected with two T valves. The first T valve opened gas flow from the oxygen tank toward the re-breathing bag and spirometer (valve 1). The seconds T valve opening gas flow from the oxygen tank to the re-breathing bag and from the re-breathing bag to the vacuum machine (valve 2). (see Picture 1)

Picture1 Gas rebreathing bag filling System



i. Turn on the main valve of the oxygen tank. (see Picture 1)
ii. Turn valve 1 to the calibration position (position 2), which delivers the delivered oxygen to the spirometer. (see Picture 1)
iii. Close the release valve. (see Picture 1)
iv. Uncap the spirometer pen and ensure that the kymograph paper is correctly aligned.
v. Press the button on the timer solenoid, which opens the timer valve for 5 seconds while delivering oxygen to the spirometer. (see Picture 1)

vi. Check the tracing on the kymograph paper for exactly 5 liters, adjust the oxygen regulator valve accordingly.
vii. Open the release valve to empty the spirometer. (see Picture 1)
viii. Close the release valve.
ix. Repeat steps v, vi, vii, and viii until exactly 5 liters is being delivered.
x. When this is accomplished the timer valve has been calibrated.
xi. Turn valve 1 to filling position (position 1) towards re-breathing bag. (see Picture 1)
xii. Attach re-breathing bag to its valve and open the valve on the re-breathing bag. (see Picture 1)
xiii. Turn valve 2 to vacuuming position (position 2). (see Picture 1)
xiv. Turn on the vacuum machine and let it vacuum completely the re-breathing bag. (see Picture 1)
xv. Close the re-breathing bag valve.
xvi. Turn off the vacuum machine.
xvii. Turn valve 2 to filling position (position 1). (see Picture 1)
xviii. Press the button on the timer solenoid to let oxygen gas flow into the re-breathing bag. (see Picture 1)
xix. Repeat steps xiv, xv, xvi, and xvii.
xx. Turn close the valve of the re-breathing bag and detach the bag from the system.
xxi. Fill up three re-breathing bags for the measurement procedure.

Calibration of system for analyzing the expired gases. The system includes oxygen and carbon dioxide analyzers with ingoing tubes for gas delivery, which are both connected with a T valve in a single outlet to which the re-breathing bag is attached.

i. Turn on both oxygen and carbon dioxide gas analyzers and let them warm up for one hour. The analyzers need to be warm in order to perform good calibration.
ii. Vacuum one 5L re-breathing bag: (1) Attach the re- breathing bag to the T valve connected to the vacuum machine. Make sure that the T valve is open toward the vacuum machine as well as the valve on the re-breathing bag itself is open. (2) Turn on the vacuum machine through the switch button and wait for the vacuum to suck out all air present in the re- breathing bag. (3) Close off the valve on the re- breathing bag. (4) Turn off the vacuum machine. (5) Remove the re breathing bag from the T valve. (see Picture 1)
iii. Using the proper valve attach the re-breathing bag to a calibration nitrogen tank and fill up the bag with pure nitrogen. Open the valve of the re-breathing bag after attaching it to the nitrogen tank, and close it off before removing the bag from the valve, preventing any room air entering the re breathing bag and keep the gas inside the bag pure nitrogen.

iv. Attach the re-breathing bag full with nitrogen to the valve connected to both oxygen and carbon dioxide analyzers.
v. Open the mediate T valve toward the oxygen analyzer.
vi. Open the valve on the re-breathing bag and push gently on it (since the oxygen analyzer do not have its own pump, help is needed to push the gas from the bag through the tube into the analyzer.) The machine will start displaying different numbers going up or down. Since there is no oxygen present in the gas the value displayed by the analyzer has to read 0.
vii. If the value of oxygen shown by the analyzer is different than zero, adjust the value to zero using the "Zero" button.
viii. Turn the T valve open towards the carbon dioxide analyzer.
ix. Turn 'ON' the pump of the carbon dioxide analyzer. The value shown on the digital display of the analyzer will start taking different numbers. Since there is no carbon dioxide present in the re-breathing bag the value on the display should read 0.
x. If the value on the display is different than zero adjust the value to 0 using the "Zero" button. Turn OFF the pump of the carbon dioxide analyzer and remove the re breathing bag from the valve connection.
xi. Vacuum the re breathing bag. (see step ii)
xii. Using the proper valve attach the re-breathing bag to the calibration gas tank and fill up the bag with the calibration gas (use calibration gas with pure oxygen). Open the valve of the re-breathing bag after attaching it to the calibration gas tank, and close it off before removing the bag from the valve, preventing any room air entering the re-breathing bag and keep the gas inside the bag the proper percentages.
xiii. Attach the re-breathing bag full with pure oxygen gas to the valve connecting to both oxygen and carbon dioxide analyzers.
xiv. See/ repeat step v.
xv. Open the valve on the re breathing bag and push gently on it (since the oxygen analyzer don't have its own pump, help is needed to push the gas from the bag through the tube into the analyzer.) The machine will start displaying different numbers going up or down. Since there is 98% oxygen present in the gas the value that has to be displayed by the analyzer has to be 98.
xvi. If the value of oxygen shown by the analyzer is different than 98, use a screwdriver to manipulate the "Span" button, to adjust the value to 98. Remove and vacuum the re-breathing bag.
xvii. Fill up the re-breathing bag with calibration gas containing 5 percent carbon dioxide.
xviii. Repeat step xiv. Turn on the T valve open towards the carbon dioxide analyzer. Turn 'ON' the analyzer's pump.
xix. The value shown on the digital display of the carbon dioxide analyzer will start taking different numbers. Since there is 5

