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THE DETERMINATION OF BODY COMPOSITION IN FEMALE ATHLETES

by

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ABSTRACT

BODY COMPOSITION IN FEMALE ATHLETES

By

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The purpose of this investigation was to determine the reliability and validity of bioelectrical impedance (BIA) and near infrared interactance (NIR) for estimating body composition in female athletes. Dual energy x-ray absorptiometry (DXA) was used as the criterion measure for fat free mass (FFM). Studies were performed on 132 college athletes (age =20.4±1.5 yr). Reliability estimates (repeat and single trial) were 0.987-0.997 for BIA (resistance and reactance) and 0.957-0.980 for NIR (optical densities). Validity of BIA and NIR was assessed by a double cross validation technique. Because correlations were high (r=0.969-0.983), and prediction errors low, a single equation was developed using all 132 subjects for both BIA and NIR. Also, an equation was developed on all subjects using height and weight only. Results from DXA analysis showed FFM =49.5±6.0 kg which corresponded to % body fat (% BF) of 20.4±3.1%. BIA predicted FFM at 49.4±5.9 kg (r=0.981, SEE=1.1, TE=1.1) and NIR prediction was 49.5±5.8 kg (r=0.975, SEE=1.2, TE=1.2). Height and weight alone predicted FFM at 49.4±5.7 kg (r=0.961, SEE=1.6, TE=1.6). When converted to % BF values, prediction errors were ~1.8% for BIA and NIR and 2.9% for height and weight. Results showed BIA and NIR to be extremely reliable and valid techniques for estimating body composition in college-aged, female athletes.

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LIST OF ABBREVIATIONS

A - cross-sectional area
BIA - bioelectric impedance analysis
BMC - bone mineral content
BMD - bone mineral density
BMI - body mass index
Ca - calcium
Db - body density
DPA - dual photon absorptiometry
DXA - dual energy x-ray absorptiometry
FFM - fat-free mass
FM - fat mass
Ht - height
L - length
MRI - magnetic resonance imaging
NIR - near infrared interactance
OD_1 - optical density 1
OD_2 - optical density 2
R - resistance
RA - ratio of attenuation
Rst - ratio of soft tissue
SD - standard deviation
SEE - standard error of the estimate
SEM - standard error of measurement
SPA - single photon absorptiometry
TBBM - total body bone mineral
TBCa - total body calcium
TBK - total body potassium
TBW - total body water
TE - total error
UWW - underwater weighing
V - volume
X_c - reactance
Z - impedance
% BF - percent body fat
% CV - percent coefficient of variation
125 I - Iodine 125
153 Gd - Gadolinium 153

CHAPTER 1

INTRODUCTION

Body composition analysis involves dividing the body into various components and estimating their volumes and percentage of total mass contained in each. Components of the body vary by classification scheme; examples are fat mass (FM), fat-free mass (FFM), bone or soft tissue. Information provided from body composition analysis is important for the female athlete, as it can be indicative of health and performance status.

Excess body fat may negatively influence sport performance by adversely affecting metabolic, mechanical, and thermoregulatory efficiency (Houtkooper & Going, 1994). Generally, high ratios of FFM to FM are favorable for an athlete, but too little body fat may result in the deterioration of both health and performance (Houtkooper & Going, 1994). Optimal body composition may vary among individuals in different sports (Heyward & Stolarczyk, 1996). Body composition analysis typically includes an estimate of an individual's % body fat (% BF), which is calculated by dividing FM by total body mass (i.e., weight). Some examples of reported values for college-aged women include: 10-15% for female runners (Robinson et al., 1995), 15-17% for female gymnasts (Kirchner, Lewis, & O'Connor, 1995), and 18-30% for female non-athletes (Brozek, Kihlberg, Taylor, & Keys, 1963). An ongoing analysis of body composition can provide useful information regarding the effectiveness of sport specific training and related conditioning programs.

Knowledge of body composition is also important in helping medical personnel in their constant surveillance of the athlete's physical and mental health. Radical changes in body composition can be indicative of serious health concerns. For example, the female athlete triad, consisting of disordered eating, amenorrhea, and osteoporosis, is prevalent in many athletes (Nattiv, Agostini, Drinkwater, & Yeager, 1991; Putukian, 1994; Smith, 1996). The triad has serious health implications, and may result in bone loss or even death (Nattiv et al, 1991; Smith, 1996). Elite or competitive athletes participating in sports in which low body weights or lean physiques are considered advantageous are at a greater risk for developing the triad (Nattiv et al 1991; Putukian, 1994).

Menstrual irregularities and decreased bone mineral density are closely related in female athletes (Fruth & Worrell, 1995; Lloyd et al., 1987). The reduction of bone density, possibly due to menstrual irregularities, may put athletes at risk of stress fractures and other injuries (Sinning & Little, 1987). Therefore, accurate assessment of body composition may be a way to detect the early signs of such health problems. For example, athletes could be tracked individually and monitored for drastic changes in, or extremely low % BF values.

Hydrodensitometry is the most widely used method for estimating % BF (Wang, Heymsfield, Aulet, Thornton, & Pierson, 1989). This method has been shown to have a measurement error of ~2-4% under the best conditions (Sanborn & Jankowski, 1994). In hydrodensitometry, body volume is typically

estimated by weighing an individual underwater (UWW). Body volume may also be obtained by estimating the weight of the water displaced, but this technique is rarely used. Body density (Db) is then estimated by dividing body mass (out of water) by body volume. Density is subsequently converted into % BF, from which FM and FFM can be calculated.

The original equation from which Db is converted into % BF was based on chemical analysis performed on five Caucasian human cadavers (Siri, 1956). These cadavers included: a 42 year old female who committed suicide, a 46 year-old male who died a week after a cerebral injury, a 35 year-old male who died of an acute heart attack (decompensation or failure, apparently after prolonged illness with mitral disease), a 25 year-old male who died of uremia (blood urea 477 mg/100 ml at death), and a 48 year-old male who died of infective endocarditis with his body wasted and grossly edematous (Keys & Brozek, 1953). Only the first two cadavers listed can be considered reasonably "normal" since the latter were in various disease states (Keys & Brozek, 1953; Brozek, Grande, Anderson, & Kemp, 1963). Based on cadaver analysis, assumptions for the density of FM was 0.90 g/cc, while FFM was 1.10 g/cc. Inherent in these assumptions is that FFM is at a constant hydration level of 73% (Pace & Rathburn, 1945).

As technology has improved, the original assumptions for UWW have been challenged (Heyward & Stolarczyk, 1996; Lohman, 1992). Variations may exist in the FFM, particularly bone mineral density. This variability may be fairly prevalent in female athletes, depending on their training and

menstrual histories (Sinning & Little, 1987). Therefore, UWW may not assess % BF accurately in female athletes. Further, UWW may not be an appropriate criterion measure when developing body composition equations for female athletes using various "field" techniques (Lohman, 1984). New, multi-component models are needed to account for interindividual variability in FFM in order to obtain more accurate estimations of body fatness (Heyward & Stolarczyk, 1996; Lohman, 1984; Withers et al., 1987).

A more valid and precise method (compared to UWW) for measuring body composition may be dual energy x-ray absorptiometry (DXA). DXA divides the body into three components: bone, fat free and bone free tissue, and fat. Originally, this method was designed to analyze bone mineral content (BMC) and bone mineral density (BMD). Precision for BMC was high, as errors were shown to be less than 1% (Mazess, Collick, Trempe, Barden, & Hanson, 1989). More recent studies have shown that DXA is highly reproducible for both BMC and body composition (Mazess, Barden, Bisek, & Hanson, 1990). Accuracy of DXA has included SEEs of 2.9%, 1.9 kg, and 2.7 kg for % BF, FM, and FFM, respectively (Svendsen, Haarbo, Hassager, & Christiansen, 1993). Studies have also shown that underwater weighing and DXA agree well at high, moderate, and low levels of body fat (Hansen et al., 1993).

The fact that DXA incorporates BMC when estimating % BF may be a major advantage in comparison to UWW. Bone comprises less than 5% of FFM, but it has the highest density. Therefore, changes in BMC can have a

great effect on the average density of FFM (Wang et al., 1989). In young adult females, variability in BMC has shown to contribute significantly to variability in body density (Bunt et al., 1990).

Although DXA is extremely valid and precise for % BF measures, this instrument is expensive, and is found primarily in clinical settings. Other electronic methods, such as bioelectric impedance analysis (BIA) and near-infrared interactance (NIR), have been developed to be used in the field. Both machines are lightweight, portable, and require little technical training for proper use. These two techniques are less costly than DXA and may provide reasonably accurate results.

BIA uses an electrical current to estimate total body water by measuring the resistance and reactance of the tissues. NIR measures the optical density of remitted light from the biceps by indirectly measuring the tissue composition of fat and water. These measures, along with height, weight, age, etc. have been used to develop equations to predict FFM, and % BF.

Despite the reasonable principles underlying BIA and NIR, neither technique has been thoroughly tested for reliability and validity in female athletes. Two equations for BIA are available for female athletes (Houtkooper, Going, Westfall, & Lohman, 1989; Lukaski & Bolonchuk, 1987). However, neither equation was based on a homogeneous athletic population and Houtkooper et al. (1989) was published only in abstract form. No equations specifically designed for female athletes are available for NIR.

Moreover, no studies using female athletes have evaluated the validity of either BIA or NIR using DXA as a criterion measure.

The purpose of this investigation is to determine the reliability and validity of BIA and NIR for estimating body composition of female athletes. The DXA technique will be used as the criterion measure. Stepwise multiple regression will be employed to develop separate equations to predict FFM using BIA and NIR. There will be no formal hypothesis presented for this thesis as it is a measurement study and not hypothesis driven research. The central questions include a) what is the degree of reliability shown by the BIA and NIR instruments and, b) what is the degree of accuracy demonstrated by the BIA and NIR when estimating FFM in female athletes?

CHAPTER 2

LITERATURE REVIEW

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Underwater Weighing

Basic Principles of UWW

The traditional "gold standard" or criterion measure for body composition analysis has been hydrodensitometry, or more specifically, UWW. This method indirectly estimates % BF from Db, which is the ratio of body mass to body volume. UWW is based on Archimedes' principle that body weight in water is equal to the weight of the water displaced during submersion, and thus, allows for the calculation of body volume (Siri, 1956). The formula for calculating Db via UWW is shown below.

Body density (Db) = body mass / body volume, where body volume, or

$$Db = \frac{Wa}{\frac{Wa - Ww}{Dw} - RLV \text{ (and intestinal gas estimate)}}$$

where Wa=weight in air, Ww=weight in water, Dw=density of water, and RLV=residual lung volume.

Body volume is equivalent to the loss of weight in water, with the appropriate temperature correction for the density of water (McArdle, Katch, & Katch, 1996). Measured density is a mean value of Db rather than the in vivo density of tissue mass; therefore, air left in the respiratory passages and lungs and gas in the gastrointestinal tract must be taken into account. Once density is determined, the Siri equation (1961) is used to convert density into percent body fat, from which FM and FFM can be calculated.

The Siri equation: % BF = (4.95 / Db) - 4.50.

FM = (Percent fat / 100) * Body mass

FFM = Body mass - Fat mass

Brozek, Grande, et al. (1963) also formulated their own equation to calculate % BF from Db.

% BF = (4.570 / Db) - 4.142

These two equations were based only on a few cadavers as previously mentioned (Keys & Brozek, 1953). The day-to-day measurement errors for the UWW technique are small. However, inherent assumptions regarding the density of FM and FFM for a given individual are unknown and probably substantial, except in young white men (Roche, 1987).

Histological Concerns with UWW

Differences in the FFM Constant

Recent advances in technology have led to the rejection of the assumption that the density of FFM is constant in all individuals. FFM consists of all tissues except fat, and includes bone, muscle, skin, and other organs. Of particular concern is the variability of bone, which is included the assumed FFM constant. Although bone comprises about 5% of lean body mass, it has by far the highest density (Wang et al. 1989).

The FFM assumption of UWW, which had been formulated from the analysis of five cadavers, has been further researched with data from the Brussels Cadaver Study (Clarys, Martin, & Drinkwater, 1984). A sample of 25 cadavers, ages 55-94 years, consisted of 12 embalmed (6 male, 6 female) and 13 unembalmed (6 male, 7 female) Belgian subjects. Skeletal density varied from 1.15-1.33 g/cc, deviating from the assumed 1.10 g/cc. Martin, Drinkwater, Clarys, and Ross (1986) provided an example to demonstrate the error in the assumption of the FFM constant. "For a typical male of whole body density 1.062 g/ml, predicted fat (by the Siri formula) would be 16.1%; however if the FFM deviates by one standard deviation from the assumed value of 1.100 g/ml, fat prediction will range from 8.5% to 22.3%" (Martin et al., 92, 1986).

Differences in BMC are also affected by other factors including age, ethnicity, and athleticism. For example, Madsen, Adams, and VanLoan (1998)

studied the effects of physical activity, body weight/composition, and muscular strength on bone density. Sixty females, ages 18-26 yr, were divided into three groups: 1) low body weight athletes involved in weight-bearing, collegiate sports; 2) matched low body weight and sedentary; and 3) average body weight and sedentary. Results indicated that the athletes had significantly greater bone density at several sites. Weight-bearing exercise and FFM were shown to enhance BMC in eumenorrheic young adult women.

Variations in BMC are not accounted for in UWW. Indeed, studies have shown that many individuals have variations in BMC that in turn will produce incorrect results for % BF as measured by UWW (Bergsma-Kadijk, Baumeister, & Deurenberg, 1996; Bunt et al. 1990; Friedl, DeLuca, Marchitelli, & Vogel, 1992; Hansen et al. 1993; Mazess, Peppler, & Gibbons, 1984; Wang et al. 1989). Based on estimates of overall variability in BMC and hydration of the fat-free body there is theoretical error of 3-4% for predicting body fatness in a population using UWW (Lukaski, 1987).

Variations of the Siri Equation

To compensate for the shortcomings of UWW mentioned above, several modifications of the Siri equation have been devised to help improve the accuracy of estimating % BF from Db measures. For instance, Schutte et al. (1984) investigated the effect of bone mineral variation on the density of lean body mass in Blacks versus Whites. They measured the density, total body water, and anthropometric dimensions in 19 white and 15 black male college students all matched for height, weight, and total body water. Among

the Blacks, the observed density was significantly greater than that predicted from anthropometry, and the lean body mass calculated from observed density was significantly greater than that calculated from total body water. For the Whites, no significant differences were noted. The authors felt that a separate formula should be used for converting density to body composition. Based on their data, the adjusted formula for converting Db to % BF in Blacks is: % BF = 100 * [(4.374/Db - 3.928].

This formula assumes a FFM of 1.113 g/cc in Blacks compared with 1.100 in Whites.

Lohman, Boileau, and Slaughter (1984) investigated the applicability of body composition constants for children. They showed that techniques used to assess body composition in adults are inadequate in youngsters due to the differences in chemical maturity between children and adults. Differences in chemical maturity included variations in bone mineral, muscle, and level of hydration which were all measured or estimated in the study. The result was that Lohman et al.'s (1984) revised Siri equation for prepubescent children became:

% BF = 100 * (5.30/Db - 4.89)

Lohman performed a similar study to investigate the FFM assumption for women (1992). The Lohman revised Siri equation for women became:

% BF = 100 * (5.05 / Db - 4.65)

In addition to a pure gender effect, females may also have variations in BMC depending on their activity levels and menstrual status (Bunt et al.,

1990; Madsen et al., 1997; Sinning & Little, 1987). There are currently no adaptations of the Siri equation for female athletes. Therefore, equations utilizing UWW as a criterion have been applied to female athletes without proof of their validity (Lohman, 1984).

Other Problems with UWW

In addition BMC variation, other factors can affect the accuracy of UWW for estimating % BF. Hydration status, inability to measure residual volume accurately, errors in intestinal gas content estimates, subjects' cooperation and understanding of test procedures, and variations in equipment and methodologies all contribute to errors in prediction of % BF by UWW (Roche, 1987).

Bergsma-Kaddijk et al. (1996) investigated the hydration of young women (aged 19-27 yr) who were weight-matched and compared with older women (aged 65-78 yr). The authors found that total body water (TBW; as measured via deuterium oxide dilution) from the younger group was significantly ($P<.001$) greater (32.5 ± 2.9 kg) than in the older subjects (29.0 ± 2.5 kg).

The effect of experimental technique on measuring body density was studied by Durnin and Satwanti (1982). Percent body fat for 6 women and 9 men, ages 17-51 yr, was estimated from UWW. Subjects were measured under a variety of conditions including maximal expiration, moderate expiration, minimal expiration, moderate inspiration, and after ingesting a light meal (~2100 kJ or ~500 kcal 1 1/2 hours before), heavy meal (~5,000-9000

kJ or ~1200-2200 kcal 1 1/2 hours before), and carbonated drink (800 ml). Results indicated that the various combinations of food/air in the lungs resulted in ~1% difference in the estimated % BF content of the subjects. Differences in % BF estimates attributed to carbonated beverages were approximately 1.5%. The authors assumed that ingestion of carbonated beverages resulted in large increases in intestinal gases, but this was not measured. The authors concluded these errors were well within the basic errors of the method. While the measurement errors introduced in this study were small, the authors noted that it is possible that extremes of all three variables acting simultaneously could influence the % BF calculations by 2-3%. The small sample size did not allow comparisons between male and female subjects.

Friedl et al. (1992) studied the reliability and accuracy of body fat estimates from a 4-component model devised from Db, TBW, and total body bone mineral (TBBM) measurements. The data indicated that this four-component model improved the accuracy of the % BF estimates. The largest error in the multicomponent model appeared to come from the UWW method used to determine Db. The most important problem was the assumed FFM hydration constant of 73.2%. Normal variation in the 10 subjects was 71-74%. This resulted in a decrease of 2.2 % BF and an increase of 3.4 % BF at the extremes of hydration.

Single Photon Absorptiometry

General Characteristics

Single photon absorptiometry (SPA), which was introduced in the early 1960s, was developed to measure the bone mass of the appendicular skeleton. The basic concept of this technique involves photon attenuation in vivo by components of tissue. A radioactive source, such as iodine 125 (125 I), emits a photon of characteristic energy. With SPA, this photon is passed transversely across a bone and loses energy proportionally to the mineral mass of the scan path (Mazess, 1971). After the photon passes across the bone, the photon energy is measured with a scintillation detector-pulse height analyzer system (Mazess, 1971).

