THE EFFECT OF BONE DENSITY ON THE ESTIMATION OF BODY FAT IN YOUNG WOMEN

BY

AURELIA M. JACOBSEN

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

© 1990

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis, to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film, and UNIVERSITY MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

THE UNIVERSITY OF MANITOBA

THE EFFECT OF BONE DENSITY ON THE ESTIMATION OF BODY FAT IN YOUNG WOMEN

by
Aurelia M. Jacobsen
B.A. University of Toronto 1971

A Thesis
Submitted to
The Faculty of Graduate Studies
In Partial Fulfillment
of the Requirements for the Degree
Master of Science

Faculty of Physical Education and Recreation Studies
© Copyright by Aurelia M. Jacobsen 1990

National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-71837-4

Canada

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Aurelia M. Jacobsen

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Aurelia M. Jacobsen

The University of Manitoba requires the signatures of all persons using or photocopying this thesis. Please sign below, and give address and date.

ABSTRACT

The effect of bone density on the estimation of body fat in young women. Jacobsen, A.M., University of Manitoba.

The purpose of this study was to assess the amount of variability in bone mineral density in young adult women and to determine the effect of this variability on whole body density and body fat as estimated by hydrostatic weighing. A literature search was undertaken to determine the coefficient of variation (CV) in young women for whole body bone density. As well, the bone density of the lumbar spine and proximal femur of 41 healthy, premenopausal females (19-48, mean age 31) was measured by dual photon absorptiometry (DPA). Whole body density and percent fat were determined by underwater weighing and extensive anthropometric measurements were taken. The variables were analyzed by correlation, factor analysis and stepwise regression to determine the interrelationships and their relationship to body density. Mathematical analysis with stepwise regression and a three component model of the fat-free mass quantified the extent of the effect of bone density on percent fat for this sample.

The literature showed that the bone density at the sites examined had different amounts of variation and the density at one site did not necessarily represent that of another site or the whole skeleton. A CV of 7% was estimated for total body bone density in a normal young women. The CV for the lumbar spine was 10.1%; femoral neck, 11.4%; Ward's triangle, 14.2% and the trochanter 12.2%. All sites were positively correlated with whole body density The correlation of the femoral neck was significant for this sample ($r=.34$, $p\leq.05$).

Factor analysis revealed that in general, skinfolds suggested fatness, DPA measures - bone density, bone breadths - bone size and muscle circumferences - muscularity. Stepwise regression identified the significant contributors to body density in this sample as fatness, primary and bone density, secondary. Stepwise regresssion to predict bone density indicated that the bone breadths were most closely associated with the DPA bone density measurements but could not be used to replace them.

Two mathematical analyses demonstrated errors in fat prediction due to bone density greater than measurement error, in 29% of the sample. The errors ranged from -4.4 to 4.7% and -6.6 to 9.1% fat in these subjects (who had >1 SD above or below the mean on femoral bone density) and were proportionately larger in lean individuals. The 5 subjects with the greatest percent fat underestimation had higher bone density at all the femoral sites ($p \leq .0001$) but not the spine. The 7 subjects with the greatest overestimation had lower bone density at both lumbar and femoral sites ($p \leq .0001$), were older (mean age 36 yrs, $p \leq .055$) and had a smaller chest girth ($p \leq .04$) than the rest of the group.

It was concluded that errors due to bone density, made prediction of percent fat from whole body density with Siri's equation which uses a constant value for the fat-free density of 1.1 g/cm^3, unsuitable for a normal group of young women. The possibility of error and the probable magnitude should be acknowledged until new equations are established for women which reflect gender differences in bone density and allow for normal variability.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis advisor Dr. A.D. Martin, for his assistance and guidance. He inspired me to take on this complex project and was confident that I would complete it. He has helped me move to a new level of understanding and to continue with the questioning process. On the practical side, he has organized the process for me in Winnipeg so that I was able to complete my degree in spite of a move to Montreal.

I am also indebted to the other members of my thesis committee: to Dr. D.T. Drinkwater, for all the advice and technical help which kept me on track; to Dr. E. Ready, for the course spent learning body composition together and to Dr. G. Sevenhusen, for his understanding about the time needed to complete this project as well as his critical comments.

Thanks are also extended to all the faculty members and staff, fellow students and subjects who contributed towards the completion of this study.

Finally, I wish to acknowledge the contribution to this project made by my family. My husband, Henrik, helped with data collection, photocopying, critical advice, and computer hardware. He took on new chores, spent more time with the children and encouraged me on through all the setbacks. My children, Peter and Paul, helped me to check figures and supported me with breakfasts and lunches. Thank you.

DEDICATION

I dedicate this thesis to my husband Henrik and my children Peter and Paul.

"Flow with whatever may happen and let your mind be free: Stay centered by accepting whatever you are doing. This is the ultimate."

Chuang-Tzu

TABLE OF CONTENTS

Page

ABSTRACT iii

ACKNOWLEDGEMENTS v

DEDICATION vi

LIST OF TABLES x

LIST OF FIGURES xiii

Chapter

1. INTRODUCTION 1
    - Statement of the Problem 7
    - Limitations 7
    - Definition of Terms 8
2. REVIEW OF RELATED LITERATURE 9
    - Introduction 9
    - Densitometry 9
    - A Two Component Model of the Body 11
    - A Three Component Model of the Fat-Free Mass 19
    - Variability of Bone Mineral in Normal Female Populations 24
3. METHODS AND PROCEDURES 42
    - Introduction 42
    - Subjects 42
    - Experimental Design 44
    - Data Collection 45

Bone Density Measurements 45

Underwater Weighing 47

Anthropometric Measurements 52

Data Analysis 54

4. RESULTS AND DISCUSSION 59

Introduction 59

Physical Characteristics of the Subjects 62

Correlation of Main Variables 68

Factor Analysis 75

Stepwise Regression to Predict Body Density and Bone Density 87

Percent Fat Differences Due to Bone Density 99

Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass 119

5. SUMMARY AND CONCLUSIONS 129

Summary 129

Conclusions 132

Recommendations 135

BIBLIOGRAPHY 136

APPENDICES

A. Numerical Estimation of Fat from Density 146

B. Example of DPA Scan Results 152

C. Example of Self Report Subject Questionnaire 156

D. Example of Description of Research Project Information to Participant and Consent Form 159

LIST OF TABLES

Table Page

2.1 Two Component Model of Body Composition 11

2.2 Percentage Composition and Densities of the Fat-Free Body Components (from Brozek et al., 1963) 14

2.3 A Three Component Model for the Fat-Free Mass (from Martin and Drinkwater, 1990, unpublished) 20

2.4 Percentage Composition and Densities of the Fat-Free Mass (from Martin and Drinkwater, 1990, unpublished)..... 22

2.5 Variability in BMC and BMD of the Radius and Ulna as Measured by SPA in Normal Young Female Populations 25

2.6 Variability in BMC and BMD of the Lumbar Spine as Measured by SPA in Normal Young Female Populations 27

2.7 Variability in BMD of the Proximal Femur as Measured by DPA in Normal Young Female Populations 29

2.8 Variability in Total Body Bone Mineral (TBBM) and Total Body Bone Density (TBBD) as Measured by DPA in Normal Young Female Populations 30

2.9 Variability in Total Body Calcium (TBCa) and Calcium Ratio (TBCa/Cap) as Measured by Neutron Activation Analysis in Normal Young Female Populations 31

2.10 Trabecular and Cortical Bone at Common Sampling Sites for Bone Mineral Measurements by SPA and DPA (from Wahner, Dunn and Riggs, 1983) 40

4.1 Physical Characteristics of the Subjects (n=41) 62

4.2 Intercorrelations of Main Variables and Correlation with Whole Body Density and Percent Fat (n=41) 68

4.3 Factor Analysis Solution Rotated to the Oblique Solution Reference Structure * - Orthotran/Varimax 77

4.4 Primary Factor Intercorrelations - Orthotran/Varimax 83

4.5 Proportionate Variance Contributions of Factors 83

4.6 Stepwise Regression to Predict Body Density with the 16 Variables from the Factor Analysis (n=41) 87

4.7 Stepwise Regression to Predict Body Density with the 5 Factors as Variables (n=41)............................... 89

4.8 Stepwise Regression to Predict Bone Density with All the Anthropometric Variables Measured (6 Skinfolds, 12 Girths, 4 Bone Breadths, 4 Muscle Circumferences and Age and Height, n=41) 92

4.9 Difference between Percent Fat Predicted by Stepwise Regression with 4 Factors and Percent Fat Predicted by Stepwise Regression with the Same Factors and Substitution of Average Values for the 2 Factor Scores on Bone Density (n=41).. 101

4.10 Subjects with the Greatest Differences (1.9-4.7 % Fat) between Percent Fat Predicted by Stepwise Regression with 4 Factors and Percent Fat Predicted by Stepwise Regression with the Same Factors and Substitution of Average Values for the 2 Factor Scores on Bone Density (n=12).. 105

4.11 Comparison of Physical Characteristics of Subjects who were Fatter with Average Bone Density in The Regression Equation based on 4 Factors (n=5) with the Rest of the Group (n=36) 108

4.12 Comparison of Physical Characteristics of Subjects who were Leaner with Average Bone Density in The Regression Equation based on 4 Factors (n=7) with the Rest of the Group (n=34) 111

4.13 Comparison of Physical Characteristics of Subjects who had Factor 5 (Spine Bone Density) Scores Greater than 1.0 (n=7) with the Rest of the Group (n=34)................. 114

4.14 Comparison of Percent Fat by Underwater Weighing with Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass (n=41) 122

4.15 Subjects with Differences Greater than 3 Percent Fat between Percent Fat Predicted by Underwater Weighing and Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass (n=12) 125

LIST OF FIGURES

Figure Page

2.1 Siri's Equation The Prediction of Body Fat from Whole Body Density (from Martin, 1984, with permission of the author)........ 17

2.2 Siri's Equation Plotted for Different Values of Fat-Free Density (from Martin, 1984,with permission of the author)........ 18

2.3 The Effect of Variation in Muscle and Bone in a Three Component Model of the Fat-Free Mass. (from Martin and Drinkwater, 1990, unpublished, with permission of the authors)........ 23

3.1 Helium Dilution Apparatus for Measuring Residual Lung Volume and Underwater Weighing Tank in the University of Manitoba Exercise Physiology Laboratory....... 51

4.1 Lumbar Spine Versus Femoral Neck Bone Density (Top) and Ward's Triangle and Trochanter Versus Femoral Neck Bone Density (Bottom) (n=41)........ 73

4.2 Femoral Bone Density Factor Versus Lumbar Spine Bone Density Factor........ 117

Chapter 1

INTRODUCTION

The determination of body composition is an important aspect of adult fitness and health. Excess fat is related to increased mortality and morbidity, particularly for diseases such as diabetes mellitus, gout, hypertension and cardiovascular disease (Seidell, Deurenberg, and Hautvast, 1987). Accurate measurements of body fat are needed to develop sound weight reduction and exercise programs. A knowledge of body composition is also important for athletes interested in maximizing their performance as excess body fat has been shown to decrease jumping ability, reduce running speed, and lower endurance (Jackson & Pollock, 1985).

Among the various methods used to measure body composition, densitometry is generally considered to be the "criterion" method or the "gold standard". Body density can be calculated from body weight and body volume which can be measured from the displacement of air or water. The most common method involves underwater (hydrostatic) weighing (Brodie, 1988; Roche, 1987; Martin, 1984; Wilmore, 1983). In the past hydrostatic weighing (HW) has been used to validate body composition techniques such as total body water and skinfolds (Keys & Brozek, 1953) and according to two recent reviews, continues to be used to validate new methods to predict body fat, such as dual photon absorptiometry, total body electrical conductivity, electrical impedance and ultrasound (Brodie, 1988; Lukaski, 1987).

Although densitometry is considered the criterion method, it is an indirect method of body composition; the only truly direct method is cadaver dissection and ether extraction and weighing of fat, which has only been performed in eight adult human cadavers (Martin and Drinkwater, 1990, unpublished). The direct method of determining body fat is obviously impossible in living individuals.

As explained by Keys and Brozek (1953), densitometry is based on the chemical model of body composition which partitions the body into total water, fat, mineral and protein mass. Fat is to be distinguished from anatomical adipose tissue and is defined as ether extractible lipid.

On the basis of direct chemical analysis of cadavers (Forbes, Cooper, and Mitchell, 1956; Widdowson, McCance, and Spray, 1951; Mitchell, Hamilton, Steggerda, and Bean, 1945) and the results of animal studies (notably, Rathbun & Pace, 1945), Keys and Brozek (1953) proposed a two component model of body composition. A "reference man" was divided into the fat-free mass and fat mass ("the obesity tissue"). This model was different from that proposed by Behnke, Feen, and Welham (1942) whose two component model, fat mass and lean body mass (LBM) included essential body fat in the LBM partition.

In 1963, Brozek and his colleagues revised the 1953 version of the Minnesota Densitometric System and on the basis of human and animal cadaver evidence established a new "reference body" with a density of 1.064 g/cm^3 and a fat content of 15.3% of body weight. The density of the fat-free mass was given as 1.10 g/cm^3 which was the same as the value determined by Behnke for LBM and as in 1953 the density of human fat was determined to be 0.9007 g/cm^3.

Wilmore explained in 1983 that the two component model of body composition was based on the following assumptions. First, the density of the body was the resultant of the densities of its components. Second, the densities of body fat and fat-free mass were known (given in 1963 by Brozek et al. and by Siri, 1956, as 0.90 g/cm^3 and 1.10 g/cm^3 respectively). Third, the densities of the components were relatively constant between individuals. Fourth, the densities of the individual portions of the fat-free mass (bone mineral, muscle and water) were constant within and among individuals and they had a constant proportional contribution to the total. In general, the individual assessed was assumed to differ from the standard reference body upon which a given equation was based only in the amount of fat.

Keys and Brozek (1953) were aware of the limitations of the densitometric system and stated that the value of the density of the fat-free body is only very "grossly a constant" and was "neither known nor in the final sense precisely knowable" (p. 266). However, they concluded that the net result of consideration of all factors of variability in the normal hydrated body as well as the errors in the estimation of body density by HW would only lead to a standard deviation of about ±0.005 in density, or perhaps ±2% fat weight.

Several studies have reported problems with the use of body density to determine body fat. Werdein and Kyle (1960) attempted to assess the validity of the fat-free body density by parallel measurements of hydrostatic weighing and total body water on both normal and abnormal subjects (osteoporotic and osteosclerotic). The fat-free mass varied considerably among normal individuals and more so in the extreme cases. The measured density of the osteoporotic individual was 1.08 g/cm^3 while the density of the osteosclerotic subject was 1.114 g/cm^3. The differences were attributed to variation in mineral mass (major factor) and muscle mass (minor factor). Bakker and Struikenkamp (1977) determined by mathematical analysis, that there was an overall uncertainty for the densitometric method of fat prediction due to interindividual variation in the density of the fat-free mass. This was expressed as a standard deviation of approximately 4% of body weight. The error was felt to be due to variation in the weight of the skeleton and fraction of fat-free mass, alteration in density of the fat-free mass in obesity, variation in the proportion of water in the fat-free mass and variation in the amount of essential lipids.

Adams and his co-workers (1982) reported an underestimation of body fat in professional football players. Of 29 players evaluated by hydrostatic weighing, eight had measured densities in excess of 1.1 g/cm^3 and thus negative percent body fat. Yet the subjects showed obvious subcutaneous fat as measured by skinfold caliper. These negative fat values were unrealistic.A certain minimum of fat is necessary to maintain life and this essential fat has been estimated by Behnke and Wilmore (1974) as 2-5% of lean body weight.