percent carbon dioxide present in the re breathing bag the value on the display should read 5.

xx. If it is not 5, a screwdriver is used to turn the node labeled as "Span" to adjust.

xxi. Calibrate both analyzers before each use throughout the day.

Measurement Procedure:

i. Water temperature was recorded to correct for water density. (see Appendix 1 for water densities)

ii. Participant was wearing swimsuit to prevent from any extra air trapped when entering water.

iii. Participant was weighed on dry land prior to entering the water to prevent elevated dry weight due to wetness.

iv. The underwater weighing scale was zeroed.

v. Participant entered water tank by placing feet on the bottom of tank adjacent to the underwater scale.

vi. Before sitting on the scale the participant was asked to put on a sinker belt of known weight (7kg), which was later subtracted from the underwater weighing obtained.

vii. Participant centered over the underwater weighing scale and sat cross-legged while holding onto the handlebars on the scale.

viii. Participant was asked to put on a nose clip.

ix. Participant was breathing through a special snorkel devise that was connected with a T valve. The T valve has an opening to the room air, second opening toward the re-breathing bag filled with pure oxygen, and opening for the snorkel.

x. The participant was asked to fully submerge under water by bringing the chest toward the knees. At this time the participant was breathing normally room air through the snorkel.

xi. The participant remained still until the underwater weighing scale is settled. The participant breathed normally during this time.

xii. After the scale was settled, the participant was asked to exhale comfortable amount of air, after which the T valve was closed and the participant was unable to breathe for approximately 5-10 seconds. At this time 3 weighing were obtained.

xiii. After the numbers for the weight of the participant were obtained the participant was asked to start breathing again and the T valve was now open toward the re-breathing bag. At this time the participant can come up from the water, and keeping the nose clip on and mouth sealed around the snorkel, took 8 to 10 slow and deep breaths in and out the re-breathing bag full with oxygen. At the last breath the participants was asked to exhale all air into the bag, after which the valve of the re- breathing bag was closed and the bag was removed from the T valve.

xiv. In this study functional residual volume is measured, since the participant is not asked to exhale all air in their lungs but only comfortable amount of air. Since the air in the lungs is measured through the re-breathing procedure, and further analyzed for oxygen and carbon dioxide it was unnecessary to ask the participant to exhale all air in the lungs. This procedure was found to be more comfortable on the participant.

xv. Three underwater weighing were performed, each using a new re-breathing bag filled with 5 liters of 98 percent oxygen.

xvi. All three bags were further analyzed for oxygen and carbon dioxide content trough gas analyzers.

Analyzing the expired gases procedure:

i. All three bags were separately analyzed for their content. The obtained percentages of oxygen and carbon dioxide were recorded to their corresponding underwater weighing. The analysis of the re-breathing bag content was performed simultaneously after the underwater weighing. All data were entered into the underwater weighing calculation sheet to determine the participant's density and percent fat.