Precision and Accuracy of SPA

There are several sources of potential error that must be controlled so that precision and accuracy are maintained. The largest source of error involves the location of the correct scanning site. The magnitude of this error is inversely proportional to the care taken in positioning the subject. Hence, error may be lessened if the subject is carefully repositioned.

Mazess (1971) used SPA to examine the intercorrelations among bone mass determinations at various sites on the human ulna, radius, humerus, and femur. Four scan determinations were made at three or four locations on various bones from the skeletal remains of 70 adult Sadlermiut Eskimos (30 male, 40 female). The relationships of local mineral content, determined by SPA, were compared with weights of individual bones.

Scans on the different bones were highly intercorrelated (r=.80 to .90), and correlation coefficients were even higher (r=.90 to .95) for different locations on the same bone. Absorptometric scans were also highly correlated with the weights (dry weight) of the bones on which they were made (r=.94). The weights of individual bones were estimated with only a 5 to 10% error; scans at several sites improved the accuracy.

In a follow-up investigation using similar methodology to the Mazess (1971) study, Mazess, Judy, Wilson, and Cameron (1973) noted that variation among seven different laboratories values were within 2% for both precision and accuracy. These results indicated that errors associated with absorptiometry were small, even among different laboratory sites.

Problems with SPA

Problems may arise with SPA due to variations in the iodine source (125 I). Dunn, Kan, and Wahner (1987) evaluated two commercial SPA instruments. Several (n=42) measurements were taken over a 138 day period using a metal bar for a standard. One SPA unit did not show any systematic dependence of measured BMC or BMD on source activity when evaluated over an entire source life. However, the other SPA showed measurements which increased quadratically and gradually with time ($r = .84$; $P < .0001$) and leveled off at about day 98. The maximum increase in BMC was ~ 2%. A drastic change in results upon replacement of the source was apparent. Specifically, the difference between the groups of five data points taken

immediately before and after source change was statistically significant ($P<.0001$) in one of the SPA machines.

Another limitation of SPA is that it cannot be used for TBBM analysis. Since the photon beam is monochromatic, only the mass of bone mineral can be determined. Thus, SPA requires that the bone be enclosed in a constant thickness of soft tissue (Peppler & Mazess, 1981). This constant thickness may help eliminate errors due to varying mineral-nonmineral composition of the bones (Mazess, 1971). However, this is not the case over the entire body, and measurement errors may result.

Dual Photon Absorptiometry

General Characteristics

Dual photon absorptiometry (DPA), first developed in the early 1980s, utilizes a different radioactive source from SPA: isotope 153 gadolinium (153 Gd). This isotope, upon decay, has a characteristic half-life of 242 days and emits two characteristic photon energies of 44 and 100 keV. Since DPA consists of two photon energies, the need for constant tissue thickness across a scan path have been eliminated and inaccessible body parts can be measured (Peppler & Mazess, 1981; Watt, 1975). Hence, whole body analysis of bone density became possible.

Having two distinct photon energies with DPA also allows the body to be divided into two components: bone and soft tissue. The bone has a constant value, but soft tissue varies as it consists of both fat and fat-free tissue. These two tissues in turn produce different attenuation coefficients

for soft tissue at both energies (Peppler & Mazess, 1981). Therefore, measurement of both TBBM and total body soft tissue analysis became possible with DPA.

Precision and Accuracy of DPA

Although both bone and soft tissue analysis are possible, DPA is inherently less precise than SPA. Errors arising from photon counting, taking optimal photon energies into account, indicate that SPA is significantly better than DPA for the measurement of TBBM (Watt, 1975).

Witt and Mazess (1978) studied the precision and accuracy of DPA using three phantoms to simulate limbs. The phantoms consisted of polymethylmethacrylate for soft tissue, and either an aluminum tube or annular cavity filled with a saturated solution of dipostassium hydrogen phosphate to simulate bone. Other phantoms were made of 11 polyethyleve bottles, ~ 7.8 cm in diameter, filled with ethanol-water solutions of different concentrations.

Precision was determined from repeat measurements of normal subjects, patients, and phantoms. The upper arm and forearm of five adults were scanned twice each week for 5 weeks; the precision of measured soft tissue content was 2-3%. The precision of the fat fraction was about ±.02 (0.5 to 0.7%), with SD of .02 to .04. Repositioning and subject motion were the greatest sources of variation and limited the precision to 2-3% in normals and 3-7% in patients; instrumental variation was 0.5% for soft tissue content. Also, DPA was found to accurately measure bone density within 1-2% for

phantoms and 2-4% in vivo (Witt & Mazess, 1978). The accuracy was 4% for typical (±10%) fluid changes observed in vivo.

Precision and accuracy of DPA were studied by Peppler and Mazess (1981). Short-term (months) precision of TBBM was about 1.5% for isolated skeletons and about 2% on normal human subjects. Long-term (years) precision on skeletons was under 3%. The precision of % BF was 0.9%, which would lead to an error of less than 1% in the TBBM. Geometry of measurements also had minimal (and correctable) influence on the accuracy of results. The accuracy (1 SEE) of TBBM on isolated skeletons (N = 5) was 36 g with a correlation coefficient of r=0.99. The accuracy of TBBM on the skeletons was interpolated to be about 1-1.5% for normal adults, 2% in older women, and 2.5% in osteoporotic females (Peppler & Mazess, 1981).

Gotfredsen, Jensen, Borg and Christiansen (1986) studied the in vivo precision of the %BF and the FFM (kg) of five healthy subjects. Precision estimates over six months were 2.5% and 2.2%, respectively. Other estimates of the FFM were performed using both the calculations by Boddy, King, and Hume (1972) and by skinfold thickness measurements (triceps and subscapular). Results were then compared to DPA measurements in 50 men and 50 women. The correlation between FFM by DPA and FFM by Boddy et al. (1972) was significant (r=0.96, SEE = 4.4%). The authors concluded that FFM measurements using DPA have precision and accuracy errors that are commensurate with a reliable estimate of the gross body composition.

Heymsfield, Wang, Heshka, Kehayias, and Pierson (1989) compared DPA measurements of bone mineral and soft tissue mass in vivo with the established methods of Db, TBW, total body potassium (TBK), total body calcium (TBCa), and nitrogen. There were 13 subjects, aged 24-94 yr. In addition to the routine scanner calibration, the authors' approach to evaluating soft tissue composition was to first scan seven different ethanol and water mixtures. Second, a beef calibration was added for conversion of the subject's ratio of soft tissue (Rst) value to % fat. Results for both the water-ethanol and beef phantom calibrations were evaluated by five consecutive runs. The Rst vs. percent ethanol and Rst vs. percent fat were highly linear with minimal standard errors. The % coefficient of variation (% CV) was calculated for each of five consecutive runs by determining the respective Rst for 50% ethanol and fat mixtures. In both cases the %CV was < 1%.

Other results indicated that DPA correlated significantly with all four methods. TBBM was highly correlated with TBCa (r=.95, P<.001). Further, the slope of TBBM vs. TBCa (0.34) regression line was similar to the that of the TBBM vs. calcium content of ashed skeleton regression line (0.34-0.38). DPA-measured fat (M±SD, 16.7±4.9 kg) correlated significantly (r= .79-.94; P<.01) with fat established by Db (16.3±5.4 kg), TBW (16.0±4.3 kg), TBK (17.7±4.6 kg, combined TBW-neutron activation (17.6±5.9 kg), and means of all four methods (16.9±4.8 kg). Thus, the authors concluded that DPA offered a new opportunity to study the human skeleton in vivo and to quantify fat by

a method independent from the classical assumption that bone represents a fixed fraction of FFM.

Problems associated with DPA

There are several potential problems/limitations associated with DPA. Some include: relatively high radiation dose (1/10 of a chest x-ray, Wang et al., 1989), long scan time (~ 1 hr, Wang et al., 1989; 20-40 min., Gluer et al., 1990), and difficulties in positioning which result in lowered precision. Mazess et al. (1990) have indicated that although the accuracy of total-body soft-tissue composition analysis using DPA appears quite good, the precision error has been relatively high. This paradox would argue against a solid endorsement for its true validity.

Source Life Variation

Another problem, which may significantly affect the precision of the DPA measurement, is the variability of source life as 153 Gd decays. Dunn et al. (1987) evaluated longitudinal measurements of bone mineral using four DPA machines. The authors looked at precision over the lifetime of a source and beyond. Repeated measures over more than one year were made on ashed bones under 20 cm of water. All instruments were assessed for the effectiveness of the manufacturer's recommended quality control program for the detection of lack of stability in the instruments. Two DPA instruments did not show any systematic dependence of measured BMC or BMD on source activity when evaluated over an entire source life.

However, one DPA instrument showed strong dependence on source strength. The 247 daily measurements of the three-bone region phantoms showed strong linear and quadratic ($P<.0001$) patterns between calibration results and time of use (source strength) for all three bone regions. Bone mass and area density from the ashed bone also showed a strong linear increase with decreasing source strength ($P<.0001$). The measured BMC increased with a decrease in source activity. The fourth DPA instrument had a significant linear decrease in BMD over a source life in the automatic mode but performed better in the manual mode. Thus, the results indicate that there may be some apparent trends with changing DPA source strength during the useful life a radiation source.

Lindsay, Fey, and Haboubi (1987) also studied the effects of source life on DPA measurements for BMD. They evaluated four separate specimens of human vertebral bone consisting of vertebral bodies L2-4 still connected. Samples were moved and repositioned between individual measurements across two source changes. On each occasion, five measurements were made of each specimen. During an 18 month period, BMD increased linearly an average of 6% for the first source. The second source had an increase in BMC of 4.7%, observed in the same time frame. At the source changes, BMD of all specimens fell by 1.8-6.2% and 1.6-4.9% for the first and second sources respectively. The vertebral measurements indicated apparent increases in bone mass by as much as 0.6% per month as a function of source life. The results indicated a decrease in the precision and, potentially, the accuracy.

Gotfredsen et al., (1986) examined the measurement of FFM and FM using DPA. The reliability of estimating the lean mass was assessed in vitro, using limb phantoms consisting of ox muscle, lard, and human bone; and in vivo with duplicate measurements on five healthy subjects. The Rst value fell with time, or with the falling activity of the source. Therefore, the authors set up a weekly calibration system using ethanol and water mixtures.

The precision and accuracy in vitro by using limb phantoms of the lean percent determination were analyzed to be 1.5% and 1.9%, respectively. The accuracy in vivo of measuring the total mass of soft tissues was approximately 1.4%, thus yielding an overall accuracy error of the LBM of about 2.5%. The precision in vivo of the lean percent and the LBM in kg of duplicate measurements on five healthy subjects was 2.5% and 2.2%, respectively.

Studies Comparing DPA with UWW

Studies Revealing Differences in BMC

Bunt et al. (1990) measured the variations in BMC in 89 young adult women, aged 25.1 ± 5.3 yr. The women varied in activity levels and menstrual status as compared to a sedentary control group. The following BMC sites were measured: lumbar vertebrae (L2-4), radial shaft (RS), femoral neck, and distal radius. These sites were measured and then evaluated for their effect on body density. Theoretical differences in body density were calculated to the ±1 and ±2 standard deviations of BMC for the population as well as for several subgroups. These subgroups included: eumenorrheic

inactive controls, recreational runners, collegiate runners, body builders, swimmers, and amenorrheic runners.

The differences in % BF for UWW (due to L2-4 and RS values for BMC) ranged from an average overestimation of 1.3% for the amenorrheic runners to an average underestimation of 1.4% for the body builders. Individual differences were more profound, with a 3.3% fat underestimation in body builders and a 3% fat overestimation in amenorrheic runners. Therefore, variability in BMC attributed to variability in Db, independent of fatness. While the impact of high or low BMC on % BF is modest for most individuals, athletes with high or low BMC values may require adjustments in equations used to convert Db to % BF.

Hansen et al. (1993) studied the body composition of 100 premenopausal, non-athletic females aged 28-39 yr. Bone mineral was found to be a significant predictor in % BF models. Standard errors of estimate decreased significantly (P <.001) from 0.0053 to 0.004 when bone mineral was included to predict body density. The females had a lower bone density compared to the assumptions inherent in the Siri equation (Siri, 1961). The authors suggested that BMC should be taken into account when converting Db to % BF.

Mazess et al. (1984) measured the FFM of 18 subjects, 14 female and 4 male, aged 23-60 yr, with both DPA and UWW. The correlation coefficient for % BF measured from DPA compared to UWW was r=0.87. The calculated % TBBM of FFM varied among subjects from 4.2 to 7.6%. Thus, TBBM was not

a constant fraction of FFM as is often assumed, nor was the percentage as low as the assumed constant of 5%. The authors concluded that skeletal variability, even in normal subjects, where mineral ranges only from 4 to 8% of the FFM, may preclude use of body density as a composition indicator unless skeletal mass is measured.

Wang et al. (1989) also emphasized the inconsistency of bone mineral on the UWW method for estimating percent body fat. Subjects included 99 males and 187 females, aged 19-94 yr. UWW assumes a constant FFM fraction of 1.10 g/cc, but results indicated an average (±sd) value of 1.09±0.01 with a range of 1.05 g/cc to 1.13 g/cc. Although bone mineral comprises a relatively small component accounting for approximately 5% of the lean body, has by far the highest density. Therefore, changes in bone mass or bone density are of proportionately great influence on the average density of FFM.

Wang et al. (1989) also evaluated measurement precision in five normal subjects, aged 31-57 yr, by performing five measurements of UWW and DPA within a three weeks period. The three measurements derived from the DPA gave the higher precision, ranging from 0.97 to 0.99, with UWW at 0.94 and 0.95.

Using DPA as a reference for BMC, the authors calculated that a 1% change in the TBBM-FFM results in a change in density of 0.01 g/cc. This would result in a change of 5% in the % BF estimate when using the Siri equation. Therefore, TBBM can significantly influence the measurement of % BF by UWW. The higher density of the skeleton and the marked

intersubject variation in TBBM again make this the source of highest variation.

Dual Energy X-ray Absorptiometry (DXA)

General Characteristics

DXA is based on the characteristic attenuation of x-rays. The dual energy source of conventional x-rays undergoes varying degrees of attenuation by human tissues such as bone and soft tissue. Attenuation is defined as the reduction in intensity of the x-ray source after it interacts with a chemical compound. Beam attenuation occurs as photons passing through tissue are absorbed or scattered by two main types of physical interaction: compton scattering and photoelectric effect (Pietrobelli, Formica, Wang, & Heymsfield, 1996). The diminished beam intensity is directly related to the specific chemical compound with which it interacts. The unattenuated energy, in the form of x-ray radiation, is determined by an external detector.

Ratio of Attenuation (RA) of DXA

When two different photons pass through an absorber, attenuation can be expressed as a ratio (RA). The attenuation of the lower energy can be expressed as a ratio to the attenuation observed at the higher energy. Each element has a characteristic mass attenuation coefficient and RA value. For example, elements found in soft tissues (e.g., Na, K, and Cl) have lower atomic numbers, and smaller RA values compared to calcium (Ca), which is primarily found in the bone (Pietrobelli et al., 1996).

Soft tissue and bone are identified by DXA-measured RA values, with the two components being distinguished simultaneously. The mass fraction of two components can be identified if each component's mass attenuation at each of the two energies is known and constant (Pietrobelli et al., 1996). First, the distinction is made between bone and non-bone; then non-bone is subsequently divided into fat and bone-free, fat-free tissue. The theoretical RA values calculated from elemental mass attenuation coefficients at assumed peak energies (of 40 & 70 keV) agree closely with RA measurements in vitro. Theoretical and in vivo measured RA values are also strongly associated (Pietrobelli et al., 1996).

Pixel Analysis of DXA

As the x-ray beam is introduced into the body, the external detector analyzes one small cross-sectional area at a time. These small cross-sectional areas are called pixels. Pixels are first separated by computing the RA value for every pixel in a total body DXA scan. Because the RA value for bone mineral is much higher than for soft tissue, bone-containing pixels will be those with the higher composite RA values. An RA value threshold must be set to distinguish between pixels that include bone mineral and those that consist of soft tissue alone.

The fat and lean composition of soft tissue pixels may be evaluated by equations. The constants in the equations, RA1 for pure fat and RA2 for pure lean tissue, are established by measuring fat and lean soft tissue samples or other materials that serve as calibration standards. Each RA for FM and FFM

is close to those based on an experimental phantom created from actual animal tissues (Pietrobelli et al., 1996).

Assumptions of pixel analysis.

Estimating soft tissue composition in bone-containing pixels is complex and requires some assumptions. The uniform distribution model, applicable to bones found in regions of the lumbar spine, has been replaced by improved algorithms such as the weighted-linear distribution model. This weighted model takes the adjacent soft tissue composition into account when calculating the soft tissue being analyzed. This model has improved algorithms and is better at estimating soft tissue in bone pixels (Pietrobelli et al., 1996).

Types of DXA Machines

All DXA machines share in common an x-ray source, detector, and calibration techniques. The three types of commercial DXA machines include: Hologic QDR, Lunar DPX, and Norland XR 26 (Jebb & Elia, 1993; Lukaski, 1993). Several approaches can be used to create the two main energy peaks. The first, Hologic, generates dual-energy x-ray spectra by pulsing the x-ray tube kV between sequential measurement points. The Hologic QDR-1000 utilizes two energy beams, 70 and 140 keV, which are alternately pulsed resulting in tube voltages of 45 and 110 keV (Kelly, Slovik, Schoenfeld, & Neer, 1988). Lunar DPX uses a constant potential generator and a Cerium K-edge filtration to generate photons at two energies (40 and 70 keV). The last, Norland XR 26, also employs a constant generator, but it operates at 100 kVp

and employs a Samarium filter with k-edge at 46.8 keV. These machines also have different detectors, calibration standards and techniques, and bone thresholds for pixels and algorithms differ also (Tothill, Avenell, & Reid, 1994).

