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**RELIABILITY OF THE BOD POD[®] COMPARED TO
TRADITIONAL REFERENCE METHODS FOR
MEASURING BODY COMPOSITION**

BY

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**A THESIS SUBMITTED TO THE DEPARTMENT OF NUTRITION
AND FOOD SCIENCE, UNIVERSITY OF GHANA, IN PARTIAL
FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF
MASTER OF PHILOSOPHY DEGREE (MPhil)**

**DEPARTMENT OF NUTRITION AND FOOD SCIENCE
UNIVERSITY OF GHANA
LEGON**

JULY 2001

ABSTRACT

In the mid 1990s, a new air-displacement plethysmograph (ADP) was developed for measuring human body composition. This device (BOD POD[®] Body Composition System) uses the relationship between the pressure and volume of air to measure the body volume of a subject seated in the test chamber. Body density (D_b) is then calculated using body mass and body volume and percent body fat (% BF) estimated using an equation such as the one by Siri (1961). This study evaluated the reliability of the BOD POD compared to dual-energy X-ray absorptiometry (DXA), underwater weighing (UWW) and isotope dilution for the measurement of percent body fat (%BF). Twenty healthy Caucasians (12 males and 8 females) aged 25 to 81 years, with mean \pm SD body mass (75.9 ± 18.1 kg), height (169.9 ± 8.3 cm) and BMI (26.1 ± 5.1 kg/m²) volunteered. %BF was measured twice on day 1, after an overnight fast and once on day 2. Also assessed was within-day and between-day reliability was assessed. The two test days were usually scheduled 5 – 8 days apart and were randomized. Results showed there were no significant differences among the three test sessions in %BF measured by any individual method, or calculated by multi-compartment models. For the within-day variability, the BOD POD had the smallest within-subject SD (0.3 %BF) compared to UWW which had the highest within-subject SD (1.0 %BF). Between days, DXA had the lowest within-subject SD (0.5 %BF) but was followed closely by the BOD POD (0.7 %BF). The actual error for estimating %BF by multi-compartment models was found to be smaller than theoretically calculated and was smallest when D_b from BOD POD was used (0.1 %BF) compared to when D_b from UWW was used (0.6 %BF). Neither subject characteristics nor the time between tests was significantly associated with variability in measured %BF

for any method either within or between-days. Moreover, the difference in oral and skin temperatures was not associated with the difference between-days in %BF measured by the BOD POD after controlling for confounding factors. Thoracic gas volume (TGV), associated with the BOD POD, and residual lung volume (RV) associated with UWW, had similar reliability; however heavier subjects had a higher RV on the second within-day trial. These findings indicate that the BOD POD is among the most reliable methods. This study supports the use of the BOD POD in clinical and nutrition research.



DECLARATION

This study was conducted and presented to the Department of Nutrition and Food Science, University of Ghana, Legon, by Alex Kojo Anderson under the supervision of Dr. Anna Larrey of the Department of Nutrition and Food Science, University of Ghana, Legon.



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DEDICATION

TO MY MOTHER, CONSTANCE QUARTEY AND MY BROTHER, CHRISTIAN ANDERSON FOR EDUCATING ME UP TO THIS LEVEL AND MY FRIEND DOREEN AMANFU FOR HER SUPPORT AND ENCOURAGEMENT ESPECIALLY WITH THIS RESEARCH IN THE U.S.

ACKNOWLEDGEMENT

I owe a great debt of gratitude to the Head and Faculty of the Department of Nutrition and Food Science, University of Ghana for honoring me with a year study in U.S. to conduct this research. My sincere thanks also go to the Chief of the Energy Metabolism Laboratory, Susan Roberts (PhD) for accepting me into her Laboratory. I gratefully acknowledge the tremendous directions, contributions and suggestions made to the success of the study by Megan McCrory (Ph.D.) who is also the Principal Investigator of the study and in whose laboratory this research study was conducted. Special thank you also goes to Anna Lartey (Ph.D.), who is my supervisor at the Department of Nutrition and Food Science, University of Ghana, Legon, for her contributions and guidelines towards the successful execution of this thesis report.

I am very grateful especially to Mr. Paul Fuss of the Energy Metabolism Lab for all the time and effort he spent in teaching me to use the different techniques and methods of the Lab for measuring body composition and all other members of the lab in diverse ways to make this study a success. I also want to thank my colleagues and the entire non-teaching staff of the Department of Nutrition and Food Science, University of Ghana for the friendship and support throughout my study.

To my grandparents, Mr. and Mrs. Cato, my brothers and sisters I say a very big thank you for your emotional and spiritual support up to this stage of my life. I gratefully acknowledge the guidance of my academic advisor, Prof. Marilyn Glater and her unrelenting effort to help whenever I need assistance. To Dr. (Mrs.) Matilda Asiedu, I say

a big thank you for all the advice and helping shape my career interest. To all friends and well-wishers I say a big thank you and God richly bless you.

Finally, I will want to thank Megan O'Neill for conducting the DXA measurements, Irene Ellis for advice and direction during isotopic analysis and all the nurses, also the staff at the admissions and recruitment departments, Dr Ed Saltzman (Study Physician) for screening subjects, Karen Leary and all the kitchen staff at the HNRC for their professional role in making this research a success.

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Chapter 1

INTRODUCTION

1.1 Background

Body composition analysis allows quantification of major structural or functional body compartments (e.g. bone, muscle and fat). For the assessment of physical, nutritional and health status of an individual, accurate methods for measuring body composition are necessary. For example information on changes in body composition in response to growth and development as well as dietary intervention, is essential for establishing the appropriate prognosis and treatment of malnourished individuals and others with chronic conditions (e.g. obesity, diabetes mellitus and coronary heart diseases).

Many methods currently used for measuring body composition are based upon two-, three- or four-compartment models that divide the human body into different compartments at the tissue level (Heymsfield et al, 1990). The basic two-compartment model assumes that the total body mass is composed of two major compartments: fat mass (FM) and fat free mass (FFM); the approach of the three-compartment model requires the measurement of either total body water (TBW) or bone mineral content in addition to body density (D_b); whereas the four-compartment model divides the body into four compartments: water, protein, minerals and fat and requires the measurement of D_b , TBW, and bone mineral content.

Among the various body composition methods that are popularly and widely used are underwater weighing (UWW), dual energy X-ray absorptiometry (DXA), and bioelectrical

impedance (BIA), the latter two exposing the subject to a small amount of radiation and electrical current respectively. Other methods include skinfold measurement, isotopic dilution, near-infrared interactance (NIR), magnetic resonance imaging (MRI), total body electrical conductivity (TOBEC) and computed tomography (CT). Many of these methods have been previously reviewed (Fuller et al, 1990; Martin and Drinkwater, 1991; Roche et al, 1991; Jebb and Elia 1993; Roubenoff et al, 1993; Van Loan, 1998).

Air displacement plethysmography (ADP) is a relatively new method for body composition measurement with little known about its reliability in comparison to reference methods. Although a variety of methods exist for body composition assessment in the study of obesity and human nutrition, their accuracy, precision, and cost can often be questioned. Furthermore, they are often difficult and inconvenient to carry out on regular basis. There is no traditional method that is widely used and accepted by both the scientific and clinical communities as providing valid and precise estimates of body composition, which is at the same time simple, quick, relatively inexpensive, and potentially applicable to a wide variety of subjects (e.g. lean and obese, younger and older, able-bodied and infirm).

1.2 Rationale of the Study

The association of percentage body fat (%BF) to the etiology of various chronic diseases (e.g. cardiovascular disease and diabetes mellitus) calls for an accurate, precise and easy to administer method of assessing body fat irrespective of age, physique and cost, and one that also guarantees the subject's or patient's comfort. The UWW method, which used to be thought of as the "gold standard," has come under criticism for some time now. The high cost involved in assembling the various compartments of an UWW system as well as the technical expertise required for its operation makes it not very available, especially to the developing world. The difficulty of the protocol and inconvenience of UWW, which limits its accessibility to certain subpopulations like children, elderly and the mentally challenged, may lead to errors in percent body fat (%BF) estimation. Although the DXA devices are easy to operate and the method non-invasive, it exposes subjects/patients to a small amount of radioactivity (equivalent to 1-2 days of natural background radiation), which also limits its use in certain subpopulations, such as children and pregnant women. Regardless of which body composition method is used, an accurate measurement of body compartments is critical. Therefore, there is a need for a non-invasive, easy and reliable method for body composition measurement, which ADP may potentially provide.

1.3 AIM

The primary purpose of this study is to evaluate the reliability of ADP compared to commonly used traditional reference methods, including DXA, UWW, TBW, and also to multi-compartment models.

1.3.1 Specific aims

1. To determine the reliability of the different methods for assessing body composition both within a day and between days.
2. To determine factors associated with within-subject differences in body composition measured by the different methods. Potential factors to be examined will include subject characteristics (age, weight, height, and BMI) and length of time between tests. Additional factors to be examined for ADP only are within-subject differences in skin, oral, and room temperatures.
3. To calculate and compare %BF from multi-compartment models of body composition (3-compartment and 4-compartment models) using both ADP and UWW for body density, and to assess the “theoretical” overall error based on the Law of Propagation of Errors in comparison to the actual errors.

1.3.2 Hypotheses

1. The within-day and between-day reliability of ADP for measuring percentage body fat (%BF) will be significantly greater than that for DXA, UWW and TBW.

2. Within-subject variations in thoracic gas volume (TGV) associated with ADP will be significantly less than within-subject variations in residual lung volume (RV) associated with UWW.
3. Within-subject variations in body composition measured by ADP will be significantly associated with within-subject variations in skin, oral and room temperatures.

1.4 Significance of the Study

It is hoped that this study will provide data on the reliability of the BOD POD compared to DXA, UWW, TBW and multi-compartment models for body composition assessment. The outcome of this study will determine with what precision body composition can be measured by the BOD POD in comparison to traditional reference methods and hence its ability to precisely quantify changes in body composition.

The present thesis represents preliminary findings using the first 20 subjects from an ongoing study at the HNRC examining the validity and reliability of the BOD POD compared to traditional reference methods in young/lean, obese, and elderly men and women.

Chapter 2

LITERATURE REVIEW

2.1 Methods for Body Composition Assessment

This section provides a brief overview of the principles, advantages and limitations of each of the methods examined in this study.

2.1.1 Underwater Weighing (UWW)

One of the most commonly used methods for measuring body composition, UWW (Behnke et al., 1942) requires the subject to be completely submerged in water (*Appendix 1*). The subject's weight under water combined with the subject's weight on land are used to compute the density of the whole body. This method relies upon Archimedes' principle, which states that a subject's loss of weight in water compared to land equals the volume of water displaced, which is in turn equivalent to the subject's body volume. Because bone and muscle are more dense than water, a person with a larger percentage of FFM will weigh more in water and have a lower %BF. Conversely, fat floats, therefore, a larger amount of FM will make the body lighter in the water, reflecting the higher %BF. Body volume is then corrected for the amount of air left in the lungs after maximally exhaling (to residual lung volume or RV, measured by gas dilution, either simultaneously with water submersion, or during a separate procedure on land). The body density is determined using the relationship between mass and volume (Density=Mass/Volume) by the following formula:

$$\text{Density (D}_b\text{)} = M_a / [((M_a - M_w) / D_w) - RV]$$

where M_a is body mass in air, M_w is body mass in water, and D_w is the temperature-corrected water density. Body density is converted to %BF by using any of the previously determined two-compartment models, such as that by Siri (1961), which assumes the densities of the FM and FFM are 0.9 g/ml and 1.1 g/ml, respectively ($\%BF = 495/D_b - 450$) or ($\%BF = 457/D_b - 414.2$, Brozek et al, 1963).

The UWW method typically requires the subject to completely submerge under water while maximally exhaling as much air from the lungs as possible. Thus, the validity of UWW, in part, depends upon how well the subject performs the maneuver: If the subject fails to exhale as much air from the lungs as possible for RV measurement on land as well as in the water, or fails to completely submerge themselves under the water, the obtained body composition estimate will be incorrect. If each test is performed correctly according to the recommended guidelines, there is a +/- 1.5% error (Siri, 1961). (Accuracy partly depends on the subject's ability to exhale the same amount of air out of the lungs both during RV measurement and during the UWW procedure itself. Since air makes the body float, failure to perform these maneuvers to the same extent will result in miscalculation of %BF). The UWW procedure is very tedious and impractical for certain categories of subjects, especially for obese, elderly and infirm individuals. Also, very obese subjects have difficulty submerging themselves completely in water because of the high adiposity, which makes them more buoyant with the excess weight around the abdomen making it hard for them to crouch and exhale all the air in their lungs. Again, even among relatively lean and motivated subjects, one can never be entirely confident that a subject has exhaled as much air as he/she needs to exhale. Although this problem can potentially be overcome by measuring the amount of air in the lungs simultaneously with water submersion, this procedure may underestimate the air in the lungs,

particularly in elderly subjects, due to closure of some pulmonary airways (“gas trapping”) while under water, rendering them inaccessible to the dilution gas being used (Dahlback and Lundgren, 1972; Robertson et al., 1978).

Advantages

1. It is traditionally considered the “gold standard” of body composition measurement.
2. It is believed to produce fairly accurate results in most subjects below age 60 years when compliance issues are followed properly.

Limitations

1. This method requires a lot of space with a high maintenance cost for the upkeep of equipment.
2. The testing process is very lengthy and tedious (45 min – 1 hr).
3. The procedure requires in-depth knowledge; hence the technician must be highly skilled to administer the tests in order to obtain accurate results.
4. Repeated measurements can be extremely arduous and difficult.
5. Being submerged under water may be difficult and produce anxiety for some subjects, especially the very obese, handicapped or the elderly, and also individuals who are uncomfortable with water submersion.
6. The assumption that FFM density is 1.1 kg/L, upon which calculations are made, does not apply to certain populations especially the elderly, children, and very obese.
7. UWW is typically only available at research institutions and colleges or universities and is therefore not suitable for clinical practice.
8. Some subjects may feel self-conscious wearing a bathing suit.

2.1.2 Air Displacement Plethysmography (ADP)

ADP is one of the newest methods available for assessing body composition (*Appendix 2*). Like UWW, body volume is measured. However, this is accomplished by displacement of air rather than water. Currently, the only available ADP system is manufactured by Life Measurement Inc (Concord, CA, USA) under the trade name BOD POD[®].

The system consists of two chambers: the test chamber (for the subject, *Appendix 2*) and the reference chamber. With the subject in the chamber, the door is closed and sealed. A diaphragm, separating the two chambers, is oscillated slightly, which alters the air volumes within the chambers and the resulting pressure changes are measured. The BOD POD measures raw (uncorrected) body volume ($V_{b_{raw}}$) under adiabatic conditions in which the air in the chambers is allowed to gain and lose heat during compression and expansion. In this case, the BOD POD utilizes the inverse relationship between pressure and volume that describes the behavior of air under adiabatic conditions (Poisson's Law, $P_1/P_2=(V_2/V_1)^\gamma$ where γ is the ratio of the specific heat of the gas at constant pressure to that of constant volume and is equal to 1.4 under these conditions). Although the BOD POD makes use of air mostly under adiabatic conditions, there is still the need to account for some isothermal air volumes. These are air near clothing, in hair, near skin and in the lungs. To minimize the effect of isothermal conditions due to clothing and hair on the head, the subject wears minimal clothing (bathing suit) and a swim cap. To account for isothermal air near the skin and in the lungs, the average volume of air in the lungs during normal tidal breathing, thoracic gas volume (TGV), is also measured by the BOD POD, and a term related to the

body surface area (SAA) is determined for each subject; the raw body volume is then corrected for these factors using the formula:

$$\text{corrected body volume (V}_b\text{)} = V_{b_{\text{raw}}} + 0.40(\text{TGV}) - \text{SAA}.$$

Once the corrected body volume is determined, D_b is calculated as mass/corrected volume and %BF is calculated using a two-compartment model, as with UWW (e.g. Siri, 1961; Brozek et al, 1963).