Martin (1984) in a comprehensive analytical chapter on densitometry, as part of the Brussels cadaver study, discussed the assumption of the constancy of the fat-free (FF) mass with reference to the above studies. He concluded that the question is not whether the FF density is constant but how variable is it. Although the Brussels study did not include chemical analysis, the variability found in the proportions of the body with adipose tissue removed, and the obtained bone density range determined by anatomical analysis combined with the cadaver evidence to date by chemical analysis, was sufficient to undermine the constancy of the density of the FF weight. Martin concluded that the variation in the FF density amounted to a standard deviation estimated at 0.02 g/cm^3 which led to large errors in predicted fat values. The most significant density variation was felt to occur in bone due to differences in bone density. The variability of the amount of muscle and the proportions of muscle and bone were also factors. On the other hand, variations in the water content of the body were felt to alter the FF density only under conditions of extreme obesity or dehydration.

Lohman (1984) proposed that when measuring body density by underwater weighing, the increase in water content in children coupled with a decrease in body mineral as compared to adult values, led to serious overestimation of body fat. The variation in the bone mineral content was determined from studies using single photon absorptiometry (SPA) of the distal radius. Assuming a mineral content of 4.6%, a water content of 77% and a protein content of 18.4% Lohman estimated the fat-free density of an eight-year old boy to be 1.085 g/cm^3 and derived a new equation for HW more appropriate for children. Lohman also expressed the need for a new equation for women based on a ten percent decrease in mineral content for women as compared to men and proposed a new "Reference Woman".

In another study, Lohman and his colleagues (1984) emphasized the need for research into the variability of the fat-free body in children, women, athletes and the elderly. These had not been well defined and appeared to indicate a larger variation than that of the standard young adult male reference man.

This view is also shared by Wilmore (1983) who stated that the density of the lean component appeared to be highly variable in younger, older and athletic populations. This could result in over or under predictions of percent body fat. He contended that research should be directed toward improving the prediction of body fat from whole body density.

Schutte and his co-workers (1984) measured density, total body water and anthropometric dimensions in black and white college students and determined that the fat-free mass of blacks was significantly denser than whites due to variation in mineral and muscle mass. A new formula for calculating percent body fat from HW was derived which indicated a fat-free body density of 1.113 g/cm^3 in blacks compared to 1.100 in whites.

Recently there has been debate over the extent of densitometric overestimation of percent body fat due to a reduction of bone mass in amenorrheic athletes. Nelson and Evans (1987) were concerned about this overestimation and found a significant negative correlation between percent body fat as estimated by hydrostatic weighing and bone mineral density of the spine. On the other hand, Sandborn and Wagner (1987) contended that the reduction in bone mass of these athletes translated into a two percent overestimation of body fat, which in their study would not significantly alter the difference between the percent fat found between amenorrheic and regular menstruating groups.

In summary, these studies show that the density of the fat-free mass is not necessarily constant and may vary according to age, race and sex. The largest source of interindividual variation is probably bone mineral content (Martin, 1984; Schutte et al., 1984; Wilmore, 1983; Brozek et al., 1963; Werdein and Kyle, 1960). However, the extent of the effect of variation in bone mineral on percent body fat as determined by HW is unclear.

Statement of the Problem

The purpose of this study was to assess the amount of variability in bone mineral density in young adult women and to determine the effect of this variability on whole body density and body fat as estimated by hydrostatic weighing.

The following hypotheses were proposed:

1. Whole body density determined by hydrostatic weighing, would be positively and significantly correlated with the bone mineral density measured at the lumbar spine and proximal femur.

2. The variation in bone density found in the subjects of this study, would result in changes of greater than five percent fat estimation as assessed by hydrostatic weighing.

Limitations

The following points should be considered when reviewing this study. The problems were addressed in more detail in the literature review.

1. The method of subject selection was by recruitment rather than random selection.

2. Dual photon absorptiometry (DPA) measures the attenuation of a dual energy beam of gamma radiation emitted from a gadolinium isotope. Although the instrument is calibrated against tissues of known densities it does not measure true density ($g/cm^{3)}$ but rather measures "areal" density (g/cm^2). Because of the dimensionality this measure is not completely independent of body size.

3. The bone density measured at one site does not necessarily represent the bone density of other sites or that of the skeleton in general.

4. Percent fat as estimated by hydrostatic weighing cannot be validated in the subjects of this study. Validation would entail dissection and chemical extraction and weighing of fat.

Definition of Terms

Percent Body Fat

This will be determined by hydrostatic weighing according to Siri's formula:

$$\% \text{ Body Fat} = \left(\frac{4.950}{D} - 4.500\right) \times 100$$

Bone Mineral Density

This will be areal bone mineral density, g/cm^2, as measured by a Lunar DP3 instrument in the lumbar spine (L2 - L4), and in the proximal femur (in the femoral neck, Ward's triangle and trochanteric region).

Chapter 2

REVIEW OF RELATED LITERATURE

Introduction

This chapter will explain in more detail the process of densitometry and the numerical estimation of body fat from whole body density as measured by hydrostatic weighing. Using the two component model, the effect of variation in whole body density will be examined with respect to Siri's equation. A three component model of the fat-free mass will be discussed as well (Martin and Drinkwater, 1990, unpublished) and used to determine the possible effect of variation in bone mineral on fat-free density.

A comprehensive literature search was performed to determine the variability of bone mineral content (BMC) and bone mineral density (BMD) as measured by single photon absorptiometry (SPA) and dual photon absorptiometry (DPA) in the lumbar spine, the proximal femur, and the radius and ulna of normal young women. As well, the variation in total body bone mineral (TBBM) and total body bone density (TBBD) by DPA and total body calcium (TBCa) by neutron activation analysis was researched in the same population group. The results will be presented and discussed. Some of the limitations outlined in Chapter 1 were addressed by detailed literature examination.

Densitometry

As explained by Behnke and Wilmore (1974), the first modern use of underwater weighing to assess fat was by the US navy, circa 1940, which measured the volume of divers in diving tanks. Using the principle of Archimedes, the volume of the body was determined by its displacement of water and the difference between the weight in air and the weight underwater (completely submerged) was the weight of the displaced volume of water. This was corrected for the density of water which at 36°C is 0.995. To eliminate the effect of variation in the amount of air in the body, the gross volume under water was corrected for the residual air in the lungs and respiratory passages. Sometimes, a correction was also made for the volume of intestinal gas which was often taken as a standard 100 ml (Brodie, 1988).

Once the volume of the body had been established whole body density was determined from the equation

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}} \quad .$$

All of the preceding steps are combined in the formula developed by Brozek, Grande, Anderson, and Keys (1963) which is presented as follows.

$$\text{Density} = \frac{M}{V} = \frac{WA}{\dfrac{WA - WW}{DW} - (RV + VI)}$$

where:

WA = weight in air

WW = weight in water

DW = density of water

RV = residual volume

VI = volume of intestinal gas

A Two Component Model of the Body

For the numerical estimation of percent body fat from whole body density a two component model has been employed by Keys and Brozek (1953) composed of fat mass and fat-free mass and Behnke et al. (1942) who partitioned the body into fat mass and lean body mass (which includes essential fat). The following numerical estimation of fat from density has been deduced from Keys and Brozek (1953) and is presented in greater detail in Appendix A.

Table 2.1

Two Component Model of Body Composition

Fat	**fat-free**
mf df	mff dff

In a two component system of:

1. different densities, df (density of fat) and dff (density of fat-free) and
2. different masses, mf (mass of fat) and mff (mass of fat-free)

A. the total mass is

$$M = mf + mff ,$$

B. and the total volume is

$$V = \frac{M}{D} = \frac{mf}{df} + \frac{mff}{dff} .$$

Rearranging B., an equation for total density can be derived.

$$D = \frac{M}{V} = \frac{mff + mf}{\dfrac{mff}{dff} + \dfrac{mf}{df}}$$

If the total mass is unity, 1 = mf + mff, and mff = 1 - mf. Then, by substituting 1 - mf for mff in the above equation for density, and by rearranging, the following equation was derived.

$$\frac{1}{D} = \frac{(1 - mf)}{dff} + \frac{mf}{df}$$

The previous formula can be rearranged further to estimate the proportional mass of the fat component (mf).

$$mf = \frac{\dfrac{dff}{D} - 1}{\dfrac{dff}{df} - 1}$$

This can also be written as

$$mf = \frac{\dfrac{dff \times df}{dff - df}}{D} - \frac{df}{dff - df} .$$

This formula describes the fraction of fat in the whole body and can be simplified as

$$mf = \frac{a}{D} - b .$$

Assuming that the density of the fat-free mass is constant and that dff = 1.1 and df = 0.9 results in the following values for a and b and Siri's formula (1956).

$$mf = \frac{4.95}{D} - 4.50$$

Percentage fat would be

$$\% \; mf = \frac{495}{D} - 450 \quad .$$

As explained in the introduction, the accurate estimation of body fat from these mathematical formulae depended on the constancy of the density of the fat and the fat-free portions (Wilmore, 1983). Behnke and Wilmore (1974) described the lipid extracted from adipose tissue at 36°C as "remarkably constant" (p. 6) in composition in man and animal and cited a value of 0.90 g/cm^3 for the density, determined by Fidanza, Keys and Anderson (1953). This is also the value used by Brozek et al. (1963). Martin (1984) noted that the density of brain derived fat was quite different. However, he concluded that, since the quantity of fat in the nervous system was only approximately 200 g, the error in using 0.90 g/cm^3 for the density of all the fat in the body was negligible.

The concern about the use of this model did not appear to be over the value used for the density of the fat mass but rather over the value of 1.10 g/cm^3 used for the density of the fat-free mass (Martin, 1984; Wilmore, 1983).

As discussed, Keys and Brozek (1953) and Brozek et al. (1963) determined the value of 1.10 g/cm^3 for the density of the fat-free mass from limited human cadaver chemical analysis, notably the studies of Forbes, Cooper, and Mitchell (1956), Widdowson, McCance, and Spray (1951), and Mitchell, Hamilton, Steggerda, and Bean (1945), and animal studies from Rathbun and Pace (1945) and others. The density of the fat-free body as calculated by the above authors is summarized in the following table.

Table 2.2

Percentage Composition and Densities of the Fat-Free Body Components (from Brozek et al., 1963)

Component	**Composition (%)**	**Density (g/cm^3)**
Water	73.8	.9937
Protein	19.4	1.340
Mineral	6.8	3.038
Total	100.0	1.100

It appeared from the literature that the assumed value of 1.10 g/cm^3 for the FF density did not apply to all subjects. Werdein and Kyle (1960) found by simultaneous measurement of total body water and body density by hydrostatic weighing, a fat-free density of 1.057 g/cm^3 for an osteoporotic person (low bone mass) and 1.189 g/cm^3 for an osteosclerotic subject (marble bone disease, high bone mass). It was concluded that the fat-free density varied considerably among normal individuals and extremely in patients with bone disease.

Wilmore and his co-workers (1974) in a study of endurance athletes aged 72-74 years, found that body fat determined by HW, was consistently greater than fat determined by anthropometry. This suggested an overestimation of fat by hydrodensitometry. On the other hand, underestimation of relative body fat has been reported by Pollock, Gettman, Jackson, Ayres, Ward, and Linnerud (1977) who found values of less than 2% body fat in five elite runners. Adams, Mottola, Bagnall, and McFadden (1982) measured eight Canadian football players with negative values of percent body fat as determined by underwater weighing. In both these studies the subjects showed measurable subcutaneous fat by skinfold caliper.

More recently, Schutte, Townsend, Hugg, Stoup, Malina, and Blomquist (1984) measured body fat in black and white college students by underwater weighing, total body water, and anthropometry. A significant difference was found between the methods of determining body fat for black athletes and fat was felt to be underestimated by densitometry. The authors determined that the fat-free mass of blacks was denser than whites and they ascribed the difference in density to differences in mineral and/or protein content. A new formula was derived for densitometry on blacks which was based on a fat-free density of 1.113 g/cm^3 for this group.

Martin (1984) has plotted Siri's equation with a constant fat density of 0.9 g/ml and constant fat-free density of 1.1 g/ml (Figure 2.1). Using this equation, a normal young female with 22% body fat would have a corresponding whole body density of 1.049 g/ml. Similarly, a normal young male with 16% fat would have a whole body density of 1.062 g/ml.

As indicated by the negative region, if whole body densities greater than 1.10 g/ml are measured, then negative values of percent body fat will be predicted. This is what happened in the study of football players by Adams et al. (1982). According to Martin (1984), the occurrence of negative fat values which must be anomalous, indicated clearly the inconstancy of the density of the fat-free mass.

For those subjects who have a fat-free density greater than 1.10 g/ml, body fat will be underestimated and the error may be highlighted by negative fat values. On the other hand, in subjects with a fat-free density of less than 1.10 g/ml, body fat will be overestimated and the error will be hidden in the positive region of the graph and may be undetected.

Martin (1984) has also plotted Siri's equation for different values of fat-free density and this is presented as Figure 2.2. If a subject had an actual fat-free density of 1.12 g/ml as compared to 1.10 g/ml, then a measured whole body density of 1.08 g/ml would correspond to a fat estimate of 19.1%.

$$\frac{458}{1.08} - 405 = 19.1 \% \text{ fat}$$

Using Siri's formula however, the percent fat would only be 8.3 which is considerably underestimated.

$$\frac{495}{1.08} - 450 = 8.3 \% \text{ fat}$$

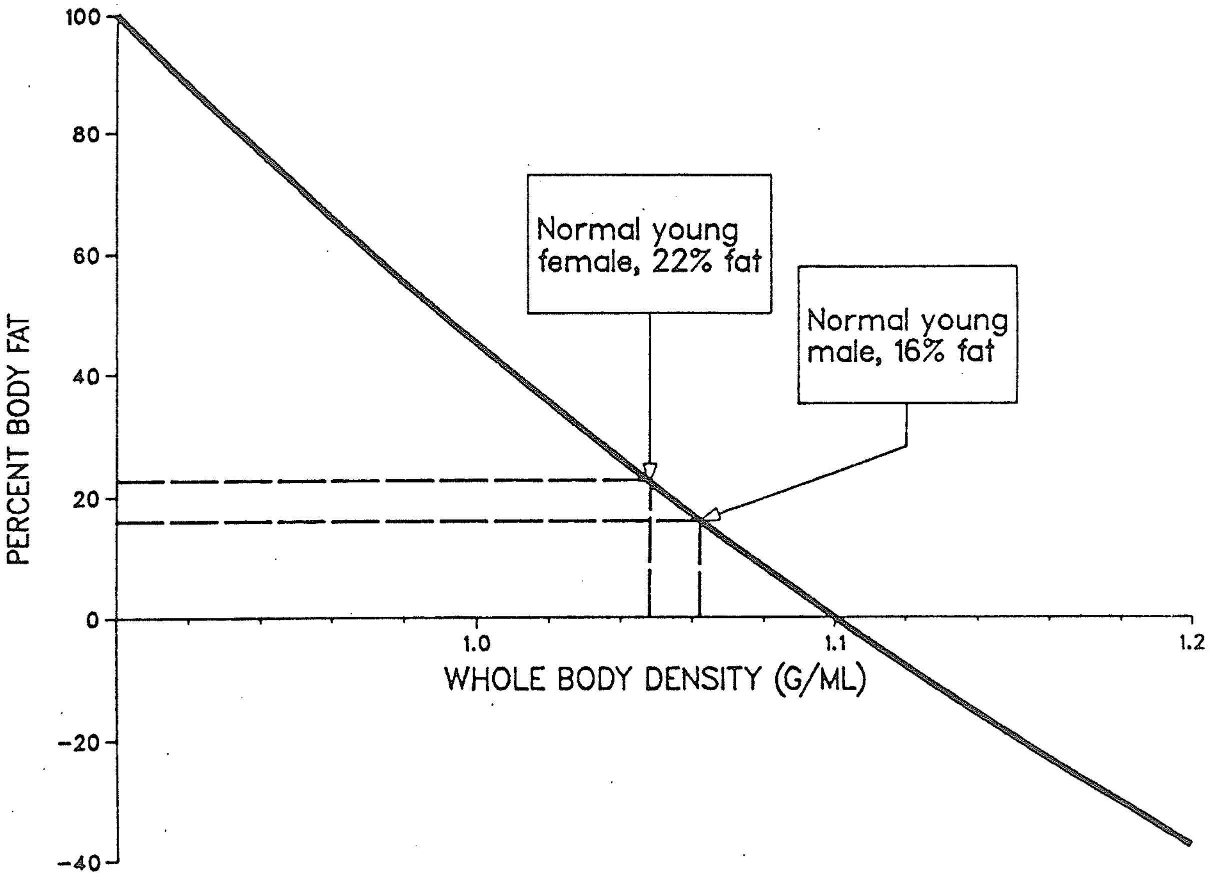


Figure 2.1

Siri's Equation: The Prediction of Body Fat from Whole Body Density (from Martin, 1984, with permission of the author)