APPENDIX D

Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) Procedures:

i. Confirm the 9 volt battery in the device is charged and ready.
ii. Encourage the participant to use the bathroom before the test.
iii. Confirm the participant has followed the pre-test guidelines (see Pretesting Guidelines)
iv. Participant lies supine on the table with right foot and right hand bare (shoe and sock removed from the right foot).
v. Identify four electrodes sites: Right Hand- Black electrode between the knuckles of the Index and Middle finger, and the Red electrode between the styloid processes of the Radius and Ulna (wrist). Right foot- Black electrode between the base of the space between the big toe and the first toe, and Red electrode between the Medial and Lateral Malleolus (ankle).
vi. Clean the electrode areas with alcohol swab, place the electrodes and connect them accordingly.
vii. Hold the BIA with both hands and instruct the participant to hold still. Press both buttons (OPERATE AND CALIBRATE) simultaneously until the electronic display reads 000. Release the Calibrate button and keep pressing on the Operate button until the digital display stops on one number. Record the number of ohms.
viii. Remove the electrodes.
ix. Insert the data into software program to obtain percent body fat.

APPENDIX E

Bod Pod

Calibration and Measurement procedure:

Bod Pod Calibration (Quality Control Procedure):

i. System Warm-Up- let the Bod Pod warm up for 30 minutes. The system should not be operated during the warm-up period. This assures the Pod Pod electrical components have reached their optimal operating temperature.
ii. Analyze Hardware- using the Quality Control > Analyze Hardware activity, one test is performed following the screen steps. Passing is defined as "PASS" next to the results being displayed. Failing is defined as "FAIL" next to the results displayed.
iii. Scale Calibration- System Setup > Calibrate Scale. Perform one test following the screen steps. Two 10kg calibration weights are used to calibrate the scale.
iv. Autorun- using Quality Control > Autorun to assess environmental and Bod Pod stability. Perform this test following the screen steps. If the first Autorun procedure fails, perform a second test.
v. Volume- assess Bod Pod volume performance using Quality Control > Volume and Calibration Volume chamber. Perform one test, following the screen steps. Passing is defined as "PASS" being displayed in the Volume results screen. Failing is defined as "FAIL" being displayed in the Volume results screen. If the test fails, perform one more test.

Bod Pod Subject and Test Preparation Procedure:

i. Height- Participant height should be measured prior to the test. Height is one of the required entries in the test.
ii. Clothing- for accurate results is important that the participant wears minimal, firm fitting clothing. For men recommended clothing is either form-fitting Speedo ® or other Lycra ®/ spandex-type swim suit, or single-layer compression shorts, without padding. For women recommended clothing is either form-fitting Speedo ® or other Lycra ®/ spandex-type swim suit, or single-layer compression shorts, without padding and single-layer (not padded) jog bra. Participant should not wear socks or any other clothing except for what is specified. Also, the participant must wear a swim cap, and all of the participant's hair should be pushed in the swim cap and any air pockets under the cap should be pushed out.
iii. Miscellaneous- the participant should use the bathroom prior to the test. All jewelry and eyeglasses should also be removed. The participant should not exercise 2 hours prior to the tests.

iv. Data Entry- The participant's first and last name are not entered during the data entry of the test because of privacy concerns, two alphanumeric entries, ID_1 and ID_2, are available in the data entry portion of a body composition test. General data about the age, height, and ethnic background is also typed into the software.
v. Participant Behavior- the participant should remain quiet, still and relaxed during the test.

Bod Pod Test Procedure:

i. Quality Control- in the first screen, the operator was reminded of Quality Control activities that have to be performed before start of the test.
ii. Volume Calibration/Enter participant data/Determine Model and Thoracic Gas volume (TGV) Method to be Used- While the volume calibration was underway, participant information was entered. Thoracic gas volume (TGV) was selected by the operator as being measured as part of the test procedure; the operator was asked to insert the disposable tube and filter in the Bod Pod test chamber.
iii. Participant Mass Measurement: During the second half of the volume calibration, the participant's mass was measured. During this step, with the exception of the swim cap, the participant was only wearing approved clothing.
iv. Begin Volume Measurement- By the time the participant's mass was measured, the volume calibration was usually finished. The participant was then asked to put on the swim cap and enter the test chamber for the body volume measurement. The operator opened the test chamber door and removed the calibration volume used during volume calibration. The operator also ensured that the participant's hair was completely contained within the swim cap and any air pockets under the cap were pushed out.
v. Volume Measurements- During each of the 2 or 3 volume measurements, pressure changes resulting from the Bod Pod's diaphragm's oscillations were measured for 50 seconds while the participant sat comfortably in the test chamber. The pressure changes in the test chamber were roughly ±0.5 cm H_2O, which were comparable to the change in pressure while moving from the first floor to the second floor in an elevator. The test chamber door must be opened between the volume measurements. If the second measurement was inconsistent with the first one, a third measurement was conducted. If three tests were performed and two consistent tests were not obtained, it was necessary to repeat the test process from the beginning.
vi. Measure Thoracic Gas Volume (TGV)- at the conclusion of the volume measurements, thoracic gas volume was measured (this option is selected at the beginning of the test). It was very important to provide clear and accurate instructions to the