It is imperative that subjects wear the appropriate minimal clothing scheme during testing (i.e., a skin tight bathing suit and swim cap), or body volume will be underestimated (Dempster and Aitkens, 1995; Fields and Goran, 2000). Subjects should also breathe normally while measurements are being made, since irregular breathing patterns may affect results.

The BOD POD offers the advantages of demanding almost no effort from the subject and requiring very little time (two 50 second measurements of body volume, plus about 1 minute to measure the amount of air in the lungs). Additionally, equipment is not difficult to operate. These features make the BOD POD suitable for testing children, elderly and disabled subjects. In addition, the chamber is 440 liters, so subjects weighing over 400 kg can be tested.

Advantages

1. It is non invasive.
2. The test procedure is very quick (5-15 min) and requires minimal compliance from the subject.

3. It can be used with different categories of individuals including obese, elderly, children and disabled.
4. A minimal level of technician skill is required for operation.

Limitation

1. Difficult to use with extremely claustrophobic (abnormal fear of confined places) individuals.
2. Some subjects have difficulty correctly performing the procedure required for TGV measurements.
3. The assumption that FFM density is 1.1 kg/L, upon which calculations are made does not apply to certain population groups especially the elderly, children, and very obese.
4. Some subjects may feel self-conscious wearing a bathing suit.

2.1.3 Isotope Dilution of Total Body Water (TBW)

Another method that is commonly used to measure body composition, though not as frequently as UWW, is isotope dilution of (TBW). The basic principle of the isotope dilution technique for body composition analysis is that the volume of a compartment can be defined as the ratio of the dose of “tracer” (i.e., a small amount of substance not normally found in the body water in substantial amounts), usually administered orally, to its concentration in that body compartment within a few hours after dose administration.

A subject’s FFM can be calculated once his/her TBW is known, by assuming that 73.2 % of the TBW is contained in the FFM (Brozek et al., 1963) using the formula $FFM = 0.732 \times TBW$ (where both TBW and FFM are in kg). This assumption is valid for most populations,

except the very obese, children or the elderly. TBW is measured by using a “tracer” substance that is given to the subject and that equilibrates in the body water over time. Tracers typically used are the stable, non-radioactive isotopes of hydrogen or oxygen: deuterium (^2H), or ^{18}O as water ($^2\text{H}_2\text{O}$ or H_2^{18}O). There are several methods and protocols available by which the tracer is administered to the subject, but the most common technique is as follows. A pre-weighed quantity of isotope is ingested by the subject. The “plateau” method assumes that after 4 to 5 hours the isotope has distributed evenly throughout the body water, and a sample of body fluid (usually urine or saliva) is obtained at that time. A fluid sample is also collected before ingestion of the tracer to determine the natural background levels of the isotope contained in the body. The second fluid sample is collected after waiting a sufficient amount of time for penetration of the tracer within the compartment of interest. According to Ellis (2000), if it is possible that a significant amount of the tracer might be excreted before equilibration is reached, then a cumulative urine sample must be collected to adjust the dose estimate. Correction is also needed for exchange of ^2H or ^{18}O with non-aqueous substances. Inherent in any tracer dilution technique are four basic assumptions: 1) the tracer is distributed only in the exchangeable pool; 2) it is equally distributed within this pool; 3) it is not metabolized during the equilibration time; and 4) tracer equilibration is achieved relatively rapidly. If any of these requirements is violated, then the ratio of the administered dose to fluid concentration must be adjusted (Ellis, 2000).

Advantages

1. It does not depend on subject’s performance.
2. It is accurate for most populations.
3. It is easy to collect samples for field studies.
4. It is non-invasive.

Limitations

1. Special instrumentation and technical training is required for the analysis, which can be very expensive.
2. There is problem of the assumed hydration factor (0.732) upon which FFM (and thereby %BF) is estimated, as it may vary with age and race, and physiologic state (e.g. extreme obesity, illness).
3. Subjects cannot ingest any food or drink during the 4 to 5 hour equilibration period after dosing. However, a small standard snack can be worked into the test protocol after 4 hours without significant effect on the results.

2.1.4 Dual Energy X-ray Absorptiometry (DXA)

DXA uses two X-ray energies to measure body fat, muscle, and bone mineral. When having the scan done, the subject lies on a scanning table in the supine position while X-ray beams at two different energies are passed through the body (thereby presenting some radiation to the subject, see *Appendices 3 and 4*). Calculation of bone mineral and lean soft tissue mass is based on computer analysis of the differential attenuation of the two X-ray beams passing through the body. It takes about 6-25 minutes depending on the type of DXA machine being used for the computer software to produce an image of the tissues. The results from the computer output may be viewed as whole body estimates of body fat, muscle, and bone mineral as well as regional body estimates.

Although DXA requires very little effort on the part of the subject, it has several limitations. Primarily, large subjects (taller than 193 cm or wider than 65 cm) cannot be tested because

the body does not fit within the scan area (Lohman, 1996). This problem of wide subjects may be overcome by scanning only one half of the body and doubling the estimate, but this procedure could be prone to errors related to accurate judgement of the midline and by the assumption that the body is bilaterally similar. The accuracy of DXA also depends on the thickness of the body, with FM being overestimated at greater thickness (Jebb et al., 1995; Laskey et al., 1992). These problems may make DXA especially unsuitable for obese subjects and accurately measuring changes in body composition over time. In addition, DXA does not distinguish well between bone and soft tissue in the thoracic region (Roubenoff et al., 1993). Although the accuracy and precision of DXA may theoretically be affected by alterations in hydration of the body (Lohman, 1996; Roubenoff et al., 1993; Horber et al., 1992; Nord and Payne, 1995) a recent review by Lohman (2000) noted that in practice, hydration probably has little effect on DXA accuracy. Some studies indicate that precision of DXA for measuring FM is not as good as UWW (coefficient of variation 2.0% vs. 0.4%) (Nelson et al., 1996), but this may no longer be the case, since DXA hardware and software is undergoing continual improvement. Several studies (Economos et al., 1997; Kistorp and Svendsen, 1997; Modlesky et al., 1996; Paton et al., 1995; Tataranni et al., 1996 and Tothill et al., 1994) report that different DXA machines do not agree, within or between manufacturers. However, DXA is quick, the subject does not have to wear a bathing suit or minimal clothing (although any jewelry must be removed), and the radiation he/she is exposed to is very low (about 1.5 mrem per scan which is equivalent to about 1 day of natural background radiation). It is important to note, however that due to the radiation involved, DXA should not be used on pregnant women, but selectively in children. Detailed reviews of the theory and methodology of DXA measurements of body composition can be found in articles by Lohman (1996) and Pietrobelli et al. (1996).

Advantages

1. DXA presents little burden to the subject.
2. It is fairly quick (6-25 min).
3. It has the ability to provide regional measurements of fat distribution.
4. It also provides information on bone mineral (important for assessment of osteoporosis).

Limitations

1. DXA is very expensive.
2. It is available only in hospitals, research facilities, or universities.
3. It is not applicable to certain population groups, e.g. pregnant women because of radiation exposure to the fetus.
4. Its use is limited in very obese and very tall people.
5. It is very bulky and immobile.
6. The subject is expected to lie still throughout the procedure.
7. DXA may not assess soft tissue accurately in the thoracic region and accuracy may also depend upon thickness (front to back) of the subject.

2.2 Multi-compartment Models of Body Composition

Theoretically, the more compartments of the fat free mass (FFM) that are measured (e.g. mineral, protein and water), the more accurate the %BF prediction will be (Heymsfield et al., 1989; Lohman, 1986). This is because of the limitations of other methods (2-compartment models) such as the assumptions that the FFM has a relatively constant water content

(73.2%) and density of 1.1 kg/L, with negligible water associated with fat stored in the adipose tissue. Because of these limitations, multi-compartment models have been developed by several investigators to account for variation in the most important compartments of the FFM. Siri (1956), recognized that the hydration fraction of FFM is very labile from day to day within an individual, and also varies among individuals. He, therefore, developed a 3-compartment model in which he added TBW to the FM and FFM. The equation for estimating %BF in the 3-compartment model was revised in 1961 because of a change in the original assumption that the combined non-fat solids density was 1.64g/ml, to one that assumes that this compartment has a density of 1.565g/ml, and is:

$$\%BF = \frac{(2.118 - 0.78w - 1.354)}{D_b} \times 100$$

where D_b = whole body density in g/ml, and w = TBW as a decimal fraction of body mass (Siri, 1961). This model has been found to improve %BF estimates over 2-compartment model, especially for children. Since this population that has much higher TBW and much lower potassium and bone mineral concentrations than adults, the 2-compartment model overestimates the FM (Lohman, 1986). Subsequently, Bunt et al (1989) utilized Siri's 3-compartment model to examine the effect of body mass fluctuations during the menstrual cycle on %BF assessed by the standard 2-compartment model of densitometry. The male control group experienced less mass fluctuations than did the females during the month, which showed up as slight difference in the calculated %BF by any of the densitometric equations. The females experienced a greater mass change that was accompanied by a 3% greater body fat according to the 2-compartment model. This, however, was only partially compensated for by using the Siri 3-compartment model.

Lohman (1986), also developed another 3-compartment model, which added mineral content instead of TBW to the 2-compartment model. In this new model the equation for %BF estimation is as follows:

$$\%BF = \frac{(6.386 + 3.96m - 6.09)}{D_b} \times 100$$

where m = mineral content of the body as a decimal fraction of body weight (estimated as bone mineral content multiplied by a correction factor of 1.279). Although this equation is useful for adult and aging population, it has not been found to be useful in children and adolescents, since the water and protein ratio changes throughout growth and thus cannot be assumed as constant as the equation supposes (Lohman, 1986).

Marked improvement in body composition assessment was realized with the development of a 4-compartment model that accounted for both the body mineral content and TBW in the same model (Heymsfield et al., 1990). %BF from this model is estimated based on the equation below:

$$\%BF = \frac{(2.747 - 0.714w + 1.129b + 1.222m - 2.0503)}{D_b} \times 100$$

where b = body mineral content and m = nonosseous mineral. Reference man's body composition data were also used in the derivation of this, including the assumption that nonosseous mineral is a constant 22.9% of the bone mineral content.

These models have the disadvantage of requiring accessibility to multiple individual methods of body composition measurements as well as an expert technical knowledge (Heymsfield et al., 1990). Additionally, although any multi-compartment model of body composition theoretically leads to greater accuracy in %BF estimation, the cumulative error associated

with measuring multiple body compartments may offset this potential advantage. (Please note, this question was examined as part of this project, see chapter 4 for results).

Advantages

1. Considered one of the most accurate methods because it involves the fewest biological assumptions. Therefore can be used as a reference method.

Limitation

1. This approach increases the time, cost, facilities and expertise required for body composition measurement.
2. It involves small radiation dose associated with measurement of bone mineral content by DXA.
3. This model is only available at selected research institutions.

2.3 Reliability of ADP

2.3.1 Within day

The within-day reliability of %BF measured by ADP has been reported to vary between 1.7 and 3.7% among different studies, as summarized in Table 2.1. Although other studies have reported the reliability of ADP but in other units (Wagner et al., 2000; Collins et al., 1999), Table 2.1 only presents studies that reported reliability as a coefficient of variation (CV). McCrory et al (1995), found no significant difference in %BF between two trials for either the ADP or UWW. They also reported that CV for ADP ($1.7 \pm 1.1\%$) was slightly lower than that for UWW ($2.3 \pm 1.9\%$), but not significantly so. Similar reliability has been reported by Miyatake et al (1999) in Japanese women; however, Sardinha and co-workers (1998) and Iwaoka et al (1999) reported somewhat higher CVs of 3.3 and 3.7%, respectively, indicating lower reliability. As discussed later in Chapter 5, these differences in CVs may partially be due to differences among studies in sample size and mean %BF.

2.3.2 Between days

Between day reliability of ADP has been reported to be on the order of 2.0 – 2.3% (Table 2.1). Nuñez et al (1998) observed a between day CV of $2.0 \pm 0.1\%$ over 4 consecutive days. Also, Levenhagen and co-workers (1998) reported between-day CV of 2.0 ± 2.1 for a 7 day interval. In the study by Miyatake et al (1999), between-day reliability of ADP was also reported to be good, with a CV of 2.3%

2.3.3 Summary

The CVs reported in the studies cited in Table 2.1 range from 1.7 to 3.7% for within-day and 2.0 to 2.3% for between-day, with means of 2.7% and 2.1%, respectively. These CV show how reliable the ADP is in measuring %BF in humans. It is important to note, however, that no study has thus far reported the reliability of ADP for obese and the elderly. In addition, factors affecting the reliability of ADP remain unknown.

2.4 Validity of ADP

The validity of ADP compared to reference methods of body composition measurement was recently reviewed by Fields et al (2002). Table 2.2 compares studies on %BF estimation by ADP and other methods from 1995 to 2000. The results from these studies provide evidence which is confirmed by Fields et al. in their review that the ADP is a valid technique for the assessment of body composition in healthy children and young to middle-aged adults. However, studies on the validity of the ADP for measuring %BF in the elderly and obese individuals are lacking.

Table 2.1: Studies on the coefficient of variation (CV) of the BOD POD for %BF measurement in adults*

First author (year)	Subjects (n)	Age (years)*	Race	Within day CV (%)*	Between day CV (%)*
McCrorry (1995)	16	20 – 56	Mixed	1.7 ± 1.1	--
Iwaoka (1998)	7	31 – 44	Japanese	3.7 ± 4.3	--
Levenhagen (1999)	20	31.1 ± 1.8	--	--	2.0 ± 2.1**
Núñez (1998)	4	20 – 86	Mixed	--	2.0 ± 0.1
Sardinha (1998)	62	37.6 ± 2.9	Caucasians	3.3**	--
Biaggi (1999)	--	--	--	2.3 ± 1.9**	--
Miyatake (1999)	5	23.6 ± 1.7	Japanese	2.5 ± 0.8	--
Miyatake (1999)	10	24.5 ± 6.1	Japanese	--	2.3 ± 0.9

* Mean ± SD is reported unless not available, otherwise minimum and maximum values are reported.

** This is an unpublished observation cited in the paper's discussion.

Table 2.2: Studies comparing %BF estimated from the BOD POD and other methods

First author (year)	Subjects	Age (years)	Gender	Race	% fat _{ADP}	% fat _{UWW}	% fat _{DXA}
Dewit 2000	22 children	8 - 12	--	--	19.9±7.7	19.2±8.7	--
	10 adults	19 - 41	--	--	20.2±9.6	23.5±8.8	--
Koda 2000	366 elderly	59.3±10.7	Females	Japanese	30.5±5.9	--	31.8±4.9
	355 elderly	59.2±11.1	Males	Japanese	22.1±5.6	--	20.9±4.5
Lockner 2000	54 children	10 - 18	--	--	23.1±8.4	26.0±8.7	25.2±9.7
Wagner 2000	30 adults	19 - 45	Males	Blacks	17.7±7.4	15.8±7.5	16.1±7.5
Biaggi 1999	24 adults	19 - 42	Females	Mixed	29.7±7.9	28.6±7.5	--
	23 adults	20 - 48	Males	Mixed	20.2±7.3	21.5±6.2	--
Collins 1999	69 adults	19.5±1.1	Males	Mixed	15.1±0.8	17.0±0.8	13.3±0.9
Levenhagen 1999	10 adults	31.3±2.4	Females	--	31.2±2.0	29.2±2.0	34.2±2.7
	10 adults	30.8±2.9	Males	--	15.7±2.1	18.6±1.9	18.6±1.9
Sardinha 1998	62 adults	37.6±2.9	Males	White	23.4±7.0	--	26.0±7.4
Nunez 1998	22 children	13.1±3.3	Females	Mixed	26.8±8.2	25.1±8.0	26.9±8.8
	26 children	12.5±3.0	Males	Mixed	20.1±9.5	19.6±7.4	20.1±8.4
	44 adults	42.7±18.4	Females	Mixed	30.0±9.1	29.7±9.4	31.0±8.5
	28 adults	38.8±16.4	Males	Mixed	19.8±8.9	20.0±8.9	20.5±8.2
McCrorry 1995	26 adults	20±42	Females	Mixed	27.4±1.4	27.1±1.3	--
	42 adults	21±56	Males	Mixed	23.7±1.0	24.4±1.0	--

Chapter 3

METHODOLOGY

3.1 Subjects and Selection Criteria

Twenty adults, aged 25–81 years volunteered to participate in this study. The sample was limited to Caucasians to prevent any possible confounding due to race. Research subjects were recruited from the greater Boston Massachusetts area in United State of America by the volunteer recruitment office of the Jean Mayer USDA Human Nutrition Research Center (HNRC) on Aging at Tufts University, Boston, via information letters to participants in previous HNRC studies, newspaper advertisements, phone calls, flyers posted throughout the University and other vantage points of Boston, and word of mouth. The subjects in the study were heterogeneous with respect to age and body mass index (BMI). All subjects were screened for certain chronic diseases (which included history of coronary artery disease, arrhythmia, congestive heart failure, cirrhosis, any condition associated with edema, ascites, stroke, inflammatory arthropathies or any conditions associated with swollen joints, chronic obstructive lung disease or history of conditions or procedures influencing lung capacity, insulin dependent diabetes, AIDS, cancer and end stage renal disease) (*Appendix 5*). If a subject was on medication, he/she must have been taking a stable dose for at least 2 months to be eligible. Those using diuretics or other medications known to influence the distribution of body water were excluded from participating in the study. Pregnant women and subjects with musculo-skeletal limitations that would render them unable to climb in and out of the UWW tank were also excluded. All subjects gave written consent before the start of testing

after being thoroughly informed of the purpose, requirements and procedures of the study. The study protocol and consent form (*Appendix 6*) were approved by the Human Investigation Review Committee of Tufts University and New England Medical Center (NEMC).