The principles are the same for each, but there are technical differences in both the hardware and software such that measurements by one instrument are not necessarily the same as those obtained by another (Jebb & Elia, 1993). No specific manufacturer is preferred (Jebb & Elia 1993). However, previous studies comparing the Norland DXA and other brands indicate that Hologic and Lunar instruments typically show a closer agreement with UWW and magnetic resonance imaging (Tothill, Hans, Avenell, McNeill, & Reid, 1996).

Formula Calculations for Hologic QDR-1000 DXA

The QDR-1000/W's computer algorithm is based on the principle that bone selectively attenuates high-energy x-ray photons, and that the bone mineral content Q of any sample point can be computed from: $Q = KH-L$; where H and L are the logarithms of the sample attenuation at high (140 kVp) and low (70kVp) energies, respectively, and the constant K depends on the tissue attenuation characteristics of the beam. In the QDR-1000/W, K is continuously measured using the "tissue" segment in the filter wheel (Hologic Operator's Manual, 1990).

Using this value of K, Q is calculated for each point scanned using the formula given above. A histogram of the Q values is compiled. Because a

large portion of the image will contain soft tissue only, this histogram will have a large peak. A threshold is chosen just above this peak, and applies that value to discriminate, point by point in the Q image, between "bone" points (whose Q is above threshold) and "non-bone" points (whose Q is below threshold).

Calibration for the Hologic QDR-1000 DXA.

Internal calibrations. The Hologic contains an internal reference system consisting of a rotating wheel composed of three sections (2 sections of epoxy-resin-based material consistent with the densities of bone and soft tissue and one section of air). This wheel automatically determines which points in the scan are bone, tissue, and air (air points are excluded from analysis of soft tissue). The Hologic DXA is calibrated daily within ±0.5% when measuring BMC of a Hologic DPA/QDR-1 anthropomorphic spine phantom containing a known weight of calcium hydroxyapatite (CaHA) (Hologic User's Manual, 1990).

Thus, before the x-ray passes through the patient, the beam is filtered through the rapidly rotating wheel. When finally intercepted by the detector, the beam contains information about the x-ray absorbing characteristics of both the patient and the calibration materials in the filter wheel. An A/D converter, fed by the detector, supplies a complex digital signal to the computer, which uses that signal both to construct the screen image and as the basis for its computations of BMC and BMD.

External calibrations. External calibration for fat and lean tissue of varying depths are performed by the tissue bar. Each scan is performed with this tissue bar at the edge of the mattress, by the feet. Tissue points and the soft tissue portions of the bone points are then compared to the soft tissue calibration phantom, or the tissue bar, to determine the fat/lean composition. This tissue bar is included for all analyses. The manufacturers were originally concerned with the longtime stability of the absolute rays. The tissue bar is composed of wedges made of aluminum and lucite (polymethylmethacrylate) calibrated against stearic acid as 100% fat, and dilute saline solution as 100% fat-free mineral-free tissue (Hologic User's Manual, 1990).

For correct reporting of the soft tissue values, the regions must be adjusted to place the soft tissue in the correct regions. Regions include: head, right arm, left arm, right leg, and left leg, and trunk.

Assumptions for the Hologic QDR-1000.

DXA has several assumptions. The first is 17.0% brain fat, which has been calculated by comparative studies (Hologic Operator's Manual, 1990). This brain fat assumption is made because the machine cannot measure the fat due to the overlying bones of the skull and the lack of adjacent soft tissue to estimate a value. The 17% brain fat assumption may vary with head size. Another assumption includes the constant hydration of FFM at 73.2% water (Pace & Rathburn, 1945). Finally, DXA accounts for bone marrow through the values derived from complex algorithms in sofware (personal communication, Hologic scientists).

Studies of the Precision of DXA

Fuller, Laskey, and Elia (1992) evaluated the precision of DXA (Lunar) for whole body and major body subregions. Subjects included 12 females and 16 males, aged 18-59 yr. Each subject was scanned twice. Following the first scan, subjects stood and walked around before being repositioned for the second scan. Components measured included: fat, fat-free soft-tissue, and bone for the whole body and subregions. These components were analyzed for precision by SDs and % CVs.

The precision (SD, % CV) for fat tissue was 0.11 kg (9.0%) for arms, 0.20 kg (3.4%) for legs, 0.25kg (4.4%) for trunk, and 0.42 kg (3.0%) for whole body. Fat-free soft tissue had precision measures of: 0.15 kg (2.8%) for arms, 0.23 kg (1.2%) for legs, 0.24 kg (1.0%) for the trunk, and 0.42 kg (0.8%) for whole body analysis. Finally, bone mineral precision was 0.01 kg (2.0%) for arms, 0.02 kg (1.4%) for legs, 0.02 (2.0%) for the trunk and 0.03 kg (0.9%) for whole body scans. In general, the larger the size of a segment was found to be, the larger was the associated absolute error, but the percentage error was smaller.

Going et al. (1993) studied the ability of DXA (Lunar, software 3.6) to detect small changes in the body composition of 9 men and 8 women, aged 19-31 yr, during a dehydration-rehydration protocol. This protocol designed to induce changes of ~ 2% of body weight. Also during this time, the subjects underwent repeat body composition measures over three consecutive days.

Results indicated that scale weight and total mass from DXA were highly correlated ($r > 0.99$); as were estimates of FFM ($r = 0.99$) and % BF ($r =$

0.97) from DXA and UWW. Changes in scale weight of ~ 1.5 kg due to fluid loss and gain were highly correlated (r=.90) with both changes in total mass and soft tissue mass from DXA. As expected, bone and FM estimates were unaffected by changes in hydration.

In contrast to the findings for bone mass and FM, DXA had a somewhat limited capacity to resolve small changes in FFM (r= 0.67) due to fluctuations in hydration for individual subjects. The changes in FFM detected by DXA accounted for only ~50% of the changes in total mass during dehydration-rehydration studies. However, mean changes in total mass, soft tissue mass, and FFM were not significantly different (P> 0.05) from changes in body weight. The average changes in body weight and fat free tissue weight agreed well ($\leq$0.9 kg). Thus, the authors concluded that DXA was able to detect small individual changes in total mass and soft tissue mass and was also useful for detecting group changes in FFM.

Haarbo, Gotfredsen, Hassager, and Christiansen (1991) studied the body composition of six subjects measured six months apart. The precision errors in vivo included the following (SD (CV%)): FM, 1.1 kg (6.4%), FFM 1.4 kg (3.1%), TBBM, 0.03 kg (1.2%), and % BF 1.6% (5.7%). None of the subjects varied their body weights by more that 4.2 kg between measurements.

Mazess et al. (1990) measured BMC and soft-tissue composition with DXA (Lunar) in 12 young adults (6 males and 6 females). Body composition was analyzed on five occasions at both medium (20 minutes) and fast (10 minutes) scanning speeds. Ten scans were made over a period of 5 to 7 days

with an average of two scans per day. There were no significant differences in mean results or in precision errors between the two speeds. The correlations between determinations using the two speeds were very high (mean r= .98, *P* <.001) for both BMC and tissue composition.

Because there were no significant differences in mean values or precision at the two scan speeds, the results were pooled. The precision error obtained from pooling results from the two speeds for total BMC and BMD averaged 50 g and 0.01 g/cm^2, or ~ 1.8% and 0.8% respectively. Precision errors attributed to: percent fat in soft tissue =1.4%, FM =1.0 kg, and FFM =0.8 kg. These results correspond to a relative error of 0.8% for total body BMD and 1.5% for FFM. These preliminary results suggest that DXA can be used to accurately estimate soft-tissue composition with better precision (1-1.5%) than was possible with DPA.

Studies of Accuracy: In Vivo analysis of DXA

Adult Applications

Haarbo et al. (1991) investigated the accuracy of DXA in vivo, comparing the components of body composition measured by DXA to DPA, TBK, and Db by UWW in 25 subjects (15 women and 10 men aged 23-41 yr). The authors found agreement between % BF and FFM by DXA and the three established modalities. Mean measurement differences were (-5.3 to -0.4%) and (-0.7 to 2.5%) for % BF and FFM, respectively. Thus, the authors concluded that DXA provides accuracy which is applicable to group research studies and probably also in clinical measurements of the single subject.

Prior et al. (1997) examined the validity of a Hologic 1000W to evaluate body composition against a four component model. This four component model consisted of TBW, Db, and TBBM as described by Lohman (1986). Subjects included 81 women (aged 20.7±2.6 yr) and 91 men (aged 21.2±2.1 yr), of which 111 were collegiate athletes and 61 were nonathletes. The authors also sought to determine whether accuracy is affected by gender, race, athletic status, or musculoskeletal development in young adults.

Results indicated a high correlation between body mass via scale weight and body mass by DXA; with r= 1.0, SEE= 0.5 kg. Also, a higher correlation and lower errors were found between % BF by DXA and % BF from the four component model (r= 0.94, SEE=2.8%, TE= 2.9%) compared to % BF by UWW versus % BF from the four component model (r= 0.91; SEE= 3.4%, TE= 3.6%). Also, mean % BF values from UWW were significantly greater than % BF from the four component model (mean diff ±SD diff = 1.2±3.4% body mass). Differences between % BF from DXA and % BF from the four component model were weakly related to body thickness (as reflected by BMI, r= -0.34) and to the percentage of water in the FFM (r= -0.51), but were not significantly affected by race, gender, athletic status, or musculoskeletal development. Thus, the authors concluded that DXA body composition estimates are accurate in young adults who vary in gender, race, athletic status, body size, musculoskeletal development, and body fatness.

Svendsen et al. (1993) evaluated the accuracy of body composition measurements in vivo by comparing DXA (Lunar, software 3.2) scans with in

vivo and chemical analysis after postmortem homogenization of seven pigs (35-95 kg). The regression lines between these measurements were not significantly different from the line of identity (P >0.05), the r values were >0.97, and the corresponding SEEs were 2.9%, 1.9 kg, and 2.7 kg for % BF, FM, and FFM, respectively.

Svendsen et al. (1993) also evaluated changes in FM and FFM which were simulated by placing 8.8 kg porcine lard on the trunk of six women. The measurements of the simulated changes in FM and FFM by DXA were not significantly different from those expected (P >0.05). However, both the measured TBBM and the number of bone pixels increased by ~7% with the lard addition. The authors could not explain the inaccuracies of the TBBM measurements, but the fact that the lard increased the number of bone pixels could indicate that the edge detection technique was influenced by the lard.

Pediatric Applications

Brunton, Bayley, and Atkinson (1993) evaluated the DXA (Hologic QDR-1000, software 6.0) for analysis of whole body composition in infants. Two specific groups were targeted: small piglets (1.6 kg) and large piglets (6.0 kg). These weights were chosen to approximate the lowest midrange weight of infants who would be of clinical interest. Precision and accuracy were estimated by scanning piglets in triplicate to calculate CVs and comparing DXA estimates with chemical analysis of whole carcass. Three consecutive whole-body scans were conducted by using the pediatric whole-body software on piglets. Generally, the piglets were not repositioned between scans unless

they became aroused during the scan, in which case they were given more anesthetic and the scan was restarted.

Results included the mean CVs for all DXA measures in small piglets and large piglets were <2.5%, except for FM, which were 6.3% and 3.5%, respectively. The authors concluded that in large piglets, DXA provided reasonable estimates of chemical analysis for BMC, FFM, FM; but only for FFM in small piglets. DXA overestimated fat with a 234.6% difference between DXA and measured fat. Also BMC was underestimated by 29.7% with DXA. The authors concluded that further improvement in accuracy is necessary before the utilization of DXA estimates of whole-body composition in small infants and animals weighing 1.6 kg.

Picaud, Rigo, Nyamugabo, Milet, and Senterre (1996) studied the reproducibility, accuracy, and precision of DXA (Hologic QDR-2000, software 5.64) by scanning 13 piglets, 1471-5507 g. Three consecutive scans were performed while the pigs were supine; there was no repositioning between each scan. Estimates of body weight, BMC, and fat content from DXA were compared with the results of carcass analysis in small piglets.

Reproducibility in DXA measurements from the pigs was 0.09% for body weight, 1.95% for BMC, and 5.35% for fat content. Results also showed that body weight determined by DXA in 13 piglets was highly correlated (r=0.999) with scale weight. BMC determined by DXA was significantly correlated with ash weight (r=0.955) and total calcium content (r=0.992). Although DXA-measured fat content was significantly correlated (r=.971) with

chemically measured fat content, DXA-measured fat content was overestimated by DXA in 13 piglets. The authors concluded that further refinements for determining fat content in preterm human infants are needed.

Studies of Accuracy: In Vitro analysis of DXA

Jebb, Goldberg, Jennings, and Elia (1995) evaluated body composition measurements with the Hologic QDR-1000W, software 5.5P and enhanced version, for effects of depth and tissue thickness. All measurements were made in the trunk portion of the scan; both oil and water as well as different meat thicknesses were measured.

All data indicated a trend in the measured fat mass with depth, such that more fat was measured at extreme depths, <10 cm and >25 cm, than at intermediate depths. In samples of meat weighing ~ 55 kg, DXA significantly underestimated the absolute FM compared with direct analysis by 5-8% or 1-4 kg of fat. However, the depths measured in this vitro system represent much greater thicknesses than measured in vivo in subjects of similar volume. Consequently, most subjects will lie in the portion of the curves in which depth has little effect on the measured BMD or FM of the body. Greater depth should only affect the grossly obese. The error at the low end of the range should contribute relatively little to the overall measurement of whole-body composition since it represents a small proportion of the total body mass.

Limitations of Dual Energy X-ray Absorptiometry

Scan Table Size

One of the most limiting features is the scanning area, which is approximately 190 cm by 60 cm. It cannot be used in the very obese, and the accuracy with which individual segments may be distinguished declines as body size increases. The limited scanning table also can exclude tall persons, and limits concerning tissue thickness can affect the precision of scan modes (Wellens et al., 1994).

Beam Hardening

The x-ray beam analysis of DXA involves the attenuation of the lower energy expressed as a ratio to the attenuation observed at the higher energy. Beam hardening may result with increasing depths of tissue. It typically occurs when lower energy photons are preferentially removed from a radiation beam compared with higher energy photons. This in turn may lead to a progressive shift in spectral distribution to higher effective energies with increasing body thickness. The polyenergetic spectrum of the DXA x-ray beam may be a potential source of error for DXA since beam hardening may result in a non-linear measurement scale for BMC.

Studies which investigate beam hardening.

Blake, McKeeney, Chaya, Ryan, and Fogelman (1992) looked at the effects of DXA beam hardening on bone density measurements. The study consisted of bone represented by layers of aluminum of linearly increased thickness that was scanned under water thicknesses ranging from 0-25 cm.

Although Hologic QDR-1000 has an internal reference wheel which provides internal standard that continuously monitors for beam hardening, the attenuation coefficients are only accurate for those introduced by wheel filters. This was significantly modified by the calibration of the QDR-1000 bone density scale with the Hologic 3-step phantom.

Beam hardening was shown to have two effects on measured BMD: 1) at a constant true BMD, measured BMD varied with water thickness, and 2) at constant water thickness, the BMD scale was not precisely linear. For conditions to approximate spine and hip studies, the maximum deviation of measured BMD from a linear scale was 0.023 g/cm^2, while the root-mean-squared deviation (0.01 g/cm^2) was comparable to the measurement precision for a spine or femoral neck scan (~ 1%). The largest error from linearity was found to occur at the thinnest water thickensses for BMD values in the range of 0.2 to 0.6 g/cm^2. The results of this study showed that for spine and hip studies, beam hardening was too small to affect clinical studies.

Tissue depth may be another potential source of error for DXA when assessing body composition. Laskey, Lyttle, Flaxman, and Barber (1992) evaluated the DXA (Lunar, 3.1) using a phantom that consisted of known but variable amounts of lard to represent FM, water to represent FFM, and lumbar spine phantom for BMC. Results showed that determinations of total tissue mass were very accurate and precise but FM was slightly overestimated (103% of the calculated value) and FFM slightly underestimated (98% of calculated value). Also, precision was excellent for all measurements but

deteriorated with increasing depths (over 22 cm) of soft tissue. This study demonstrated that in vitro bone mineral measurements are affected by depth and composition of the subject and will be least accurate for obese subjects.

Assumptions for Tissue Surrounding Bone

As previously mentioned, pixels are first divided into bone and non-bone. The non-bone pixels, containing mostly soft tissue, are subsequently divided into fat and fat-free tissue. Bone pixels also contain some amount of soft tissue as well. But since DXA has only two photon energies, only two body compartments can be evaluated, independent of thickness. It has been estimated that 40 to 45% of the 21,000 pixels in a typical whole-body scan contain bone in addition to soft tissue, and these pixels therefore are excluded from the calculation of values for soft tissues (Lohman, 1996). Attempts to add a third photon have been rejected by the nature of the relationships between attenuation, energy, and atomic number (Tothill et al., 1994). Hence, DXA is unable to evaluate tissue thickness under or overlying bone. For these pixels assumptions are necessary regarding the amount of soft tissue in each pixel.

Because of the potential limitation discussed above, it is necessary for DXA manufacturers to make assumptions about the distribution of fat. Some software packages use the lumbar spine as the only calibration sample. The assumption is that there is a constant amount of soft tissue surrounding the vertebral bone This calibration in turn, provides a constant to bone pixels in which soft tissue is unable to be divided into FM and FFM. However, there is

non-uniformity for the amount of soft tissue surrounding the bone, causing unpredictable errors to occur in the determination of BMD in the lumbar spine (Tothill & Pye, 1992).

For whole body scanning, more complex assumptions are necessary. Enhanced software versions use complex algorithms to calculate bone pixel soft tissue by taking surrounding non-bone pixels tissue amount into account. Dual energy measurements allow the determination of the proportion of fat in non-bone areas (60% of pixels) and these assessments are used to extrapolate or interpolate over bone (Tothill et al., 1994).

Studies investigating the tissue assumption.