participant on how to conduct the thoracic gas volume measurement. All participants were read out the same instructions of how to perform the thoracic gas volume measurement, which are included in the Bod Pod user manual (page 85). If the participant was unable to perform the thoracic gas volume measurement properly the measurement was repeated. If the thoracic gas volume measurement was unattained after the fifth trial, a predicted measurement of TGV was performed.

vii. Receive/ Write out Test Results- at the conclusion of the test, results were printed out.

APPENDIX F

DEXA

Calibration and Operation of the DEXA.

DEXA Calibration and Quality Assurance:

Automated test program with complete mechanicals and electronics tests and global measurement calibration Automated QA Trending with complete storage was used for calibration. After the calibration was completed the results were printed out and stored.

DEXA Test Procedure:

i. Before the test started the physician has to input the patient's information such as name, date of birth, gender, height, weight and ethnicity. The participant also had to provide a DEXA prescription prior to the test.

ii. Following the patients was be instructed to eliminate all metal from his or her body including jewelry or any clothing containing metal.

iii. The patient was asked to lie down on the DEXA table, and the technician positioned the participant on the table, fitting all body parts within the lines drawn on the edges of the table. Legs are strapped together at two locations: above the ankle and knee.

iv. When the patient was prepared the test was started the top portion of the DEXA started sliding from the head of the patient towards the legs. This part of the equipment was not in any physical contact with the body of the patient.

v. The whole procedure took about 10-12 minutes, where the actual scanning took about 8.5 to 9 minutes.

vi. The patient had to be very still during the scanning time, no speaking or moving was allowed.

vii. DEXA divided the body into 16 regions which include head, left arm, right arm, left leg, right leg, left trunk, right trunk, total left and total right side, arms, legs, trunk, ribs, pelvis, spine and total. For each one of these regions the DEXA gave bone mineral density in grams per centimeter square. The DEXA also provided percent tissue fat for the legs, trunk and total body as well as total mass in kilograms, fat in grams, lean mass in grams, and BMC in grams. A colorful chart showed the patient current bone mineral density level. The report also included a skeletal picture of the subject as well as fat tissue outline image.

viii. The printouts were attached to the score sheet.

APPENDIX G

Ultrasound

The detailed description of using and measuring with the BodyMetrx:

i. Ultrasound Data Entry: Participant's first and last name initials are entered, date of birth, gender, athletic type, height (in), current body weight (lbs) are entered.
ii. Ultrasound Set Up- after the participant's profile is created; from the Measurement View 7 site Jackson and Pollack equation is chosen from the Current Formula Used drop down menu.
   - Jackson & Pollack Σ 7 sites (Lange) for male:
   - $Db(g/cc) = 1.112 - 0.00043499(\Sigma 7SF) + 0.00000055(\Sigma 7SF)^2 - 0.00028826(Age)$
   - Siri (men): $\% BF = [(4.95/Db) - 4.50] \times 100$
   - Jackson & Pollack Σ 7 sites (Lange) for female:
   - $Db(g/cc) = 1.097 - 0.00046971(\Sigma 7SF) + 0.00000056(\Sigma 7SF)^2 - 0.00012828(Age)$
   - Siri (women): $\% BF = [(4.96/Db) - 4.51] \times 100$
iii. Ultrasound Calibration- the ultrasound is calibrated by pressing and holding the button on the top of the device for 2 seconds, while not touching the ultrasound to any surface.
iv. Ultrasound Test Procedure:
v. The same 7 sites used in the skinfold procedure were used for measurement sites for the ultrasound.
vi. Before taking a measurement a small amount of the Ultrasound Gel was applied to the center of the device.
vii. The device was placed on the site to be measured and it was gently rubbed on the skin to eventually spread the gel. The button on the top of the ultrasound was then pressed and held for 2 seconds. A red light on the top of the device glow when taking the measurement. The device was only gently touching the skin. There was no angle between the skin and the ultrasound. Each site was measured two consecutive times before moving to the next site of measurement.