3.2 Experimental Design

Subjects arrived at HNRC around 7:00-8:00 am having fasted overnight (i.e. not eaten since midnight the previous day) and reported to the nurses' station. The subject changed into a "johnny and robe" (light hospital clothing) and voided before his/her vital signs (weight, height, blood pressure and body temperature) were taken. The subject was then given a weighed amount of deuterium (for TBW measurement) to drink by the nurse in charge of the study on that day. The subject was then given over to the researchers for the body composition measurements, which included DXA, ADP and UWW usually in that order (see *Appendix 10* for sample schedule). While these measurements were ongoing, the subject provided urine sample at 3¹/₂, 4¹/₄ and 5 hours after deuterium dosing. Subjects were not allowed to ingest any food or drink while measurements were ongoing until the end of the study though a small snack (banana) was allowed after the 4¹/₄-hour time point if necessary. The study lasted until approximately 1:30 pm after which an optional lunch was provided to the volunteer.

The study involved two testing days, 5-8 days apart, with the test day order randomized. On "day 1," measurements were made in duplicate (except for TBW) while measurements were made only once on "day 2". Subjects were asked not to change their usual eating or exercise

habits between the two test days in order to minimize changes in body composition during this time.

3.3 Instrumentation and Body Composition Measurements

3.3.1 Anthropometry

Body mass was measured using an electronic weighing scale (TOLEDO, model 15S, Burlington, MA) while the subject was in johnny and robe. The mass of the johnny and robe was subtracted from the subject's mass measured to obtain the nude mass. Height was measured using a wall-mounted stadiometer (Seca, Germany) to the nearest 0.25 cm.

3.3.2 Total Body Water

TBW was determined by the isotope dilution technique using deuterium oxide ($^2\text{H}_2\text{O}$). Briefly, $^2\text{H}_2\text{O}$ was orally administered after collection of a baseline urine sample. The dose contained 10% of pure (99.9%) $^2\text{H}_2\text{O}$ (Cambridge Isotope Laboratories Inc., Andover, Massachusetts) in distilled water, which was prepared in batches prior to the study (*Appendices 11 and 12*). In brief, the pure $^2\text{H}_2\text{O}$ was diluted in distilled water to 10% of $^2\text{H}_2\text{O}$ by mass. Samples of the distilled water used for the dilution and the diluted dose were stored at -70°C for later analysis with each urine sample set. The dose for the first visit of the two testing days was 1.0g per kg body mass and for the second visit 0.8g per kg body mass irrespective of the study day (i.e., whether study day 1 or day 2), weighed to the nearest 0.001g. The container was rinsed with two 25ml portions of tap water and drank by the subject in order to ensure that all of the $^2\text{H}_2\text{O}$ dose was consumed.

Urine samples were collected at approximately 3¹/₂, 4¹/₄ and 5 hours post dose. The exact time and total volume voided was recorded at each void, and 9 ml of urine was saved from the later two voids in sealed Cryos cryogenic vials (Vanguard International, Inc., Neptune, NJ). The tubes were then labeled and stored -70°C until later analysis at the Mass Spectrometry Laboratory at the HNRC. The analysis of deuterium enrichment in baseline and 5-hour urine samples was accomplished by H₂-H₂O equilibration in the presence of platinum catalyst, followed by analysis of the equilibrated H₂ on a PDZ-Europa Hydra Continuous Flow, Gas Chromatograph Isotope Ratio Mass Spectrometer System (Crewe, England). All samples were analyzed in triplicate using the off-line zinc reduction method.

Isotope dilution spaces were calculated using the computer program “DLW” (Dallal, 1991). The peak isotopic abundances were specified as the measure of zero time intercept of the isotopic curve. In correcting for the isotopic exchange with non-aqueous organic compounds, TBW was then calculated as the ²H₂O dilution space at 5hr after the dosing divided by 1.04 (Racette, 1994). The TBW obtained from the dilution space was then used to calculate FFM assuming a hydration coefficient of 0.732 for FFM (Pace, 1945), and %BF was subsequently calculated as [(body mass – FFM)/body mass]*100%.

3.3.3 Air-Displacement Plethysmography

Body composition by ADP was evaluated using a BOD POD[®] Body Composition System with software version 1.69 (Life Measurement, Inc., Concord, CA, USA). The BOD POD was calibrated according to the manufacturer’s instructions with the chamber empty and using the 50-litre calibration cylinder before each test. After the calibration was completed, the test procedures were fully explained to the subject. Body mass was measured using the

electronic scale connected to the BOD POD to the nearest 0.001 kg. The subject, in swimsuit (males: Speedo; females: 1 piece) and swim cap only, then sat in the test chamber with the door closed for two raw body volume ($V_{b_{raw}}$) measurements. During these measurements, the volunteer breathed normally for about 50s. The testing chamber door was opened between trials. If both $V_{b_{raw}}$ were within 0.2% or 150ml, whichever was smaller, then the two trials were accepted and averaged. Otherwise a third trial was performed and the two trials that were closest were averaged. The subject was then connected to a breathing tube internal to the system to measure TGV, and was instructed to breathe normally. Then, after two to three breathing cycles, a valve in the circuit momentarily occluded the airway during mid-exhalation for 2 seconds, at which point the volunteer was signaled to make two or three light puffs by alternately contracting and relaxing the diaphragm. This action produced small pressure fluctuations in the airway and chamber pressures that were used to determine TGV. For TGV to be considered successful, two criteria had to be met. First, the figure of merit had to be <1.0 . The figure of merit is a theoretical value that indicates compliance with TGV measurement procedure. A figure of merit of less than 1.0 indicates good agreement between chamber pressure and the airway pressure in the tube, while a merit >1.0 indicates poor compliance (usually due to lack of tight seal of the lips around the tube) (Dempster and Aitkens, 1995). Second, airway pressure had to be below 35 cm H₂O. If the airway pressure is greater than 35 cm H₂O, there may have been closure of the glottis (i.e., a Valsalva maneuver), which leads to falsely measured low TGV values.

D_b from the BOD POD was calculated using the equation

$$D_b = M/(V_{b_{raw}} + 0.40TGV - SAA)$$

where SAA and 0.40TGV are used to correct for the isothermal conditions in the lungs and near the skin's surface and M is the mass of the subject measured with the electronic scale connected to the BOD POD. Percent body fat was then calculated using the Siri (1961) formula [%BF = $(495/D_b) - 450$].

3.3.3.1 Temperature Measurements

Room, skin and oral temperatures were also measured during BOD POD testing.

Room temperature was measured in triplicate to the nearest 0.1°C using a Traceable® Monitoring Thermometer (Friendswood, Texas). For this measurement, the thermometer was placed in close proximity to the BOD POD.

Skin temperature was measured in triplicate using a Tele-Thermometer (YSI model 46 TUC, Yellow Springs, Ohio). A temperature probe (made of a disk-shaped stainless steel sensor attached to a 10-foot long vinyl-jacketed lead and terminated with a 7mm jack) was connected to the thermometer. The sensor was placed at the back of the subject's left hand and secured with medical tape. The skin temperature measurement was allowed to stabilize for 3 minutes and read to the nearest 0.1°C.

Oral temperature was measured in triplicate to the nearest 0.01°F using a Digital Basal Thermometer (MABIS HEALTHCARE INC. Lake Forest, IL) as outlined in the user's manual. The thermometer was turned on and the thermometer probe tip placed under the tongue close to the heat pocket. The thermometer was held under the tongue with mouth

closed for about 1 – 2 minutes until the oral temperature stabilized (indicated by 4 beep tones). The thermometer was then removed and temperature recorded.

3.3.4 Dual Energy X-Ray Absorptiometry

DXA measurements were performed in the DXA Laboratory at NEMC. Measurements were made while subjects lay in the supine position using a Hologic QDR-2000, software version 5.73A (Hologic, Waltham, MA) in the array scan mode after having removed all metal objects worn and while wearing a hospital gown. One whole body scan took approximately 6 minutes and the radiation exposure was 3.6 μ SV (equivalent to 1 – 2 days of natural background radiation). During measurement, a step phantom of known absorptive properties provided by the manufacturer for calibration was placed on the subject's right, parallel with the back edge of the mattress with the tall end of the phantom level with the subject's feet. This was scanned alongside the subject to serve as an external standard.

On days in which two scans were done, subjects got off of the scanning table between tests and were repositioned to ensure independency of each of the two tests from the other on that day. The same technician positioned the volunteers, performed the scans, and executed the analysis according to the operator's manual, using the standard analysis protocol provided by the manufacturer.

3.3.5 Underwater Weighing

UWW was performed using a rectangular shaped steel tank filled with 95 – 100 °F water to measure body volume. After entering the UWW tank and removing trapped air in the

swimsuit, instructions were given to the subject. The subject then sat in a chair (that was resting on a weighing scale) and voluntarily submerged him/herself completely under water while maximally exhaling so that body mass in water could be measured. The scale reading was obtained after all the air bubbles from exhalation disappeared. This procedure was repeated until three measurements of %BF using a predicted value for residual lung volume (RV) agreed within $\pm 1\%$ (note that measured RV was used in the final calculation; see below). This usually required 3 - 6 trials within a session.

RV was measured on land by nitrogen washout with a Sensormedics Vmax 229 (Yorba Linda, CA) and software version 3.2. The flow sensor and oxygen and carbon dioxide analyzers were calibrated according to the manufacturer's specifications. Measurements were made with the subject in a sitting position until there were two values within ± 150 ml in a session, which usually required 2-4 trials. The two values were averaged and used in the calculation of D_b .

Body mass in water obtained during each of the trials was corrected for the tare weight. D_b was calculated using the formula; $M_a / [(M_a - M_w) / D_w] - RV$ where M_a is mass in air during the BOD POD trials, M_w is subject's mass in water, and D_w is the temperature-corrected water density. The Siri (1961) formula was then used to calculate %BF from body density.

3.3.6 Multi-compartment Models

The 3-compartment model divides the human body into fat, water, and FFM, which is assumed to have a constant ratio of protein to mineral. This model used data on body density

(D_b) from either the BOD POD or UWW, and TBW as a fraction of body mass. %BF was calculated using Siri's 3-compartment model equation (see chapter 2).

For the 4-compartment model, the human body is divided into fat, water, protein, and mineral, thereby further avoiding the assumption that the ratio between mineral and protein in FFM is constant. The 4-compartment model used D_b (from either BOD POD or UWW) and both bone mineral content (from DXA) and TBW expressed as a fraction of body mass. %BF here was calculated using Heymsfield's 4-compartment model (see chapter 2).

3.4 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 10.1 for Windows, (SPSS Inc., Chicago, USA) and Systat version 9.0 for Windows (SYSTAT, Inc., Evanston, IL). Data are reported as means \pm SD. Repeated measures analysis of variance (ANOVA) was used to examine trends in %BF and lung volumes. Trial was the repeated measure (day 1 Trial 1, day 1 Trial 2, day 2) and day order was the between subject factor (i.e., whether the subject's first test day was day 1 or day 2).

Within-day and between-day reliability was assessed by calculating average within-subject SDs and CVs for each of the body composition methods, including the multi-compartment models. In addition, the theoretical overall error (repeatability) of the 3-compartment model was calculated as:

$$CV_{\text{Theoretical}} = [CV_{\text{UWW or BOD POD}}^2 + CV_{\text{TBW}}^2]^{0.5}$$

while that for the 4-compartment model was calculated as:

$$CV_{\text{Theoretical}} = [CV^2_{\text{UWW or BOD POD}} + CV^2_{\text{rBW}} + CV^2_{\text{DXA}}]^{0.5}.$$

These theoretical errors were then compared with the actual errors (CV) of the multi-compartment models, and the individual methods.

To determine, associations among within subject differences (day 1 – day 2) in %BF and lung volumes and several potential factors, Pearson product-moment correlations among variables were calculated. These factors included age, weight, height, BMI, time between tests, and for the BOD POD, within-subject differences (day 1 – day 2) in skin, oral and room temperatures. Multiple regression analysis was also used to determine predictors of within-subject variability, so that potential confounding factors could be controlled.

For all tests, statistical significance was accepted at $p \leq 0.05$

Chapter 4

RESULTS

4.1 Descriptive characteristics

The physical characteristics of the subjects are presented in Table 4.1. The subjects ranged in age from 25 – 81 years, and mean age was similar between males and females. As shown by the large between-subject SDs, the subjects were heterogeneous with respect to all physical characteristics.

4.2 Within-day reliability of %BF

Percent body fat determined by the individual methods, as well as calculated for multi-compartment models, on the same day are presented in Table 4.2. As shown, there was no significant difference between the first and second sessions in %BF for any of the methods. The mean within-subject SD for the different methods ranged from 0.1 to 1.0 %BF. Of the three individual methods under study, the BOD POD had the smallest SD but was followed closely by DXA, and UWW had the largest SD. Regarding the 3- and 4-compartment models, using D_b from the BOD POD resulted in smaller within-subject SDs than using D_b from UWW. Furthermore, the 3- and 4-compartment models calculated using D_b from UWW resulted in within-subject SDs that were similar to using either the BOD POD or DXA alone. The within-subject CVs showed a similar pattern of within-day variability to that of the SDs.

Table 4.1 Physical characteristics of subjects¹

	Females (n = 8)	Males (n = 12)	Total (n = 20)
Age (yrs)	45.5 ± 24.3	51.9 ± 21.0	49.3 ± 21.9
Weight (kg)	63.6 ± 13.4	84.2 ± 16.3	76.0 ± 18.0
Height (cm)	162.5 ± 4.5	174.8 ± 6.2	169.9 ± 8.3
BMI (kg/m ²)	24.1 ± 4.7	27.5 ± 5.0	26.2 ± 5.1

¹ Mean ± SD

Table 4.2 Within-day variability in %BF by the different methods^{1,2}

	Session 1	Session 2	SD	CV (%)
DXA	30.8 ± 10.4	31.0 ± 10.2	0.4 ± 0.3	1.6 ± 1.1
BOD POD	28.5 ± 9.7	28.5 ± 9.7	0.3 ± 0.2	1.3 ± 1.3
UWW	27.0 ± 10.5	26.4 ± 10.6	1.0 ± 0.6	4.2 ± 2.9
3-C BOD POD	29.0 ± 9.0	29.0 ± 9.1	0.1 ± 0.1	0.5 ± 0.5
3-C UWW	28.4 ± 9.4	28.1 ± 9.4	0.4 ± 0.3	1.6 ± 1.0
4-C BOD POD	27.7 ± 8.3	27.8 ± 8.3	0.1 ± 0.1	0.6 ± 0.6
4-C UWW	27.0 ± 8.7	26.7 ± 8.7	0.5 ± 0.3	2.0 ± 1.3

¹ Mean ± SD; DXA, dual-energy X-ray absorptiometry; BOD POD, BOD POD body composition system; UWW, underwater weighing; 3-C BOD POD, 3-compartment model using body density from BOD POD; 3-C UWW, 3-compartment model using body density from UWW; 4-C BOD POD, 4-compartment model using body density from BOD POD; 4-C UWW, 4-compartment model using body density from UWW. Note that for the 3-C and 4-C models, the between-day CV for TBW as a fraction of body weight was used in the calculations (since TBW measurement was conducted only once each day).