Snead, Birge, and Kohrt (1993) studied the body composition of 113 women and 72 men, aged 21-81 yr, by DXA (Hologic QDR-1000) and UWW. This large sample was subdivided into three smaller groups: young (21-39 yr), middle (40-59 yr), and older aged subjects ($\geq$ 60 yr). The study purpose was to determine whether % BF is overestimated in older people by UWW because of age-related decreases in BMC. Measures of % BF by UWW and DXA, respectively, were not different in young people [17.6 $\pm$ 6.4 vs. 17.6 $\pm$ 7.2%, NS]. Values were slightly, yet significantly different in middle-aged subjects [25.5 $\pm$ 6.4 vs. 24.1 $\pm$ 6.7%, $P < 0.05$]. The largest overestimation of % BF by UWW was in older subjects [34.9 $\pm$ 7.9 vs. 30.8 $\pm$ 8.7 %, $P > 0.05$]. Thus, the study results confirmed the hypothesis that UWW loses its sensitivity for % BF estimation as BMC varies from the assumed value.

The authors also assessed DXA's prediction of % BF in nine subjects with packets of lard (2-3 kg) overlying either the trunk or thigh regions. Only 55% of the exogenous fat was identified as fat when it was in the trunk region compared with 96% when it was positioned over the legs. These data suggested that the age-related increase in upper body fat tissue may be underestimated by DXA. The authors concluded that although UWW and DXA % BF differences were small, there was a widening discrepancy with advancing age, which possibly may be due to an underestimation of fat by DXA in the trunk region.

Another study examining profiles of fat distribution was done by Tothill et al., (1996). They compared total and regional fat by DXA (Norland, software 2.4), UWW, and magnetic resonance imaging (MRI) in 13 pre-menopausal women, aged 20-51 yr. Results showed poor agreement between methods; the mean values of % BF include: DXA= 40.0%, UWW= 28.6%, and MRI= 23.0% (although MRI excluded the head, forearms and feet, estimated from the DXA measurements to contain 8% of the body fat). Reasons for the differences between DXA and MRI were sought.

Evidence from in vivo comparisons and phantom measurements suggested fat calibration errors in the Norland DXA (over a three year period) which contributed to MRI/DXA differences. Profiles of fat distribution along the body showed variations in the DXA/magnetic resonance imaging ratio, particularly of the chest, with the DXA pattern thought to be less accurate. However, the authors stated that previous comparisons between the Norland

DXA and other brands indicate that Hologic and Lunar instruments would show a closer agreement with MRI and UWW.

Assumption of Constant Hydration by DXA

As previously mentioned, DXA assumes that 73.2% of the LBM is water (Pace & Rathburn, 1945). This assumption could be a potential source of error as FFM may differ due to fluid retention or dehydration. Hemodialysis is a convenient model for assessing the influence of changes in hydration of the FFM on the estimates of other compartments. While bone and fat compartments are held constant, a known amount of fluid is removed from the FFM during dialysis. The salt-containing ultrafiltrate has the radiologic properties of pure fat free tissue (Abrahamsen, Hansen, Hogsberg, Pedersen, & Beck-Neilsen, 1996; Horber, Thomi, Casez, Fonteille, & Jaeger 1992).

Studies examining the constant hydration assumption by DXA.

Horber et al. (1992) used DXA to measure the body composition 7 patients (5 males and 2 females, aged 23-75 yr) on maintenance dialysis with DXA (Lunar DPX, software 3.1) . Patients were scanned immediately before and within 1 hour following hemodialysis. Results showed that FFM decreased in all segments of the body as a consequence of the removal of 0.9 kg-4.4 kg of salt-containing fluid by hemodialysis. The decrease in FFM ($P <$ 0.01) accounted for 94 $\pm$ 5% of the changes in body weight as a consequence of dialysis therapy. As expected, the amount of weight lost and the change in FFM observed during dialysis were strongly correlated ($r = 0.94$, ; $P < 0.006$).

Conversely, apparent changes in FM (0.12 $\pm$ 0.17 kg), and BMC (0.0 $\pm$ 0.01 kg) were not significantly different from zero.

An investigation similar to the Horber et al. (1992) study was performed by Abrahamsen et al. (1996). The authors evaluated the use of Hologic QDR-2000 to determine whether DXA was sensitive to soft tissue changes during hemodialysis. DXA was performed on 19 hemodialysis patients (9 women and 10 men, aged 26-73 yr) before and after removing 0.9-4.3L of ultrafiltrate.

Results of reduction in FFM measured by DXA were highly correlated with the ultrafiltrate, as determined by the reduction in gravimetric weight ($r = 0.975$, $P < 0.0001$; SEE±233 g). Whole body BMC was estimated to be slightly (~0.6%), yet significantly ($P<0.05$) reduced after dialysis, but % BF, BMD, and lumbar BMD did not change. Therefore, DXA accurately reflected the changes in ultrafiltrate even when a large proportion of the changes occurred in the trunk. Once again, this study showed that the accuracy of DXA measurements was unaffected by hydration status.

Studies Comparing Types of DXAs and Software

Different Types of DXAs

A lack of comparability of DXA results among machines of different manufacturers because of variations in calibration procedures, photon energies, and algorithms used to estimate body composition from the absorption data (Wellens et al, 1994). Tothill et al. (1994) studied the precision and accuracy of whole body BMC in three commercially available DXA

machines: Hologic, Lunar, and Norland absorptiometers. Assessment of comparability was made in vivo on 6 women and 5 men, covering a range of size and adiposity. Precision was also evaluated in vitro through repeated measurements of a phantom in various configurations. In vivo precision was measured by making two consecutive measurements for each subject with repositioning in between.

Results showed good precision for whole body BMC; CVs include 0.7% for Hologic, 1.4% and 1.0% for Norland MkII and Norland XR36, respectively. BMC as measured by each machine was compared by linear regression. The correlations of BMC measurements were high, with Lunar vs. Hologic at 0.974, Norland XR 36 vs. Hologic was 0.978, and finally Norland XR 36 vs. Lunar was 0.930. However, there were significant differences between instruments made by different manufacturers. Slopes of regression lines suggested differences of calibration of up to 8%; SEE values ranged from 110 to 190 g, with maximum deviations from regression of 17%.

Several factors may account for the differences between manufacturers. One may be that different manufacturers utilize different calibration devices. Another may involve machine algorithm variations on the fat distribution model. Also, regional differences in BMC, particularly in the trunk, were due in part to Hologic having higher bone threshold than the other two machines. Finally, the dependency of measurement on the thickness of the subject may vary. Overall, the authors warn that these differences preclude the interchangeability of results from different instruments; caution is needed

even when comparing regional versus whole body measurements from the same machine.

Different Software Versions

Changes in computer software within a given DXA machine could also affect measurement reliability and validity. Van Loan, Keim, Berg, & Mayclin (1995) compared two different software packages, 3.4 and 3.6R, in the Lunar DXA. Fifteen women, aged 20-40 yr, were evaluated. All women were involved in a weight loss program and had % BF values from 26%-40%.

Total body mass measured via DXA 3.4 (76.3 kg) and 3.6R (76.5 kg) were comparable to subjects' scale weight (76.5 kg). BMC and BMD measures were higher (by 5.5% and 1.8%, respectively $P < 0.01$) with software version 3.4 as compared to 3.6R. Also, a larger FFM (by 1,115 g) and a smaller FM (by 1,492 g) were observed with 3.4 compared to 3.6R. Percent fat estimated by UWW, DXA 3.4 and DXA 3.6R were 38.1%, 39.9%, and 41.9%, respectively. The authors concluded that different software versions used on a given machine could indeed affect the measurement of total body mass, FM, FFM, and BMC values. This is likely due to differences in algorithms among various sofware packages.

Ellis, Shypailo, Pratt, and Pond (1994) looked at the accuracy of body composition measurements by DXA (QDR-2000, adult whole body, single beam, analysis versions 5.56 & 5.57) as compared to total carcass chemical analysis in 16 pigs with a weight range of 5-35 kg. Also, body composition

measurements were performed on 18 boys, aged 4-12 yr, using the same two DXA software versions.

Results showed that both software versions accurately predicted body weight, although there were significant differences in the partitioning between BMC, non-bone lean tissue, and BF compartments. All estimates of body composition were highly correlated ($r \geq 0.99$) with the results of the direct chemical reference method. Standard errors of estimate were 226-271 g for body weight, 387-429 g for FM, 3.5-4.3 kg for FFM, and 35.4-36.5 g for BMC.

The two different software versions produced a shift in the partitioning of the nonbone mass of ~ 0.4 kg. Specifically, version 5.57 resulted in a reduction of the % BF DXA estimate and an equal increase in the DXA estimate of FFM. Furthermore, the mean BMC value for version 5.57 was increased by ~ 65 g or 10% relative to version 5.56.

Comparability of Hologic DXAs

A study examining in vitro comparability was done by Renchen, Murano, Drinkwater, & Chestnut (1991). Six Hologic QDR-1000 DXA bone densitometers located across the United States were used. Nine successive scans were acquired on each machine using a single anthropomorphic lumbar spine phantom, manufactured by Hologic. Values for BMC, area, and BMD were recorded. Means, SD, and CVs were calculated for each machine. All the CVs (BMC, area, BMD) were less than 1% (range 0.3%-0.6%). The CV of the means at the six sites were 0.4%, 0.6%, and 0.5% for BMC, area, and BMD, respectively. Also, the difference between the highest and lowest

means of the individual machines was only 1.1%, 1.31%, and 1.07% for BMC, area, and BMD respectively. Thus, the authors concluded that these small variations between the DXA systems (all using the same software) are encouraging for researchers involved in multi-center trials in which data are pooled.

Differences between Dual Photon Absorptiometry and Dual Energy X-ray Absorptiometry

Source Differences: X-ray Versus Radionuclide

There have been several source differences between DPA and DXA. DPA employed 153 Gd, which is an isotope that emits two characteristic photons upon decay. The natural process of radioisotope decay is not easily controlled, making the radionuclide source in DPA unstable. As previously discussed, problems arise due to drifting of results as the source decays. DXA, on the other hand, utilized x-rays instead of a radioisotope as its energy source. The x-rays generated by the DXA machine are more easily controlled, thus providing a more stable source (Cullum, Ell, & Ryder, 1989; Pietrobelli et al., 1996).

Also, photon flux is greater from the x-ray source in DXA than the radioisotope source used in DPA. This increased flux allows for decreased scanning time, improved resolution, and better precision of the image (Wahner, Dunn, Brown, Morin, & Riggs, 1988; Kelly et al., 1988). Precision in vivo has been shown to be 2-3% for DPA compared to 1% for DXA (Cullum et al., 1989). Another advantage of DXA is the smaller radiation exposure

compared to DPA. DPA radiation exposure for a whole body scan is ~2.0 mR (Wang et al., 1989) in contrast with the Hologic DXA which is <1.0 mR for a whole body scan (Hologic Operator's Manual, 1990).

Precision of DPA vs. DXA

Cullum et al. (1989) evaluated DPA compared to the Hologic QDR-1000 for bone density measurements. Precision of DXA was also determined in vitro by performing 10 repeat scans on a series of rectangular slabs of different density bone-equivalent material placed in a variety of water depths, 2.0 cm to 30.0 cm. In vivo precision was also done scanning nine patients on ten occasions, over a period of 4-6 weeks.

Results of both the in vitro and in vivo measurements were better than a CV of 1%. The only larger CV was from the in vitro analysis of varying thicknesses. At 20 cm depth the CV was 0.71%, while 25 and 30 cm were 1.43% and 3.19% respectively. Since precision was limited by the accuracy of the detection of the bone edge and the reproducibility of object positioning, the better resolution of DXA provided more precise results. The authors concluded that DXA showed significant improvement over DPA in image quality.

Gluer et al., (1990) also compared DPA with the Hologic QDR-1000 for bone density measurements of the spine and hip. For in vitro studies, an anthropomorphic spine phantom, lucite blocks, and variable thickness mixtures were used as reference materials. Short-term in vivo analysis involved two consecutive exams of 10 healthy adults, aged 21-40 yr, with

repositioning between scans. Long-term precision of the spine-scanning mode was analyzed by scanning an X-Caliber (Hologic) spine phantom. This phantom was analyzed once per day with both DPA and DXA over a nine month period. During that time, there was one source change for the DPA scanner.

Results from this study indicated that DXA had excellent precision in vitro as compared to DPA. Specifically, long term precision error in vitro was 1.30% in DPA and 0.44% in DXA. However, both DPA and DXA were highly correlated in vivo: r= 0.98 for the spine and r= 0.95 for the femoral neck. Small differences between the two machines were explained by differences in hardware, or resolution, and software, or bone edge definition. The scanning time for both spine and hip measurements was reduced from 20-40 minutes for DPA to 6-7 minutes for DXA. Thus. the authors concluded that the precision, spatial resolution, and scanning time of DXA are significant improvements over DPA.

Kelly et al. (1988) also evaluated the bone density of the lumbar spine for both DPA and DXA. The lumbar spine BMD was measured by DPA (Lunar) and by DXA (Hologic QDR-1000) in 85 patients, aged 21-78 yr. Each patient was measured once by DPA and twice by DXA on the same day with repositioning between patients. Long term stability was evaluated by making serial measurements of a spine phantom of known elemental composition.

The spinal BMD measurements with DPA and DXA were linearly related and highly correlated (r=.98) over a range of bone densities, from

severely osteopenic to high normal. Compared to DXA, DPA values were three times as variable for 170 days and increased 1.0% after a software change. Measurement time by DXA was 5-8 min. for the lumbar spine, with a maximum radiation exposure of 3 mrem, which was significantly less than corresponding DPA values. Therefore, the authors concluded that DXA is the superior method for spinal BMD measurements since DXA was faster than DPA, involved less radiation exposure, provided greater image resolution, and better short- and long-term reproducibility.

Pacifici et al. (1988) assessed the short-term precision of the Hologic QDR-1000, and also compared DPA to DXA. Short-term precision was done by performing three DXA measurements of the lumbar spine in 19 healthy women, aged 34 ± 9.3 yr, over a three week period. In addition, both DPA and DXA measurements were obtained in 52 women, aged 54.3 ±12.5 yr.

Results of short-term precision included DXA measurements at a CV of 1.0%. Also, a significant correlation was found between DPA and DXA (r= 0.94; P <0.001). From the shape of the regression line, it appeared that DPA values were approximately 6.8% higher than DXA values. Hence, a conversion factor of -6.8% was used to convert DXA to DPA values. Both had similar rates of bone loss; 0.66% for DXA and 0.57% for DPA. Hence, the authors concluded that DXA was a precise method for vertebral bone mass measurements.

Strause, Bracker, Saltman, Sartoris, and Kerr (1989) also investigated lumbar spine bone measurements using both DPA and DXA (Hologic QDR-

1000). Subjects included 131 postmenopausal women, over 55 yr of age and free from other major risk factors for osteoporosis. Bone mineral densities were determined for 4 individual levels in the lumbar spine (L1-L4). All subjects were scanned by both DPA and DXA, with measurements performed within 15 minutes of each other.

Results showed a high correlation (r= 0.918) between BMD measurements of the lumbar by DPA and DXA. However, regression data suggested an interlevel difference between DPA and DXA, with BMD values derived by DPA consistently higher than those obtained by DXA. This discrepancy may have been due to poor image resolution which in turn may have produced inaccurate determination of vertebral edges. The authors felt that vertebral bone of the spine may have been underestimated by DPA computer analysis, leading to a lower BMD value from the DPA instrument.

Wahner et al. (1988) also measured the lumbar spine using DPA and DXA. Long-term precision was assessed through spine phantom analysis performed once weekly with the DPA instrument and once or twice daily with the DXA instrument and over a period of 4 to 6 months. For estimation of short-term precision, duplicate scans on the lumbar spine were made in and 5 volunteers with DPA and 15 volunteers with DXA.

Wahner et al.'s results showed good correlation (r= 0.988) between DPA and DXA. DPA had a longer scanning time and a lower image resolution than the DXA system. Long-term precision had CVs for BMD of 1.5% for the DPA instrument (n=20) and 0.4% for DXA (n=214). Short-term precision as a

mean percentage difference between the two scans was 1.7% for DPA (range, 0.2 to 4.4%; N= 5) and 1.0% (range, 0 to 3.1%; N= 15) for DXA. Overall, DXA instruments showed better precision in a spine phantom and reduced influence of thickness for patient measurement. Patients' BMD values were consistently lower with the DXA instrument apparently because of better accuracy in the specific measurement area. The authors concluded that DXA instrument is a major advance in bone mineral absorptiometry and provides improved, yet less expensive, measurements in research and clinical applications.

Studies comparing DXA with UWW

Bergsma-Kaddijk et al. (1996) measured the body composition of 20 young females (aged 19-27 yr) and 18 elderly females (aged 65-78 yr) using both UWW and DXA (Lunar, software 3.1). The BMC of the young women was 2644 ± 274 g, while older women had a BMC of 1987 ± 234 g ($P<.001$). The mineral fraction of FFM was also significantly different ($P<.001$) with older women having values of 6.0±0.6% and younger women having 7.5±0.5%. These BMC differences resulted in apparent differences in % BF as estimated by UWW. The % BF of the young women was underestimated, while it was overestimated in the elderly subjects.

Hansen et al. (1993) evaluated the body composition of 100 premenopausal, non-athletic women aged 28-39 yr. The authors used four predictor methods including DXA (Lunar, 3.1), BMI, skinfolds, and BIA. The criterion method was UWW. The primary purpose was to compare the

prediction of % BF from body density determined by DXA versus UWW. The secondary purpose was to examine the effect of bone mineral on the body density measures obtained from UWW using a multicomponent approach to further evaluate DXA.

DXA correlated well with UWW (r=.91) and had a SEE of 2.4%. When % BF was estimated from other methods, larger SEEs were obtained: 3.0% for skinfolds, 3.3% for BMI, and 2.9% for BIA plus weight. Also, individual body fat values derived from UWW were corrected for bone mineral variation. Bone mineral was found to be a significant predictor for the % BF fat models of DXA and skinfolds. The sum of 4 skinfolds predicted body density by UWW with a SEE of .0068 g/cc, but when corrected for bone mineral this decreased to .0056. DXA also had a lower SEE when corrected bone mineral was added, from .0053 to .004. The authors concluded that the DXA method was extremely precise and highly correlated with FFM and % BF.

Morrison et al. (1994) studied body composition in 31 Black and 38 White girls, aged 9-16 years. DXA measurements of FFM and % BF were made using Hologic QDR-1000. Corresponding FFM and % BF from UWW were calculated by the Siri equation, using the model of Lohman (1986) for White girls only.