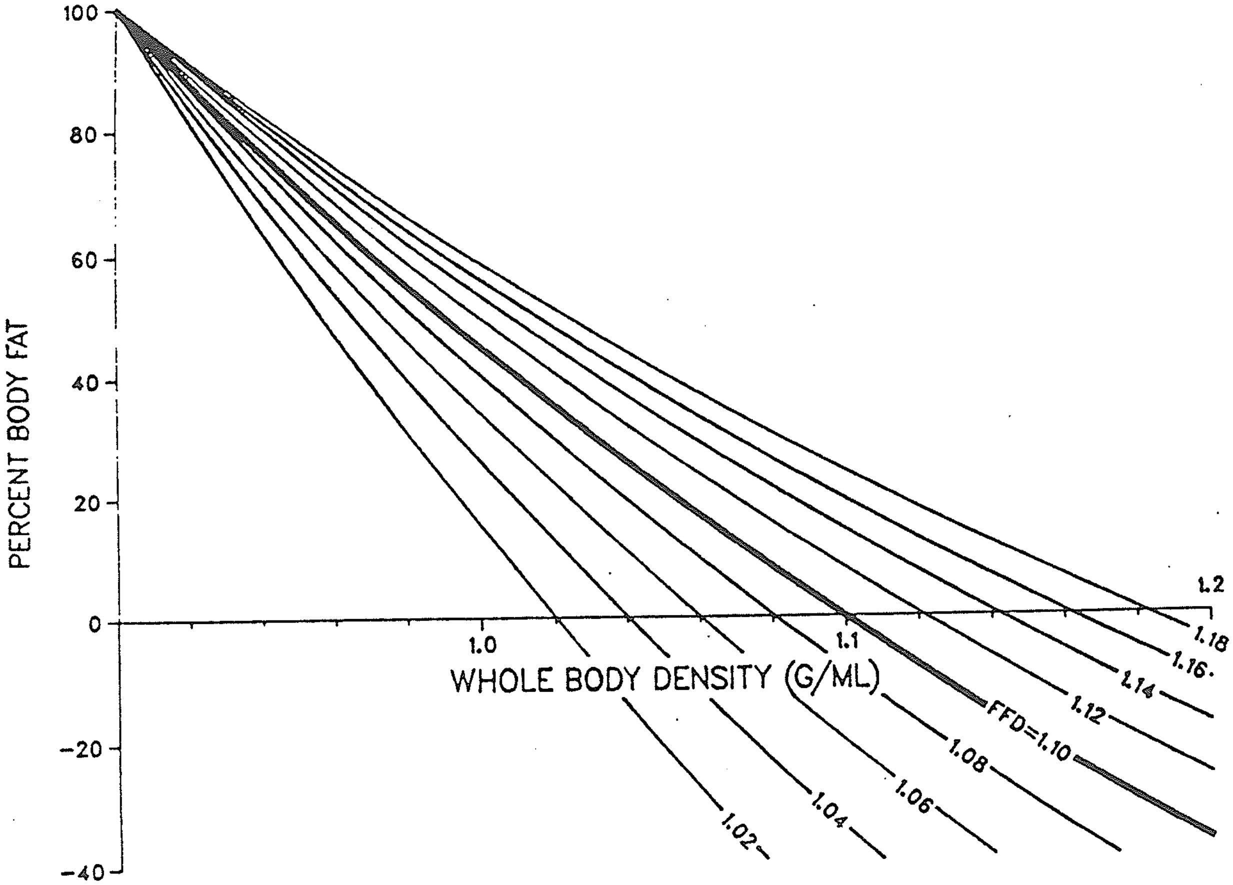


Figure 2.1

Siri's Equation Plotted for Different Values of Fat-Free Density (from Martin, 1984, with permission of the author)

Conversely, if the actual fat-free density was 1.08 g/ml, then a measured whole body density of 1.06 g/ml would correspond to a percent body fat of 9.4. Using Siri's formula, percent fat would have been 17.0, a large overestimation. This analysis showed that small changes in the fat-free density could alter the percent fat as calculated by Siri's formula appreciably.

A Three Component Model of the Fat-Free Mass

A review of the literature showed in general that differences in bone mineral were felt to be the most significant determinant of variation in fat-free tissue density (Schutte et al., 1984; Martin, 1984; Bakker & Struikenkamp, 1977; Werdein & Kyle, 1960; Keys & Brozek, 1953). Lohman (1984) contended however, that variation in the water content of the fat-free body exerted the greatest influence, especially in children. Wilmore (1983), felt that for older and younger populations differences in bone mineral and total body water might have varied the fat-free mass while for athletes the variation might have been due to differences in the densities and, or proportions of the fat-free mass.

In a recent study by Drinkwater, Plato, Lakatta, Goldberg and Andres (1987, unpublished), the effect of variation in bone mineral density (BMD) on body density as determined by hydrostatic weighing was examined in twenty nine active men (mean age, 68 years). BMD was determined by SPA and DPA at five different sites. All sites were positively correlated with whole body density. Using multiple regression analysis, fatness indicators (skinfolds and girths) were found to account for 44% of the explained variance and the trochanter mineral density for an additional 30%. When whole body density was corrected for BMD, corrections made to the percentage fat values determined by hydrostatic weighing ranged from +11.9% to -7.8%. This study suggested that in older lean individuals, variations in BMD contributed significantly to total body density.

To investigate the effects of variation in the proportions and densities of the components of the fat-free (FF) mass, a theoretical model has been devised by Martin and Drinkwater (1990, unpublished). This is presented here to examine the effect of varying FF muscle and FF bone fractions as well as the density of FF bone on the overall fat-free density.

Table 2.3

A Three Component Model for the Fat-Free Mass (from Martin and Drinkwater, 1990, unpublished)

Fat Mass	**Fat-Free Mass**		
	Muscle	Bone	Residual
	dM	dB	dR
	mM	mB	mR

Using this model the fat-free mass (FFM) is further divided into three components:

1.fat-free muscle, with a density, dM, and constituting a fraction, mM, of the fat-free mass,

2. fat-free bone, with density, dB, and fraction, mB, and

3. fat-free residual, with density, dR. and fraction, mR.

Using these components, an equation was derived as follows, to describe the fat-free mass.

From the formula,

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

If mass = 1, then

$$\frac{1}{\text{Density}} = \text{Volume}$$

and

$$\frac{1}{FFD} = \frac{mM}{dM} + \frac{mB}{dB} + \frac{mR}{dR} \quad .$$

Since mM + mB + mR = 1,then mR = 1 - mM - mB.

Substituting for mR the following equation was derived

$$\frac{1}{FFD} = \frac{mM}{dM} + \frac{mB}{dB} + \frac{1 - mM - mB}{dR}$$

and by rearranging it became

$$FFD = \frac{dMdBdR}{mMdB(dR - dM) + mBdM(dR - dB) + dMdB} \quad .$$

Using mean values from cadaver studies, the following fractions and densities were determined.

Table 2.4

Percentage Composition and Densities of the Fat-Free Mass (from Martin and Drinkwater, 1990, unpublished)

Component	**Composition (%)**	**Density (g/ml)**
Muscle	50.0	1.07
Bone	15.6	1.43
Residual	34.4	1.034
Total	100.0	1.10

These values were used in the foregoing equation to give a FFD of 1.10 g/ml. (The value for dR was determined by elimination.) Using the estimated values and the equation, the authors were able to look at the effects on fat-free density of varying the densities and the fraction of the components by 10% around the mean values. Varying the fat-free bone density through the estimated range had the strongest effect on the fat-free density.

The effect of variation in both muscle and bone, and bone density is presented in Figure 2.3. This mathematical analysis showed that the greatest value of FFD occurred when the upper limits for mM, mB and dB occurred together (muscle as 60% of FFM and bone as 18.7% of FFM). On the other hand, the minimum values of FFD occurred when the minimum values of mM (40%) and mB (12.5%) occurred together. Thus, varying both

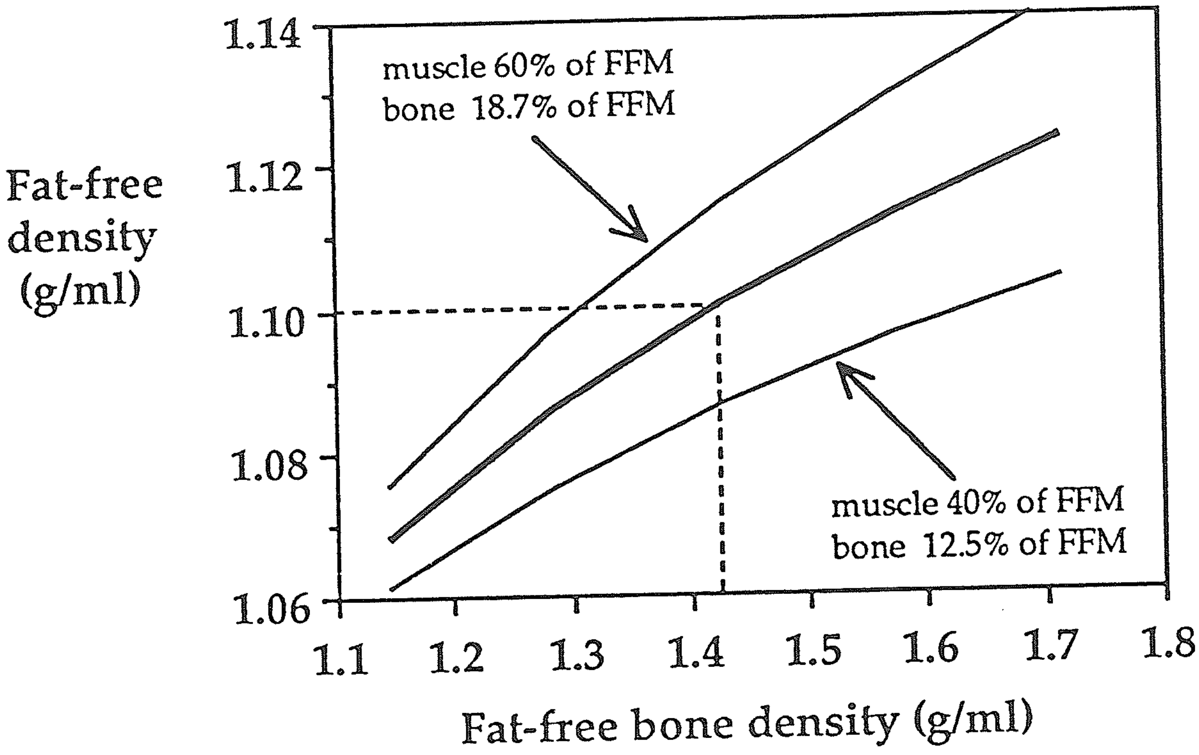


Figure 2.3

The Effect of Variation in Muscle and Bone in a Three Component Model of the Fat-Free Mass. (from Martin and Drinkwater, 1990, unpublished, with permission of the authors) the muscle and bone fraction 10% about their means in relation to a similar 10% variation in bone density, resulted in a range of fat-free density from 1.061 g/ml to 1.142 g/ml.

Variability of Bone Mineral in Normal Female Populations

To determine the amount of variability found in bone mineral content (BMC) and bone mineral density (BMD) as assessed by single photon absorptiometry (SPA) and dual photon absorptiometry (DPA), a literature review was performed on studies with normal female populations. Since the development and widespread use of DPA is fairly recent (Goodwin, 1987) most of these occurred in the last two decades.

The instruments for measuring bone density have been used mainly in a clinical setting, and the focus has been on the study of older osteoporotic populations of women. It was difficult to find studies which involved only young adult women and women without pathologies. Some studies did not report mean values and standard deviations (S.D.). In one case values were estimated from a graph. The coefficient of variation (C.V.) is the S.D. expressed as a percentage of the mean. The results of the literature review are summarized in the following tables.

Table 2.5

Variability in BMC and BMD of the Radius and Ulna as Measured by SPA in Normal Young Female Populations

Authors	n	Age (yrs)		Value		CV(%)
		Mean	Range	Mean	Std. Dev.	
1. BMC				**g/cm**		
Mazess & Christiansen 1982 USA 1.& Denmark 2.	165	27.0	20-44	.964	.099	10.3
	90	32.5	20-44	.940	.104	11.1
Nilas et al. 1988 Denmark 3.	23		21-30	40.5	5.1	12.6
	32		31-40	40.3	4.3	10.7
	29		41-50	41.2	4.2	10.2
	23		21-30	40.2	5.5	13.7
	32		31-40	40.2	4.7	11.7
	29		41-50	41.1	5.5	13.4
2. BMD				**g/cm^2**		
Boyd et al. 1974 Canada 4.	34	17.0	11-20	.740	.060	8.1
	71	24.6	21-30	.750	.060	8.0
	14	35.5	31-40	.760	.050	6.6
	26	46.7	41-50	.750	.060	8.0
				mg/ml		
Nordin et al. 1985 Australia 5.	77	43.3	22-59	469	51	10.9

Table 2.5 cont.

Authors	n	Age (yrs)		Value		CV(%)
		Mean	Range	Mean	Std. Dev.	
2. BMD cont.				g/cm^2		
Nilas et al.	23	25	21-30	1.458	.118	8.1
1988	32	35	31-40	1.470	.135	9.2
Denmark [3]	29	47	41-50	1.434	.148	10.3
	23	25	21-30	1.091	.139	12.7
	32	35	31-40	1.080	.121	11.2
	29	47	41-50	1.056	.164	15.5

[1] Site was radius midshaft; [2] Site was combined distal radius and ulna; [3] The first 3 values were for the combined proximal radius and ulna site. The last 3 values were for the combined distal radius and ulna. The same subjects were measured for BMC and BMD at both sites.[4] Sites were radius shaft; [5] Site was combined distal radius and ulna. Values were reported as FMD (mg/ml), forearm mineral content divided by cross sectional area.

Table 2.6

Variability in BMC and BMD of the Lumbar Spine as Measured by DPA in Normal Young Female Populations

Authors	n	Age (yrs)		Value		CV(%)
		Mean	Range	Mean	Std. Dev.	
1. BMC				**g/cm**		
Lindquist et al. 1981 Sweden [1]	20	46.0		3.91	0.65	16.6
Krolner 1982 Denmark [2]	32	36.0	19-51	3.877	0.160	4.1
				g hydroxyapatite		
Geusens et al	28	24.4	20-29	48.1	7.1	14.8
1986	34	35.2	30-39	46.4	6.7	14.4
Belgium [3]	42	44.6	40-49	46.2	5.9	12.8
				g/cm		
Nilas et al.	23		21-30	46.5	7.6	16.3
1988	32		31-40	46.5	7.7	16.6
Denmark [4]	29		41-50	46.1	6.0	13.0
2. BMD				**g/cm^2**		
Riggs et al. 1980 USA [5]	42		20-50	1.5706	.1549	9.9
Tothill et al. 1983 Scotland [6]	24	41.2		.940	.157	16.7

Table 2.6 cont.