² %BF did not differ significantly between sessions for any method.

4.3 Between-day reliability of %BF

Percent body fat determined by the individual methods and calculated using multi-compartment models on the two study days are shown in Table 4.3. There was no significant difference in %BF measured on the two days for any method. The between-day, within-subject SD for the different methods ranged from 0.5 to 1.7 %BF. Of the four individual methods under study, DXA had the lowest SD, followed closely by the BOD POD. Like for the within-day comparison, UWW had the highest SD. Regarding the 3- and 4-compartment models, using D_b from the BOD POD again resulted in smaller SDs than using D_b from UWW. Additionally, the 3- and 4-compartment models calculated using D_b from BOD POD resulted in within-subject SDs that were similar to the BOD POD or DXA alone but lower than using UWW or TBW alone. The within-subject CVs showed a similar pattern of between-day variability to that of the SDs. For all the methods studied, the between-day SDs were higher than within-day SD measurements reported in Table 4.2.

4.4 Reliability of RV and TGV

The mean figures of merit for within and between days for measurement of TGV during BOD POD testing were 0.24 ± 0.24 and 0.22 ± 0.24 respectively, and for all individuals were below 1.0. The mean airway pressures during TGV measurement were 16.0 ± 6.8 cm H₂O and 16.1 ± 7.4 cm H₂O within and between days respectively, and for all individuals were below 35 cm H₂O. Thus, acceptance criteria for TGV measurement was adhered to.

Table 4.3 Between-day variability in %BF from the different methods^{1,2}

	Day 1	Day 2	SD	CV (%)
DXA	30.8 ± 10.4	30.6 ± 10.4	0.5 ± 0.4	1.9 ± 1.7
BOD POD	28.5 ± 9.7	28.6 ± 10.0	0.7 ± 0.7	2.7 ± 2.9
UWW	27.0 ± 10.5	26.4 ± 10.9	1.7 ± 1.4	7.4 ± 7.0
TBW	29.4 ± 8.8	29.1 ± 8.9	0.9 ± 0.7	3.6 ± 2.3
3-C BOD POD	29.0 ± 9.0	28.9 ± 9.3	0.6 ± 0.5	2.4 ± 2.2
3-C UWW	28.4 ± 9.4	28.0 ± 9.6	1.0 ± 0.9	4.1 ± 3.5
4-C BOD POD	27.7 ± 8.3	27.6 ± 8.5	0.6 ± 0.5	2.6 ± 2.1
4-C UWW	27.0 ± 8.7	26.4 ± 8.9	1.2 ± 1.0	4.8 ± 4.1

¹ Mean ± SD; DXA, dual-energy X-ray absorptiometry; BOD POD, BOD POD body composition system; UWW, underwater weighing; 3-C BOD POD, 3-compartment model using density from BOD POD; 3-C UWW, 3-compartment model using density from UWW; 4-C BOD POD, 4-compartment model using density from BOD POD; 4-C UWW, 4-compartment model using density from UWW.

² %BF did not differ significantly between days for any method.

Acceptance criteria for RV tests was also met for the majority of individuals (data not shown).

Since the variability in UWW and BOD POD in part depends upon the variability in the respective lung volume measurements, within-day and between-day repeatability in RV and TGV was also examined (Table 4.4). Neither RV nor TGV differed significantly within or between days. Additionally, the SDs for RV and TGV were similar to each other both within and between days, though as expected, the SDs for both were higher between days than within days. When within-subject variability was examined as CV, RV showed higher variability than TGV.

Table 4.4 Within and between day variability of lung volume measurements associated with UWW and BOD POD^{1,2}

	Day 1		Day 2		Within-day		Between-day	
	Session 1	Session 2	Mean	SD	SD	CV (%)	SD	CV (%)
*RV (n=19)	1.91 ± 0.69	1.99 ± 0.78	1.98 ± 0.59	0.10 ± 0.01	0.20 ± 0.17	5.6 ± 4.9	0.20 ± 0.17	11.3 ± 9.4
*TGV (n=19)	3.51 ± 1.18	3.51 ± 1.11	3.56 ± 1.15	0.11 ± 0.01	0.19 ± 0.18	0.0 ± 0.2	0.19 ± 0.18	7.7 ± 11.5

¹ Mean ± SD; RV, Residual lung volume associated with UWW; TGV, Thoracic gas volume associated with BOD POD. All measurements are in L except where indicated.

² Neither RV nor TGV differed significantly across the 3 test sessi4.5 Error in measuring %BF with multi-compartment models

4.5 Errors in measuring %BF with multi-compartment models

Table 4.5 presents propagated theoretical versus actual errors in measuring %BF with multi-compartment models, expressed as CVs. Theoretical estimates ranged from 1.3 to 1.7% for within-day and 1.3 to 2.1% for between-day. However, actual within-day and between-day CVs were much smaller, ranging from 0.1 to 0.5%, and 0.6 to 1.1% for within-day and between-day errors, respectively. Of further note, for both within-day and between-day comparisons, theoretical errors for the 3-compartment models were lower than for 4-compartment models. However, actual errors were similar between 3-compartment and 4-compartment models. Finally, theoretical errors for the models incorporating D_b from BOD POD and UWW were similar, but actual errors were smaller for those models incorporating the BOD POD.

4.6 Potential factors associated with measured %BF variability

The mean between-day time interval between the two test days was 7.7 ± 4.9 days. This time interval included one subject whose interval was 28 days due to illness after the first visit and re-scheduling problems thereafter. The mean time between the two test days reduced to 6.6 ± 0.8 days when this subject was excluded. The mean time between the two within-day sessions was 8.3 ± 0.7 minutes for DXA, 12.7 ± 3.4 minutes for BOD POD and 55.0 ± 21.0 minutes for UWW. As shown in Figure 4.1, the time between test days (day 2 – day 1) was not significantly associated with the variability in %BF ($\Delta\%BF$; day 1 – day 2) measured by

Table 4.5 Propagated theoretical versus actual error of multi-compartment models for determining %BF¹

	<u>Within day CV (%)</u>		<u>Between day CV (%)</u>	
	Theoretical ²	Actual	Theoretical ²	Actual
3-C BOD POD	1.3	0.1	1.3	0.6
3-C UWW	1.3	0.4	1.4	1.0
4-C BOD POD	1.6	0.1	2.0	0.6
4-C UWW	1.7	0.5	2.1	1.1

¹3-C BOD POD = 3-compartment model using body density from BOD POD.

3-C UWW = 3-compartment model using body density from UWW.

4-C BOD POD = 4-compartment model using body density from BOD POD.

4-C UWW = 4-compartment model using body density from UWW.

²Theoretical error was calculated as = $[CV^2 \text{ of } D_b \text{ from UWW or BOD POD} + CV^2 \text{ of } w]^{0.5}$ for 3-compartment models and $[CV^2 \text{ of } D_b \text{ from UWW or BOD POD} + CV^2 \text{ of } w + CV^2 \text{ of } bmc]^{0.5}$ for 4-compartment models, where D_b is body density, w is TBW expressed as a decimal fraction of body mass and bmc is bone mineral content measured by DXA, expressed as a decimal fraction of body mass. Note that within-day theoretical errors were calculated using the between-day CV for w (since TBW measurement was conducted only once each day)

any method. Similarly, the time between tests on the same day was also not associated with %BF variability for any method (data not shown).

Subject characteristics, including body mass, height, BMI and age, were not significantly associated with either between-day or within-day differences in %BF by any of the methods (data not shown).

Figure 4.2 shows the associations of temperature differences with measured %BF variability between the two test days. There were associations between differences in oral and skin temperatures, but not differences in room temperature, with the difference in %BF. However, the skin temperature association no longer existed after controlling for the time between tests in multiple regression analysis. Additionally, after controlling for differences in skin and room temperatures, and the time interval between test days, the difference in oral temperature was only marginally associated with the difference in %BF ($p = 0.06$)

4.7 Potential factors associated with measured lung volume variability

Figure 4.3 shows the relationship for the within-day difference in RV with body mass ($r = -0.56$; $p = 0.013$) where both differences are calculated as session 1 – session 2. There were also relationships between within-day differences in RV and height ($r = -0.595$; $p = 0.007$) and BMI ($r = -0.415$; $p = 0.077$). These relationships indicate that larger subjects had higher measured RV on the second within-day trial. However, between-day differences in RV were

not significantly related to subject characteristics. Regarding TGV, there was no significant association between within-day or between-day TGVs and either subject characteristics or temperature variations during BOD POD testing.

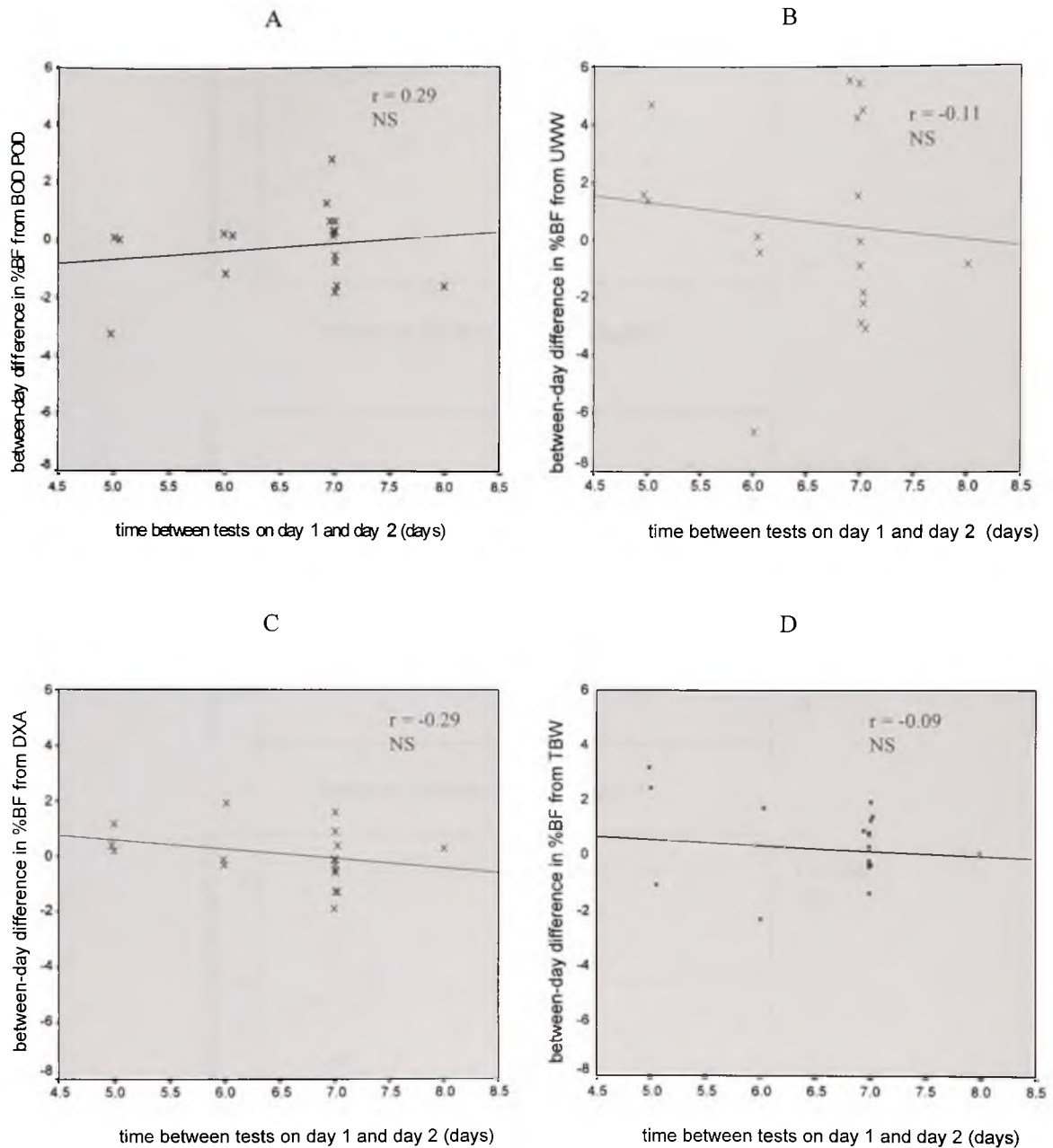


Figure 4.1 Associations of time between tests with variability in measured %BF

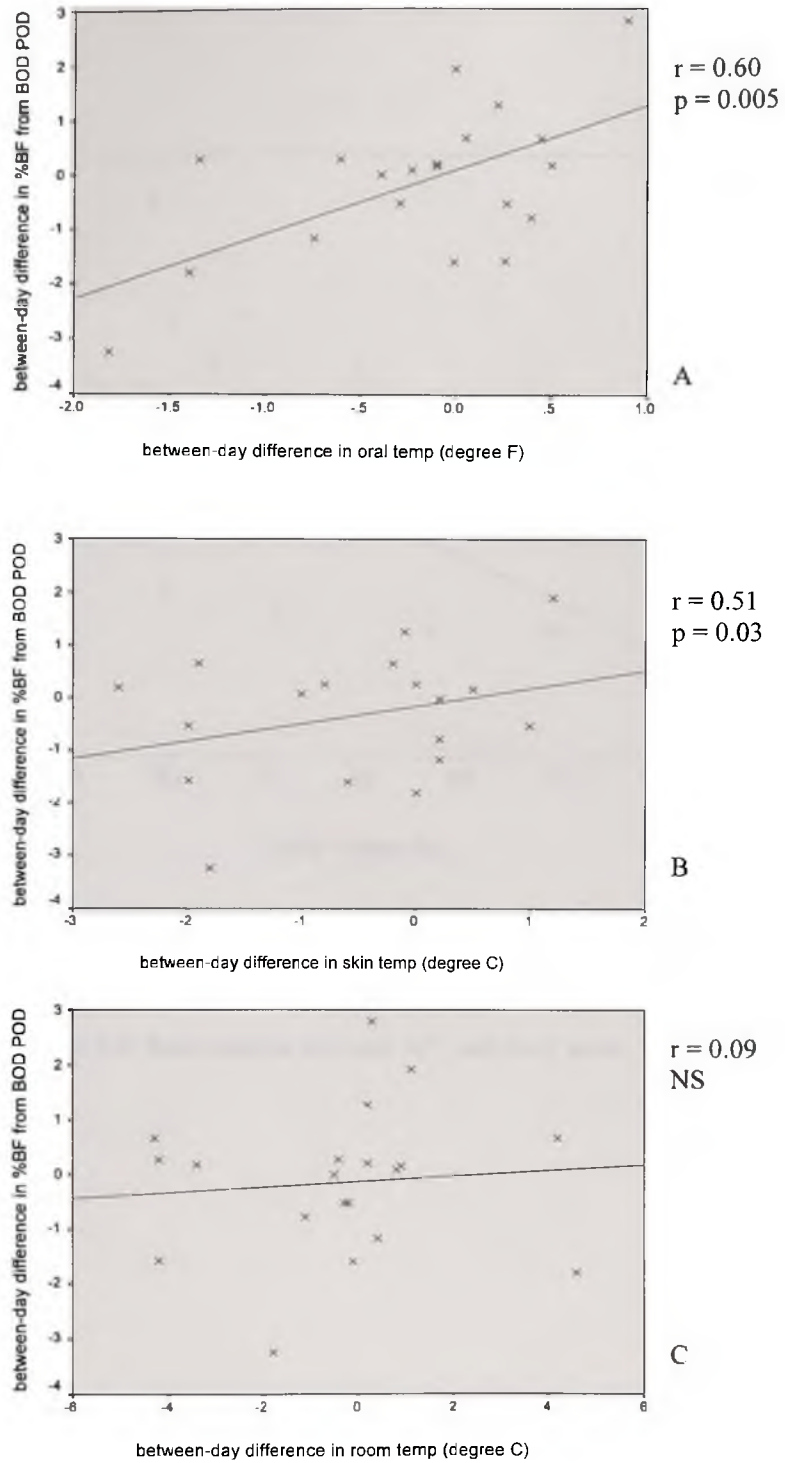


Figure 4.2 Associations of temperature differences with variability in measured %BF by BOD POD