The two-component model of UWW significantly overestimated % BF in both White and Black girls compared to estimates from DXA. In White girls, % BF values measured from UWW and DXA averaged 28.4% and 25.3%, respectively. For Blacks, average % BF values for UWW and DXA

were 28.7% and 27.9%, respectively. The authors felt that since the BMC density of FFM increased or approached adult status in black girls, differences between UWW and DXA decreased. Moreover, the inability of UWW to account for BMC in these girls can be corrected either by including it in a multicomponent model or by using the DXA technique.

Pritchard et al. (1993) evaluated DXA as a method of measuring % BF. Their purpose was to a) compare the precision of the Hologic QDR-1000 with the Lunar DPX and b) compare both DXA machines to three other body composition modalities (UWW, skinfolds, and BIA). Ten Hologic DXA scans were performed on one subject to assess precision. The CV was 1.8% for % BF, 0.6% for FFM, and 2.1% for FM.

Other results showed that DXA machines were highly correlated with r= 0.986, $P<0.00001$. Also, based on the observation of 12 subjects, correlations of QDR and DPX with UWW for % BF were high: r= 0.916 for QDR and r= 0.913 for DPX. The authors performed a limits of agreement estimate which showed a between-method difference of + 1.3% for the QDR compared with UWW. The DPX showed a between-method difference of + 4.8% for % BF compared with UWW. Correlations between both DXAs and other methods were also high. For QDR: r= 0.824 with skinfolds and r= 0.972 with BIA. For the DPX, correlations were r = 0.923 for skinfolds and r = 0.910 for BIA. The authors concluded that both DXAs measured % BF with greater precision than UWW as reflected by the coefficient of variability and the high correlation with other methods.

Wellens et al. (1994) evaluated the body composition of 78 women and 50 men, aged 18-67 yr, by using DXA (Lunar, software 3.4), UWW, and TBW. The three body composition methods were compared to determine the degree of agreement among methods, and reasons for discrepancies were explored.

Results showed that in both men and women there were no significant inter-method differences for % BF and FFM estimates between UWW and DXA. On the other hand, % BF measures in women were significantly less with TBW compared to UWW in women (mean difference 2.7 ±4.2%) and in men (mean difference 2.2 $\pm$ 3.2%). Thus, for each sex, statistical analysis indicate that body composition estimates show less agreement between TBW and either UWW and DXA.

Bioelectric Impedance Analysis

Bioelectric impedance analysis (BIA) uses an electrical current to estimate total body water by measuring the resistance and reactance of the tissues. Resistance (R) is a measure of pure opposition to the current flow through the body. Reactance (X_c) is the opposition to current flow caused by the capacitance produced by the cell membrane. The impedance (Z) is a function of the resistance and reactance where $Z = \sqrt{(R^2 + X_c^2)}$ (Heyward & Stolarczyk, 1996).

Basic Principles of BIA

Thomasett (1962) was the first to establish the basic foundation of BIA principles. With this method, a low-level current is sent through the body via surface electrodes. Subsequently, the BIA analyzer measures body

impedance, or resistance to current flow. Biological tissues act as conductors or insulators, and the flow of current through the body will follow the path of least resistance. Impedance is based upon the nature of the conduction of an applied electrical current in an organism. An individual's TBW can be estimated because the electrolytes in body water are excellent conductors of electrical current.

When TBW volume is large, current flows more easily through a given body with little resistance. On the other hand, resistance to current flow will be greater in individuals with larger FM, given that adipose tissue is a poor conductor due to its relatively small water content. Because the water content of FFM is relatively large (73% water), FFM can be predicted from TBW estimates.

To measure total body impedance, a low-level excitation current (500 - 800 uA) at 50 kHz is used. At low frequencies (~1 kHz), the current passes through the extracellular fluids only. At higher currents (500 - 800 uA), the current penetrates cell membranes and passes through the intracellular fluid, as well as the extracellular fluid (Lukaski, 1987). Given that fat is a poor conductor of electrical current, the total body impedance primarily reflects the volumes of water and muscle compartments comprising the FFM and the extracellular water volume (Kushner, 1992).

Also, impedance is a function of resistance and reactance, where

$Z = \sqrt{(R^2 + X_c^2)}$

As stated earlier, R is a measure of pure opposition to current flow through the body and X_c is the opposition to current flow caused by capacitance produced by the cell membrane (Kushner, 1992). Since the size of R is much larger than X_c when measuring whole body impedance, R is usually a better predictor of FFM and TBW than Z (Lohman, 1992). For these reasons, the resistance index (height $(Ht^2)/R$), instead of Ht^2/Z, has been used in many BIA models to predict FFM or TBW (Lohman, 1992).

Advantages of Using BIA

The BIA modality offers a number of potential advantages to other body composition techniques including the following:

1) requires minimal operator training and is easy to perform
2) is generally more comfortable than other methods and requires minimal intrusion on an individual's privacy
3) is safe, relatively inexpensive, and portable
4) can be used to estimate the body composition of obese individuals (There is less observer error associated with BIA than skinfold thicknesses, especially in the obese; Segal, Van Loan, Fitzgerald, Hodgdon, & Van Itallie, 1988).

Assumptions of BIA

There are a few assumptions inherent in the BIA method of body composition analysis. One assumption is that the human body is shaped like a perfect cylinder with a uniform length and cross-sectional area. This assumption is not exactly true. The human body more closely resembles five

cylinders connected in series versus one large uniform cylinder (Kushner, 1992). Also, these cylinders are not uniform in length or cross-sectional area, therefore, resistance to current flow through these segments will differ.

Another assumption is that at a fixed frequency (50 kHz), the impedance (Z) to the current flow through the body is directly related to the length (L) of the conductor and inversely related to its cross-sectional area (A) (Heyward & Stolarczyk, 1996). That is,

$Z = p\,(L/A)$,

where p is the specific resistivity of the body's tissues and is assumed to be constant. To express this relationship in terms of Z and the body's volume, instead of its cross-sectional area, the equation is multiplied by L/L.

$Z = p\,(L/A) * (L / L)$

Since A * L is equal to volume, the equation can be rearranged to be:

$V = p\,L^2 / Z$

Thus, the volume of the FFM or TBW of the body is directly related to L^2, or height-squared (Ht^2).

Problems with BIA Assumptions

The basic BIA assumptions are simple ideas which do not accommodate for the complexity of the human body. The application of the BIA equation may not be perfect because of the complex geometric shape of the body (Van Loan, 1990). Also, the specific resistivity (p) of the body's tissues is not constant and has been shown to vary among body segments. Specific resistivity differences exist due to changes in tissue composition,

hydration levels, and electrolyte concentration (Kushner, 1992). For example, Chumlea, Baumgartner, and Roche (1988) reported that the specific resistivity of the trunk is two to three times greater than that of the extremities. The result would be that the current would flow with least resistance in the limb areas, particularly in the arms.

Studies Examining the Reliability and Validity of BIA

Hoffer, Meador, Simpson (1969) estimated TBW from tritium dilution space in 20 healthy volunteers and 34 patients. Four surface electrodes were used, two placed on the dorsal surface of the right hand and two placed on the flexor surface of the left foot; with 100 uA AC, at 100kHz. In both subject groups, Ht^2/Z was the best predictor ($r= 0.92$; $P<0.001$) of TBW. Thus, the authors reported a strong relationship between total body impedance measures and TBW, suggesting that BIA may be a valuable tool for analyzing body composition and assessing TBW in the clinical setting.

Jackson, Pollock, Graves, and Mahar (1988) studied the reliability and validity of BIA in the determination of body composition; they also compared its accuracy with the results obtained by standard anthropometric methods. Subjects included 44 women and 24 men, each having % BF measured via UWW, skinfolds, and BIA. Each subject was tested four times by two testers on two different days. An additional 26 men and 38 women were tested once and combined with the data used for the reliability analysis to cross-validate BIA estimates of % BF with UWW % BF.

The % BFs from BIA, skinfold, and UWW were found to be reliable (R_{xx}= 0.957-0.987, with SEM= 0.9 to 1.5% fat). The cross-validation correlations for BIA determinations of % BF ranged from 0.71 to 0.76, which were significantly lower than those obtained using the sum of seven skinfolds equations (r_{xy}= 0.92 for men and 0.88 for women). Correlations between the weight-to-height ratio for BMI and UWW % BF were 0.75 and 0.74 for men and women, respectively. The SEE for the two BIA models ranged from 4.6% to 6.4 % BF compared with 2.9% and 3.6% BF for the skinfold equations. Thus, the authors concluded that the BIA method for measuring body composition was comparable to the BMI method, but less accurate than skinfold analysis, with height and weight accounting for most of the variance in the BIA equation.

Lukaski, Johnson, Bolonchuk, and Lykken (1985) used BIA to develop models for estimated the FFM and TBW in 37 men, aged 28.8 ± 7.1 yr. Current source was 800 uA at 50 kHz. FFM was also assessed by UWW, with TBW determined by deuterium dilution and total body potassium (TBK) from whole body counting.

Results produced test-retest correlation coefficients of 0.99 for a single R measurement; the reliability coefficient for a single R measurement over 5 days was 0.99. Because R and Z were equally correlated with TBW (r= 0.99) and X_c is not as strongly related to Z (r= 0.70), the investigators included only R as the BIA variable in the prediction of FFM and TBW. Further, linear relationships were found between R values and FFM (r= -0.86), TBW (r= -

0.86), and TBK (r= -0.79). Significant ($P < 0.01$) increases in the correlation coefficients were observed when the predictor Ht^2/R was regressed against criterion measures of FFM (r= 0.98), TBW (r= 0.95), and TBK (r= 0.96). Thus, the authors concluded that BIA is reliable and valid for the estimation of human body composition.

Segal et al. (1988) provided validation of BIA for body composition estimation. At four laboratories, UWW-determined body composition was compared with BIA in 1069 men and 498 women aged 17-62 yr (% BF ranged from 3-56%). Among the labs, some regression coefficients differed, but these differences were eliminated after adjusting for subjects' body fatness differences among labs. Data were pooled to derive fatness-specific equations for predicting FFM by UWW. The resulting correlations ranged from 0.907 to 0.952 with SEEs of 1.97 to 3.03 kg. Therefore, the authors concluded that BIA was valid estimator of body composition and that the precision of predicting FFM from impedance can be enhanced by sex- and fatness-specific equations.

Studies Assessing the Sensitivity for BIA Detection of Body Composition Changes

Ross, Leger, Marin, and Roy (1989) studied the sensitivity of BIA to detect changes in body composition. UWW and BIA techniques were used on male subjects (n=17) before and after a 10-week diet and exercise regimen. A control group of 20 males was also studied. The authors found that for both FFM and % BF, the Lukaski et al. (1985) and Segal et al. (1988) BIA equations produced values that were not significantly different ($P > 0.05$) from UWW in

both pre- and postregimen values. Thus, the authors concluded that their study findings suggest that the BIA method with these equations is a valid means of predicting changes in % BF as measured by UWW and the Siri equation. It is possible that 10 weeks was not sufficient to effect a %BF change that could be detected by any of the modalities used.

Studies Analyzing Different Variables

Kushner and Schoeller (1986) identified factors and conditions which may require standardization during BIA testing for healthy subjects and those in a medical setting. Some of these include: accurate measurement of height and weight, posture (supine) and time (e.g., time of day when measurements were taken, were they standing before, for how long?), abduction of limbs (due to their geometric shape, the extremities contribute ~90% of whole-body Z), consumption of food or beverage (could influence Z by changing TBW and extracellular water volumes), and recent exercise. In terms of positioning, the wrist electrode is the single most important site, since the arm appears to make the largest contribution to the total measurement (Jebb & Elia, 1993).

Chumlea, Guo, Cockram and Siervogel (1996) studied FFM and % BF by DXA and total-body and segmental impedance measures taken at 16 frequencies (from 5 to 1300 kHz) in a sample of white men and women aged 18-30 yr. Results showed that the segmental impedance spectrum variables for FM and % BF and the ratios of low- to high- frequency impedance from

the trunk were significantly associated with total body fatness as measured by DXA.

Gleichauf and Row (1989) assessed the reliability of resistance (R) and body composition estimates measured by BIA in 25 women during their menstrual cycles. To examine the effect of the menstrual cycle on BIA measures, each subject's cycle was divided into four stages: 1) menses, 2) follicular, 3) postovulatory, and 4) premenstrual. Weight and BIA were measured daily for one cycle and sodium intake was recorded. The precision of BIA was examined, and average resistance, weight, Na intake, and subjects' calculated body composition during the four phases of menses were compared using paired *t* tests.

Small, yet significant differences were observed between phases 1 and 2 for R ($P < 0.001$), weight ($P < 0.05$), and FFM ($P < 0.05$). Differences were also observed between phases 2 and 4 for R ($P < 0.05$), and weight ($P < 0.05$). No significant differences were observed for % BF. Changes in body weight ($P < 0.001$) associated with Na intake explained a significant proportion of error in R measures. The authors concluded that on average, BIA reliably measures body composition variables, regardless of menstrual status. However, for a given woman, alterations in hydration status (due to menstrual phase) may affect her results.

Another factor that may affect reliability of BIA is skin temperature. Gudivaka, Schoeller, and Kushner (1996) evaluated the effect of skin temperature on multifrequency BIA and the prediction of body water

compartments. Skin temperature was raised over 50 minutes from a baseline of 29.3 ± 2.1 °C to 35.8 ± 0.6 °C in 6 adults. Then, skin temperature was cooled for 20 minutes to 26.9 ± 1.3 °C. All heating and cooling was done by using temperature controlled blankets.

Impedance has been shown to vary inversely with changes in skin temperature across all frequencies (5-500 kHz). Thus, controlled ambient and skin temperatures should be included in the standardization of BIA measurements. Gudivaka et al. (1996) found that errors in predicted TBW are <1% within an ambient temperature range of 22.3 to 27.7 °C (72.1-81.9 °F).

Sources of Error for BIA

There may be several sources of error affecting the reliability and accuracy of BIA. Subject factors such as eating, drinking, and exercising may alter hydration state and thereby affect impedance measures and FFM estimates. Technician skill may also lead to incorrect measurements. However, skill should not be a major source of error, provided that the technician follows standardized procedures. The proximal sensor electrodes, in particular, need to be correctly positioned at the wrist and ankle. A 1 cm displacement of the sensor electrodes may result in a 2% change in R (Elsen, Siu, Pineda, & Solomons, 1987). Other potential sources of error include environmental factors such as ambient temperature and relative humidity.

BIA Prediction Models

The BIA method has been used to generate mathematical models relating impedance measurement to body composition variables. BIA

prediction models are generally one of two types. The first is population-specific. This type of equation is developed using a homogeneous sample in order to predict accurately to a like population. For example, individuals could be grouped by age, ethnicity, gender, physical activity level, level of body fatness, etc., and specific equations developed for each. These equations are only valid for, and should only be applied to, individuals whose physical characteristics are representative of the population subgroup.

The other type of BIA prediction equation is one that can be generalized to a larger population. These equations are developed for heterogeneous populations which may vary, for example, in age, gender, and body fatness. This approach can account for biological variability among population subgroups by including factors such as age and gender as predictor variables in BIA equations.

Regardless of the approach, the predictive accuracy of BIA equations typically is improved by including body weight, along with Ht^2, and R, and X_c in the BIA regression model. Since many of the assumptions previously mentioned may not be accurate, including body weight in the equation may be one way of accounting for the complex geometric shape of the body, as well as individual differences in trunk size (Kushner, 1992). To date, there are only a few BIA equations developed against multi-component models to derive reference measures of FFM (Guo, Roche, and Houtkooper, 1989; Lohman, 1992).

Body weight and height are significantly correlated with each body composition variable. It has been argued by some investigators that height and weight account for the majority of the BIA prediction of FFM or TBW (Mazess, 1991). Regardless, in studies where a heterogeneous population is represented, Ht^2/R appears to be the single best predictive variable for FFM or TBW (Kushner, 1992).

Studies Involving Female Athletes

According to Heyward and Stolarczyk (1996) there are two BIA equations that are appropriate for female athletes. One equation, developed by Lukaski and Bolonchuk (1987) was originally formulated using males and females with varying activity levels. Subjects included 312 individuals, aged 18-73 yr. Of these, 151 subjects were used to develop an impedance model for predicting FFM. The rest, aged 19-50 yr, were assigned to another group whose data were used to cross-validate the model.

Stepwise multiple regression analysis was performed to identify the best prediction equation which follows:

$FFM = (0.734 * Ht^2/R) + (0.116 * Wt) + (0.096 * X_c) + (0.878 * Gender) - 4.03$

($r^2 = 0.988$, SEE = 2.06 kg); gender is coded as 1=male, 0=female

The cross-validation produced a significant ($P < 0.0001$) correlation between FFM by UWW and FFM by BIA (r= 0.970, SEE=2.29 kg). Statistical analyses of the regression coefficients of this line indicated that the slope was not significantly different from one (F= 0.06, $P = 0.81$) and the intercept was similar to zero (F= 0.02, $P = 0.98$). Thus, the regression line was similar to the

line of identity. The authors concluded that BIA provides valid and acceptable estimates of body composition in healthy individuals.

Lukaski, Bolonchuk, Siders, and Hall (1990) used the equation discussed above on a group of 104 male and female college athletes. All athletes underwent UWW and BIA under controlled conditions (measurements made two hours after consuming a light meal and no preceding exercise) and uncontrolled conditions (measurements made without regard to preceding exercise, level of hydration, or eating).

Results showed that the relationships between FFM measured by UWW and BIA in both controlled (r= 0.988, SEE= 2.04 kg) and uncontrolled (r= 0.977, SEE= 2.85 kg) groups predicted FFM with no significant differences ($P > 0.05$) from the lines of identity. When comparing % BF measured by UWW and BIA, the regression line under controlled conditions was similar to the line of identity (r= 0.874, SEE= 2.81%). However, the relationship between % BF by UWW and BIA under uncontrolled conditions was different ($P < 0.05$) from the line of identity (r= 0.758, SEE=3.76%). Although only athletes were used as subjects, Lukaski et al. (1990) concluded that these findings indicated the need for controlled measurement conditions to obtain valid body composition estimates using the TBW method in "healthy people".