After completing the measurements of all seven sites the body fat percentage was displayed on the result screen. The percent body fat obtained was recorded on the results sheet.

APPENDIX H

Near Infrared Reactance

Testing Procedure:
Participant's body weight and height were measured before the test, as well as age, gender, date of birth, frame size, physical activity frequency, intensity, and duration.

Calibration Procedure:

i. The device did not require any warm-up time.
ii. Adjust Zero- put the Light Wand into Optical Stand and align the silver strip on both parts to match, as they are lying on flat surface. Press the Adjust Zero button. The procedure takes few seconds and when ready the display starts flashing "Ready".

Participant Data Entry:

i. Press: Select Program > Other [3] > New Subject.
ii. Enter ID number.
iii. Enter participant's gender using Male and Female buttons.
iv. Enter participant's date of birth.
v. Enter participant's height in inches.
vi. Enter body frame size using Small, Medium, or Large buttons. The body frame size is measured by taking the ankle girth at the smallest point above the ankle with measuring tape as tight as possible. The ankle girth for each gender defined in the user manual follow:

<u>Body Frame Size</u>

	Small Frame	Medium Frame	Large Frame
Men	< 8 inches	8 to 9.25 inches	> 9.25 inches
Women	< 7.5 inches	7.5 to 8.75 inches	> 8.75 inches

vii. Enter Frequency value- how often is physical activity performed:

<u>Physical Activity Frequency</u>

Press 5	Daily or almost daily (6 or 7 times per week)
Press 4	3 to 5 times per week
Press 3	1 or 2 times per week
Press 2	A few times per month
Press 1	Less than once per month

viii. Enter Intensity value- how intensive is the workout:

<u>Intensity Value</u>

Press 5	Aerobic activities that result in sustained heavy breathing and perspiration (e.g. high impact aerobics, running, speed swimming, distance cycling).
Press 4	Intermittent aerobic activities that result in sustain heavy breathing and perspiration (e.g. tennis, racquet-ball, squash).
Press 3	Moderate aerobic activities (e.g. normal bike riding, jogging, low impact aerobics).
Press 2	Moderate aerobic activity (e.g. recreational volleyball, moderate speed

	walking).
Press 1	Light aerobic activity (e.g. normal walking, golfing)

iii. Enter Time- how long did a single physical activity session continued:

Physical Activity Duration

Press 4	Over 30 minutes
Press 3	20 to 30 minutes
Press 2	10 to 20 minutes
Press 1	Under 10 minutes

iv. Enter participant's weight in pounds.

v. The display reads: "Ready to Read".

Testing Procedure:

i. The participant was asked to sit on a chair and rest his/ her dominant arm on a flat surface with his/her palm up. The arm was slightly bent at the elbow.

ii. Establish the correct placement of the Light Wand: Measuring tape was used to find the mid- distance between the underarm crease and the acromion crease of the elbow, to locate the belly of the bicep muscle. A small line on the side of the arm adjacent to the mid-point of the bicep was marked with pen.

iii. The Light Wand was placed into the Light Shield (allowing the Light Wand to protrude from the edge of the shield by about ¼ of an inch). The silver stripe on the Light Wand was lined up with the silver stripe on the Light Shield.

iv. The participant was instructed to place his/her dominant arm on a table with the palm in supine position, and to relax the biceps of the arm. The Light Wand was placed on the center of the belly of the biceps so that the silver strip of the Light Shield was pointing directly toward the shoulder.

v. The top of the Light Wand was pressed firmly, using enough force to leave a slight ring on the arm when the Light Wand was removed. The Light Shield was folded around the arm to block the external light.

vi. The Measurement Button on the top of the Light Wand was pressed. The display showed "Reading…" during the optical measurement. When the measurement was completed the display read "Remove & Replace". When this message appears on the display, the Light Wand was removed and replaced on the same point on the biceps for a second measurement. Press the Measurement Button again, and the display showed "Reading…".

vii. Percent body fat will be printed and displayed.

APPENDIX I

Equipment, Company Addresses, and Information about Equipment.

1. Weight Scale
   Healthometer- kilo-pound beam

2. Height Scale
   Novel Products, Inc.,
   Rockton, Illinois
   PAT. #DES 290237

3. Skinfolds
   Lange skinfold calipers
   Cambridge Scientific Industries
   527 Poplar Street
   Cambridge, MD 21613
   Lange skinfold caliper- this calipers meets the specifications of the Food and Nutrition Board of the National Research Council of the United States. The pressure in this type of calipers is 10 g/mm^2 (10 grams of pressure for each square millimeter of caliper jaw surface) and the jaw surface of this model calipers is 30 mm^2, which is total of 580g of pressure.