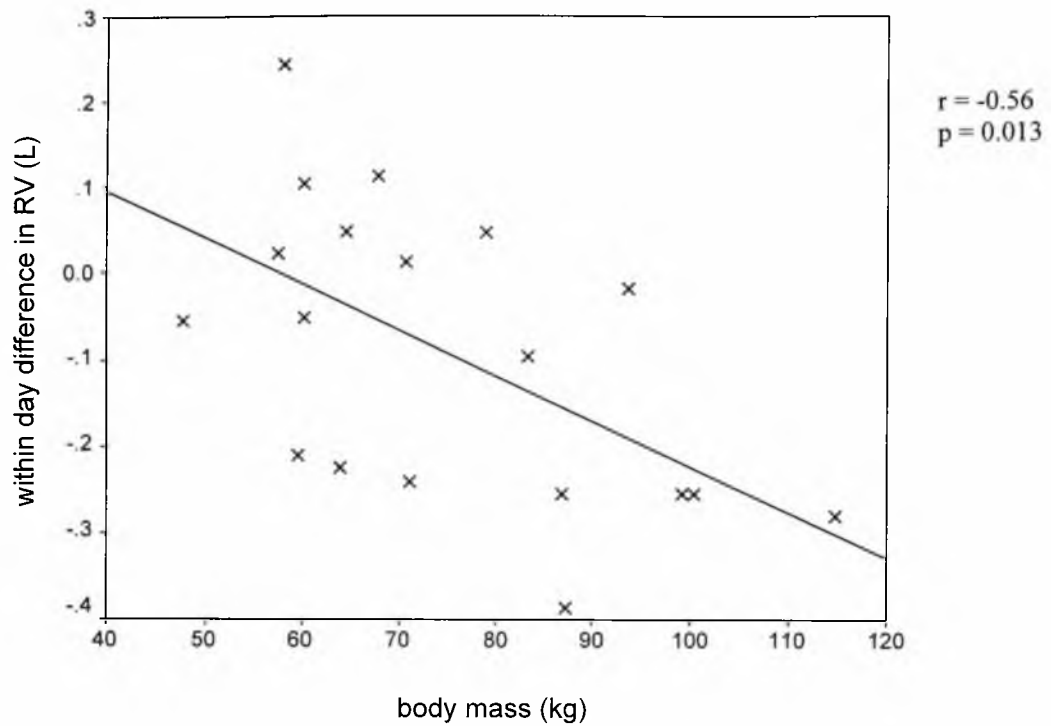


Figure 4.3 Relationship between RV and body mass

Chapter 5

DISCUSSION

In this study, %BF was determined by DXA, BOD POD, UWW and TBW by isotope dilution using deuterium-enriched water ($^2\text{H}_2\text{O}$). Both within-day variability, primarily signifying technical or machine variability, and between-day variability, indicating technical as well as human biological day-to-day variability, were measured. The major findings were that the within-day variability in %BF measured with the BOD POD was the lowest while its between-day variability in %BF was the second lowest, closely following DXA. Furthermore, multi-compartment models incorporating D_b from the BOD POD had much better reliability than those incorporating D_b from UWW. Finally, the between-day reliabilities of both the BOD POD and DXA alone were similar to those of multi-compartment models incorporating D_b from the BOD POD.

5.1 Reliability of methods

5.1.1 *Within-day reliability*

The within-day reliability of the individual methods was very good. Among the methods studied, the BOD POD had the lowest within-subject SD, but was followed closely by DXA, and UWW had the highest SD. The differences in variability in %BF measurements for the within-day two sessions between BOD POD and UWW may have been due to the greater difficulty in performing the maneuvers required for UWW (including exhaling as much air as

possible both in water and on land) in comparison with BOD POD, which requires almost no physical effort from the subject.

The within-day reliability of %BF measured by the BOD POD averaged $1.3 \pm 1.3\%$ (mean \pm SD). This CV is slightly better than the previously reported range of 1.7 to 3.7% (McCrorry et al., 1995; Iwaoka et al., 1998; Miyatake et al., 1999; and Wagner et al., 1999). The improved CV observed in this study for the BOD POD may be due to a number of factors, including differences in sample size and the mean %BF in this study compared to other studies. For example, in the study by McCrorry et al (1995), a mean %BF of about 26% was observed for the 68 subjects studied compared with a mean of about 29 %BF in this study. They observed within-day CV of 1.7% based on 16 subjects compared to a CV of 1.3% based on 20 subjects in the present study. Since the CV is calculated as the SD divided by the mean, a higher %BF would lead to a lower CV for a given SD. Iwaoka and colleagues (1998) also observed a CV of 3.7% based on 7 subjects with average %BF of 14.9%. A very small sample size such as this is likely to lead to a high CV, since the lower the sample size, the more likely it is that there will be high variability in the measured variable, and hence a higher SD which then translates into a higher CV for a given mean.

The within-day CV (4.2%) observed for UWW was very close to the 4.3% reported by Iwaoka et al (1998) but higher than the 2.3% reported by McCrorry et al (1995). While these differences among study CVs could be due to differences in sample size and mean %BF, they could also be due to differences in criteria for accepting the results of UWW sessions as well as differences in methods for measuring RV by the different laboratories.

For the DXA, our within-day CV (1.6%) was higher than the 1.0% within-day CVs reported by Kelly et al (1991) and Economos et al (1997) for 13 and 5 subjects, respectively. However, the average %BF for the subjects in those studies was not reported. The difference in CVs in this study compared to the others could be due to differences in the type of DXA machine used (i.e., manufacturer and models) as well as software versions. In this study, a Hologic QDR-2000 was used compared with a Hologic QDR-1000 used in the study by Kelly et al (1991) and Lunar DPX used in the study by Economos et al (1997). However, Economos et al (1997) also reported higher CVs (2.5 and 3.9%) on different Lunar DXAs.

For the multi-compartment models, the within-day variability using D_b from the BOD POD was lower than when using D_b from UWW, with mean SDs of 0.1 and 0.4 for the 3-compartment models, and 0.1 and 0.5 for the 4-compartment models, respectively. These respective SDs were also lower than those observed for the individual BOD POD and UWW measurements. The differences in SDs could be due to differences in mean %BF for the two studies.

It was also observed that within-day errors of 3- and 4-compartment models were smaller than for 2-compartment models. This trend was also reported by Wells and Fuller (in press). The within-day errors in estimating %BF from multi-compartment models were lower than those for 2-compartment models possibly due to cancellation of errors in the individual methods when incorporated into the multi-compartment models. For example, the 3-compartment models, the D_b term is positive while the w term is negative. Similarly, in the 4-

compartment model, the w term is negative, while the D_b , b and m terms are all positive. Therefore, the positive and negative terms cancel each other out when the multi-compartment models are computed.

5.1.2 Between-day reliability

The between-day variability of the individual methods was good. Overall, DXA performed better than the other methods, but the between-day SDs of the BOD POD and DXA were very close. Like the within-day measurements, UWW had the highest variability of the four methods studied.

The between-day CV for measuring %BF with the BOD POD of 2.7% observed in this study was higher than the 2.0% and 2.3% previously reported by Nuñez et al (1999) and Miyatake et al (1999), respectively. The CV of 1.9% observed in this study for %BF by DXA measurement was much lower than the range of 2.1% to 7.9% CV reported by Economos et al (1997), Jensen et al (1993) Hansen et al (1993), Russel-Aulet et al (1991), and Mazess et al (1990) (all using Lunar DPX machines), but lower than the 1.4 %BF reported by Pritchard et al (1993) who used a Hologic QDR-1000. In a study by Friedl et al (1992) over a 1-week period, DXA had a between-subject SD of 0.5 for %BF measurement which was the same as that observed in the present study, even though the two studies used DXAs made by different manufacturers. For UWW, the difference in our SD (1.7 %BF) compared with the SD of 1.0 %BF reported by Friedl and colleagues (1992) could be due to the different criteria in

accepting UWW measurements as well as the different methods used for RV measurements by the individual laboratories.

The between-day SDs for the 3- and 4-compartment models using D_b from BOD POD were the same (both 0.6 %BF) and very close to that for DXA and the BOD POD alone (0.5 and 0.7 %BF, respectively). Regarding the SDs using D_b from UWW, there was slight difference between the 3- and 4-compartment models (1.0 versus 1.2 %BF), but both values were lower than the SD for the between-day variation in UWW alone (1.7 %BF). The SDs observed in this study for both the 3- and 4-compartment models using D_b from UWW were very close to the 1.1 %BF reported by Friedl et al (1992) from their data for 3- and 4-compartment models.

5.2 Factors affecting reliability of body composition methods

For all methods, time between tests both within and between-days was not significantly related to the difference in %BF. This shows that over the short term of this study, time was not a factor affecting the observed within-individual differences in %BF.

The time between the BOD POD tests was dependent on how early the temperatures stabilized, especially oral and skin temperatures (approximately 1 and 3 minutes, respectively). It should be noted that in this study we chose to measure these temperatures; however, temperature measurement is not part of the usual BOD POD protocol, and typically the time between BOD POD test would be even less than in this study. For DXA, the time

interval between tests 1 and 2 was primarily due to the subject getting off the scanning table and being repositioned by the technician. UWW had the most time interval between sessions 1 and 2, due to two important factors. First, there were about 4 to 8 trials needed for measuring body mass in the water within a session in order to obtain 3 trials within 1.0 %BF, which was the long-standing criterion in the Energy Metabolism Laboratory for accepting the trials within a session. Second, the RV sessions were conducted between the two UWW sessions. As mentioned earlier in Chapter 3, the RV measurements continued until two values within 150 ml variation were obtained. Each single RV trial lasted between 2 and 8 minutes, with 3-4 minutes rest in between trials.

There were significant associations between skin and oral temperatures and differences in %BF measured with the BOD POD, as indicated by Pearson correlation coefficients. It can be seen that as temperature increased there was a decrease in %BF measured with the BOD POD. This observation could be due to a corresponding decrease in body volume as temperature increases subsequently increase body density, resulting in a decrease in %BF measured. However, the association with the skin temperature difference disappeared after controlling for the time between test days, and the oral temperature difference was only significant after controlling for variations in other temperatures and the period between test days.

The relationship between the within-day difference in RV and body mass was inverse, with larger subjects having a higher RV in the second session. This suggests that subjects with greater body mass could not exhale as much air during the second session of RV

measurements as they did during the first session, possibly due to fatigue. However, the within-subject SD for RV was comparable to that of TGV, indicating that the greater variability in UWW %BF compared to BOD POD %BF may have been due to greater variability in measuring body volume in water (by UWW) compared to in air (by BOD POD). This possibility will be examined in future analyses. In the case of DXA, the major technical errors associated with within-subject variations include consistency in positioning the subject on the scanning table.

The within-day variability observed in this study as well as other studies will likely be due purely to technical variability and minimal biological variability. This is because the times between sessions of trials within a day are very short (\approx 10 minutes to 1 hour), so that the possibility of any biological or physiological changes in body composition will be negligible. The repeatability of the BOD POD depends on the precision with which both body volume and TGV are measured in successive tests. For UWW, the degree at which the subjects consistently exhale maximally during RV measurement and under water are very important contributors to the within-subject variability.

The between-day differences in %BF observed in this study are due to technical variability as well as usual day-to-day biological variability. Biological variability was minimized, however, by instructing subjects to maintain their usual eating and exercise habits throughout the 5–8 day study period.

5.3 Comparison of 3-compartment to 4-compartment models

The within-subject SDs for the 4-compartment models were higher than that of the 3-compartment models for the within-day variability even though the 4-compartment model is believed to have the greatest accuracy in estimating %BF because of minimal reliance on biological assumptions, and hence improved reproducibility of the model irrespective of the biological variability in the composition of FFM (Friedl et al., 1992). This observation could be attributed to the additional bone mineral content as a decimal fraction of body mass to the 4-compartment model, which also carries its own error.

The observation that 3-compartment models have similar errors to that of 4-compartment models between-days could be explained by the low variability in bone content over a short period of time (5-8 days) compared to the high day-to-day variability in TBW as reported by Siri (1961) and confirmed by Friedl and colleagues (1992) and this study. It is therefore important to note that these findings suggest that when using multi-compartment models to measure short-term changes in %BF, only a 3-compartment model and not 4-compartment model is needed.

5.4 Error of the multi-compartment models based on the Law of Propagation of Errors

Actual errors were much lower than the theoretical errors computed for the multi-compartment models for both within-day and between-day CVs. This implies that multi-compartment models using D_b from either the BOD POD or UWW have the ability to

measure much smaller changes in %BF than would be predicted based on the errors of the individual methods alone. However, using a multi-compartment model incorporating D_b from the BOD POD would allow detection of a minimal change in %BF that is nearly half that of a multi-compartment model incorporating D_b from UWW (e.g., 0.6 %BF versus 1.1 %BF). It should also be noted, however, that using a 3- or 4-compartment model improves precision for detecting %BF changes by UWW alone, but not the BOD POD or DXA alone. Therefore, based on these preliminary data, either the BOD POD or DXA alone may be sufficient for measuring small, short-term changes in %BF, and multi-compartment models are not needed. Nonetheless, 3- and 4-compartment models may still be necessary if absolute accuracy in quantifying body composition at a single time point is the primary goal.

Chapter 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study indicate that the BOD POD is among the most reliable methods for measuring %BF, and therefore is a suitable alternative to traditional reference methods for measuring body composition in clinical settings and for nutrition research.

Multi-compartment models were found to have better repeatability than each of the individual methods for measuring %BF both within and between days. Moreover, the actual errors in estimating %BF by multi-compartment models were lower than the theoretically calculated errors.

Within-subject differences in %BF measured by the individual methods were not time dependent, nor were they related to subject characteristics. However, the within-day variability in RV was significantly related to body mass, with heavier subjects having a lower RV in the second session.

Differences in oral and skin temperatures were not associated with differences in %BF measured by the BOD POD over time after controlling for confounders.

The BOD POD is therefore recommended for routine research and clinical use based on its superior repeatability, non-invasive nature, and ease and convenience to operate. It requires minimal compliance from the subject and comparatively little operator expertise during testing. Furthermore, since both the BOD POD and UWW measure body density, the BOD POD does so with less error and burden to the subject. It is therefore recommended that the BOD POD be used instead of UWW whenever possible.

6.2 Recommendations

Further studies are required to assess the reliability and validity of the BOD POD in measuring %BF in different populations, including the elderly, children and adolescents, extremely obese patients, patients with special nutritional needs, pregnant women and people of different ethnic origin.

Further research is required on the effect of alternative clothing (i.e. other than that recommended by the manufacturer) on the reliability of measuring %BF.

Additionally, there is need for assessment of further studies examining what both the physical and physiologic factors that can cause the discrepancies in %BF measured by different methods.