The second BIA equation for female athletes was developed by Houtkooper et al. (1989). The authors actually developed their equation on a group of 45 active and inactive females, aged 18-38 yr. FFM was determined

by UWW and the Siri equation, also correcting for BMC by dual and single photon absorptiometry. Results from an equation which apparently included R, X_c, and Wt showed an $r^2 = 0.83$, with SEE of 1.7 kg. However, the specific equation was not included in the publication, which was only in abstract form. Moreover, Heyward and Storlarczyk (1996) recommend this "equation" for female athletes in their book. Unfortunately, the equation included in the book is incorrect. However, the correct formula was published correctly in another body composition text (Lohman, 1992).

Near Infrared Interactance

Near infrared interactance (NIR), another method to estimate body composition, is relatively new. NIR was originally used to measure the protein, fat, and water content of agricultural products. Conway, Norris, and Bodwell (1984) applied this technology to study human body composition. They used an expensive, high-precision (6 nm) computerized spectrophotometer to measure % BF in 20 males and 33 females, aged 23-65 yr. The % BFs from NIR were compared to results from measurements of deuterium oxide dilution (r= 0.94), skinfolds (r= 0.90), and ultrasound (r= 0.89). The authors concluded that the noninvasive NIR technique was safe, rapid, easy to use, and may also be useful to predict % BFs in the obese.

After the Conway et al. (1984) study, a low-cost, portable, wide-slit spectrophotometer was designed by computer simulation for work in the field (Conway & Norris, 1987). Shortly after, a commercial NIR analyzer called the Futrex-5000 was developed and marketed based on the results of

these first two studies (Conway et al., 1984; Conway & Norris, 1987). The NIR method involves a wand which emits infrared light. The wand is typically placed over the belly of an individual's biceps brachii muscle and optical densities of remitted light from various tissues are measured.

Basic Principles of NIR

NIR works on the following principles. When electromagnetic radiation strikes a material, the energy is reflected, absorbed, or transmitted depending on the scattering and absorption properties of the sample. A probe, or wand emits electromagnetic radiation to a selected site on the body, collects interactive energy (which is the combination of reflected and scattered energy), and conducts it to the detector. This remitted energy is represented through optical density (OD) values. Subsequently, % BF may be determined from an equation which includes these optical densities. Interactance data are calculated by the instrument as the ratio of the energy received from a scan site to the energy received from a calibration standard, a one centimeter thick Teflon block. Thus, changes in optical density are calculated by:

Δ OD = OD standard - OD measured from body site

Assumptions of NIR

As with other body composition methodologies, there are assumptions associated with the NIR technique. One assumption is that the degree of infrared light absorbed and reflected is related both to the composition of the tissues and to the specific wavelength of the near-infrared light. Conway et al. (1984) demonstrated that peak absorption wavelengths are 930 nm for pure fat

and 970 nm for pure water. Also, the shape of the interactance curve at these two wavelengths is a function of the amount of fat and water present in the measured sample. The Futrex-5000 emits two wavelengths, 940 nm (resulting in OD_1) and 950 nm (resulting in OD_2), and then measures the amount of light reflected by the underlying tissues (Futrex, 1988). Therefore, OD changes are measured by:

Δ OD_1= OD standard - OD_1 from measured site

Δ OD_2= OD standard - OD_2 from measured site

It is not clear why the manufacturer selected these wavelengths (940 nm and 950 nm) instead of the wavelengths identifying pure fat and water (930 nm and 970 nm, respectively) (Heyward & Stolarczyk, 1996). Perhaps this was done because the body is composed of neither pure fat nor pure water, but a combination of the two.

Another concept central to current NIR methodology is that optical densities are linearly related to subcutaneous fat at the biceps site and total body fatness. To examine this, Quatrochi et al. (1992) examined OD as a function of skinfold thicknesses at different sites including: pectoral, midaxillary, biceps, triceps, subscapular, abdominal, suprailliac, thigh, and calf. Results indicated that OD values are significantly correlated ($P < 0.05$) with skinfolds, age and % BF (via UWW) in the pectoral, biceps, subscapular, and abdominal regions. The highest correlations included the pectoral (skinfold- 0.61, age- 0.53, and % BF- 0.70) and biceps regions (skinfold- 0.66, age- 0.42, and % BF- 0.71). While none of the relationships were extremely

high, the OD measures at the biceps appeared to correlate the best with skinfold thickness measures.

Elia, Parkinson, and Diaz (1992) also examined different body sites for correlations between optical densities and skinfolds at the site. Correlations for % BF were highest for the bicep at r= -0.71 for OD_1 and r= -0.75 for OD_2. This was in comparison to the triceps (r= -0.54 for OD_1 and r= -0.61 for OD_2), thigh (r= -0.36 for OD_1 and r= -0.41 for OD_2), biceps and triceps (r= -0.70 for OD_1 and r= -0.75 for OD_2, and biceps, triceps and thigh (r= -0.65 for OD_1 and r= -0.69 for OD_2).

In addition, Hortobagyi, Israel, Houmard, McCammon, and O'Brien (1992) examined NIR using multiple sites. Correlation coefficients indicated that the bicep was the best site for NIR measurement of optical density (when compared to skinfold thicknesses), although no site had particularly high correlations. Some examples of the correlations included: biceps at r= -0.67 for OD_1 and r= -0.68 for OD_2, triceps at r= -0.47 for OD_1 and r= -0.48 for OD_2, and the thigh at r= -0.45 for OD_1 and r= -0.40 for OD_2.

Besides the biceps being apparently the most valid body site to measure, there is an assumption that NIR light penetrates a depth of 4 cm and is reflected off the bone and back to the detector. This claim, which is made by the manufacturer, has not been proven through research. The measured optical density of the reflected light radiation is influenced by the specific absorption characteristics of the underlying tissue. Hence, the OD measures of NIR are supposed to measure both subcutaneous and intramuscular fat.

Even if this depth penetration is true, since skin and fat layers differ among individuals, the predictive value of resultant measures may be compromised.

Supporting this concept, Quatrochi et al. (1992) noted that the relationship between OD and skinfolds was strongest in the lean, young women; with the technique possibly underestimating the true fatness of older, more obese women. Also, Elia et al. (1992) commented that NIR was found to underestimate % BF increasingly as the degree of adiposity increased. Thus, differences apparently exist in the layering of skin and fat in individuals of various % BFs.

Reliability and Validity of NIR

Quatrochi et al. (1992) examined the reliability of optical density measures for NIR. The average reliability coefficients for ODs measured across two days ranged from r= 0.76 for midaxillary OD, r= 0.79 for thigh, r= 0.85 for calf, r= 0.87 for suprailiac, r= 0.88 for triceps, r= 0.93 for subscapular, r= 0.96 biceps and abdominal, r= 0.99 for pectoral.

Israel et al. (1989) evaluated the body composition techniques of NIR, three-, and seven-site skinfold equations (Jackson & Pollock, 1978), and BMI in 80 physically active males (average age was 25.8 yr). The criterion method used was UWW. The equation for NIR was provided by the manufacturer (Futrex, 1988). In addition to OD measures, the equation includes height, weight, age, gender, and activity level. Results showed correlations of r= 0.79 for NIR, r= 0.90 for seven-site skinfolds, r= 0.87 for three-site skinfolds, and r= 0.68 for BMI. Mean % BFs included: and 9.8% for NIR (SEE= 4.16%), 12.9% for

UWW, 12.9% for seven-site skinfolds (SEE= 2.9%), and 12.3% for three-site skinfolds (SEE= 3.4%). Important contributors for the NIR equation were OD_1, activity level, and weight. Age and height contributed less. From the results of the correlations and SEEs, the authors concluded that NIR did not accurately estimate body composition in white males.

Elia et al. (1990) investigated the body composition of 15 males and 14 females, aged 18-40 yr, using the following methods: NIR, whole-body impedance/resistance, skinfold thicknesses, and BMI. All techniques were compared to UWW. Results showed that NIR was highly correlated with UWW for males: FM (kg) (r=0.90, SEE± 2.29), % BF (r= 0.80, SEE± 3.10), and FFM (kg) (r= 0.91, SEE± 2.43). For females, NIR correlations with UWW included: FM (kg) (r= 0.93, SEE± 2.35), % BF (r= 0.82, SEE± 4.33), and FFM (kg) (r= 0.61, SEE± 2.60). However, BMI also correlated well with UWW for males: FM (r= 0.84, SEE± 2.80), % BF (r= 0.71, SEE± 3.66), and FFM (r= 0.88, SEE± 2.81). For females BMI correlations with UWW included: FM (r=0.97, SEE± 2.35), % BF (r= 0.82, SEE± 4.33), and FFM (r=0.62, SEE± 2.58). Thus, the authors concluded NIR had little or no advantage over other simple anthropometric techniques in evaluating body composition.

Hortobagyi et al. (1992) evaluated body composition using several techniques including NIR, UWW, and skinfolds with seven sites (Jackson & Pollock, 1978). Subjects included 171 men, with 52 black and 119 white males (aged 22.6±7.6 yr). Results showed the reliability for repeated NIR trials included: 0.97 for the biceps, chest, and abdomen; 0.95 for the supraillium;

0.87 for the subscapula; 0.73 for the thigh; 0.70 for the axilla; and 0.65 for the triceps.

Also, mean % BF for the criterion method of UWW was 13.4%. The average % BF for NIR using a combination of eight sites was significantly underestimated at 12.7%, r= 0.71, SEE= 3.64%. In addition, the average % BF by NIR using just the biceps underestimated % BF even more at 8.9%. However, the % BF average for skinfolds was much closer at 13.7%; r= 0.94, SEE= 2.9%. The authors summarized that when compared to the criterion of UWW, the error of prediction for NIR could reach ~ 4%. Thus, the authors concluded that NIR should not be used as an alternative to skinfolds in body composition analysis.

McLean and Skinner (1992) studied the body composition of 30 males (aged 33±7 yr) and 31 females (aged 31±6 yr) using UWW, NIR, and skinfolds (Golding, Myers, & Sinning, 1982). UWW was used as the criterion method. Mean % BFs for all 61 subjects included UWW with 20.8% ±8.2, NIR at 20.8% ±5.5, and skinfolds at 22.0% ±8.5. The correlation between UWW and NIR was r= 0.81, SEE= 4.8%; while the correlation between UWW and skinfolds was 0.94, SEE= 2.8%. Hence, skinfolds more accurately measured % BF than NIR when compared to the UWW % BF criterion.

Sources of Error for NIR

A major drawback to the ultrasonic and infrared interactance approaches is the dependence upon regional adipose distribution to predict total body fat (Lukaski, 1987). Also, as stated previously, NIR has been found

to be less accurate for obese individuals (Elia et al., 1990; Quatrochi et al., 1992) compared to lean subjects. For example, Elia et al. (1990) found that NIR underestimation of % BF (compared to UWW) increased as a function of adiposity. Elia et al. (1990) also stated that this underestimation was marked (16% body weight) in a small and separate group of grossly obese women, BMI > 50 kg/m^2. McLean and Skinner (1992) noted that NIR overestimated % BF in lean subjects with <8% BF and underestimated it in subjects with >30% body fat.

Also, errors may be occur if the infrared wand is not adequately shielded from extraneous light and the pressure exerted on the instrument may influence the recorded spectra (Jebb & Elia, 1993). Other possible sources of error may include subject factors such as skin color and hydration status (Elia et al., 1990; Heyward & Stolarczyk, 1996).

NIR Prediction Models

There has been little published research done with the NIR technique. The studies that have been done indicate an extremely strong relationship (r= 0.99) between OD_1 and OD_2 at the biceps site, with only small difference between average OD_1 and OD_2 measurements (Israel et al., 1989). Hence, only one OD typically enters in a prediction model. At present there are no NIR equations based on multi-component model estimates of % BF (Heyward & Stolarczyk, 1996).

Body Composition Literature Review Summary

Body composition has evolved with changes in technology and the advance of scientific research. Original techniques (e.g., UWW) are constantly being refined as investigators strive to achieve more accurate measures of body compartments. While UWW remains the popular criterion measure among a majority of researchers, advanced techniques are being developed with increased sensitivity to detect changes in body composition for wider varieties of population groups.

Single photon absorptiometry was developed to quantify appendicular bone mass. Precision and accuracy were both good but only certain sites could be measured versus whole body bone mineral content. Subsequently, DPA was developed to measure total body bone mineral content. The dual photon source also provided the ability to analyze fat mass and bone-free, fat-free mass. This enabled clinicians and researchers to measure three components of the body with DPA. However, the dual source was produced by 153 gadolinium, a radioactive compound. This isotope produced two characteristic photon energies which were difficult to control and standardize over time. Thus, DPA had only average precision and accuracy.

Replacement of the radionuclide source with an x-ray source resulted in the development of DXA. The evolution of DXA represented a major advance in the field of body composition analysis. Bone, fat, and fat-free, bone-free tissue could be directly analyzed with high precision and accuracy. This direct analysis of FFM by DXA offers a major advantage over UWW.

That is, the DXA technique eliminates the need to convert Db to % BF as is the case with UWW (which compounds the measurement error in body composition estimates).

Direct measurement of bone mass may be advantageous when studying female athletes. Female athletes may have a wider than average range of bone densities depending on menstrual history and training. The UWW technique assumes a constant FFM; with bone mass remaining constant as part of this assumption. Therefore, variations in bone density are not taken into account with UWW, while they are accounted for by DXA.

Although DXA has been shown to be both precise and accurate, this instrument is expensive and limited to clinical settings. In addition, other obstacles may include the lack of portability and the need for trained technicians. Thus, body composition field techniques are more desirable for coaches, trainers, and medical staff. However, these field techniques include various measured variables that require the development of regression equations to estimate body composition. Two commonly used field techniques include BIA and NIR. The validity of these techniques depends on the appropriateness of the regression equation for the population being tested. This equation should be based on a multi-component model to account for individual differences.

Since female athletes may differ in their bone mineral content, DXA appears to be an appropriate criterion method for this athletic population. In the present investigation, DXA was used as the criterion method to compare

to body composition estimates via BIA and NIR. Specifically, stepwise multiple regression was employed to develop separate equations (using the double cross validation procedure) to predict FFM using BIA and NIR. The purpose of this investigation was to determine the reliability and validity of BIA and NIR for estimating body composition of female athletes.

CHAPTER 3

METHODS

Subjects

We invited athletes from all Michigan State University (MSU) women's varsity sports to participate. These sports included basketball, crew, cross country, field hockey, golf, gymnastics, soccer, softball, swimming and diving, tennis, track and field, and volleyball. Athletes were recruited through the MSU athletic department from each team's coaches and athletic trainers. VanLoan and Mayclin (1992) have shown that one hundred to four hundred athletes are needed to form an equation which would be representative of this particular population.

The study was approved by the MSU committee on research involving human subjects (UCRIHS) and written informed consent was obtained from each participant. Although the medical staff expressed an interest in knowing some of the study results, care was taken to ensure that the athletes understood that their results would remain confidential unless they informed us otherwise.

Instrumentation

Dual energy X-ray absorptiometry (DXA)

The DXA instrument is a Hologic QDR-1000W, software 6.10. The instrument works on the physics principle that as x-rays pass through the body, the exiting attenuated signal is exponentially related to the path length, tissue density, and energy of the x-ray. The x-ray source, mounted beneath

the patient, generates a narrow, tightly collimated beam of x-rays which pass through the patient at rapidly switched x-ray voltage energies of 70 and 140 kVp. The transmitted intensity at each energy is measured by a radiation detector mounted on a C-frame (movable arm) directly above the x-ray source. During a scan, the C-arm oscillates rapidly in the transverse direction while slowly moving longitudinally.

Participants lie supine on the scanning bed and were scanned from head to toe in approximately 20 minutes. The instrument can be operated to examine the whole body or any region of the body, including arms, legs, head, and trunk. The area of the scanning table includes maximal values of 190.5 cm length and 63.0 cm width (Hologic Operator's Manual, 1990). Line spacing is the amount of scanning arm steps between each pass of the scanner arm, and is fixed at 1.303 cm for whole body scans. Resolution is the space between samples along each line and is .205 cm.

As the x-ray beam is introduced into the body, the external detector analyzes one small cross-sectional area (1 x 1 mm area) at a time. These small cross-sectional areas are called pixels. For the analysis, each pixel in the image is determined to be one of two components: bone or soft tissue. Spatial threshold is the outline around bone and determines the mass of the bone and tissue in adjacent areas.

Those pixels which do not contain bone are then re-analyzed as two compartments containing lean and fat tissues. The distribution of the lean and fat tissues overlying the pixels which contain bone is then estimated by

interpolation from the adjacent bone-free pixels. This is true of the "enhanced" software, 6.10. Therefore, DXA divides the body into three components: total body bone mineral, or ash; fat-free, mineral free soft tissue; and fat.

Bioelectric Impedance Analysis (BIA)

BIA (RJL analyzer; Detroit, MI) induces a small current through the body, providing resistance and reactance values (both measured in Ohms) from bodily tissues. This current measures TBW which is indicative of FFM (Hoffer et al., 1969). Individuals of a given height and weight will often different resistance to the current, based on the fat content of their bodies (Lukaski et al., 1985).

Procedures were performed based on methods described by Heyward and Stolarczyk (1996) in a setting of ambient temperature (~25 °C). For the BIA method, the athlete lay supine on a mattress, with the arms abducted 45 degrees from the legs (which are less abducted than the arms). All measurements are performed on the right side of the body and skin electrode sites were cleaned thoroughly with an alcohol pad.

The RJL analyzer was employed which delivers an alternating current of 800 μA at a fixed 50 kHz frequency (Heyward & Stolarczyk, 1996). Two electrodes induce the current source. Placements for these current source electrodes include: one electrode placed on the dorsal surface of the wrist so that the upper border of the electrode bisects the head of the ulna (sensor electrode, proximal); and the other electrode put on the dorsal surface of the

ankle so that the upper border of the electrode bisects the medial and lateral malleoli. This current is detected by the sensor electrodes which are located proximally to the source electrodes just described. The placement of the sensor electrodes includes: the base of the second metacarpal and phalangeal joints of the hand and foot. Also, there should be at least 5 cm between the proximal and distal electrodes (Heyward & Stolarczyk, 1996).