4. Bioelectrical impedance analysis
   ELG III Metabolic Analyzer healthPort
   HealthPort Corporation
   7909 SW Cirrus Drive
   Beaverton, OR 97008
   Operating current: 800 microamps
   Operating frequency: 50 kiloherts
   Operating voltage: 6.0-9.0 volts
   Low-battery Indicator: 6.2 volts
   Average battery life: 500 tests/readings
   Output range: 001 – 999 ohms
   Number of leads: Tetrapolar
   Calibration balance: 000

5. Bod Pod
   Bod Pod ® Body Composition Tracking System Software
   Life Measurement, Inc.
   1850 Bates Ave , Concord, CA, 94520, USA
   Temperature range: $70° - 90°F (21° - 32°C)$
   Temperature Range Between Calibration and Volume
   Measurements: $\pm 0.9°F (\pm 0.5°C)$
   Relative Humidity: 20-70% (Non-Condensing)

Relative Humidity Variation Between Calibration and Volume
Measurements: ±5%
Dimensions:
   Height: 61in (155cm)
   Weight: 310lbs (141kg)
   Depth: 52in (132cm)
   Width: 35in (81cm)
Scale:
Tanita Corporation, Japan (Model BWB-627-A), modified by Life Measurements, Inc.
Capacity: 440lbs (200kg)

6. Underwater Weighing
   a. Infrared Gas Analyzer; Anard AR 400 Series
      Anard Inc. Santa Barbara, CA.
      Model AR- 411
      Serial # 2386
      Power 117; Volts 60; Hz 1.5 amps
   b. Servomex ® Oxygen Analyzer 570A
      Sybron Servomex Company ins.
   c. Mattler-Toledo, Inc.,
      Toledo Scale
      Industrial Products
      350 W. Wilson Bridge Road
      Worthington, Ohio 43085
   d. 8806 Printer
      Temperature of operation: $41°F - 113°F(5° - 45°C)$
      Relative Humidity of operation: 10%-95%
      8142 Digital Indicator
      1. Serial Number 4336508-4ZU
      2. Serial Number 4336511-4ZU

      Temperature of operation: $14°F - 104°F(-10° - +40°C)$
      Relative Humidity of operation: 0%-95%
   e. Analog Load Cells:
      Artech Industries, Inc.,
      Part No.20210-100, Class III
      Capacity: 100lbs (45.5kg)

7. Ultrasound
   IntelaMetrix Inc.,
   6246 Preston Ave., Livemore, CA 94551
   Ultrasound BodyMetrix™ BX2000 IntelaMetrix version 70605
   Serial number:07020038, Certified & tested July 12, 2007- Approved
   Operating Temperature: $32° - 140°F(0° - 60°C)$
   Operating Humidity: 5%-95%
   Windows XP operating system

Minimum of 256MB of RAM, 512 MB recommended
1024 x 768 display
CD ROM Drive
1 USB port minimum, 2 recommended for BodyView Live

8. Near infrared
   Furtex Inc.,
   130 Western Maryland Parkway
   Hagestown, MD 21740
   Futrex- 6100 A/ZL Infrared Advanced Body composition Analyzer Version 1.0
   Measuring Principle: Near Infrared Interactance Technique based on technology from the United States department of Agriculture
   Measuring Range: 3% - 45%
   Age Limits: >6 years old

9. Dual energy X-ray absorptiometry
   Lunar Prodigy
   enCORE 2006
   version 10.50.086
   General Electric Company
   726 Heartland Trail
   Madison, WI 53717-1915 USA
   Windows XP Professional
   Intel processor computer and printer
   Power: 230/240 VAC ±10%, 10A, 50/60Hz
   Ambient Temperature: $64° - 80°F (18° - 27°C)$
   Relative Humidity: 20%- 80%, non-considery
   Dimensions (L x H x W) and weight: 263 x 111 x 128cm, 272kg (full)
   Vinyl table pad
   Magnification: None- Object-plane measured
   X-ray characteristics: Contrast potential source at 76kV. Dose efficient K-edge filter.
   Scanning method: Narrow FanBeam (4,5° angle) with SmartFan, MVIR and TruView algorithms.

10. Magnetic resonance imaging
    Philips Intera 3.0T MRI

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