REFERENCES

- Behnke AR, Feen BG, Welham WC.** The specific gravity of healthy men. *JAMA* 118:495-501, (1942).
- Biaggi RR, Vollman MW, Nies MA, Brenner CE, Flakoll PJ, Levenhagen DK, Sun M, Karabulut Z and Chen KY.** Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults. *Am J Clin Nutr* 69:898-903, (1999).
- Brozek J, Grande F, Anderson JT, Keys A.** Densitometric analysis of body composition: revision and some quantitative assumptions. *Ann NY Acad Sci* 110:113-40, (1963).
- Bunt JC, Lohman TG, Boileau RA.** Impact of total body water fluctuations on estimation of body fat from body density. *Med Sci Sports & Exerc.* 21: 96-100, (1989).
- Collins MA, Millard-Stafford ML, Sparling PB, Snow TK, Rosskoff LB, Webb SA and Omer J.** Evaluation of the BOD POD for Assessing Body Fat in Collegiate football players. *Med Sci Sports & Exerc.* pp:1350-56, (1999).
- Dahlback GO, Lundgren CEG.** Pulmonary air-trapping induced by water immersion. *Aerospace Med* 43:768-74, (1972).
- Dallal GE and Roberts S.** "DLW" A computer program for the calculation of total energy expenditure in doubly labeled water studies. *Comp Biomedics* 24:143-151, (1991).
- Dempster P, Aitkens S.** A new air displacement method for the determination of human body composition. *Med Sci Sports Exerc* 27:1692-7, (1995).
- Dewit O, Fuller NJ, Fewtrell MS, Elia M and Wells JCK.** Whole Body Air Displacement Plethysmography Compared with Hydrodensitometry for Body Composition Analysis. *Arch Dis Child.* 82:159-164, (2000).

- Economos CD, Nelson ME, Fiatarone MA, Dallal GE, Heymsfield SB, Wang J, Yasumara, MaR, Vaswani AN, Russell-Aulet M, Pierson RN.** A multi-center comparison of dual energy X-ray absorptiometers: In vivo and in vitro soft tissue measurement. *Eur J Clin Nutr* 51:312-17, (1997).
- Ellis KJ.** Human Body Composition: In Vivo Methods. *Physiol Rev* 80; (2):655, (2000).
- Fields DA, Goran MI, McCrory MA.** Body composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr* 75:453-67, (2002).
- Friedl KE, Deluca JP, Marchitelli LJ, Vogel JA.** Reliability of body-fat estimations from a four-compartment model by using density, body water, and bone mineral measurements. *Am J Clin Nutr* 55:764-70, (1992).
- Fuller MF, Fowler PA, McNeil G and Foster MA.** Body Composition: the precision and accuracy of new methods and their suitability for longitudinal studies. *Proc Nutr Soc* 49: 423-436, (1990).
- Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M.** Four-compartment model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass, *Clin Sci* 82:687-93, (1992).
- Hansen NJ, Lohman TG and Going S.** Prediction of body composition in premenopausal females from dual-energy X-ray absorptiometry. *J Appl Physiol.* 75:1637-1641, (1993).
- Heymsfield, S.B., Wang J, Lichtman S, Kamen Y, Kehayias J and Pierson RN.** Body composition in elderly subjects: a critical appraisal of clinical methodology. *Am. J. Clin. Nutr.* 50: 1167-1175, (1989).

Heymsfield SB, Lichtman S, Baumgartner RN, Wang J, Kamen Y, Aliprantis A and

Pierson RN. Body composition of humans: comparison of two improved four-compartment models that differ in expense, technical complexity, and radiation exposure. *Am J Clin Nutr* 52:52-8, (1990).

Horber FF, Thomi F, Casez JP, Fonteille J, Jaeger P. Impact of hydration status on

body composition as measured by dual energy x-ray absorptiometry in normal volunteers and patients on haemodialysis. *Br J Radiol* 65:895-900, (1992).

Iwaoka H, Yokoyama T, Nakayama T, Matsumura Y, Yoshitake Y, Fuchi T, Yoshike

N, Tanaka H. Determination of percent body fat by the newly developed sulfur hexafluoride dilution method and air- displacement plethysmography. *J Nutr Sci Vitaminol* 44:561-568, (1998).

Jebb S and Elia M. Techniques for the measurement of body composition: a practical

guide. *Int J of Obes.* 17:611-621, (1993).

Jebb SA, Goldberg GR, Jennings G, Elia M. Dual-energy X-ray absorptiometry

measurements of body composition: effects of depth and tissue thickness, including comparisons with direct analysis. *Clin Sci* 88:319-324, (1995).

Jensen MD, Kanaley JA, Roust LR, OBrien PC, Braun JS, Dunn WL, Wahner HW.

Assessment of body composition with use of Dual-Energy X-ray Absorptiometry: Evaluation and comparison with other methods. *Mayo. Clin. Proc.* 68:867-873, (1993).

Kistorp CN, Svendsen OL. Body composition analysis by dual energy X-ray absorptiometry

in female diabetics differ between manufacturers. *Eur J Clin Nutr* 51:449-54, (1997).

- Kelly TL, Richardson TL, Berger N, Steiger P, and Stein JA.** Assessment of body composition by dual-energy X-ray absorptiometry using a simultaneously scanned calibration standard. *Med.Sci. Sports. Exerc.* 23:4, (1991).
- Koda M, Ando F, Niino N, Tsuzuku S and Shimokata H.** Comparison Between the Air Displacement Method and Dual Energy X-Ray Absorptiometry for Estimation of Body Fat. *J Epidemiol.* 10: S82-S89, (2000).
- Laskey MA, Lyttle KD, Flarman ME, Barber RW.** The influence of tissue depth and composition on the performance of the Lunar dual energy x-ray absorptiometer whole body scanning mode. *Eur J Clin Nutr* 46:39-45, (1992).
- Levengagen DK, Borel MJ, Welch DC, Piasecki JH.** A comparison of air-displacement plethysmography with three other techniques to determine body fat in healthy adults. *Journal of Parenteral and Enteral Nutrition.* 23:(5):293-9, (1999).
- Lockner DW, Heyward VH, Baumgartner RN, and Jenkins KA.** Comparison of Air-Displacement Plethysmography, Hydrodensitometry, and Dual X-ray Absorptiometry for Assessing Body Composition of Children 10 to 18 years of Age. *Annals New York Academy of Sciences.* 904:72-8, (2000).
- Lohman TG.** Dual Energy X-Ray Absorptiometry. In: *Human Body Composition*, Roche AF, Heymsfield SB, Lohman TG, eds. Champaign, IL, Human Kinetics, 1996, pp. 63-78.
- Lohman, T.G.** Applicability of body composition techniques and constants for children youth. In: *Exercise and Sports Science Reviews.* Vol 14, K.B. Pandolf, ed. New York: MacMillan, 1986, pp. 330-341.

- Martin AD and Drinkwater DT.** Variability in the measurements of body fat. *Sports Medicine* 11(5):277-288, (1991)
- Mazess R, Barden HS, Bisek JP and Hansen J.** Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J. Clin Nutr* 51:1106-1112, (1990).
- McCrorry MA, Gomez TD, Bernauer EM, Molé PA.** Evaluation of a new air displacement plethysmograph for measuring human body composition. *Med Sci Sports Exerc* 27(12):1686-91, (1995).
- Miyatake N, Nonaka K and Fujii M.** A new air displacement plethysmograph for the determination of Japanese body composition. *Diabetes, Obesity and Metabolism*, 1: 347-351, (1999).
- Modlesky CM, Lewis RD, Yetman KA, Rose B, Roszkopf LB, Snow TK, Sparling PB.** Comparison of body composition and bone mineral measurements from two DXA instruments in young men. *Am J Clin Nutr* 64:669-76, (1996).
- Nelson ME, Fiatarone MA, Layne JE, Trice I, Economos CD, Fielding RA, Ma R, Nord RH, Payne RK.** DXA vs. underwater weighing: comparison of strengths and weaknesses. *Asia Pacific J of Clin Nutr* 4:173-5, (1995).
- Nunez C, Kovera AJ, Pietrobelli A, Heshka S, Horlick M, Kehayias JJ, Wang Z, Heydsfield SB.** Body composition in children and adults by air-displacement plethysmography. *Eur J Clin Nutr* 53:382-387, (1999).
- Paton NIJ, Macallan DC, Jebb SA, Pazianas M, Griffin GE.** Dual-energy X-ray absorptiometry results differ between machines (letter). *Lancet* 346:899, (1995).

- Pritchard JE, Nowson CA, Strauss BJ, Carlson JS, Kaymakci B, Wark JD.** Evaluation of dual energy X-ray absorptiometry as a method of measurement of body fat. *Eur J Clin Nutr* 47: 216-228, (1993).
- Racette SB, Scheoller DA, Luke AH, et al.** Relative dilution spaces of ^2H and ^{18}O -labeled water in humans. *Am J Physiol* 267:E585-590, (1994).
- Robertson CH, Engle CM, Bradley ME.** Lung volume in man immersed to the neck: dilution and plethysmographic techniques. *J Appl Physiol: Respirat Environ Exercise Physiol* 44:679-82, (1978).
- Roche AF, Guo S, Siervogel RM.** New field methods for the study of body composition. *Nuevas Perspectivas en Anthropologia. Granada* 819-827, (1991).
- Roubenoff R, Kehayias JJ, Dawson-Hughes B, Heymsfield SB.** Use of dual-energy x-ray absorptiometry in body-composition studies: not yet a "gold standard." *Am J Clin Nutr* 58:589-91, (1993).
- Russell-Aulet M, Wang J, Thornton J, and Pierson RN.** Comparison of dual-photon absorptiometry systems for total body bone and soft tissue measurements: Dual-energy X-rays versus Gadolinium 153. *J Bone Min Res*, 6:411-415, (1991).
- Sardinha LB, Lohman TG, Teixeira PJ, Guedes DP, Going SB.** Comparison of air-displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men. *Am J Clin Nutr* 68:786-93, (1998).
- Schoeller DA.** Hydrometry. In: *Human Body Composition*, Roche AF, Heymsfield SB, Lohman TG, eds. Champaign, IL, Human Kinetics, 1996, pp. 25-44.
- Siri W.** The gross composition of the body. *Adv. Biol. Med. Phys.* 4:239-280, (1956).

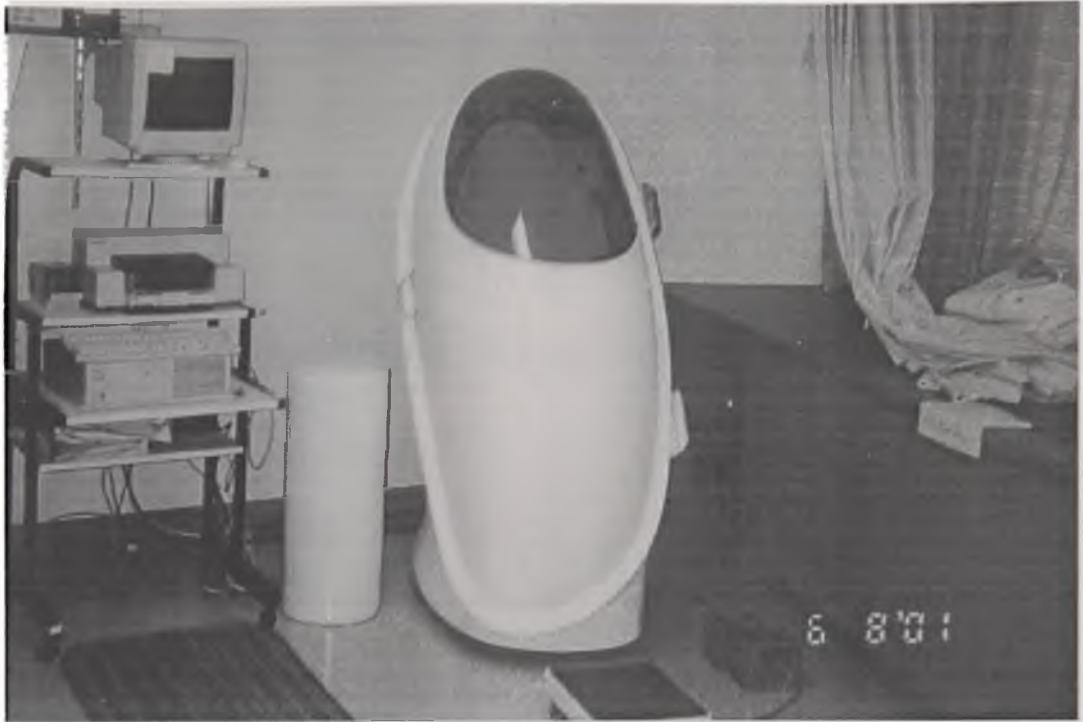
- Siri WE.** Body composition from fluid spaces and density: Analysis of methods. In: Techniques for measuring body composition, J Brozek and A Henschel, eds. Washington, DC: NAS/NRC, 1961, pp. 223-224.
- Tataranni PA, Pettitt DJ, Ravussin E.** Dual energy X-ray absorptiometry: inter-machine variability. *Int J Obes* 20:1048-50, (1996).
- Tothill P, Avenell A, Love J, Reid DM.** Comparisons between Hologic, Lunar and Norland dual-energy X-ray absorptiometers and other techniques used for whole body soft-tissue measurements. *Eur J Clin Nutr* 48:781-94, (1994).
- Van Loan MD.** Is dual-energy X-ray absorptiometry ready for prime time in the clinical evaluation of body composition? *Am J Clin Nutr* 68:1155-6, (1998).
- Wagner DR, Heyward VH, and Gibson AL.** Validation of air-displacement plethysmography for assessing body composition. *Med Sci Sports Exerc* 32(7):1339-1344, (2000).
- Wells JCK and Fuller NJ.** Precision of measurement and body size in whole-body air-displacement plethysmography. (In press).

Appendix 1



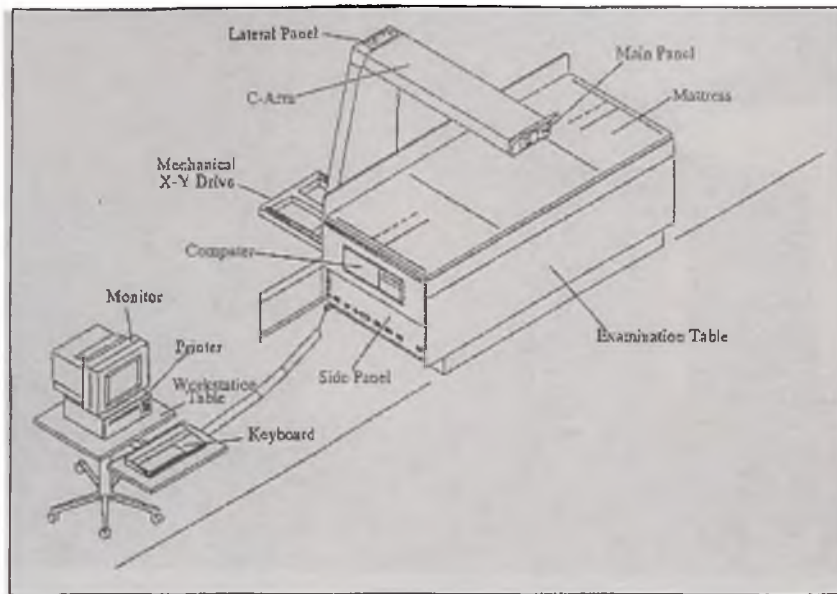
Subjects in underwater weighing tank at the HNRC

Appendix 2



The BOD POD® Body Composition System at the HNRC

Appendix 3



Components of the dual X-ray absorptiometry machine (QDR 2000, Hologic)

Diagram taken from operators manual of QDR-2000, Hologic, Waltham, MA.

Appendix 4



Subject undergoing DXA measurement at the New England Medical Center

Appendix 5

**USDA HUMAN NUTRITION RESEARCH CENTER ON AGING
AT TUFTS UNIVERSITY
711 Washington Street
Boston MA 02111
1-800-738-7555**

HEALTH QUESTIONNAIRE

The following questions are designed to obtain a thorough preliminary medical history. The information you provide will help us to make the best determination about your eligibility for a particular study or studies. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide, will be kept confidential except where required by law.