The lead wires are attached to specific electrodes. Red lead wires are attached to the wrist and ankle, with the red lead head attached to the wrist and the black lead head attached to the hand. Black lead wires are attached to the ankle and foot, with the red lead head attached to the ankle and the black lead head attached to the foot. Once the subject is properly set up, current is activated and then resistance and reactance values are recorded.

Near-Infrared Interactance (NIR)

NIR (Futrex, Inc.; Gaithersburg, MD), involves a wand, emitting infrared light, which is placed over the participant's biceps while she is seated. The assumption is that the degree of infrared light absorbed and reflected is related to both the composition of the tissues through which the light is being passed and the specific wavelength being emitted from the light (Conway et al., 1984). The peak absorption wavelengths for pure fat and pure water are 930 nm and 970 nm, respectively. The Futrex-5000 emits two wavelengths, 940 nm and 950 nm from the wand and subsequently measures the amount of light reflected by the underlying tissues as OD_1 and OD_2 (Futrex, 1988).

Measurements were made in accordance with the manufacturer's instructions (Futrex, 1990). Each subject was seated with her right arm extended (palm up) and resting comfortably on a table. The NIR wand was placed halfway between the antecubital fossa (of the elbow) and the acromion (the armpit). The light wand was held perpendicular to the measurement site. Caution was used to make sure that the shield flaps were pressed to the skin so that no light would penetrate and affect the optical density measurements. The same amount of pressure was exerted on the wand for each subject, since all measurements were performed by the same investigator.

To obtain the optical density measurements of OD_1 and OD_2 the "Clear" button was pushed, and the numbers 881 were entered into the NIR instrument (Heyward & Stolarczyk, 1996). Also, before each NIR measurement, the wand was calibrated with a teflon standard 1 cm thick. Optical densities were determined by:

ΔOD_1 = OD standard- OD_1 subject

ΔOD_2 = OD standard- OD_2 subject

Data Collection Procedures

Testing occurred in the rheumatology clinic, located in the St. Lawrence Health Science Pavilion (East Lansing, MI). On arriving, each female was measured for body composition by each of the three methods: DXA, BIA, and NIR. Trained investigators operated each station, and the athletes rotated through the stations. First, each subject had her standing

height and weight measured using a pre-calibrated beam balance scale and stadiometer. Next, each subject performed repeat BIA and NIR tests. The order of tests was BIA, NIR, repeat BIA, repeat NIR. Finally, all subjects underwent a single DXA analysis. The DXA machine was calibrated daily to a lumbar spinal phantom for bone density, and a tissue bar was also included in every scan for soft tissue analysis of FFM.

Clothing

Athletes wore the same long t-shirt with a sports bra and shorts for all methods of body composition except the DXA analysis. For the DXA measurement, only the long t-shirt and sports bra were worn. It is important that clothing be standardized and minimal for the DXA scan as this will contribute to the attenuation (Jebb & Elia, 1993). Cotton, for example, has an attenuation similar to that of fat (Jebb & Elia, 1993).

Pre-test Guidelines

Each athlete was given a set of written guidelines to adhere to before her designated testing date. These guidelines were distributed at either a team meeting or in the mail. The guidelines included (Heyward & Stolarczyk, 1996):

1. No large meals 4 hours before the test
2. No heavy exercise 12 hours before the test
3. Urinate immediately before the test
4. No alcohol consumption 48 hours before the test
5. No diuretic medications 7 days before the test

6. Drink 1% of body weight, or ~2 eight ounce glasses of water, 2 hours before the test

Statistical Analyses

Descriptive statistics (mean, standard deviation, and range) were calculated for each varsity team studied. Variables included age (yr), height (cm), weight (kg), body mass index (BMI), FFM (from DXA), and % BF (from DXA). Those individuals who were athletes, yet not members of a varsity team were included in a category termed "other". They were recruited via personal communication with the investigator. Also, if less than 10 members of a given varsity team were tested, then the subjects' results were added to the "other" category. While team values were calculated for descriptive and general comparison purposes, all reliability and validity analyses were conducted on total sample as a whole.

Reliability

BIA and NIR analysis were performed twice on each subject. Dependent variables included resistance (R) and reactance (X_c) for the BIA method (measured in ohms), and the two optical density values (OD_1 and OD_2) obtained with NIR (measured in nm). Intraclass correlation coefficients were determined for each BIA and NIR variable, using repeated measures analysis of variance (ANOVA) where $R_{xx'} = (MS_s - MS_e) / MS_s$ for a reliability estimate given both trials, and single = $(MS_s - MS_e) / (MS_s + MS_e)$ for an estimate given a single trial. MS_s was the mean square for subjects, and MS_e was the mean square for error (Baumgartner & Jackson, 1987). Standard

errors of measurement were also calculated according to the formula SEM = $S_x \sqrt{(1 - R_{xx'})}$ where SEM = the standard error of measurement, S_x was the standard deviation of the test measures, and $R_{xx'}$ was the reliability coefficient for the test scores. The SEM values were calculated in both absolute and relative (%) terms.

Validity

The FFM value measured by DXA was used as the criterion measure. Stepwise multiple regression was used to derive an equation for both BIA and NIR estimates of FFM. Predictor variables entered into the BIA equation were height, weight, R, and X_c. For the NIR equation, predictor variables included height, weight, OD_1, and OD_2. In all cases the R, X_c, OD_1, and OD_2 values used in the regression analysis represented the average of the repeat test values.

Prediction equations for BIA and NIR were developed using a double cross validation technique (Kerlinger & Pedhazur, 1973). That is, the subjects were split into two samples (based on odd or even identification numbers) and an equation was developed for each, with the opposite group being used to cross validate each equation. If the equations proved to be similar (evaluated by comparison of multiple r values, and visible inspection of graphs), groups were combined and a single equation was developed using the entire sample.

In addition to correlation and regression techniques, error analysis was also performed. Standard errors of estimate (SEE) were calculated (SEE = $s_y \sqrt{1}$

- r^2) and used as an error of prediction of DXA derived FFM using the BIA and NIR estimates. Total error (TE) was also calculated and used as an indication of standard deviation of error relative to the line of identity ($TE = \sqrt{(\Sigma(y - y')^2/n)}$) (Lohman, 1981).

CHAPTER 4

RESULTS

A total 135 female athletes between the ages of 18-27 years were recruited as subjects. The data of three subjects were dropped from the study; two subjects were too tall for the DXA scanner and the other one was suspected of having an eating disorder. Therefore, the final subject total for this study was 132 athletes. All but 14 subjects were varsity athletes currently participating at MSU. The "nonvarsity" athletes included four ice hockey players from the MSU ice hockey club team and ten former competitive athletes who were still physically active. Minimum activity for these additional athletes included five or more days of vigorous activity per week, 30 minutes per bout of exercise.

The total number of athletes studied represented approximately 65% of all members of women's varsity teams during the length of the study. Very few potential subjects (<10) who were told about the study actually refused to participate. The major reason for nonparticipation was a time conflict with the available testing schedule. Three teams, basketball, golf, and tennis were not included due to time and scheduling conflicts. Eight subjects were Black, one was Asian, and the rest were Caucasian.

Table 1 shows descriptive data for the subjects organized by teams. It should be pointed out that diving (n=3), swimming (n=6), track (sprinters and field events; n=8), volleyball (n=4) team data were merged with the "other" group due to small numbers. Also, cross country runners and track and field

athletes who specialized in the 800 m run (or longer) were combined in a group called "distance runners".

Reliability analysis is presented in Table 2. Reliability coefficients were extremely high for all measures when analyzed for both multiple and single trials. Specifically, BIA reliability ranged from R_{xx} = .987 - .997, while NIR values ranged from R_{xx} = .957 - .980. In addition to high reliability for both multiple and single trials, SEM values were low for all variables mentioned.

To assess the validity of BIA and NIR techniques, the 132 subjects were divided into two groups of 66, based on whether the subject identification number was odd or even. First, we calculated separate equations for BIA and NIR with the even numbered subjects. Then cross-validation was performed using the odd numbered subjects. Next, we did exactly the opposite, formulating additional equations for BIA and NIR using the odd subjects as the validation sample while the even subjects were used for cross-validation. Due to a few numbers out of order, two "odd" numbered subjects were randomly chosen and added to the "even" group. Thus, validity analysis was initially performed using two groups (EVEN and ODD) of 66 subjects each.

Table 3 shows BIA and NIR equations derived from EVEN numbered subjects. Also included in Table 3 are results of the cross validation sample (ODD numbered subjects). That is, subjects in the ODD group were used to predict FFM using the equation developed on the EVEN group subjects. Table 4 shows the equations developed with the ODD group subjects, and cross validated on their EVEN counterparts.

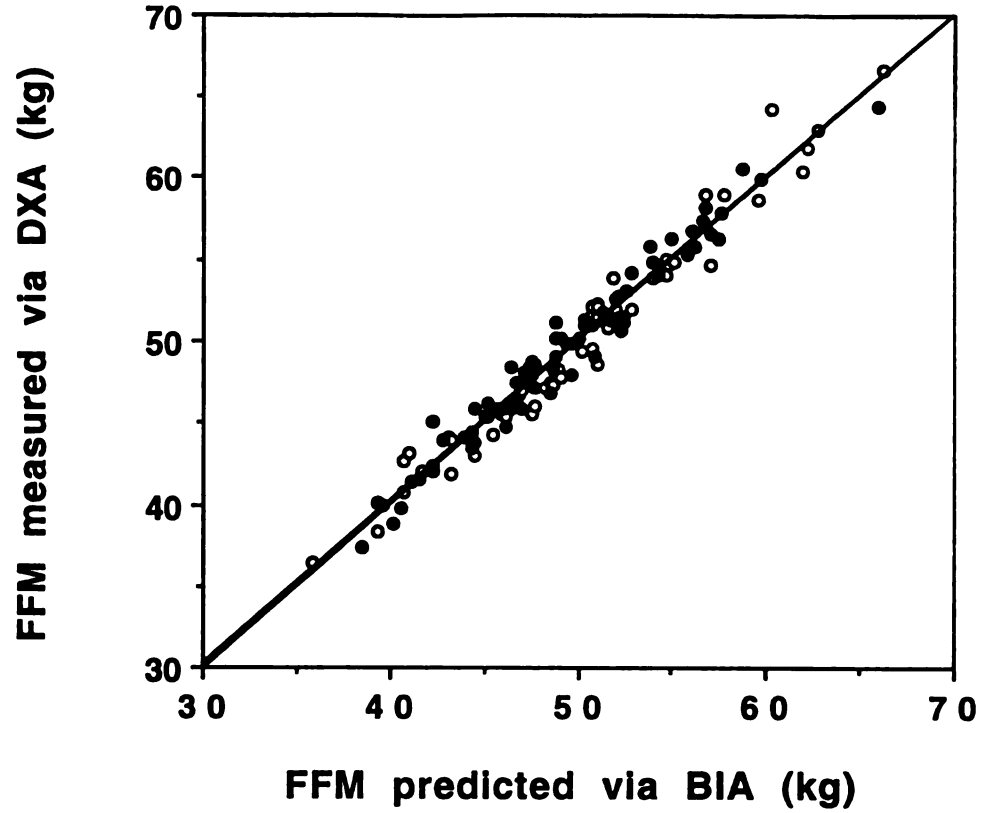
It is apparent from Tables 3 and 4 that the validity coefficients (r values), SEE, and TE were similar between EVEN and ODD samples, and that the cross validations showed similar results. Figure 1a and 1b presents a graphic representation of the closeness of the equations developed using the EVEN vs. ODD subjects. It is apparent that the regression lines were virtually identical, with deviation from the line of identities being similar for both samples. Thus, a single equation using all 132 subjects was developed for BIA and NIR prediction of FFM. In addition, height and weight alone were used to develop an equation. All three equations, validity coefficients, and error analyses can be seen in Table 5.

Figure 1a: FFM measured via DXA (kg) vs. FFM measured via BIA (kg)

Figure 1b: FFM measured via DXA (kg) vs. FFM measured via NIR (kg)

Legend- Even subjects are represented by hollow dots

Odd subjects are represented by filled dots


FFM measured via DXA (kg)
70
60
50
40
30
30
40
50
60
70
FFM predicted via BIA (kg)

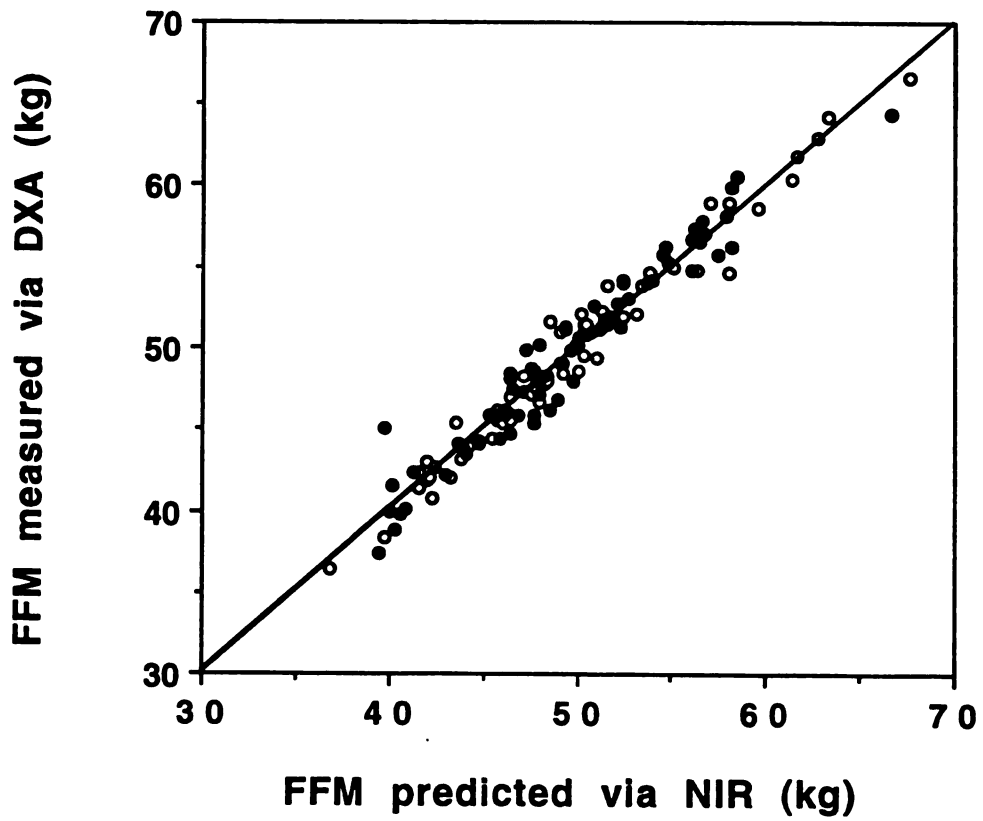

FFM measured via DXA (kg)
70
60
50
40
30
30
40
50
60
70
FFM predicted via NIR (kg)

Table 1: Age and anthropometric subject data (N=132)

Sport	Age (yr)	Ht (cm)	Wt (kg)	BMI	DXA (kg)	Wt DXA FFM (kg)	DXA % Fat
Crew (n=22)							
Mean	20.4	168.7	69.0	23.9	68.1	53.1	21.9
SD	1.9	7.4	7.8	1.6	7.7	5.4	2.3
Min	18.5	157.0	55.1	21.3	54.4	42.9	17.3
Max	27.2	182.0	90.9	28.1	89.5	64.4	28.1
Distance Runners (n=24)							
Mean	20.3	165.1	57.3	21.0	56.4	46.0	18.3
SD	1.1	6.6	5.0	1.2	5.0	3.8	2.7
Min	18.6	153.0	50.0	19.1	49.2	39.9	13.2
Max	22.5	175.0	68.2	24.8	67.6	53.1	23.9
Field Hockey (n=10)							
Mean	19.8	165.7	62.7	22.7	61.8	48.9	20.9
SD	1.2	7.5	10.6	2.4	10.6	8.5	4.1
Min	18.6	155.5	46.0	18.2	45.3	37.3	14.3
Max	21.6	180.0	81.2	26.8	80.6	64.2	27.3
Gymnastics (n=15)							
Mean	19.8	157.7	57.6	23.1	56.8	45.9	19.1
SD	1.0	5.6	7.2	1.7	7.1	5.4	2.2
Min	18.5	146.5	44.5	20.4	43.8	36.4	15.9
Max	21.6	166.0	68.8	25.9	68.0	54.7	24.2
Soccer (n=10)							
Mean	19.8	168.2	65.4	23.1	64.5	50.4	21.8
SD	0.9	4.9	7.7	1.7	7.7	5.8	2.7
Min	18.5	160.0	51.3	20.0	50.6	41.3	18.4
Max	21.3	177.5	81.2	25.8	80.2	61.8	26.3
Softball (n=17)							
Mean	20.4	170.2	66.8	23.0	66.4	52.4	20.9
SD	1.4	6.6	10.0	3.0	9.0	5.9	3.9
Min	18.1	160.0	46.8	17.6	54.6	44.2	15.5
Max	23.8	181.0	89.5	30.3	88.5	66.6	27.3
Other (n=34)							
Mean	21.1	166.8	63.4	22.7	62.5	49.6	20.5
SD	1.9	7.6	7.5	1.8	7.4	5.4	2.7
Min	18.5	152.0	49.0	19.4	48.3	38.3	14.7
Max	25.6	187.0	79.4	27.8	78.6	60.5	26.1
Total (N=132)							
Mean	20.4	166.4	63.1	22.7	62.3	49.5	20.4
SD	1.5	7.6	8.7	2.1	8.5	6.0	3.1
Min	18.1	146.5	44.5	17.6	43.8	36.4	13.2
Max	27.1	187.0	90.9	30.3	89.5	66.6	28.1

Table 2: Reliability estimates for BIA and NIR procedures (N=132)

Variable	R_{xx}	SEM	SEM%	Single	SEM	SEM%
R (ohms)	.997	3.8	0.7	.994	5.4	1.0
X_c (ohms)	.993	0.8	1.3	.987	1.1	1.8
OD1 (nm)	.978	0.016	19.5	.957	0.022	27.1
OD2 (nm)	.980	0.017	21.3	.961	0.024	29.6