Authors	n	Age (yrs)		Value		CV(%)
		Mean	Range	Mean	Std. Dev.	
2. BMD cont.				g/cm^2		
Aloia et al.	159	50.4	20-75	.662	.007	13.3
1985						
USA [7]						
Hansson &	20		35-39	.932	.191	20.5
Roos	23		40-44	.880	.178	20.2
1986						
Sweden [8]						
Nilas &	15	32	29-34	1.04	.16	15.4
Christiansen	15	40	35-44	1.03	.12	11.7
1986						
Denmark [9]						
Mazess et al.	164		20-29	1.24	.13	10.5
1987	248		30-39	1.26	.13	10.3
USA [10]	58		40-49	1.21	.12	9.9
Mazess et al.	281		20-40	1.28	.12	9.4
1988						
USA [11]						
Nilas et al.	23	25	21-30	1.030	.124	12.0
1988	32	35	31-40	1.015	.138	13.6
Denmark [4]	29	47	41-50	.992	.118	11.9

[1] Site was L3, random sample; [2] Site was sum of L2.L3, and L4.; [3] Sites were L2-L4, lumbar BMC given as grams hydroxyapatite; [4] Sites were L2-L4. The same subjects were measured for BMC and BMD; [5] Site was L1-L4, values reported were linear regression predicted mean and residual S.D.;[6] Site was mean L2-L4; [7] Site was mean L2-L4, values reported were mean and standard error of the mean, calculated S.D. was .088; [8] Sites were L3, random sample; [9] Sites were L2-L4, DPA instrument was from Lunar, USA; [10] Sites were L2-L4; [11] Sites were L2-L4.

Table 2.7

Variability in BMD of the Proximal Femur as Measured by DPA in Normal Young Female Populations

Authors	**n**	**Age (yrs)**	**Site**	**Value (g/cm^2)**		**CV(%)**
		Range		**Mean**	**Std. Dev.**	
Mazess et al.	90	20-29	F. Neck	1.01	0.10	9.9
1987	132	30-39	F. Neck	0.99	0.12	12.1
USA	60	40-49	F. Neck	0.88	0.10	11.4
	90	20-29	Wards t.	0.93	0.10	10.8
	132	30-39	Wards t.	0.91	0.14	15.4
	60	40-49	Wards t.	0.76	0.11	14.5
	90	20-29	Troch.	0.82	0.09	11.0
	132	30-39	Troch.	0.80	0.12	15.0
	60	40-49	Troch.	0.72	0.10 [1.]	13.9

[1.] Skew significant at $P < .05$

Table 2.8

Variability in Total Body Bone Mineral (TBBM) and Total Body Bone Density (TBBD) as Measured by DPA in Normal Young Female Populations

Authors	n	Age (yrs)		Value		CV(%)
		Mean	Range	Mean	Std. Dev.	
1. TBBM				g		
Mazess et al. 1981 USA [1]	7	young	adults	2746		25.4
Nilas et al. 1986 Denmark [2]	161	50.1	45-54	2156	322	14.9
	161	same	subjects	2156	126	5.8
Gotfredsen et al. 1987 Denmark [3]	23		21-30	2366		18.0
	32		31-40	2346		15.0
	29		41-50	2307		13.0
Nilas & Christiansen 1987 Denmark	15	32	29-34	2422	395	16.1
	15	40	35-44	2304	307	13.3
2. TBBD				g/cm^2		
Gotfredsen et al. 1987 Denmark [3]	23		21-30	1.063		7.0
	32		31-40	1.062		7.0
	29		41-50	1.054		9.0

[1] This study included both males and females and the large C.V. was ascribed to this. The authors contended that the usual age and sex specific variations are about 12-15%. The C.V. was reported but not the S.D., nor age; [2] The subjects were early post menopausal. 126 is the S.D. after normalization for local index of body size; [3] The same subjects were used to calculate both TBBM and TBBD. The C.V. was reported but not the S.D.

Table 2.9

Variability in Total Body Calcium (TBCa) and Calcium Ratio (TBCa/Cap) as Measured by Neutron Activation Analysis in Normal Young Female Populations

Authors	**n**	**Age (yrs)**		**Value**		**CV(%)**
		Mean	**Range**	**Mean**	**Std. Dev.**	
1. TBCa				**g**		
Ellis & Cohen 1975 USA [1]	5	48.2	36-61	846	106.4	12.6
Cohn et al.	6	33.5	30-39	785		4.5
1976 USA [2].	11	44.1	40-49	849		8.7
Ott et al.	9		21-30	840	53	6.3
1983 USA [3]	7		31-40	835	108	12.9
Yasmura et al. 1987 USA	51	42		898	99	11.0
2. TBCa/Cap						
Ellis & Cohen 1975 USA [1]	5	48.2	36-61	.988	.022	2.2
Cohn et al.	6	33.5	30-39	1.014		5.7
1976 USA [2]	11	44.1	40-49	.991		6.5
Harrison et al	13	29	20-37	.99	.12	12.1
1979 Canada [4]	13	47	38-55	.96	.10	10.4
Ott et al.	9		21-30	1.01	.06	5.9
1983 USA [3].	7		31-40	1.00	.10	10.0

KEY: **TBCa/Cap,** Cap is predicted normal calcium; [1] Same subjects used for TBCa and TBCa/Cap measurements; [2] Same subjects used for TBCa and TBCa/Cap measurements. C.V. reported but not Std. Dev.; [3] Same subjects used for TBCa and TBCa/Cap measurements; [4] This study used a different index (CaBI, calcium bone index) and measured body calcium only in the central skeleton.

As described in Table 2.5 the coefficient of variation (C.V.) for the BMD of the radius and ulna ranged from 6.6% to 15.5%. The amount of variation appeared to be related to the site measured.The Canadian study by Boyd, Cameron, McIntosh, and Walker (1974) measured the distal radius shaft and had C.V. values around 8%. The Danish study by Nilas, Gotfredsen, Hadberg and Christiansen (1988) measured two forearm sites in the same subjects. The coefficient of variation for the proximal radius and ulna ranged from 8.1% to 10.3% while the C.V. for the distal radius and ulna was higher and ranged from 11.2% to 15.5%. The authors contended that the proximal site contained approximately 85% cortical bone while the distal site was composed of 50% cortical and 50% trabecular bone.

In Table 2.6 the C.V. for the BMD of the lumbar spine ranged from 4.1% to 20.5%. The low value found by Krolner (1982) could reflect the method of calculation (sum of L2, L3, and L4 versus the mean of L2 - L4 used in most of the other studies). The values of the large American study by Mazess, Bardev, Ettinger, Johnston, Dawson-Hughes, Baron, Powell and Notelovitz (1987) were around 10%. The two Danish studies by Nilas et al. (1988 and 1986) showed a lower amount of bone density for the same age group and a higher C.V. than the American study (from 11.7 to 15.4). This might have been due to differences in instruments, site selection, and calculation of mean values. The Swedish study by Hansen and Roos (1986) showed the highest variability. This was probably due to the fact that only one vertebra was measured (L3) rather than four or five.

It is possible that the larger range of variation found with DPA of the spine as compared to the radius and ulna might be due to the greater proportion of trabecular bone in the lumbar vertebrae. According to Wahner, Dunn and Riggs (1984) trabecular bone had a higher bone turnover rate and might be more sensitive to loss in osteoporosis. On the other hand, the radius shaft had a higher proportion of cortical bone and showed a greater correlation to the total body bone density (TBBD) as measured by DPA and total body calcium (TBCa) as measured by neutron activation analysis (Ott, Kilcoyne and Chestnut, 1988; Mazess, Peppler, Harrison and McNeill, 1981). Consequently, the C.V. for the radius shaft may be more representative of the total skeleton.

Table 2.7 showed that the C.V. for the BMD of the proximal femur ranged from 9.9% to 15.4%. The values were all from the large American study by Mazess et al. (1987). The range at the femoral neck was slightly smaller (9.9% to 12.1%) then at the other two sites and this may reflect the fact that the percentage of cortical bone at that site is reputedly higher than at the Ward's triangle or the trochanteric site. According to Wahner, Dunn and Riggs (1983), the ratio is 75% cortical to 25% trabecular bone at the femoral neck.

The values presented represented the variability in bone mineral in a population of normal adult premenopausal women. There was evidence that there was a higher range of variability in postmenopausal women and in abnormal populations. Seldin, Esser and Alderson (1988), measured 181 American women aged 1-83 (mean age 52) with suspected abnormalities (138 - suspected osteoporosis; 37 - hyperparathyroidism, 14 - anorexia nervosa). The coefficient of variation for the lumbar spine was 20.2%, for the femoral neck 20.0% and for the radius 19.6%. As Seldin and his colleagues pointed out, the high degree of variability associated with a large

SEE made it difficult to determine with confidence (i.e. a small 95% confidence interval) the mineral content of any other part of the skeleton than the one being measured.

The studies were from several countries.- USA, Sweden, Denmark, Belgium, Scotland and Australia. Only two Canadian studies on bone mineral in normal female populations were found. It is possible that there are differences in BMC among the countries and in patterns of variation. Mazess and Christiansen (1982) compared bone mineral results from Denmark and the U.S. SPA on the radius shaft with an American instrument was compared with SPA on the distal radius and ulna with a Danish instrument (n = 34 males and females). The correlation between the two procedures was high (r = 0.93 SEE = 8%). On the basis of regression analysis larger samples were compared and Danish females, particularly between 45-80 years of age, had significantly lower BMC (7%) and significantly greater age associated bone decrease (12% versus 9% per decade).

It was hard to determine the estimated variability in a population of normal Canadian women, while considering the preceding observations on variation among the sites measured and between cortical and trabecular bone, random sampling, and differences between populations from country to country. The large Canadian study by Boyd et al. (1974) and the American ones by Mazess et al. (1987 and 1988) should be more heavily weighted in an analysis of the overall magnitude of variation in a normal Canadian population. In the former study, the mean value for the C.V. for the age groups listed for the radius shaft was 7.6%. In the latter studies, the mean C.V. for the lumbar spine was 10%, for the femoral neck 11.1%, for Ward's triangle 13.6%, and for the trochanter 13.3%. Mazess and his colleagues (1987), noted that the larger variance observed in some other studies might be due to technical variance (instrument difficulty and the use of different scanning alogarithms).

Table 2.8 summarized the literature on total body bone mineral (TBBM) and total body bone density (TBBD) as measured by DPA in normal female populations. Unfortunately, there were no large scale Canadian or North American studies found, presumably because the method was newer, more costly and time consuming than DPA of isolated body sites. The coefficients of variation for TBBM ranged from 5.8% to 25.4%. For the Danish study by Gotfredsen, Hadberg, Nilas, and Christiansen (1987) the variability of TBBD was consistently almost half of that for TBBM measured on the same subjects, which indicated that TBBD corrected for variation in total body size. Also Nilas, Gotfredsen and Christiansen (1986) reduced the variation for TBBM considerably when they normalized for body size. The coefficient of variation for TBBD for the study by Gotfredsen et al. (1987) ranged from 7.0% to 9.0%. The mean value was 7.7%. This was smaller than the variation seen for the lumbar spine (10%) and proximal femur (11.1% to 13.6%) but comparable to the C.V. for the radius shaft (7.6%).

Table 2.9 showed the variability in normal female populations in total body calcium (TBCa) by total body neutron activation analysis (TBNAA) and partial neutron activation analysis. A literature search was done in this area to further define the variation in the bone mineral density of the whole skeleton. This method involved a larger dose of radiation than DPA and was not as widely used (Murby and Fogelman, 1987). No consensus had been reached on how to normalize the raw data for size. The measured

TBCa was expressed in terms of a predicted normal calcium (Cap) and this ratio was used to normalize results. Cohn, Vaswani, Zanzi and Ellis (1976) used total body potassium measurements and height to normalize for size and adjusted for sex and age. Thus, the ratio did not really represent the density of the bone mineral present. On the other hand, Harrison, McNeill, Hitchman and Britt, (1979) normalized the calcium measured for body size by relating the content to the cube of the subject's height. This gave the calcium bone index, CaB1. Although height cubed was not a perfect estimate of body volume, this was perhaps closer to a bone density measure than the TBCa/Cap. Unfortunately, this study measured body calcium only in the central skeleton and showed a higher variation (mean.11.3%) than the studies using TBCa/Cap ratios. The mean C.V. for TBCa/Cap ratios was 6.1% which was lower than the mean for TBBD (7.7%). Some of the difference in variation might be ascribed to the smaller sample size of the TBCa/Cap studies. There would be more variation in a larger sample.

The limitations imposed by the use of dual photon absorptiometry (DPA) to measure bone mineral density (BMD) must also be addressed. As explained by Goodwin (1987) the density as measured by DPA was given in units of g/cm^2. This BMD was not actual density which would be in units of g/ml or g/cm^3 but instead represented the total mineral in one column 1 cm in cross sectional area. Depth of measurement was not included. However, the accuracy of DPA has been assessed by comparing the BMD measured on excised bones with the actual weight of ashed samples. By this method Goodwin (1987) reported an accuracy (SEE) of 1.2% to 5%. Reproducibility in scanning patients was given as a range from 1.35% (C.V.) on repeat measurements on the same day to about 4% for measurements six months apart by the same author.

The limitations and strengths of DPA can be illustrated by comparison with another method, dual energy quantitative computed tomography (DEQCT) which was able to provide a direct density measurement and could distinguish cortical from trabecular bone (Goodwin, 1987). In the spine QCT was used to measure a selected volume of the trabecular bone in the vertebral body whereas DPA measured a projected area of both cortical and trabecular bone including the posterior elements of vertebrae (Eriksson,Isberg and Lindgren, 1988). The accuracy of QCT when compared to ash weights ranged from SEE 5-20% for SEQCT (single energy) and SEE 3-7% for DEQCT, inaccuracy due mainly to the amount of fat in bone. Although DEQCT was more accurate, it reduced precision threefold, increased radiation dose twofold and was generally not recommended for clinical applications on most CT scanners (Genant et al., 1987).

Genant and his colleagues (1987) measured the lumbar spine of 40 early postmenopausal women and 68 postmenopausal osteoporotic women with both single and dual QCT and DPA. Their results showed good correlations between SEQCT or DEQCT and DPA in early postmenopausal women ($r = 0.87$ and $r = 0.82$) and moderate correlations ($r = 0.53$ and $r = 0.42$) in postmenopausal osteoporotic women. It was interesting to note that the range of values and coefficients of variation of trabecular bone density measured by QCT were greater than those of integral bone content measured by DPA, both across and within the two populations. In general, DPA appeared as accurate and precise as QCT and measured bone mineral density with a smaller radiation dose.

Wasserman and Barzel (1987) observed that studies with QCT and SPA and DPA are limited by the fact that bone density observed at one site does not necessarily reflect that of other sites or that of the skeleton in general. Ideally, the subjects in this study would be measured for total body bone density (TBBD). This can be done in about 70 minutes with DPA. However this was not possible for this study and instead the bone mineral density was measured at the lumbar spine (L1-L4) and at three sites on the proximal femur.

In a study on 7 young adult subjects Mazess et al. (1981) determined that the BMC of the lumbar spine was only moderately correlated with trunk Ca (as measured by partial neutron activation), radius BMC and total body bone mineral (TBBM) assessed by DPA (r = .82, SEE 18%). It was noted that the total spine was 10% and the lumbar spine 3% of total skeletal weight. On the other hand, the radius shaft bone mineral was highly correlated with the TBBM (r = 0.97, SEE 9%) and TBCa (r = 0.98, SEE 6%). The authors suggested TBBM could be approximated by multiplying the radius BMC by 2500.

The differences in correlation of the two sites might be due to the different percentages of cortical and trabecular bone at the lumbar spine and the radius shaft. According to Ott, Kilcoyne and Chestnut (1988) and Wahner, Dunn and Riggs (1983), the skeleton was composed of 80% cortical bone and 20% trabecular bone, the latter being located mainly in the axial skeleton. The long bones were predominantly cortical bone.

Ott, Kilcoyne, and Chestnut, (1988) assessed bone mass via neutron activation analysis (total body calcium, TBC), SPA (BMC of the radius) and DPA (BMC of the lumbar spine) and QCT of the spine in 122 women with postmenopausal osteoporosis. All methods correlated significantly with each other (r = 0.33 - 0.76) and the correlations were not significantly different when the bone mass measurements were normalized for age and height. The best correlation with TBC (r = 0.76) was with SPA of the radius which the authors concluded occurred because most of the skeleton consisted of cortical bone. The correlation of DPA of the spine with TBC was 0.69 and the correlation with QCT of central vertebra 0.56 and integral vertebra 0.68. These correlations were deemed weaker because DPA and QCT measured more trabecular bone. Multiple linear regression analysis of TBC on SPA, QCT and DPA suggested that TBC could be predicted more reliably by measurements of both cortical and trabecular bone and that different women had different proportions of cortical to trabecular bone. The approximate contribution of the cortical and trabecular components of bone at five SPA and DPA scanning sites is summarized in Table 2.10.