INSTRUCTIONS:

In the three spaces below, please indicate by number the research studies that interest you, beginning with your first choice. If a label with your name is affixed below, please make any necessary corrections. If no label is affixed, please PRINT your name and address in the space provided.

Please PRINT your answers to all questions in INK. For those questions requiring further information, be as complete and specific as possible. Additional space for comments is provided on the last page of the questionnaire.

I am interested in these studies: 1. _____ 2. _____ 3. _____

Name: _____

Street Address:

City, State, Zip:

Telephone number: Home () _____ Work () _____

Do you mind being called at work? Yes ___ No ___

Date of Birth: _____ Age: _____ M ___ F

Social Security Number:

Do you have health insurance? Yes ___ No

Are you currently participating in research studies outside the Center? No ___ Yes ___

If yes, please explain: _____

If there are specific times over the next 6 months when you would be unavailable to participate in a study, please indicate:

DEMOGRAPHICS

1. MARITAL STATUS

_____ Married _____ Separated _____ Single, never married
 _____ Divorced _____ Widowed

2. LIVING SITUATION: Where do you live? (Check one.)

_____ House _____ Apartment _____ Shelter _____ Dormitory _____ Other

If other, please explain:

3. RACE: (please indicate): _____

4. OCCUPATION

Current occupation (if applicable):

Occupation at retirement:

5. EDUCATION

Last grade completed in elementary or secondary school: _____

Education since leaving elementary or secondary school:

_____ None _____ Four year college
 _____ Vocational school _____ Graduate school
 _____ Community or junior college _____ Professional school

PERSONAL HEALTH HISTORY

1. Height: _____ ft. _____ inches Weight: _____ lbs.
 (without shoes) (without shoes)

2. Are you allergic to, sensitive to, or intolerant of any foods or medications? No ___ Yes

Food:

Medication:

Seasonal Allergies: (what season?)

Other: (soap, tape, latex, lotions, etc.)

3. When did you last see your physician or other health care provider?
(gynecologist, eye doctor, etc.)

Date: _____ Reason: _____

4. Do you have any chronic illnesses? If yes, please explain: No ___ Yes ___

5. Have you ever been hospitalized or had surgery? No ___ Yes ___
Please list all hospitalizations and surgeries: _____

6. Are you currently taking any medication either prescribed by a doctor or purchased over the counter at least once a week? Please include regular use of pain relievers, aspirin, eye drops, creams, sleeping pills, antacids. No ___ Yes ___

<u>Drug Name</u>	<u>Amount</u>	<u>How Often</u>	<u>How Long</u>	<u>Reason</u>
(ex. Advil)	2 tablets	every day	one year	joint pain)

- a. _____
b. _____
c. _____
d. _____
e. _____

7. Are you currently taking any vitamins, minerals, herbs, or health food supplements once per week on a regular basis? No ___ Yes ___

<u>Supplement Name</u>	<u>Amount</u>	<u>How Often</u>	<u>How Long</u>	<u>Reason Prescribed</u>
------------------------	---------------	------------------	-----------------	--------------------------

MD/Self

- a. _____
b. _____
c. _____
d. _____
e. _____

8. Would you be willing to stop your vitamins, minerals, herbs,

- or health food supplements if needed while participating in a study? No__ Yes__
9. Do you currently smoke or use tobacco? No__ Yes__
How much? _____ For how long?
- a. Have you ever smoked? No__ Yes__
If yes, how long ago did you quit? _____
10. Are you currently following a special diet? (i.e., vegetarian, diabetic, low fat, lactose free) If yes, what kind? _____

- a. Is this diet being prescribed by your health care provider? No__ Yes__
11. Where do you most often obtain your meals? (Check all that apply.)
() Home () Home, with home delivered meals () Work
() Restaurants () Congregate meal sites () Other
12. If accepted for a study, would you be willing to follow a diet that may vary from your current food intake? No__ Yes__
13. Have you had a weight loss or gain in the last 6 months? No__ Yes__
If yes, how much? _____ lbs. Gain _____ Loss _____
17. How many meals and/or snacks per day do you usually eat?
Meals: _____ Time of day:
Snacks: _____ Time of day:
18. Do you currently participate in regular physical activity? No__ Yes__
If yes, how often and what type? _____
19. Do you have any condition that would prevent you from being physically active? If yes, please explain: _____ No__ Yes__
20. Have you ever received counseling or psychotherapy on an outpatient or inpatient basis? If yes, explain: _____ No__ Yes__
21. Do you currently drink alcohol? If yes, how much? No__ Yes__
Per day: _____ Per week: _____
22. Have you ever had a drinking problem? If yes, please explain. No__ Yes__

**The following is a list of health conditions.
Check yes or no and circle those conditions that apply to you.**

**Please indicate when you had the problem and any treatment,
hospital stay, and/or follow-up required.**

31. Breasts: lumps, nipple discharge, pain, discomfort, and lumpectomy, No ___ Yes ___
mastectomy, If yes, explain: _____
- Date of last mammogram _____
32. Respiratory: cough, shortness of breath, asthma, wheezing, No ___ Yes ___
bronchitis, pneumonia, emphysema, tuberculosis or a positive TB test.
If yes, explain: _____
- Last chest x-ray: _____
33. Heart: chest pain or pressure, murmur, palpitations, No ___ Yes ___
irregular heart beat, rheumatic fever, mitral valve prolapse, history of
coronary heart disease, heart attack, congestive heart failure.
If yes, explain: _____
- Last electrocardiogram: _____
34. Blood Pressure: high or low. If yes, explain: _____ No ___ Yes ___
- Last reading, if known: _____
37. Intestine: constipation, diarrhea, hernia, change in bowel habits, No ___ Yes ___
irritable bowel disorder, colitis, polyps. If yes, explain: _____
38. Do you use any type of aid such as laxatives, suppositories or enemas No ___ Yes ___
to regulate your bowel habits?
39. Have you ever had any form of cancer, skin or other? No ___ Yes ___
If yes, explain: _____
40. Liver, Gallbladder: hepatitis, gallstones, cirrhosis. : No ___ Yes ___
If yes, explain: _____
41. Urinary: frequent urination, incontinence urgency, burning, blood in No ___ Yes ___
urine, infection, kidney stones. If yes, explain: _____
42. Circulation: leg cramps or pain in hands or feet, blood clots, phlebitis. No ___ Yes ___
If yes, explain: _____
43. Muscles, Bones, Joints: joint pain, swelling, weakness, No ___ Yes ___
disc disease, arthritis, tendinitis, bursitis, gout, backache, osteoporosis.
If yes, explain: _____

44. Have you ever had a broken bone, stress fracture, or fractured a vertebra in your back?
 No ___ Yes ___ If yes, explain: _____

45. Neurological: seizure, stroke, paralysis, fainting, weakness, numbness, tingling, tremors, memory loss? No ___ Yes ___
 loss. If yes, explain: _____

46. Do you have any problems with your memory? No ___ Yes ___

48. Glands: diabetes or high blood sugar, over or underactive thyroid, excessive hunger, thirst. If yes, explain: _____ No ___ Yes ___

49. Eating disorders: anorexia, bulimia, binge eating. No ___ Yes ___
 If yes, explain: _____

50. Do you mind having your blood drawn? No ___ Yes ___

51. Are you a blood, plasma, platelet donor? No ___ Yes ___
 If yes, please give date of last donation. _____

52. FOR MALES ONLY:

Have you ever had prostate problems, enlargement, incontinence of urine or stool, hernias, testicular pain, lumps, discharge from or sores on penis, sexually transmitted illness? No ___ Yes ___
 If yes, explain: _____

53. FOR FEMALES ONLY:

Have you ever had menstrual problems, vaginal discharge, irregular bleeding, sexually transmitted illness, incontinence of urine or stool? No ___ Yes ___
 If yes, explain: _____

Are you still menstruating? No ___ Yes ___

If no, what was your age at menopause? _____

If menopausal, are you currently on estrogen replacement therapy? No ___ Yes ___

Last PAP smear: _____

Number of pregnancies: _____

Number of births: _____

COMMENTS:

Please provide the following information regarding your primary care doctor in the event that your screening test results need to be sent to him/her for review.

Name

Address (street)

(city, state, zip code)

() _____ ()
Telephone Number (include area codes) Fax Number

SIGNATURE:

DATE:

Rev. 12/00

FOR FURTHER INFORMATION CALL: (617) 556-3300

Appendix 6

INFORMED CONSENT FORM

USDA Human Nutrition Research Center on Aging at Tufts University

- Study Title:** Evaluation of the BOD POD Air Displacement Plethysmograph for Measurement of Body Composition and Changes in Body Composition - Study I/ II: Measurements in Young vs Older and Lean vs Obese Individuals
- Principal Investigator:** Megan A. McCrory, PhD
- Co-Investigators:** Susan B. Roberts, PhD
Virginia A. Hughes, MS
Edward Saltzman, MD
- Physician:** Edward Saltzman, MD

Purpose of Study

I understand that I am being asked to participate, as a volunteer, in a research study at the Human Nutrition Research Center (HNRC) on Aging at Tufts University. The purpose of this study is to compare several different methods for their ability to measure body composition (how much fat and lean tissue my body contains) and changes in body composition due to weight gain. To simulate weight gain, I will hold small objects of known weight during some of the body composition tests.

I have provided to the best of my knowledge a complete history of all my medical problems, medications, and vaccination history. To my knowledge I am free from any serious medical disorder including insulin dependent diabetes, active cancer or AIDS. I will avoid taking any new non-prescription medications, vitamins, or nutritional supplements during this study without informing the study physician. To my knowledge I am not pregnant.

I understand that my eligibility for this study may not be confirmed until the study physician, Dr Saltzman, reviews my Health History Questionnaire and performs a physical examination if he believes it necessary. I understand that if I am still menstruating, I may be asked to undergo a pregnancy test about a week before the study begins.

Study Procedures

I understand that if I choose to participate the study will take place on two different days, 5-8 days apart. Each test day will last for approximately 5-6 hours and will include several body composition tests and a possible physical examination (on the first day only). I understand that I can refuse to participate in any of the tests listed in the consent form. On each day after I am finished with all the tests, if I desire I may eat a lunch provided by the HNRC (this is not part of the study).

I will report to the HNRC at the dates and times designated by the investigators. The night before my scheduled test days at the HNRC, I will eat a large meal but will avoid consuming gas-producing foods such as beans, broccoli and cabbage. Also, the day before my test day I will not consume more than one serving of an alcoholic beverage, which is either 12 ounces of beer (1.5 cups), 4 ounces of wine (0.5 cups), or 1 ounce of hard liquor (1 Tablespoon or 1 shot glass). In addition, I will not consume more than 1 cup of caffeinated coffee or 1 can of caffeinated soda or other caffeine-containing beverage. I will finish eating my last meal by midnight, and will report to the HNRC the next morning at the scheduled time without eating or drinking anything except water that morning. I understand that before arriving at the HNRC that morning, I should drink enough water to quench my thirst, but no more than this. I also understand that I should not exercise vigorously in the morning before arriving at the HNRC. In addition, I will provide four urine samples before the optional lunch at specified times as part of the body composition measurements.

General health examination

I will fill out a general health history questionnaire which will be reviewed by the study physician, Dr Saltzman. A physical examination may be performed at Dr Saltzman's discretion, and a pregnancy test may also be performed.

The body composition measurements to be made

1. Dual energy x-ray absorptiometry: My body composition will be measured using the dual energy x-ray absorptiometry method. This method involves a small radiation dose, and will measure my body fat distribution as well as my bone density and the total amount of fat in my body. For this measurement I will lie on a padded table while the measurement is being made for approximately 15 minutes. This measurement will be performed twice on one day and once on another day for a total of three tests. The total radiation dose is 3 mrem (1 mrem per test), which is equivalent to 3 days of natural background radiation exposure. I will be able to get up and walk around between each measurement.
2. Underwater weighing: My weight will be measured while I am submerged in water and this information will be used to calculate the total amount of fat in my body. For this test I will sit on a seat which resides on a scale in a tank of warm water. During this time, I will be asked to breath out as much air as I possibly can and my weight will be measured while I am totally submerged in water. This will take a total of about 10 seconds. This will be repeated 4-5 times to get an accurate measurement. In addition to this, I will breathe through a machine that will measure the total amount of air my lungs can hold, and the amount of air remaining in my lungs after I breathe out as much air as I possibly can. The entire test procedure (being weighed in water plus lung volume measurement) will be performed twice on one day and once on the other day for a total of three tests. In addition, on one of these days the lung volume measurement will also be carried out while I am in the water immersed only up to my neck.

If deemed necessary by the principal investigator or his/her representative, I will be asked to wear a harness while in the underwater weighing tank. This harness will be used in the event

that I need assistance to exit the tank. There is a chance that I could get bruised using this alternative exit procedure.

3. **Weight and height:** My body weight and height will be measured using anthropometry. My height will be measured twice on each day, and my weight will be measured several times each day.

4. **Total body water by deuterium dilution:** My body water content will be measured. I will drink a small glass of water that contains about 1 teaspoon (5 grams) of deuterium oxide, which is a normal compartment of water. I will be asked to provide urine specimens before the drink and at 3, 4 and 5 hours afterwards. There is no radiation exposure or other risk during this procedure. This test will be performed only once on each day.

5. **BOD POD air displacement method:** My body composition will be measured using the BOD POD air displacement method. During a single BOD POD test sequence I will sit comfortably in a chamber with a big window for less than 1 minute on 2-3 occasions. During this time I will be breathing normal room air and very small air pressure changes inside the chamber will be made, but they are so small (< 1 cubic centimeter of water) that I will not be able to feel anything. The door will be opened between each 1-minute measurement to allow fresh room air into the chamber. After this, the amount of air in my lungs during normal breathing will be measured while I am in the chamber. For this lung test, I will be breathing room air through a snorkel device and then the airway will be closed for 2 seconds. While the airway is closed, I will gently blow against the occlusion. This lung test may be repeated several times. I understand that this measurement is completely safe. This entire test sequence will be performed two to five times each day. Some of these tests will be performed while I am holding small objects weighing between approximately 1-5 kg. In addition, I may be asked to perform this test several more times while wearing different clothing (bathing suit, different shorts and exercise tops, and a paper examination gown like is given in a doctor's office). My oral and skin temperatures may also be measured during this test session.

6. **Waist and hip circumferences:** The distance around my waist and hips will be measured with a tape measure while I am in my bathing suit in order to get an accurate measure. These will be measured twice on one day, and once on another day.

Questionnaires

As part of the screening procedure, I will complete a simple Health History Questionnaire which will ask information about my health, diet, physical activity and lifestyle. The total time it will take to complete this questionnaire is about 10 minutes.

Risks

There is a small radiation exposure from the dual x-ray absorptiometry. This radiation exposure is not expected to result in any observable effects. The total radiation dose I will receive is 3 mrem, which is equivalent to 3 days of natural background radiation exposure. Also, I may feel short of breath during the measurement of my body composition by

underwater weighing, although only for a short time. There is also a chance that I could get bruised by the harness if it is used during the underwater weighing procedure. Also, because the air displacement test is inside an enclosed chamber, I may feel some discomfort during this test. This discomfort may be minimized by the presence of a large window on the door of the chamber.

Benefits

I understand that there is no direct medical benefit to me from the study. However, I will be informed of any adverse health problems found during the course of the study.

Stipend

I will be paid \$75.00 for completing the study. If for some reason I only complete one day of the study, I will be paid half this amount (\$37.50).

Phone numbers

I have been told that I can reach the Principal Investigator or any of the Co-investigators at any time of the day or night if I have any questions or problems related to the study.

Dr Megan McCrory	work (617) 556-3313, home (781) 631-1416
Dr Susan Roberts	work (617) 556-3238, home (617) 244-0951
Virginia Hughes	work (617) 556-3079, home (978) 356-1420
Dr Edward Saltzman	work (617) 556-3245, home (781) 237-7326

PARTICIPANT'S STATEMENT

I have read this consent form and have discussed with Dr. Saltzman or his/her representative the procedures described above. I have been given the opportunity to ask questions, which have been answered to my satisfaction. I understand that any questions that I might have will be answered verbally or, if I prefer, with a written statement.