BIA = bioelectrical impedance analysis using resistance (R) and reactance (X_c) measures

NIR= near-infrared interactance using two optical density (OD) measures

R_{xx}= the intraclass correlation coefficient for reliability of multiple trials

Single= the reliability estimate of a single trial

SEM= standard error of measurement

Table 3: Prediction Equations for FFM using EVEN numbered subjects (n=66)

DXA (measured FFM = 49.5±6.3 kg)

BIA

FFM = (0.272 * ht) + (0.461 * wt) - (0.036 * R) + (0.101 * X_c) - 11.567

predicted FFM = 49.6±6.2 kg r = 0.964 SEE = 1.2 TE = 1.2

(cross validation using ODD numbered subjects)

predicted FFM = 49.3±5.6 r = 0.983 SEE = 1.1 TE = 1.2

NIR

FFM = (0.065 * ht) + (0.72 * wt) - (14.334 * OD2) - 5.673

predicted FFM = 49.5±6.2 kg r = 0.984 SEE = 1.1 TE = 1.1

(cross validation using ODD numbered subjects)

predicted FFM = 49.6±5.5 r = 0.965 SEE = 1.5 TE = 1.7

FFM	=	fat free mass
BIA	=	bioelectrical impedance analysis using resistance (R) and reactance (X_c) measures
NIR	=	near-infrared interactance using two optical density (OD) measures
r	=	validity coefficient
SEE	=	standard error of estimate
TE	=	total error

Table 4: Prediction Equations for FFM using ODD numbered subjects (n=66)

DXA (measured FFM = 49.5±5.8 kg)

BIA

FFM = (0.284 * ht) + (0.38 * wt) - (0.037 * R) + (0.096 * X_c) - 7.85

predicted FFM = 49.4±5.7 kg r = 0.983 SEE = 1.1 TE = 1.1

(cross validation using EVEN numbered subjects)

predicted FFM = 49.6±6.3 kg r = 0.982 SEE = 1.2 TE = 1.3

NIR

FFM = (0.015 * ht) + (0.565 * wt) - (9.549 * OD_2) - 10.346

predicted FFM = 49.4±5.6 kg r = 0.969 SEE = 1.5 TE = 1.4

(cross validation using EVEN numbered subjects)

predicted FFM = 49.3±6.3 kg r = 0.981 SEE = 1.2 TE = 1.4

FFM = fat free mass
BIA = bioelectrical impedance analysis using resistance (R) and reactance (X_c) measures
NIR = near-infrared interactance using two optical density (OD) measures
r = validity coefficient
SEE = standard error of estimate
TE = total error

Table 5: Prediction Equations for FFM using ALL subjects (N=132)

DXA (measured FFM = 49.5±6.0 kg)

BIA

FFM = (0.282 * ht) + (0.415 * wt) - (0.037 * R) + (0.096 * X_c) - 9.734

predicted FFM = 49.4±5.9 kg r = 0.981 SEE = 1.1 TE = 1.1

NIR

FFM = (0.111 * ht) + (0.641 * wt) - (12.397 * OD_2) - 8.423

predicted FFM = 49.5±5.8 kg r = 0.975 SEE = 1.2 TE = 1.2

Height and Weight

FFM = (0.143 * ht) + (0.565 * wt) - 10.03

predicted FFM = 49.4±5.7 kg r = 0.961 SEE = 1.6 TE = 1.6

FFM = fat free mass
BIA = bioelectrical impedance analysis using resistance (R) and reactance (X_c) measures
NIR = near-infrared interactance using two optical density (OD) measures
r = validity coefficient
SEE = standard error of estimate
TE = total error

CHAPTER 5

DISCUSSION

Accurate body composition assessment is beneficial for female athletes, as it can be indicative of health and performance status. Our data provide measured (DXA) and predicted (BIA and NIR) FFM and % BFs for the individual sports of crew, distance runners, field hockey, gymnastics, soccer, softball, and other female athletes. The study purpose was to determine whether the BIA and NIR field techniques could provide both reliable and valid estimates of body composition in this population.

In this study, both BIA and NIR techniques were used to predict FFM as compared to a value obtained from a criterion measurement instrument (DXA) that can separate fat free bone and soft tissue from fat tissue. In theory, BIA measures the conductivity of TBW and electrolytes which are found exclusively in the FFM. The NIR is designed to differentiate between the water and fat contents of the various body compartments by the amount of light that is absorbed and reflected from an infrared source. Both TBW and electrolytes have been shown to be highly correlated to FFM (Kushner, 1992).

While FFM may be the appropriate variable to be measured and predicted in this investigation, most body composition studies in the literature have centered around an individual's % BF. This allows a more logical point of comparison when discussing differences among groups that differ widely in absolute body mass.

As can be seen in Table 1, there was little variability in either FFM or % BF measures when making comparisons either between and within our different sport teams. As a group, distance runners had the lowest values (FFM=46.0±3.8 kg; %BF=18.3±2.7%) and crew had the highest (FFM=53.1±5.4 kg; %BF=21.9±2.3%). On the whole, FFM measured by DXA averaged 49.5±6.0 kg and %BF averaged 20.4±3.1 % in the 132 athletes we tested. Indeed, even the 14 "other" non-varsity athletes had similar values, supporting their inclusion in our sample.

Many body composition studies have been performed with female athletes. For instance, elite runners (training distance of 64.5±15.8 miles/week) have been measured at 14.3% with UWW (Graves, Pollock, & Sparling, 1987). Picard, Kyle, Gremion, Gerbase, and Slosman (1997) used DXA to determine body composition in elite runners (mileage values of 82±6.4 km/week) and reported average BF was 14.8%. The athletes in these two studies had somewhat lower % BFs than our distance runners who averaged 18.3±2.7%. This difference may be due to lower mileage run by college distance runners versus highly elite runners who are more seasoned and typically have more rigorous training schedules.

Withers et al. (1987) investigated the % BFs of female soccer and softball players using UWW. Their results showed an average of 22% for soccer players and 19.1% for softball athletes. These values are consistent with our data, which showed BF values of 21.8% for soccer players and 20.9% for softball.

The body composition of gymnasts has been studied also. Sinning (1978) found a mean value of 15.3% in champion gymnasts using UWW. In contrast, Kirchner et al. (1995) found an average value of 17.0% for collegiate gymnasts. Our results agree more with the Kirchner et al. study for our gymnasts averaged 19.1 %BF. A possible reason for the difference in % BFs may include that Sinning's gymnasts were all collegiate champions, and were taller and lighter than our sample.

Sinning and Wilson (1984) measured body composition (UWW and skinfolds) in a group of 79 women who were similar to our overall study sample. Their subjects included women who participated in a variety of intercollegiate sports at Kent State University. The average % BF for these athletes was 20.1%, while the average % BF for athletes in our study was 20.4%.

Although most other studies have utilized UWW as the criterion, DXA has been shown to correlate well with UWW for eumenorrheic female subjects (Hansen et al, 1993). Also, given the BMC assessment included in all DXA body composition estimates, it is likely to be a more widely used criterion measure than UWW in the future (Heyward & Solarczyk, 1996). Further contributing to DXA's validity, studies have shown high correlations between scale weight and DXA (Going et al., 1993; Prior et al., 1997) and also DXA and a four component model (Prior et al., 1997). It is of interest to note that in our study, DXA values for weight were highly correlated with (r=0.999) and only slightly above (<1%) scale weight values as seen in Figure 2.

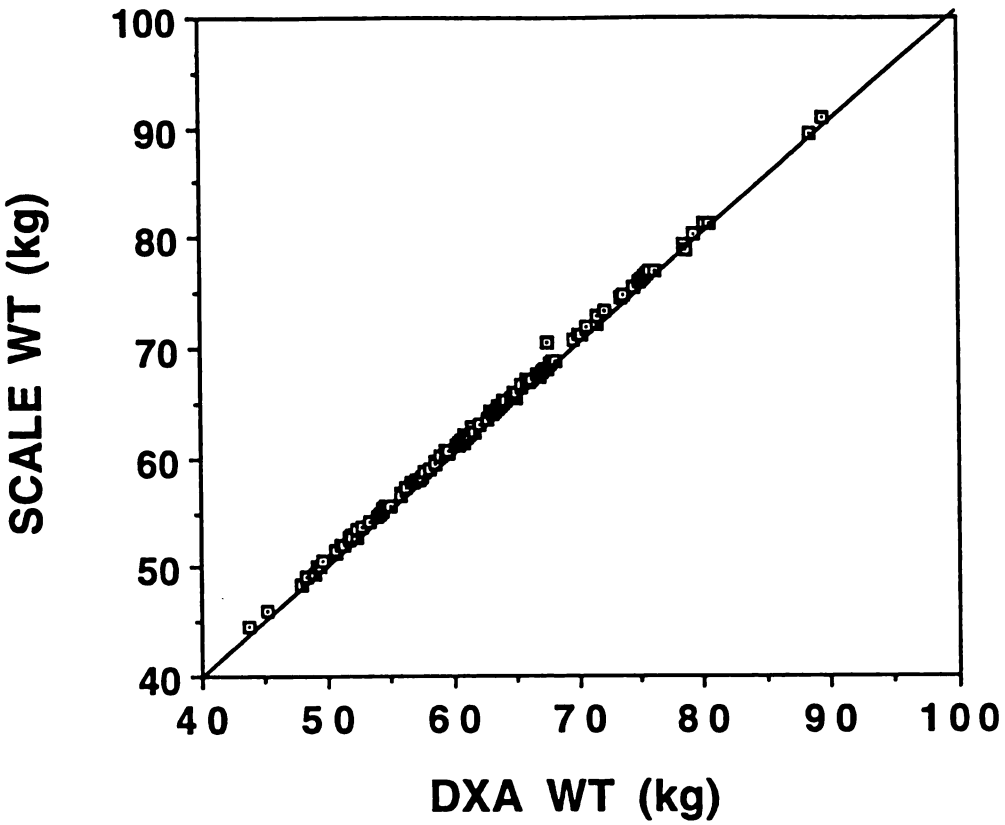


Figure 2: Scale weight (kg) vs. DXA weight (kg)

This overestimation was small and should not have contributed significant error to our body composition estimates.

DXA is believed to be a more sensitive measurement technique for body composition than UWW because it is theoretically independent of compartmental assumptions (Lukaski, 1987). However, DXA is an expensive device (~$60,000) and is not readily available to trainers, coaches, or even most medical staff. Therefore, other methods are frequently utilized in field settings. These techniques, if shown to be reliable and valid, are more

desirable due to their reduced cost, portability, and little operational training required.

The field techniques employed in this study include BIA and NIR. The reliabilities of BIA and NIR variables were assessed by calculating multiple and single trial reliability coefficients. Single trial reliability for BIA was high at 0.994 and 0.987 for R and X_c, respectively. Also, the single trial reliability for NIR was also high at values of 0.957 and 0.961 for OD_1 and OD_2, respectively. Previous investigations of the reliability of R in BIA studies have shown similar results. Some of these include: 0.957 for men and 0.967 for women (Jackson et al., 1988); 0.99 for men (Lukaski et al., 1985); and 0.907 for women and 0.946 for men (Segal et al., 1988). Limited research has been performed investigating the reliability of OD measures used with NIR. However, studies have shown reliability for the biceps site to be 0.96 (Quatrochi et al., 1992) and 0.97 (Hortobagyi et al., 1992). These values are similar to the values found in the present study.

The high single reliability coefficients found in this study indicate that only a single trial is necessary when utilizing the field techniques of BIA and NIR. This is important for the busy sports training staff faced with caring for hundreds of athletes on a daily basis. However, it is important to note that the high reliability coefficients obtained in this study were likely a function of the investigator paying close attention to manufacturers' instructions regarding measurement techniques.

The accuracy of results in these field techniques depends on specific equations developed for each technique. Our equation for BIA predicted FFM and used height, weight, resistance, and reactance. Our NIR equation also predicted FFM and included the variables of height, weight, and OD_2. OD_1 was also a high predictor of FFM, but slightly less than, and also highly correlated with, OD_2. Consequently, it was not entered into the equation. Equations developed for specific populations (such as a similar age group and activity level) improve the accuracy of predicting body composition in a comparative population. Additionally, cross-validation of these equations is important to test for accuracy.

To illustrate the point discussed above, Sinning and Wilson (1984) studied the validity of body composition analysis using several skinfold equations in a group of females athletes (n=79), age 17.8 to 22.5 yr. Only two of the nine equations tested were found to be in agreement with values obtained via UWW. The authors pointed out that the failure of all of these equations to be acceptable emphasized the need for further cross-validation studies.

In another study, Pichard et al., (1997) studied the relationship between FFM and FM calculated in elite female runners with 12 different BIA formulas reported in the literature. The criterion measure was DXA (Hologic QDR-2000, software 5.54). There were 17 elite runners included in the study, age 15-39 yr, and 17 female control subjects who were age and height matched. The authors discovered that those equations that performed well in the controls gave poor results in the elite runners. Likewise, the formulas that

were more predictive in elite runners were less accurate with control subjects. This suggests that the subjects represented two distinct groups that must be evaluated with population specific prediction equations.

In our study, we tested 132 female athletes. All had equivalently high levels of physical activity which included daily (or twice daily) sessions of weight training and aerobic conditioning in both the on- and off-season.

Tables 3 and 4 show that all correlations were extremely high, and similar for both BIA (r=0.964-0.983) and NIR (r=0.956-0.954) whether validation or cross validation samples were used. Similarly, SEE and TE values were very low and consistent in all cases (1.1-1.4 kg). Additionally, a graph comparing the two equations (Figure 1) indicated virtually identical lines for both BIA and NIR. This graph added further support for combining both samples and developing a single equation on all 132 subjects. Not surprisingly, the single sample regression lines for BIA and NIR (Table 5) were nearly identical to all others developed from the split sample.

Pichard et al. (1997) used 12 BIA equations, including two provided by the manufacturer (RJL Systems; Detroit, MI) and nine other equations commonly found in the literature. The best equation for elite runners was found to be RJL Systems-2 equation which had FFM results of r= 0.90, SEE= 2.0 kg, TE= 2.1 kg. We tested this equation with our data (N=132) which resulted in an average FFM of 48.7 kg, r= 0.94 SEE= 2.0 kg, TE= 2.7 kg. While the correlation using the RJL equation on our subjects was high, FFM was

underpredicted and error terms were nearly twice as high as those in our BIA equation.

As previously mentioned, only two published BIA equations exist which have specifically included female athletes in the validation samples (Houtkooper et al., 1989; Lukaski & Bolonchuk, 1987). Again, we tested both equations using our subject data. Results for the Houtkooper et al. (1989) equation included: average FFM= 49.3 kg, r= 0.935, SEE= 2.0 kg, and TE= 2.2. Also, results for Lukaski & Bolonchuk (1987) were: average FFM= 46.8 kg, r= 0.94, SEE= 2.0 kg, and TE= 3.4 kg. As was the case with the RJL equation, our equations had slightly higher correlation values, and lower SEE and TE. One possible explanation for these differences may be that our study utilized female athletes exclusively while the other equations were developed using both active and inactive female subjects.

As stated earlier, no published research study has formulated an equation for female athletes using NIR to assess body composition. Authors of other NIR studies suggested not to use the Futrex formulas provided by the manufacturer (Heyward & Stolarczyk, 1996). Thus, we are the first to develop an equation for female athletes using NIR. From our data, the NIR technique was shown to be as valid as BIA for predicting FFM in this population.

Some investigators have questioned the ability of field techniques such as BIA and NIR to add significantly to body composition estimates. Specifically, how much do the R, X_c, and OD values add to height and weight when developing appropriate prediction equations? To address this issue, we

developed an equation based only on the heights and weights of our athletes (Table 5). Our results showed a predicted FFM value of 49.4 kg, r= 0.961, SEE= 1.7, and TE= 1.6. Given this finding, the results for BIA and NIR may appear less impressive. The predictive ability of this height and weight regression equation may be more of a function of the homogeneity of our population rather than an inadequacy of the field techniques. However, this same homogeneity likely attributed to the accuracy of our BIA and NIR equations as well.

The following example utilizing TE analysis may put the issue utility of BIA or NIR vs. height and weight into perspective. For example, DXA values averaged 62.2 kg for weight, 49.5 kg for FFM, and 20.4% for BF. The BIA equation predicted a FFM of 49.4 kg, with TE= 1.1. The NIR equation yielded a FFM value of 49.5 kg, with TE= 1.2 kg. Finally, the height and weight equation predicts FFM to be 49.4 kg, with TE = 1.7. All three predictions appear very similar. However, by converting FFM predictions into % BF values, further comparisons can be made. For example, FFM from BIA ranges from 48.3-50.5 kg, which corresponds to a % BF of 22.3-18.8. Compared to DXA values, the TE (deviation around the line of identity) for BIA % BF was 1.9%. Similarly, NIR FFM is 48.3-50.7, with % BF 22.3-18.5. Thus, NIR results in a 1.8% BF error. Lastly, height and weight predict FFM to be 47.7-51.1, which corresponds to a % BF 23.3-17.8. Hence, height and weight yields a 2.9% error for % BF (~33% more error than BIA and NIR equation).

One might argue that an increase in predictive accuracy for BF of only 1% is not worth the trouble and expense of using techniques such as BIA and NIR. Indeed, each apparatus may cost from $2,000 - $3,000 or more. However, the reality is that these techniques are being used all over the U.S. and the world. Practically speaking, it may be difficult to convince coaches and medical staff that this loss of accuracy is balanced by the money and time saved. This is a decision that will certainly involve more study to completely resolve. In the meantime, our data show that the BIA and NIR techniques are extremely reliable and valid for estimating body composition values in collegiate female athletes. The equations developed in this study were shown to be slightly better predictors than using height and weight alone.

Recommendations for Future Studies

1. Measure body composition with the additional method of UWW as a further validation for DXA.
2. Measure changes in body composition with DXA, BIA, and NIR. For example, before and after a sports season, weight loss, or maturation (i.e. freshman to senior years in college).
3. Recruit female athletes from other sports teams not included in this study.
4. Cross-validate the BIA and NIR equations on an athletic population in other sites.
5. Perform additional reliability and validity studies with NIR.
6. Evaluate individual differences in body composition based on menstrual patterns, type of exercise, and diet.

CHAPTER 6

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