Table 2.10

Trabecular and Cortical Bone at Common Sampling Sites for Bone Mineral Measurements by SPA and DPA (from Wahner, Dunn and Riggs, 1983)

Bone	**Site**	**% Cortical**	**% Trabecular**
Radius	midshaft	>90	<10
	distal	75	25
Femur	cervical	75	25
	inter-trochanteric	50	50
Spine	lumbar	50	50

These values agreed with those given by Riggs, Wahner, Seeman, Offord and Dunn, (1982) except for the lumbar spine which Riggs contended was greater than 60% trabecular bone. On the other hand, Genant, Block, Steiger, Gluer and Smith, (1987) using QCT claimed that the lumbar vertebrae contained substantial amounts of compact (cortical) bone, 60-80%, with only 20-40% high turnover trabecular bone. It was difficult to combine the results of the literature search into these various methods and sites to get an estimate of the variability of the total body bone density in normal young Canadian women. All the sites showed different amounts of variation and the measurements at one site did not necessarily represent that of another site or the skeleton as a whole.

The studies which used whole body DPA were few and largely European. The Danish study by Gotfredsen et al. (1987) showed a mean C.V. of 7.7% for TBBD. This variation was comparable to the variation of the

BMD measured by SPA at the radial shaft which showed a mean C.V. of 7.6%. This might have been because the radius shaft contained approximately the same proportion of cortical bone as the whole skeleton (80%). On the other hand, the sites at the lumbar spine and proximal femur which had been measured in larger populations of women showed a mean C.V. of around 10% to 13.6%. One of these sites, the femoral neck, was estimated to contain approximately the same proportion of cortical bone as the whole skeleton.

Because of the independence of the sites, the heaviest weighting was given to the studies measuring TBBD. Since some of the variation could be attributed to technical error of measurement, a value of 7% was estimated for the coefficient of variation for whole body bone density in a normal young female Canadian population. There would be more variation in a randomly selected population which contained both males and females, with normal and abnormal bone density.

Chapter 3

METHODS AND PROCEDURES

Introduction

This chapter is separated into four sections: subjects, experimental design, data collection and data analysis. The first section, subjects, describes the method of selection and the characteristics of the subjects chosen. The second section, experimental design, explains the type of research carried out. The third section, data collection, describes the methods used to determine whole body density, percent fat, bone mineral density and anthropometric measurements, and the fourth and final section, data analysis, explains the statistical analysis used to interpret the data collected.

Subjects

The subjects were 41 normal, healthy, premenopausal female adults aged 19 to 48 years, engaged in varying levels of physical activity. Subjects were screened by means of a subject status questionnaire administered before their inclusion into the study. The subjects were asked to report if they had the following health conditions which might affect the calculation of residual lung volume and percent fat by underwater weighing or put them at risk of injury when measurements were taken: heart trouble, high blood pressure, fainting or dizziness, fear of submersion under water, respiratory disorders such as asthma or breathing difficulties, and smoking. They were also asked to report any drug use known to affect calcium metabolism such as anticonvulsants, corticosteroids, and estrogens and any other medications used. They were asked if they had any chronic diseases affecting bone such as diabetes, alcoholism, osteoporosis and renal disease, and if they had recently been immobilized for a month or more. Finally, they were asked to report if they were pregnant, postmenopausal or had had a hysterectomy. If they had any of the foregoing conditions they were excluded from the study.

Prior to inclusion into the study they also received a description of the research project and signed a consent form. These forms explained the tests which were carried out and informed the subjects as to the possible risks present during testing. Copies of these forms and the health status questionnaire are included in the appendix. The research proposal was approved by the Committee on Research Involving Human Subjects.

Most of the subjects were recruited from another study at the University of Manitoba which was assessing the effect of intense physical training on the menstrual cycle and bone density. The control group subjects (normals) were approached and asked to participate in this study as well. It was practical and cost efficient to use the same subjects for both studies. Both research projects used the same bone density equipment for measurement, at the same location and within approximately the same time frame. The anthropometric measurements were also the same and performed by the same team of researchers for both studies.

The subjects were recruited from the University of Manitoba staff and students and Winnipeg athletic organizations and community clubs. They formed one group and underwent all the measurements. This group initially consisted of 45 subjects. However, 4 of these were excluded from the study due to measurement errors discovered during data analysis. Each error involved a different method. The first subject excluded had an extremely high DPA bone density measurement on only the trochanteric site. For this subject, the value at this site was almost twice as high as the mean value for the group while the values for other femoral sites were only slightly above average. This error was believed to be due to movement and rotation of the femur during measurement which decreased the area measured but not the bone mineral content. The second subject was excluded due to the inaccuracy of skinfold measurements. It was difficult to measure this subject due to fatness. The third subject gained 8 pounds between the time the anthropometric measurements were taken and the underwater weighing was done to calculate body density and percent fat. Unfortunately it was impossible to remeasure these two subjects. The fourth subject had an unreasonably high residual lung volume measurement by helium dilution (1.9 litres). The percent fat estimated using this residual lung volume (9%) was unreasonably low when compared to the percent fat predicted with the estimate of residual lung volume based on vital capacity (20%) and percent fat estimated using skinfold measurements (14%). The very high residual lung volume measurement by helium dilution was believed to be due to asthma which was reported when the underwater weighing was completed.

Experimental Design

This study can be described as correlational or analytical research. The data were collected in a cross sectional manner. The bone density of the subjects, body density and percent fat by hydrostatic weighing, as well as anthropometric measurements were determined. These were analyzed by various correlational techniques to determine the relationship between variables. Each subject was only tested once and there was no follow up testing except in the case of the reliability check.

Data Collection

Bone Density Measurements

Bone mineral content (BMC, g) and areal bone mineral density (BMD, g/cm^2) were determined by a Lunar Corporation Dual Photon Absorptiometer, Model DP3 (Lunar Radiation, Madison, Wisconsin). The measurements were performed at St. Boniface General Hospital, Winnipeg, Manitoba, from September 1988 to April 1989, by a technician trained by the hospital in use of the equipment. The following sites were measured: the femoral neck, Ward's triangle, and the trochanteric region in the proximal femur, and vertebrae L1 to L4, in the lumbar spine.

A Gadolinium 153 radiation source with two distinct energies (44 and 100 ke V) was used for the DPA. The absorption of radiation measured at the two energies allowed comparison with standard absorption factors and allowed the readings to be transformed into indicators of density. Although the instrument is calibrated against tissues of known densities it does not measure true density (g/cm$^{3)}$ but rather measures "areal" density (g/cm^2). Because of the dimensionality this measure is not completely independent of body size.

The radiation dose to the skin and ovaries was < 200 and 100 Gy respectively (Wahner, Dunn and Riggs, 1984). The marrow doses of DPA were 1000 times lower than that from computed tomography (Mazess et al., 1987). The direction of scanning was perpendicular to the long axis of the body. The source, which was under the subject who laid on a table, provided a pencil-like beam which was detected by a sodium iodide crystal and a photomultiplier. Scans were made across the table with line lengths of about 10 cm and from 2-5 cm between the lines (Goodwin, 1987). Scan speed or collimation was varied as the source strength decayed.

Results were displayed on a computer screen and were available as a computer printout. A sample scan printout is included in the appendix. The method was outlined in detail by Goodwin (1987) and Wahner et al. (1984).

The accuracy of DPA has been assessed by comparing the BMC measured on excised bones with the actual weight of ashed samples. By this method Goodwin (1987) reported an accuracy (SEE) of 1.2% to 5% while Gotfredsen, Podenphant, Norgaard, Nilas, Nielsen, and Christiansen, (1988) determined a systematic error of 10% underestimation in in vitro studies. Reproducibility in scanning patients was given as range between 1.3% and 2.3% (C.V.) for short term and 2.3 to 4.0% for long term by Goodwin (1987) and Gluer, Steiger and Genant, (1988).

The scanner used at St. Boniface Hospital was calibrated daily for precision and reliability by scanning of a phantom. The short term reproducibility of the DPA measurements for the spine and proximal femur was investigated in 29 females, aged 19 to 46, by Lesnick Smith (1989, unpublished).These subjects were part of the concurrent study on bone density and amenorrhea and some of them were part of this study as well. Precision, described as a percentage, was defined as 1 standard deviation of the differences between repeated measurements divided by the mean of those measurements. Precision results for 19 immediate repeated measurements were: L2 to L4, 2.04%; femoral neck, 3.12%; Ward's triangle, 3.39% and trochanteric region, 4.74%. Precision results for 13 short term repeated measurements were: L2 to L4, 2.98%; femoral neck,3.84%; Ward's triangle,7.17% and trochanteric region, 4.52%. Lesnick Smith concluded that the precision indices found were higher than previously reported and indicated that short term reproducibility was variable with the least precision occurring in the Ward's triangle and the trochanteric region.

Underwater Weighing

Whole body density was determined by hydrodensitometry. Underwater weighing was performed in a fiberglass tank at the University of Manitoba, Exercise Physiology laboratory from May to July 1989. The subjects were suspended underwater from a harness and were weighed by a hanging Chatillon scale (capacity, 15 kg). This scale was calibrated with weights obtained from the engineering department of the University of Manitoba prior to testing. Five to seven weighings were performed and the mean of the highest three weighings was used to record the weight underwater. Prior to each submersion, the subjects were asked to blow out all the air they could. They were weighed immediately afterwards, with their lungs emptied of all air possible, fully submerged in a semi-prone position. They were encouraged to exhale maximally each time and to the same extent and in the same position as for the residual volume measurements with helium dilution which were taken out of the water. If the subjects floated on the surface of the water for the initial trials, a belt with lead weights was worn to ensure complete submersion. The weight ot the belt and the harness underwater was recorded and added into the calculation. Water temperature was kept constant at 34 to 36°C and was recorded for each subject. A sample underwater weighing worksheet is included in the appendix.

Residual lung volume was determined out of the water by the helium dilution method as originally proposed by Willmon and Behnke (1948). A minimum of two trials were taken. One was taken before the underwater weighing and one was taken after. If the residual volume calculated on the second trial was different from the first trial by 100ml a third, and fourth trial if necessary, was taken immediately. The mean of the two closest trials was used as the final value which was then corrected to body temperature, pressure, saturated. A sample residual lung volume worksheet is included in the appendix as well as a detailed outline of the procedure.

The helium dilution equipment was assembled for this study. Pilot tests were performed on University of Manitoba staff and students prior to the start of this research. As well, before and after the study the validity and reliability of the method was checked with 48 trials with a rubber bag. The bag was filled with varying known amounts of room air (.5L, 1.0L, and 1.5L) and then the volume was predicted using the helium dilution method. The mean differences between the known and predicted amounts for all three volumes were negligible (0.4ml to 6ml) and the intraclass correlation for reliability on split halves trials with the bag (all 3 volumes combined) was 0.99. The calculations for these tests are described in detail in the appendix.

The helium dilution method was also validated against the nitrogen washout procedure for determining residual lung volume through the University of Manitoba physiology department. Six subjects, 3 males and 3 females (subjects in this study) had their residual lung volume measured by both methods in June and July 1989. The correlation between the two methods was 0.77. Only one trial was performed for the nitrogen washout method and two or more trials for the helium dilution method. The results are reported in more detail in the appendix.

The reliability of the helium dilution method for determining residual lung volume was assessed on repeated trials (successive or taken before or after underwater weighing on the subjects of this study (n=56 and included retest subjects). The intraclass correlation for reliability was 0.99. The results are reported in more detail in the appendix.

The subjects were instructed to report for weighing dressed in bathing suits and were to refrain from eating for 3 to 4 hours prior to testing. They were also asked to urinate and defecate before reporting to the lab. Dry weight on a scale was recorded prior to testing. A Digi electronic platform scale from Japan was used (capacity, 300 lbs).

Total body density was calculated from the following formula:

$$D = \frac{WA}{\dfrac{WA - WW + WH}{DW} - (RV + IG)}$$

where:

D = whole body density (kg/l)

WA = weight in air (kg)

WW = weight in water (kg)

WH = weight of harness (kg)

DW = density of water (0.994 kg/l at 34 to 36°C)

RV = residual lung volume (l)

IG = intestinal gas (estimated at 0.1l)

Percent body fat was calculated from Siri's equation:

$$\% \text{ Fat} = \left(\frac{4.95}{D} - 4.50\right) \times 100$$

The accuracy of the method of hydrodensitometry to estimate body fat has not been established in humans and is influenced by the assumptions outlined in this thesis. Durnin and Satwanti (1982), determined the effect on percent fat by underwater weighing of maximal, moderate and minimal expiration, moderate inspiration, light and heavy meals and carbonated drink. Variations in expiration and inspiration and food consumption before weighing caused about 1% difference in estimated fat content. The carbonated drink resulted in the largest difference, 1.5% fat. However the authors observed that these errors were well within the basic errors of the method.

According to Jackson (1984) the reliability of the method of underwater weighing to determine fat was quite high and varied from 1.3 to 1.8% fat. Mendez and Lukaski (1981) reported the reliability of body fat measurements as 0.32 - 0.73% body fat. Reliability of measurement in this study was assessed by repeated measurement on ten subjects over the course of the study. The intraclass correlation for the repeated trials was .99. The mean fat difference between the two trials was -0.7%. The calculations are included in the appendix.

Photographs of the helium dilution apparatus for measuring residual lung volume and the fiberglass underwater weighing tank, used for this study, are presented in figure 3.1.

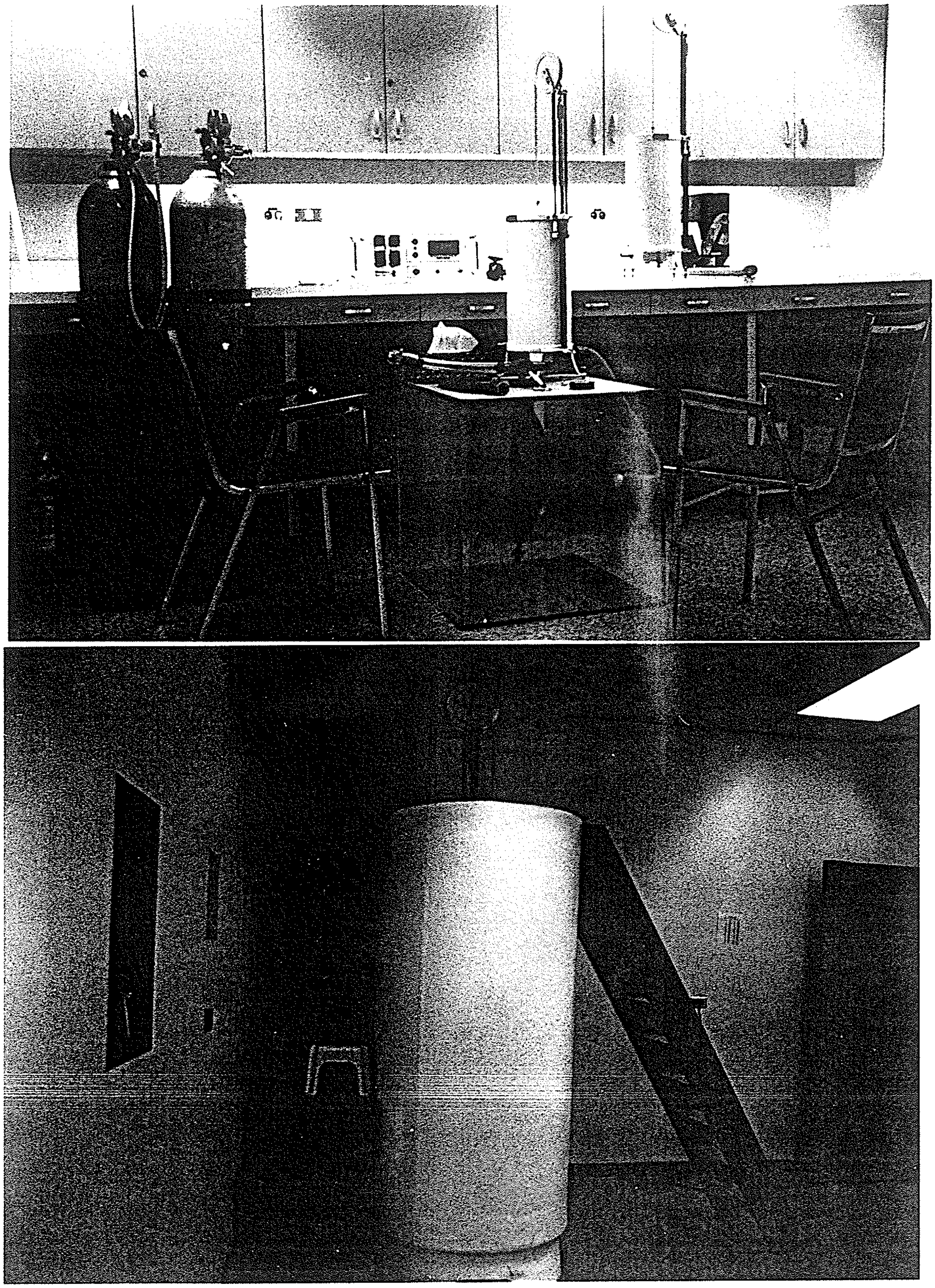


Figure 3.1

Helium Dilution Apparatus for Measuring Residual Lung Volume and Underwater Weighing Tank in the University of Manitoba Exercise Physiology Laboratory.