I understand that I will be informed of any new findings developed during the course of this research study. I understand that my participation is voluntary. I understand that I may refuse to participate in this study. I also understand that if, for any reason, I wish to discontinue my participation in this study at any time, I will be free to do so, and this will have no effect on my future care or treatment by my physicians or this hospital.

I understand that if I discontinue my participation in this study, I will be paid up to the day I withdraw, and the amount will be proportional to the time I have spent in the study. Also, the Investigator or the Institution may decide, at any time and for any reason, that my participation in the study will be terminated. In this event, I will be paid up to the day of the termination and the amount will be proportional to the time I have spent in the study.

I understand that in the event I become ill or am injured as a result of participating in this research study, medical care will be provided to me. However, such medical care will not be

provided free of charge, even if the injury or illness is a direct result of this research study. I understand that no funds to provide financial compensation for research-related injury or illness are available.

If I have any questions concerning my rights as a research subject in this study, I may contact the Human Investigation Review Committee at (617) 636-7512.

I have been fully informed of the above-described study with its risks and benefits and I hereby consent to the procedures set forth above. I have received a copy of this signed consent form.

I understand that as a participant in this study, my identity and my medical records and data relating to this research study will be kept confidential, except as required by law and except for inspectors by the study sponsor.

Date

Participant

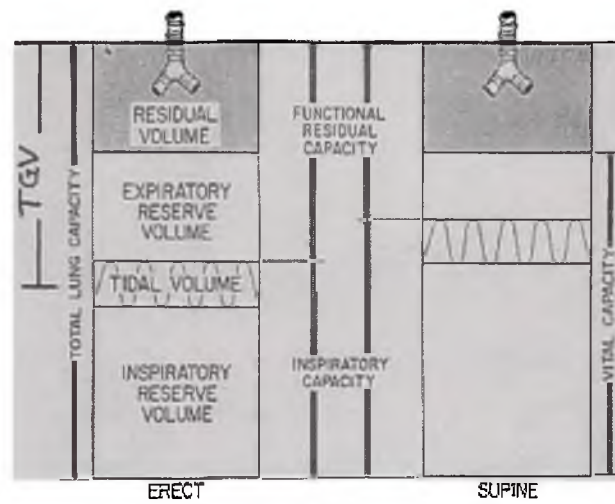
I have fully explained to _____ the nature and purpose of the above-described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

Principal Investigator or Representative

Date

Witness

Appendix 7

**Lung volume and its subdivisions**

Picture taken from Respiratory Physiology by N. Balfour Slonim and Lyle H. Hamilton.
The C.V. Mosby Company. St Louis, Missouri. Fourth Edition, 1981; Pg 53

Appendix 8



Subjects performing residual lung volume measurements at the HNRC

Appendix 9



Subjects undergoing body composition measurement in the BOD POD at the HNRC

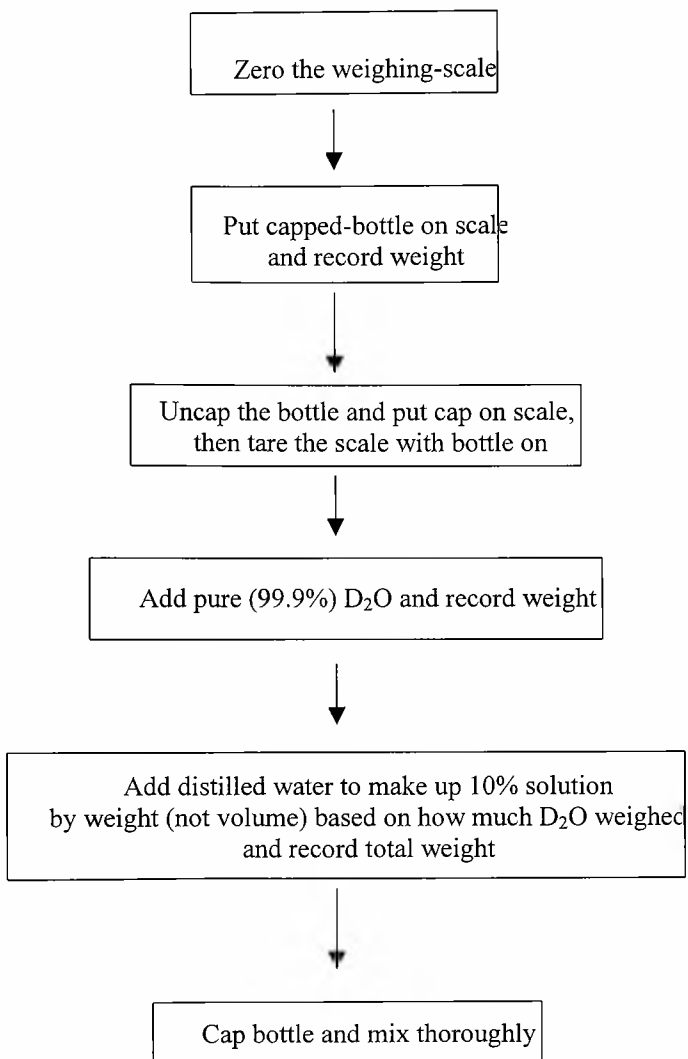
Appendix 10

Schedule for study

Estimated Time	Event	Person doing measurement	Where
8:00 am	Check in at nurses' station, get vitals	Nurse on duty	HNRC 12
8:25	BIA and circumferences	Megan M.	HNRC 12
8:40	Baseline urine specimen	Nurse on duty	HNRC 12
8:45	Dose D2O	Nurse on duty	HNRC 12
9:00	DXA (two)	Megan O'Neill	NEMC Pratt 617
9:35	BOD POD (two)	Megan McCrory	HNRC 13–BP rm
10:15	RV on land #1	Nick/Alex	HNRC 13-back lab
10:50	UWW (use weight on BP scale) #1	Nick/Alex	HNRC 13-uwv rm
11:15	RV on land #2–new file/recalibrate	Nick/Alex	HNRC 13-back lab
11:50	UWW (use weight on BP scale) #2	Nick/Alex	
12:15 pm	use bathroom (3:30 hr discard)	Nurse on duty	HNRC 12
1:00	use bathroom (4:15 hr specimen)	Nurse on duty	HNRC 12
between these two urine specimens	If volunteer is super-duper hungry, e.g., feeling really lightheaded or faint, he/she can have a banana from the kitchen, but not if he/she can wait		
1:45	use bathroom (5 hr specimen)	Nurse on duty	HNRC 12
1:50	lunch		HNRC 11

Appendix 11

Preparation of the deuterium dose



Store some amount of the prepared dose in “NUNC” tube for future analysis with specimen.

Appendix 12

How to weigh out the Deuterium Dose--Study 1461

1. Make label for dose bottle: include volunteer's name, test date, study 1461, dose amount per kg body weight, HNRC id number. Be sure to put label on bottle BEFORE beginning the weighing process.
2. Get deuterium dosing form and calculator.
3. Calculate dose amount needed and record on form. For body weight, always use screening value.

* If first test occasion, need 1.0 g deuterium/kg body weight
**If second test occasion, need 0.8 g deuterium/kg body weight
4. Turn on and tare the scale on lab bench in room 605.
5. After putting label on the dose bottle, weigh and record the weight of empty container + lid.
6. Tare the empty container without the lid.
7. Gently invert large bottle containing the dose mixture at least 10 times. This is because the heavier molecules sink to the bottom, but we want to be sure the volunteer gets a uniformly mixed dose.
8. Carefully pour dose into the dose bottle while it is on the scale. Pour it in until you get just a little more than the amount you will need (from calculation). Then use a clean disposable transfer pipette to remove extra amount and plunge it back into the large dose mixture bottle. Throw away pipette after use. Record the exact weight (you don't need to weight out exactly what you calculated, just get in very close range)—note you will not really use this number; it is just for verifying the weight you will calculate in step 12.
9. Tare scale with nothing on it.
10. Re-weigh the dose bottle (container, lid + dose) and record on form.
11. Use calculator to calculate amount of dose weighed out; record on form next to "weight of dose only".

Appendix 13

PROTOCOL #1461: Body Composition of Adults

DEUTERIUM DOSING FORM

Please fill in all information that is not shaded.

Name: _____

Date: _____

Dose Lot#	<input type="text"/>	<input type="text"/>
Calculated dose (gm): Body Wt (kg) x _____ of bulk dose	_____	
Weight of empty container + lid	_____	
Weight of container, lid + dose	_____	
Weight of dose only	<input type="text"/>	<input type="text"/>

Fasting body weight (kg) _____

Dose administered by _____

Time dose given _____

Did subject consume all the dose and the two 25ml Tap rinses? Y or N

Was there any loss of dose? Y or N

TIMES OF URINE SAMPLES:

(2-4.5ml NUNC-Put time and total void volume on NUNC tube)

****USE CLEAN-DRY URINE HAT/URINAL FOR EACH SAMPLE TIME**

*** MEASURE TOTAL VOID IN GRADUATED CYLINDER AFTER NUNC TAKEN**

<u>TIME</u>	<u>TOTAL VOLUME</u>
Baseline sample _____	Baseline sample _____
3° 30" sample _____ (Don't save)	3° 30" sample _____
4° 15" sample _____	4° 15" sample _____
5° sample _____	5° sample _____

(URINE SAMPLE TIMES APPROXIMATE)

2/28/01

Dosing sheet for total body water

Appendix 14

NEMC-BOSTON OBESITY/NUTRITION CENTER



May 4 08:57 2001 (333 x 152)
Hologic QDR-2000 (S/N 2495)
Enhanced Array Whole Body US.73A

M05040102 Fri May 4 08:48 2001
Name:
Comment: Baseline for 1461
I.D.: Sex: F
S.S.#: Ethnic:
ZIP Code: Height: 5' 6"
Scan Code: AHB Weight: 222
BirthDate: 04/17/65 Age: 36
Physician: MCCRORY
Image not for diagnostic use

TOTAL BMC and BMD CV is < 1.0%
C.F. 1.008 1.065 1.000

Region	Area (cm2)	BMC (grams)	BMD (gms/cm2)
L Arm	223.68	169.04	0.756
R Arm	265.77	211.38	0.795
L Ribs	147.52	100.23	0.679
R Ribs	159.14	100.37	0.631
T Spine	149.12	134.64	0.903
L Spine	53.31	61.05	1.145
Pelvis	208.05	277.61	1.334
L Leg	406.06	559.46	1.400
R Leg	432.53	566.55	1.310
SubTot	2046.01	2190.34	1.071
Head	229.29	584.30	2.548
TOTAL	2275.30	2774.64	1.219

HOLOGIC

NEMC-BOSTON OBESITY/NUTRITION CENTER

Hologic QDR-2000 (S/N 2495)
Enhanced Array Whole Body US.73A
May 4 08:57 2001

TEAR754
P.S. 68.00% 0(10.00)%

M05040102 Fri May 4 08:48 2001
Name: McCrory, Megan
Comment: Baseline for 1461
I.D.: Sex: F
S.S.#: Ethnic:
ZIP Code: Height: 5' 6"
Scan Code: AHB Weight: 222
BirthDate: 04/17/65 Age: 36
Physician: MCCRORY

Region	BMC (grams)	Fat (grams)	Lean (grams)	Lean+BMC (grams)	Total (grams)	% Fat (%)
L Arm	169.0	2940.6	1245.6	1414.6	4355.2	67.5
R Arm	211.4	3290.8	1862.3	2073.7	5364.5	61.3
Trunk	673.9	22385.6	20625.3	21299.2	44204.8	51.9
L Leg	569.5	9229.9	7373.2	7942.6	17172.5	53.7
R Leg	566.6	9277.6	7715.1	8281.6	17559.3	52.0
SubTot	2190.3	47724.5	30021.4	41011.7	88736.3	53.8
Head	584.3	866.5	3231.7	3816.0	4684.5	16.5
TOTAL	2774.6	48593.1	42053.8	44827.7	93428.7	52.0

*assumes 17.0% brain fat
LDM 73.2% water

HOLOGIC

Print out from dual-energy X-ray absorptiometry

Appendix 15

BOD POD data sheet for Study 1461

Name _____
 ID _____
 Height (cm) _____
 Sex M F (circle one)
 Date of birth _____

Day 1
 Testers (1) _____ (2) _____
 Time _____
 Date _____
 Age _____ y

TGV trials (need 2 within 10%) ***DO ONLY IF 1ST VISIT**

no BFB	1	2	3	4	5	6	7	8	9	10
Merit										
Airway Pressure										
MeasLV										
Tidal Volume										
TGV@FRC										

	Pre-test temps	1st test	2 nd test
Clothing worn			
Room temp			
Oral temp			
Skin temp			
Time of weight			
Body vol 1			
Body vol 2			
Body vol 3 (if needed)			
Mean body vol			
Merit (<1.0)			
Airway Pressure (<35)			
# times to get LV			
Weight (kg)			
Raw volume			
Area artifact			
Measured LV			
Predicted LV			
Tidal volume			
TGV@FRC			
Body vol			
Density			
% body fat			
Fat weight			
Lean weight			

Volunteer's degree of comfort, where 1 = uncomfortable and 5 = relaxed

	<u>Volunteer's own assessment (ask verbally):</u>					
(uncomfortable)	1	2	3	4	5	(relaxed)
	<u>Tester's assessment:</u>					
(uncomfortable)	1	2	3	4	5	(relaxed)

Appendix 16

Underwater Weighing Data Sheet—Study 1461

Day: _____ Testers: (1) _____ (2) _____
 Name: _____ Date: _____
 ID: _____ Age: _____ years
 Height (cm): _____ (measured on 13th flr) Sex: M F (circle one)
 Weight (kg): _____ (from BOD POD scale) Date of birth: _____
 Type of attire worn for test: _____

A. Do usual UWW procedure #1 Time at tare: _____ Water Temp (°F): _____

(1) Zero scale (initial): _____ (kg) {range 0.0 to 0.1}

(2) Tare mass: _____ (kg)
 Is nose clip worn for measurement? Yes No (circle one)
 Nose clip included with tare? Yes No (circle one)

(3) Determine density → set predicted RV to Crapo ****Repeat measurements of body density until at least 3 values for percent body fat agree within 1.0% (high value to low value).**

Any comments:

(4) Re-check tare mass: _____ (kg) {goal: <0.02kg from initial reading}.

(5) Volunteer's degree of comfort, where 1 = uncomfortable and 5 = relaxed
Volunteer's own assessment (ask verbally):
 (uncomfortable) 1 2 3 4 5 (relaxed)
Tester's assessment:
 (uncomfortable) 1 2 3 4 5 (relaxed)

(6) ******Save and print calibration information and UWW trials data**

B. Do usual UWW procedure #2 Time at tare: _____ Water Temp (°F): _____

(1) Zero scale (initial): _____ (kg) {range 0.0 to 0.1}

(2) Tare mass: _____ (kg)
 Is nose clip worn for measurement? Yes No (circle one)
 Nose clip included with tare? Yes No (circle one)

(3) Determine density → set predicted RV to Crapo ****Repeat measurements of body density until at least 3 values for percent body fat agree within 1.0% (high value to low value).**

Any comments:

- (4) Re-check tare mass: _____ (kg) {goal: <0.02kg from initial reading}.
- (5) Volunteer's degree of comfort, where 1 = uncomfortable and 5 = relaxed
- | | | | | | | |
|-----------------|---|---|---|---|---|-----------|
| | <u>Volunteer's own assessment (ask verbally):</u> | | | | | |
| (uncomfortable) | 1 | 2 | 3 | 4 | 5 | (relaxed) |
| | <u>Tester's assessment:</u> | | | | | |
| (uncomfortable) | 1 | 2 | 3 | 4 | 5 | (relaxed) |
- (6) ******Save and print calibration information and UWW trials data**