Anthropometric Measurements

Anthropometric measurements were taken on the same day or prior to underwater weighing in the University of Manitoba exercise physiology laboratory. If subjects had gained or lost more than 2 kg of weight since they had been measured their anthropometric measurements were redone. The following measurements were taken:

1. Body size; height (cm) and weight (kg).
2. Skinfolds (mm); triceps, subscapular, iliac crest, ,abdominal (umbilical), front thigh and medial calf.
3. Girths (cm); arm, forearm, wrist, chest, waist, abdominal, gluteal, upper thigh, mid thigh, calf, head and neck.
4. Bone Breadths (cm); humerus, wrist, femur, and ankle.

Body height was measured with the subject in the standing position with the head in the Frankfort plane, without shoes and against the wall to the nearest 0.1 cm.

The body weight of the subject in bathing attire was measured on a Digi electronic scale from Japan, to the nearest 0.1 kg.

Skinfolds were taken at the various sites indicated with a Harpenden skinfold caliper (H.E. Morse Co., England). Two or three measurements were taken at each site and the mean of the two closest used as the designated value. The location of the sites were as outlined by Lohman, Roche and Martorell in the "Anthropometric Standardization Reference Manual" (1988) and the measurements were taken to the nearest 0.1 mm.

Two or three girth measurements were also taken and measured with a metal tape (Lufkin Executive Thinline, USA) to the nearest 0.1 cm. The mean of the two closest measurements was used as the final value.

Similarly, the bone breadths at the various sites were measured by a Siber-Hegner aluminum caliper from Switzerland and the mean of the two closest measurements taken to the nearest 0.1 cm was used.

Muscle circumferences were calculated for the arm, forearm, midthigh and calf using the limb girths and skinfolds. The formula used was:

muscle circumference (cm) = limb girth (cm) - p (skinfold, mm/10).

This formula assumed that the cross-sectional tissue boundaries were circular and that the skinfold caliper reading was twice the skinfold thickness (Martin, 1984). The forearm muscle circumference was not corrected for a skinfold. The corresponding girths and skinfolds for the other sites were midarm and triceps, midthigh and midthigh, and midcalf and midcalf.

The recordings were made by a pair of researchers, with one person measuring and one person recording. All the body sites were measured once, and then remeasured a second and a third time as necessary. To ensure intertester reliability, the same teams were kept throughout the time course of the study and the same testers were used for both this study and the one on bone density and intense physical activity. To establish credibility as an anthropometrist, a directed study was undertaken in body composition and over thirty hours were spent learning techniques in anthropometry workshops at the University of Manitoba and practical experience with an adult fitness organization before this research was undertaken.

In anthropometry, interobserver errors in general, are the most problematic. Improper skinfold site selection caused the greatest variation among observers (Bennett and Osborne, 1986; Pollock and Jacobson, 1984). Oppliger, Looney and Tipton (1987), concluded that the variance due to investigators was less than 5% and skinfold measurements were deemed highly reliable under attainable conditions, given experienced investigators. In this study and the concurrent one, the testers and recorders were trained anthropometrists (staff) and students and were the same throughout the course of the research. A copy of the anthropometric worksheet is included in the appendix.

Data Analysis

At the conclusion of all the testing the raw data from the various worksheets and the computer printouts on bone density were entered onto a computer spreadsheet. The computer was an Apple Macintosh Plus and the program Microsoft Excel Version 1.5. Using Excel, mean values were determined for the raw data. For example, the mean value for the two triceps skinfold measurements recorded for each subject was calculated. These mean values were then transferred to a statistical program, Statview II for data analysis and to Cricket Graph for graphical presentation.

Using Statview II, mean values, standard deviations and coefficients of variation were determined using the total n of 41 subjects, for all the variables measured. This information was presented in a subject table and compared to the values in the literature. Then, the data were analyzed by Pearson product moment correlation (r). Mean values of whole body density as determined by HW, were correlated with all the measurements taken and a correlation matrix calculated.

Through Statview II, a factor analysis was used to identify clusters of variables, such that each cluster represented a common underlying biological factor related to whole body density. This analysis lent support to the three component model of the FFM, identified how much of the variation in the body composition of the subjects was due to these factors, and reduced the number of variables for subsequent regression analysis. It also provided an opportunity to evaluate the data on the basis of most of the measurements done. The ability to use more data increased the generalizability of the results. The factor analysis program provided factor scores for each subject, on each factor, which were added to the database and used in further analysis. Because these factor scores were composite measurements (for example, the femoral bone density factor) they were more stable in statistical analysis than the various individual measurements of body composition they represented. They were not as prone to unusual results due to individual body composition patterning that could be obtained by use of a single measure to represent a group of measurements (for example, by use of the bone density measurement at the femoral neck to represent the bone density at all the three femoral sites).

The principal factor procedure used was iterated principal axis, a common factor procedure which is different from principal components analysis. A common factor method was recommended by Gorsuch (1984) as the most suitable for a small sample size and when the number of items used was less than 40. According to the author, if the communalities were high and the number of variables reasonably large any of the principal factor analyses would result in the same factors. However, he preferred to begin with a common factor model. The principal component model uses the correlation matrix with unities as the diagonal elements. This procedure attempts to account for all the variance of each variable and assumes that all the variance is relevant and error free. On the other hand, the common factor method estimates the communalities and does not assume the the variables are error free. This concept was deemed more appropriate for the social sciences. As well, Gorsuch pointed out, if the communalities were actually unities then the estimates would be unities and and then the common factor analysis automatically would become a component analysis.

For this study the squared multiple correlations were used as the initial communality estimates. These were modified with each iteration until they stabilized. The extraction rule was the default (number of eigenvalues >1) and the transformation methods were the oblique solution - orthotran and the orthogonal solution - varimax. For the final factor solution the oblique solution was chosen as most suitable for these data. When the factors were designated as oblique or correlated, the variable complexity approached the ideal simple structure (i.e. each variable was associated with only one factor) more closely than for the orthogonal solution. It was more realistic to assume that these body factors should be correlated since they were all part of the same physical sphere.

Using the Statview II program, two separate stepwise regression equations were created to predict whole body density. One was created with the variables entered into the factor analysis and another with the factor scores which were composites of the same variables. Multiple stepwise regression was chosen because it computed the most frugal solution with the smallest number of independent variables. The regression equations were formed to determine the extent of the effect of bone density on body density and to identify the variables that described whole body density best.

Furthermore, the regression equation with the factor scores was used in a mathematical analysis to quantify the effect of bone density on body density for the subjects of this study. In the latter analysis, the percent fat estimated from body density predicted by stepwise regression with 4 factors was compared to the percent fat estimated from body density predicted with the same factors and substitution of average values for the 2 bone density factor scores.

An analysis of variance was performed to compare the subjects who had the largest percent fat differences on the foregoing mathematical analysis using the regression equations, with the rest of the group. This was also calculated with Statview II. The ANOVA was done to identify with anthropometric variables the subjects most at risk for error in percent fat estimation with underwater weighing due to their bone density. It was also used to identify suitable anthropometric measurements which could more easily and economically give an indication of bone density than DPA.

Finally, on the Excel spreadsheet, using the three component mathematical model of the FFM, a new fat-free density was calculated for each subject. A mean value of 7% was used to represent the coefficient of variation for whole body bone density in this sample. This was the value determined from the literature review and was based on studies using SPA and DPA which measured areal bone density in g/cm^2. It was assumed that there was a similar amount of variation in true body bone density which is expressed as g/cm^3. Using the equation generated for total body fat-free density in the three component model of the fat-free mass, the mean value for the bone density component was varied for each subject according to their individual variation from the mean on the proximal femur density factor and a new individual fat-free density was created which was different from 1.1 g/cm^3. This new fat-free density was combined with the value of .9 g/cm^3 for the density of fat and the whole body density as determined by underwater weighing to arrive at a new value of percent fat estimation which incorporated individual bone density variation.

Chapter 4

RESULTS AND DISCUSSION

Introduction

The results of the investigation of the effect of bone density on the estimation of body fat in young women are presented and explained under the following headings:

1. Physical Characteristics of the Subjects Mean values, standard deviations, ranges and coefficients of variation for all the variables measured are presented (table 4.1) and the bone density measurements taken are compared with those of the literature review;

2. Correlation of Main Variables Sixteen main variables were correlated with each other and whole body density and the relationships between variables are presented (table 4.2) and discussed. The relationship between the femoral neck and the other three bone density sites is also presented in graphical form (figure 4.1);

3. Factor Analysis The variables were analyzed by factor analysis to identify the independent sources of body composition variance, to support the three component model of the fat-free mass with factors that represented the biological characteristics outlined and to refine and reduce the set of variables for subsequent analysis. The factor analysis solution, the factor intercorrelations and the proportionate variance contributions of the factors are presented in table form (tables 4.3, 4.4 and 4.5);

4. Stepwise Regression to Predict Body Density and Bone Density The main variables and the factors from the preceding analysis were entered into separate stepwise regressions to predict body density. This was done to identify the variables which were most important to body density, to

determine the extent of the effect of bone density on body density and to create an equation which could be used to quantify the extent of this effect. All the anthropometric measurements taken were also used in separate stepwise regressions to predict the four bone density measurements. This was done to identify any anthropometric variables which could be substituted for the more costly DPA bone density measurements. All the aforementioned separate regression equations are presented in table form and explained (tables 4.6, 4.7 and 4.8);

5. Percent Fat Differences Due to Bone Density As discussed, a regression equation was created using the factor scores to predict body density. This was modified to include both bone density factors and percent fat, based on this body density, was estimated using Siri's equation. Average values were substituted for the bone density factor scores in the equation and a new body density and percent fat were calculated and compared to the original. The results of this analysis on percent fat differences due to bone density for all the subjects and for those with the greatest differences is presented in two separate tables (4.9 and 4.10). As well, the hip density factor versus the spine density factor is graphed (figure 4.2) and discussed. An analysis of variance was done to compare the the subjects with the greatest differences with the rest of the group and the comparison of selected physical characteristics is made in table form (tables 4.11 and 4.12). This was done to identify the physical characteristics of the subjects most at risk for error in percent fat calculation due to bone density and to isolate any anthropometric variables which could be substituted for the DPA bone density measurements;

6. Percent Fat Adjusted for Bone Density via the Three Component Model for the Fat-Free Mass Using a mean value of 7% coefficient of variation for whole body bone density (determined from the literature review) the mean value for the bone density component in the three component model was varied for each subject according to their individual variation from the mean on the hip density factor. A new fat-free density was calculated and combined with the whole body density determined by underwater weighing to arrive at a new value of percent fat estimation which incorporated individual bone density variation. The results of this analysis are summarized in two tables. The first one (table 4.14) is a comparison of the percent fat calculated by underwater weighing and Siri's formula, with the percent fat adjusted for bone density via the three component model for the fat-free mass for all the subjects and the second one (table 4.15) presents only the subjects with differences greater than three percent fat. This analysis was another way of quantifying the magnitude of errors in percent fat estimation due to bone density in a normal group of women and provided a more accurate estimation of percent fat for the individuals within the sample.

Physical Characteristics of the Subjects

Table 4.1

Physical Characteristics of the Subjects (n=41)

Variable	**Mean**	**S.D.**	**Range**	**C.V. (%)**
Age (yrs)	31	8	19-48	27.0
Height (cm)	166.5	7.0	151.1-182.8	4.2
Weight (kg)	57.62	5.06	46.70-69.65	8.8
Weight underwater (kg)	1.98	.64	.54-3.27	32.2
Vital capacity (VC) (l)	3.87	.50	2.60-5.00	13.0
Res. lung vol.% of VC (l)	1.03	.17	.79-1.49	16.0
Res. lung vol He.dil. (l)	1.17	.34	.49-1.89	28.1
Body density (kg/l)	1.054	.013	1.026-1.079	1.2
Body fat (%)	19.7	5.8	9.0-32.4	29.6
Body volume (l)	54.7	5.06	43.65-66.43	9.3
Fat mass (kg)	11.47	3.80	5.29-18.96	33.1
Fat-free mass (kg)	46.15	4.32	36.91-54.23	9.4
Bone density (g/cm^2)				
Lumbar spine	1.233	.125	.968-1.510	10.1
Femoral neck	1.035	.118	.719-1.353	11.4
Ward's triangle	.948	.135	.598-1.356	14.2
Trochanter	.861	.106	.560-1.088	12.2

Table 4.1 continued.

Variable	Mean	S.D.	Range	C.V. (%)
Skinfolds (SF) (mm)				
Triceps	13.1	4.6	6.5-24.9	34.9
Subscapular	10.7	5.0	5.0-26.4	47.1
Iliac crest	11.1	6.1	3.0-30.5	55.2
Abdominal	11.5	6.7	3.5-36.2	58.5
Thigh	25.7	9.8	9.1-48.7	38.3
Midcalf	13.7	6.6	4.6-36.0	48.5
Sum of 6 SF	85.8	34.0	37.2-169.1	39.6
Girths (G) (cm)				
Arm	26.4	1.8	20.9-30.1	6.7
Forearm	23.5	.9	21.6-25.4	3.9
Wrist	15.0	.6	13.4-16.3	4.0
Chest	84.8	3.4	78.2-92.4	4.0
Waist	67.42	3.5	60.0-76.5	5.2
Umbilical	73.7	6.0	64.3-92.0	8.1
Gluteal	93.5	4.2	84.0-101.9	4.5
Upper thigh	55.0	2.8	49.4-60.9	5.1
Mid thigh	51.3	2.9	45.5-58.9	5.7
Calf	35.4	1.6	31.8-39.1	4.5
Head	55.1	1.2	52.6-57.8	2.2
Neck	31.5	1.0	29.6-33.6	3.1

Table 4.1 continued.

Variable	Mean	S.D.	Range	C.V. (%)
Muscle circ. (MC) (cm)				
Arm	22.2	1.5	18.6-26.1	6.8
Forearm	23.5	.9	21.6-25.4	3.9
Thigh	43.2	3.3	34.5-49.8	7.7
Calf	31.1	2.6	24.5-36.7	8.2
Bone breadths (BB) (cm)				
Humerus	6.3	.3	5.5-7.0	4.8
Wrist	5.1	.3	4.1-5.5	6.0
Femur	9.0	.4	8.2-10.1	4.2
Ankle	6.8	.4	6.1-7.6	5.6

Table 4.1 presents the physical characteristics of the sample. The muscle circumferences and percent fat are derived values. There was a diverse range of age, 19-48 years, with a mean age of 31 years. The range encompassed approximately 30 years and covered three of the ten year age groups (20-29,30-39,and 40-49) commonly used by the bone density researchers cited in the literature review. If the sample had been larger it might have been useful to divide it into the same ten year groups to determine if there were age related changes in the relationship between bone density and body fat within this range and at the extremes.

The coefficient of variation for the residual lung volume measured by helium dilution (28%) was almost twice that of the estimate based on the measurement of vital capacity and there was no significant correlation (R=.28) found between the two methods for the subjects of this study. It is recommended that underwater weighing be accompanied wherever possible by an actual measure of residual lung volume.

There was a wide range of body fat measured and the coefficient of variation was 29.6%. Almost the same variation (29.9%) in percent fat estimated by underwater weighing was reported for 249 women, aged 18 to 55 years (mean age 31.4 years), measured by Jackson, Pollock and Ward (1980). This group was slightly fatter however, and had a mean value for percent fat of 24.1%. The mean value for this study was 19.7% fat.

The mean value found for bone density of the lumbar spine (1.23 g/cm^2, C.V. 10.1%) was very close to that reported by Mazess, Barden, Ettinger, Johnston, Dawson-Hughes, Baron, Powell and Notelovitz, (1987) and Mazess, Barden and Ettinger (1988) for two very large sample groups of American women. These two studies were discussed in the literature review. In the 1987 study the values reported were: for 164 women aged 20-29, 1.24 g/cm^2; for 248 women aged 30-39, 1.26 g/cm^2, and for 58 women aged 40-49, 1.21 g/cm^2. The coefficients of variation were 10.5, 10.3, and 9.9% respectively. In the 1988 study of 281 women aged 20-40 years the mean value reported by Mazess et al. was 1.28 g/cm^2, C.V. 9.4%. This mean value was slightly higher than found in this study and this may have been due to a younger mean age of the sample. Mean ages were not reported for the American studies.

The same study by Mazess et al. (1987) reported mean values and standard deviations for the same three proximal femur sites measured in this study. The mean value found for the femoral neck in this study (1.04 g/cm^2, C.V. 11.4%) was slightly higher than that found by Mazess et al. but was still comparable as was the coefficient of variation. The values reported by Mazess et al. were: for 90 women aged 20-29, 1.01 g/cm^2; for 132 women aged 30-39, .99 g/cm^2, and for 60 women aged 40-49, .88 g/cm^2. The coefficients of variation were 9.9, 12.1 and 11.4 respectively.

The mean value found for the Ward's triangle in this study (.95 g/cm^2, C.V. 14.2%) was also higher than those reported by Mazess et al. (1987) for the sample groups listed above. The values and coefficients of variation for these same subjects were: .93 g/cm^2,10.8%; .91 g/cm^2, 15.4%; .76 g/cm^2, 14.5%. It should be noted that the coefficient of variation for this site was the highest of the four sites measured and that it was the site which showed the least precision of measurement in the current study.

The value found in this study for the trochanteric region was, like the other proximal femur sites measured, slightly higher than those of the large American study. It was .86 g/cm^2 and the C.V. was 12.2%. Those reported in the 1987 study cited above were: .82, .80 and .72 g/cm^2 and the coefficients of variation were 11.0, 15.0, and 13.9% respectively. In summary, the mean value and the coefficient of variation determined for the bone density at the lumbar spine was very close to those reported by Mazess et al. (1987 and 1988). The mean bone density values at all the proximal femur sites measured were slightly higher than the mean American values but they were very comparable as were the coefficients of variation.

Table 4.1 shows the diverse coefficients of variation for the measurements taken. The skinfolds showed the most variation ranging from 34.9% to 58.5%. There was also a comparable amount of variation in weight underwater, body fat, and fat mass. All of these were measures of fatness. The variation was lower for the bone density measurements (10.1 to 14.2%) and lower still for the girths, muscle circumferences and bone breadths. The variation for these last three was similar and ranged from 2.2 to 8.2%. There were also different coefficients of variation for height (4.2%) and weight (8.8%). There would be more variation in a random sample and one that included males and females and older and younger subjects. These different variations should be considered when devising a model for the proportions, masses and densities of the fat and fat-free body.

Correlation of Main Variables

Table 4.2

Intercorrelations of Main Variables and Correlation with Whole Body Density and Percent Fat (n=41)

	SF Tri	SF Sub	SF Ili	SF Abd	SF Thi	MC Arm	MC Thi	MC Caf	BB Hum
SF Triceps	**1.00**								
SF Subscap	**.82**	**1.00**							
SF Iliac cr	**.72**	**.88**	**1.00**						
SF Abdom	**.69**	**.84**	**.86**	**1.00**					
SF Thigh	**.79**	.71	**.63**	**.55**	**1.00**				
MC Arm	-.28	-.20	-.19	-.10	-.31	**1.00**			
MC Thigh	**-.34**	**-.37**	**-.33**	-.25	**-.59**	**.41**	**1.00**		
MC Calf	**-.67**	**-.70**	**-.66**	**-.67**	**-.61**	**.36**	.55	**1.00**	
BB Hum	-.24	-.22	-.24	-.18	-.20	**.35**	**.36**	**.46**	**1.00**
BB Wrist	-.26	**-.37**	**-.35**	**-.43**	-.22	.28	.27	.47	.58
BB Femur	-.06	-.16	-.15	-.15	.07	**.33**	.28	**.34**	**.68**
BB Ankle	-.29	**-.37**	**-.37**	**-.34**	-.26	.25	**.40**	**.56**	**.64**
BD Lumbar	-.07	-.05	.10	.01	.03	**.40**	.18	.26	.29
BD Femoral	-.24	-.22	-.07	-.19	**-.31**	.19	**.31**	**.31**	.16
BD Ward's t	-.12	-.11	.09	-.05	-.24	.24	**.32**	.21	.11
BD Troch	-.14	-.11	.01	-.05	-.20	.22	**.31**	.26	.07
Body Dens	**-.80**	**-.78**	**-.67**	**-.73**	**-.73**	.24	.30	**.70**	.10
Percent Fat	**.80**	**.79**	**.67**	**.73**	**.73**	-.24	-.30	**-.70**	-.10

	BB Wri	BB Fem	BB Ank	BD Lum	BD Fem	BD Ward	BD Troc	Body Dens	% Fat
BB Wrist	**1.00**								
BB Femur	**.65**	**1.00**							
BB Ankle	**.67**	.76	**1.00**						
BD Lumbar	**.35**	**.49**	.29	**1.00**					
BD Femoral	.41	.24	.23	**.49**	**1.00**				
BD Ward's t	**.38**	.20	.14	**.55**	**.92**	**1.00**			
BD Troch	.15	.10	.04	**.50**	**.82**	.78	**1.00**		
Body Dens	.29	-.05	.16	.07	**.34**	.25	.30	**1.00**	
Percent Fat	-.29	.04	-.17	-.08	**-.35**	-.26	-.30	**-1.00**	**1.00**

KEY: SF, skinfold (mm); MC, muscle circumference (cm); BB, bone breadth (cm); BD, bone density (g/cm^2); Body Density (kg/l); Fat (%); Correlations > .308 are in bold face type - $p \leq .05$

Table 4.2 shows the correlation between the main variables. The relationships between the variables and their relationship to whole body density are discussed with reference to the following measurement groups; skinfolds, muscle circumferences, bone breadths and bone densities.

Skinfolds

The five skinfolds were highly intercorrelated and as a group had the highest positive correlation with percent fat and and the highest inverse correlation with whole body density. The perfect inverse correlation between percent fat and whole body density is an artifact of Siri's formula for the calculation of percent fat from body density which assumes that only fatness influences body density. In this sample, the skinfolds had the highest correlations with body density but they were not the only group with significant correlations with body density.

As a group the skinfolds were also significantly negatively correlated with with the muscle circumferences of the thigh and especially the calf. As the muscle circumferences went up skinfold fat went down. This relationship indicates an important connection between muscularity and body density, and therefore fatness. In this sample muscular individuals were leaner.

Only the skinfolds which represented central body fatness (subscapular, iliac crest and abdominal) were inversely correlated with the bone breadths at the wrist and ankle. Why central fatness indicators were related to peripheral bone breadths is unclear.

The only connection between skinfolds and bone density was a negative relationship between the bone density of the femoral neck and the thigh skinfold. This correlation was only just significant (r=.31) but indicated that the subjects with less thigh fat had higher femoral bone density. The femoral neck bone density was also correlated to the same extent (r=.31) but positively with the muscle circumference of the thigh and the calf. Because of the relationship between skinfold fat and thigh muscularity in this sample the conclusion made was that thigh muscularity was associated with less skinfold fat and a higher femoral bone density for the subjects in this study. The relationships are only just significant however. Further analysis with a group of highly muscular women might show a stronger relationship.

Muscle Circumferences

The muscle circumferences were used as indicators of muscularity. As a group they were also intercorrelated but not as highly as the skinfolds. Even the intercorrelation between the calf and thigh was only .55. These sites were more independent and this may have been due to the specificity of physical training done. The muscle circumference (MC) of the calf was strongly positively correlated with body density (r=.70). In this sample as body density increased so did calf MC. As discussed, this showed again the connection between muscularity and fatness in this sample.

The muscle circumferences were also correlated with some of the bone breadths. The bone breadth at the humerus was the only one correlated significantly with all the muscle circumferences. Also, the bone breadth at the femur was correlated significantly with the MC of the arm and the calf but surprisingly not the thigh. This relationship is unclear.

Bone Breadths

The bone breadths as a group were highly intercorrelated, more so than the muscle circumferences and less than the skinfolds. Their relationships with the skinfolds and muscle circumferences have been discussed. They were the only group not correlated significantly with body density. The bone breadth (BB) at the wrist had the highest correlation with body density (r=.29). The bone breadth at the wrist was also correlated significantly with the bone density measured at the lumbar spine (r=.34), the femoral neck (r=.41) and Ward's triangle (r=.38). The bone breadth at the wrist was different from the other BB sites in that it measured not one but two bones. The only other BB correlated with bone density was the femoral bone breadth which was again associated with the lumbar spine bone density (r=.49) and not the proximal femur.

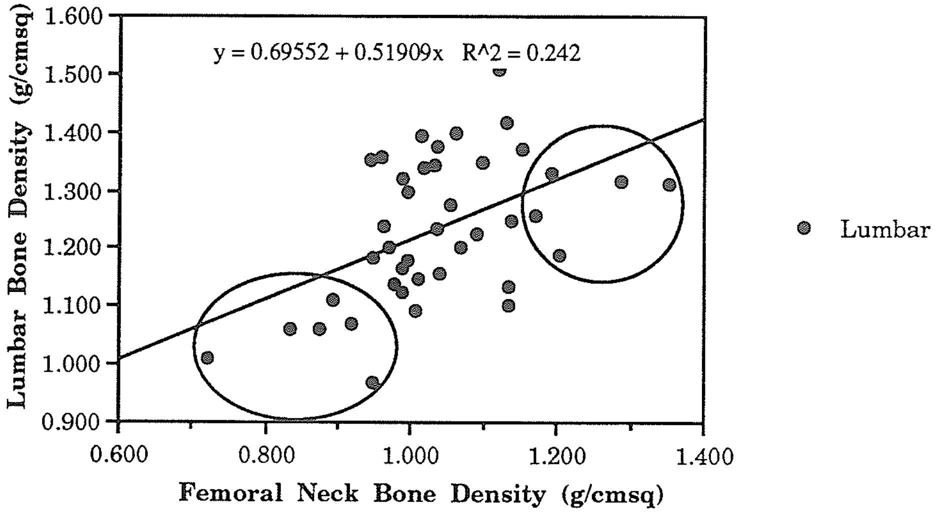
Bone Densities

The bone densities at the four sites measured were intercorrelated. There were really only 2 principal sites of measurement, the lumbar spine and the proximal femur and the intercorrelations reflected this split. The lumbar spine was moderately correlated with all the hip sites. The correlation with the femoral neck and trochanter was almost the same (r=.49 for the former and r=.50 for the latter). The correlation with the Ward's triangle was the highest (r=.55). This might have been because both the lumbar spine and the Wards triangle had higher proportions of trabecular bone than the other sites measured and/or were influenced by the same factors which promoted bone density.

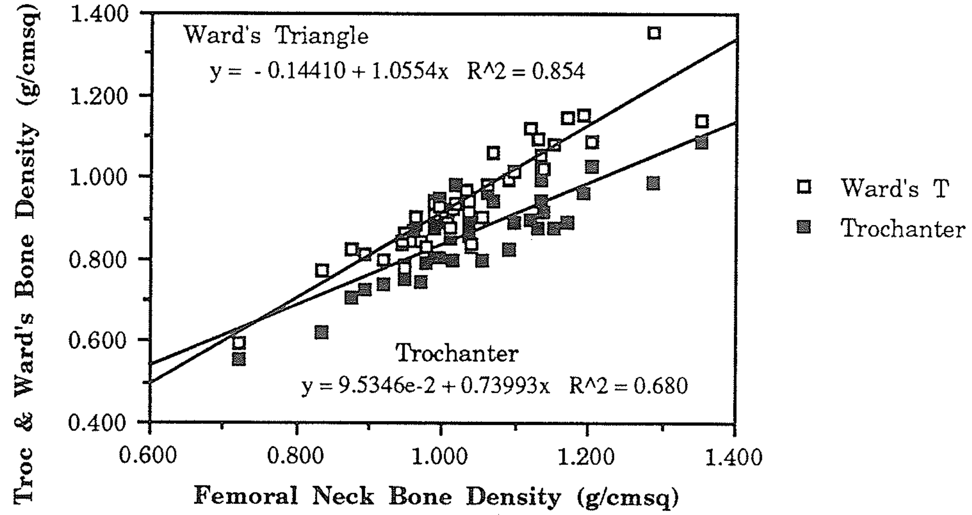
The three proximal femur sites were highly intercorrelated and could be considered as one site. The highest correlation (r=.92) was between the Ward's triangle and the femoral neck. This was not surprising since the triangle was part of the femoral neck site.

Only one bone density site was significantly correlated with the whole body density measured. This was the femoral neck (r=.34) and the correlation was positive. This may be because this site had the greatest proportion of cortical bone of the sites measured (approximately 75% according to Wahner et al., 1983) and thus was the best representative in this sample of whole body bone density. The proportion of cortical to trabecular bone in the whole skeleton is believed to be 80:20 (Ott et al., 1988 and Wahner et al., 1983). This significant correlation showed that bone density made a contribution to body density in this sample. As expected, it was not as high as that of fat. The magnitude of the relationships between fatness, bone density and whole body density were further explored through factor and regression analysis.

The other hip sites had similar positive correlations which indicated that they had similar proportions of cortical and trabecular bone and/or were subject to the same factors which influenced bone density. On the other hand the correlation of the lumbar spine density and whole body density was very weak (r=.07). This may be because the lumbar spine area measured contained a greater proportion of trabecular bone than the proximal femur and thus did not contribute much to overall bone density. The relationship could also be an artifact of this particular sample.



Lumbar Spine vs Femoral Neck Bone Density



Ward's Triangle and Trochanter vs Femoral Neck Bone Density

Figure 4.1

Lumbar Spine Versus Femoral Neck Bone Density (Top) and Ward's Triangle and Trochanter Versus Femoral Neck Bone Density (Bottom) (n=41)

In figure 4.1, the relationship between the femoral neck bone density and the bone density at the other sites was examined graphically. First, the lumbar spine bone density was plotted against the femoral neck bone density. This relationship was positive and linear but only moderately so (r=.49). Three different groupings were visually evident. The first group (n=6) contained those subjects who had both low femoral neck and lumbar spine bone density. The middle group (n=30) showed a lot of scatter about the line and represented those who had moderate and moderately high femoral neck bone density. These subjects had average or higher or lower than average lumbar bone density. The last group (n=5) contained the subjects with the highest femoral neck bone density. These last 5 subjects had average or lower than average lumbar bone density. It appeared from this analysis that high femoral neck bone density was not necessarily accompanied by high lumbar spine density.

In contrast, the relationship between the femoral neck bone density and the other two proximal femur sites was strongly linear and there was little scatter about the lines. The relationship with the Ward's triangle was the strongest because the triangle was part of the femoral neck. Different groupings were not visually evident and because of the high intercorrelations these three could be considered one proximal femoral site.

Factor Analysis

Introduction

The variables were analyzed by factor analysis to identify the independent sources of body composition variance and to further explore and quantify the relationships indicated by the correlation analysis, between skinfold measurements, muscle circumferences, bone density and bone breadth measurements. It was expected that these variables would represent factors that had true biological counterparts and that the factors could be used to describe and analyze the variability in the body composition of the subjects.

Factor analysis can perhaps best be understood from a geometric viewpoint. According to Gorsuch (1983), to represent factors and variables geometrically the factors are identified as the axes and the variables are vectors in Cartesian coordinate space. Variables can be plotted alone first and then factors and weights can be determined as additional steps. When a variable is physically close to a factor they are highly correlated. When the factors are uncorrelated with each other they form 90 degree angles with each other and are called orthogonal. In the correlated component model, the axes or factors form an oblique angle and are thus correlated and referred to as oblique factors. Once the factors have been added they need not remain in the same position but can be shifted or rotated to any position felt appropriate. In the common factor model the geometric representation of the variables proceeds from the correlation matrix between them and the initial communalities of the variables are estimated. (The communality of a variable is the proportion of the variable's variance accounted for by the common factors).

The factor procedure chosen for this study was the Iterated Principal Axis, a common factor model. It was chosen instead of a principal component analysis for the reasons outlined in the earlier discussion on the data analysis. The initial estimates of the communalities were the squared multiple correlations. The original intention was to use all the variables measured for the factor analysis, but there were too many variables for too few subjects and so the girths were omitted. The muscle circumferences, however, were based on the girth measurements and they were included.

Using the Kaiser Index, which provided a measure of variable sampling adequacy, the number of variables was reduced to the 16 with the highest measure of sampling adequacy. The final total matrix sampling adequacy was .80 which showed that the variables were highly suitable for factor analysis and the accompanying Bartlett Test of Sphericity that the correlations in the matrix were statistically significant ($p \leq .0001$). This was another benefit of the factor analysis procedure. It allowed the systematic reduction of the number of variables to those which explained best the independent sources of variation and thus provided the maximum information from the minimum number of variables. It was also a way to group correlated variables so they could be used as a single variable in future analysis. This was particularly useful in this study because it allowed the bone density measurements at the four sites to be combined into one bone density factor. The factor analysis provided Z scores for each subject, on each factor, which were added to the database and used in the subsequent stepwise regression analysis. As discussed in the data analysis, the ability to use more data increased the generalizability of results. As well, the use of the factor scores which were composite measurements, in the statistical analysis, provided more stability than the use of a single measurement.

Table 4.3

Factor Analysis Solution Rotated to the Oblique Solution Reference Structure * - Orthotran/Varimax

Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Skinfolds (mm):					
Triceps	**.71**	-3.03E-4	.10	-.17	-.10
Subscapular	**.85**	1.23E-3	-.01	.01	-.04
Iliac Crest	**.82**	.15	-.07	.01	.02
Abdominal	**.86**	-.01	-.08	.20	.04
Thigh	**.50**	-.11	.13	**-.55**	.13
Muscle Circumfer. (cm):					
Arm	-.03	.01	.11	.30	**.35**
Thigh	-.06	.13	.17	**.56**	-.02
Calf	**-.51**	.04	.22	.21	.11
Bone Breadths (cm):					
Humerus	.04	-.08	**.64**	.20	.04
Wrist	-.16	.20	**.65**	-.09	-.07
Femur	.11	-2.19E-4	**.82**	-.07	.13
Ankle	-.08	-.03	**.77**	.11	-.10
Bone Density (g/cm^2):					
Lumbar spine	.07	**.35**	.17	-.13	**.57**
Femoral neck	-.04	**.95**	.10	-1.92E-4	-.13
Ward's triangle	.09	**.88**	.05	.06	-6.58E-5
Trochanter	.02	**.75**	-.12	.08	.12

* (The Reference Structure defines loadings that are correlations. Loadings >.31 are in bold face type-p ≤.05)

The factor analysis solution is presented in table 4.3. The oblique solution has identified 3 main factors and 2 minor ones. Each factor and the relationships between them are discussed separately.

Factor 1: Fatness

The first major factor represented fatness and was the most highly correlated but inversely, with body density (r=-.73). For this sample, it was described best by skinfold measurements and also by calf muscle circumference (MC). The correlations with the factor for these variables were in opposite directions i.e. high skinfold fat was associated with low calf MC. That the calf muscle circumference was closely associated with fat was borne out by an ANOVA of the leanest (n=12), intermediate (n=23), and fattest (n=10) subjects on calf MC. There were significant differences between the 3 groups ($p \leq .0001$). The leanest had the highest calf MC, intermediate had intermediate and the fattest had the lowest of the group. Since the calf muscle circumference was used as an indicator of muscularity it was surprising to find it could also be used as an indicator of fatness albeit not as strong an index as the skinfolds. This pointed to a close relationship between leanness and muscularity and may be related to a genetic tendency of mesomorphic people to be lean. This relationship was also evident, to a lesser degree, with the thigh muscle circumference. Why the calf MC was singled out in this sample (that is, high calf muscularity in this sample was strongly associated with leanness) was unclear. Probably this was activity related, in that physical activity that promoted calf muscle such as running, contributed more to allover body leanness than activity that promoted upper body strength such as weight lifting.

Factor 2: Proximal Femoral Bone Density

The second major factor represented bone density at the proximal femur and the correlation with body density was r=.15. It was best described by the three femoral bone density sites but was also associated to a much lesser extent, but significantly with the lumbar spine. The site with the highest correlation with this factor was the femoral neck. This may be because it contained the greatest proportion of cortical bone of the all the sites. This factor was seen as the best representative of overall body bone density for this sample and was used for the subsequent analysis of the effect of bone density on percent fat prediction.

The other variables associated with the femoral bone density factor were the wrist bone breadth,,the iliac crest skinfold and the thigh muscle circumference. For all, the correlations with the factor were positive but not significant for this sample size. As discussed, the wrist bone breadth might have been a strong indicator because it included two bones. It should also be remembered that the literature review showed that the bone density measured at the distal radius and the radius shaft had the highest correlation with total body bone mineral. The wrist breadth might be an indicator of overall body bone density. The iliac crest skinfold and the thigh muscle circumference are both lower body measures and logically could be associated with femoral bone density. Also, the direction of the association of these two with this factor points to the possible positive influence of lower torso body fatness and thigh muscularity on femoral bone density. The connection between muscularity and bone density could be explored with a group of highly muscular individuals. Further research with diverse sample groups may clarify the connection between wrist breadth, iliac crest skinfold, thigh muscle circumference and femoral bone density. However, for this study, the factor analysis showed that there was no anthropometric variable that could be substituted for the DPA femoral bone density measurements in this study.

Factor 3: Bone Size

The third major factor represented bone size and was described best by the 4 bone breadth measurements. There was some intercorrelation with factor 2 (femoral bone density) The trochanter bone density was correlated negatively with bone size and the correlation of this factor with body density was negative as well, r=-19. Why the trochanteric site was correlated with the bone size factor in a different direction from the other bone density sites is unclear. Although all the bone density correlations with this factor were non-significant, factor 3 showed some association with the bone density variables which was logical since both were measurements of bone. However,the bone size factor described a different dimension of bone and might be used to analyze the fraction of skeletal mass in the three component model.

Perhaps the strongest association of this factor with a group other than the bone breadths was with the muscle circumferences, particularily the thigh and calf. All the correlations were positive. This relationship between muscularity and bone size might indicate some positive effect of the mass of muscle on bone size. On the other hand the relationships between the bone size factor and the skinfolds were not as clear. A positive relationship was only evident in the case of peripheral body fat.

Factor 4: Thigh Muscularity

The first minor factor, factor 4, represented thigh muscularity. It was correlated positively with whole body density (r=.13). It was described best by presence of muscular circumference of the thigh and absence of thigh skinfold. The correlations of these two with the factor were not as strong as the correlations of the other variables with their factors. Nevertheless, it appeared to be the strongest indicator of overall body muscle in this sample because of its association with the muscle circumference of the arm and calf.

What had been expected was that the muscle circumferences would emerge together as a major separate factor. Instead, the calf muscle circumference loaded with the fatness factor, the thigh muscle circumference appeared as a separate minor factor and the arm muscle circumference loaded with another minor factor, spine bone density. It appeared that muscularity was too closely linked with the other factors to be considered a major independent source of body composition variance in this sample. Although muscularity and fatness are most certainly strongly linked, part of the connection may have been due to the use of anthropometric measurements based on girths and skinfolds to represent muscularity. The communality summary for the factor analysis showed that only 41% of the variance of the arm MC and only 57% of the variance of the thigh MC was accounted for by the factors. It would have been valuable to include other indicators of body muscle which were not anthropometricaly based such as urinary creatinine. These indicators should also represent both central and peripheral musculature.

Factor 5: Spine Bone Density

The last minor factor, factor 5, represented spine bone density. The correlation with whole body density was r=.06. That spine density appeared as an separate factor, again showed the independence of the femoral and lumbar sites. As illustrated graphically in figure 4.1 high bone density at one site did not guarantee high bone density at the other site. There was significant intercorrelation however, between the two main bone density sites, because the lumbar spine bone density also loaded with factor two.

The other variable that loaded with factor 5 was the arm muscle circumference. This showed that upper body muscle was associated in a positive manner with the bone density at the lumbar spine which was similar to the relationship seen between thigh muscle circumference and femoral bone density. Unlike the situation for factor 2 femoral bone density however, here there was an anthropometric variable that was significantly associated with the bone density at the lumbar spine. It appeared from the correlation analysis and the literature that the lumbar bone density did not contribute much to the overall body bone density and so the arm muscle circumference could not be used in this sample to predict bone density. Further research could be done with osteoporotic populations of women to see if it could be used as indicator of spine bone density for that group.

The primary factor intercorrelations and the proportionate variance contributions of the factors are given in the following tables.

Table 4.4

Primary Factor Intercorrelations - Orthotran/Varimax

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Factor 1	1				
Factor 2	-.16	1			
Factor 3	-.29	.19	1		
Factor 4	**-.37**	.20	.21	1	
Factor 5	-.04	.29	**.33**	.24	1

Correlations >.31 are in bold face type-p ≤.05

Table 4.5

Proportionate Variance Contributions of Factors

		Oblique	
	Direct	**Joint**	**Total**
Factor 1	.38	-2.31E-3	.37
Factor 2	.26	-2.68E-3	.26
Factor 3	.26	3.29E-3	.27
Factor 4	.10	-.07	.04
Factor 5	.06	3.71E-3	.07

Primary Factor Intercorrelations

The primary factor intercorrelations are presented in table 4.4. If the factor intercorrelations had been larger than .50 this would have indicated that more factors should have been extracted initially. It appeared that the number of factors was appropriate for these variables.

Factor 1, fatness was negatively intercorrelated with all the other factors. The strongest relationship was with factor 4,.thigh muscularity. This again showed the inverse connection between fatness and muscularity. As fatness increased, thigh muscularity decreased. There was also an inverse correlation between fatness and bone density and bone size but this relationship was not significant for this sample size.

The other factors were positively intercorrelated with each other There was a significant intercorrelation between factor 3, bone size and factor 5, spine bone density. The factor analysis solution showed that the lumbar spine loaded the highest of the bone density sites with factor 3. This suggested that perhaps some of the bone breadths could be used to indicate lumbar spine density.

This analysis showed the intercorrelations of these 5 factors which represented part of the fat and fat-free body. Although the intercorrelations were small, their existence should be acknowledged when dividing the human body into arbitrary fat and fat-free components.

Proportionate Variance Contributions of the Factors

The proportionate variance contributions of the factors are shown in table 4.5. The direct proportionate contribution of a factor represents the independent proportion of common variance that factor accounts for, and the joint contribution deals with the variance that is common to more than one factor. The greater the proportionate variance contribution of the factor the more important the factor is in explaining the intercorrelations of the variables. All the joint contributions were negligible, except for factor 4, thigh muscularity. The joint contribution for this factor suggested that 7% of the common variance could be attributed to the covariation of two factors (factor 1, fatness and factor 4, thigh muscularity). The negligible joint contributions put into perspective the importance of the intercorrelations between the variables, i.e. except in the case of factor 4, they did not greatly affect the final total variance contribution.

Factor 1 (fatness) accounted for the greatest single proportion of common variance of these variables but it was less than half. Factor 2 (femoral bone density) and factor 3 (bone size) were the next most important and accounted for approximately the same amount each. These were both measures of bone and together explained 53% of the variance. To these could be added the variance contribution of factor 5, also a bone density factor. Together these three accounted for 60% of the variance. The two bone density factors accounted for 33% of the variance. In contrast, the muscularity factor accounted for only 4% of the total.

Summary

The factor analysis identified 5 factors. The three major ones represented fatness, proximal femoral bone density and bone size. The two minor ones represented thigh muscularity and spine bone density. For this sample the variables chosen appeared to be good representatives of the biological factors of fatness, bone size and bone density and to a lesser extent muscularity.

In general, the factors were independent of each other. The greatest intercorrelation was between muscularity and fatness and a close relationship was indicated for these two in this sample.

The proportionate variance contributions of the factors identified the independent sources of body composition variance. Fatness accounted for the greatest single source of variance (37%). Taken together, the 3 bone factors accounted for 60% of the variance.

The correlation of the factors with whole body density indicated that there might be factors other than fatness which would affect whole body density. The femoral density factor had the highest positive correlation with whole body density (r=.15) and this was followed by the thigh muscularity factor (r=.13). On the other hand, the bone size factor was correlated negatively with whole body density (r=-.19). To determine which of these factors influenced the whole body density of the sample and the magnitude of the effect on whole body density two separate stepwise regression analyses to predict whole body density were performed. The first one used the 16 variables entered into the factor analysis and the second one, the factor scores which represented these same variables. The results of these analyses are presented in the following tables.

All the variables measured were entered into 4 separate stepwise regression analyses to predict the bone density measured by DPA at the four sites. This was done to identify single or multiple anthropometric measurements that could be used as an indication of bone density in the absence of dual photon absorptiometry measurements. The results of these analyses are also presented in the following section.

Stepwise Regression to Predict Body Density and Bone Density

Table 4.6

Stepwise Regression to Predict Body Density with the 16 Variables from the Factor Analysis (n=41)

Step No. 1 Variable Entered: Triceps Skinfold (mm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.796		.634	.625	.008	67.639

Step No. 2 Variable Entered: Abdominal Skinfold (mm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.727	.833	.694	.678	.007	43.033

(Last Step) Step No. 3 Variable Entered: Trochanter Bone Density (g/cm^2)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.295	.857	.734	.713	.007	34.094

Variables in Equation

Variable	**Co-efficient**	**Std. Error**	**Std. Co-eff.**	**F to Remove**
Intercept	1.060			
Triceps SF	-.001	.0003	-.520	19.153
Abdomin SF	-.001	.0002	-.357	9.214
Troc.BD	.025	.0106	.204	5.660

Table 4.6 illustrates the stepwise regression to predict body density with the 16 variables from the factor analysis. This analysis was done to

identify which variables influenced whole body density in this sample and to determine the extent of the effect. The multiple stepwise regression used computed the most frugal solution with the smallest number of independent variables. This was statistically important to the study which had a modest n of 41 subjects. As each variable was entered into the equation the procedure selected as the next variable, the one with the highest partial correlation with the dependent variable. The partial correlation was the correlation between the independent and dependent variable with the effects of the other independent variables partialled out.

The first step identified the triceps skinfold as the single best predictor of body density in this sample. The second variable was also a skinfold,the abdominal one. These two skinfolds measured two different aspects of fatness. The triceps skinfold represented peripheral fatness and the abdominal one central fatness. This established fatness as the biggest influence on whole body density in this sample.

The trochanter (femoral) bone density was chosen as the last variable. This confirmed the importance of bone density to the overall body density of this sample of women. It was surprising that the trochanteric site was stepped in instead of the femoral neck because the latter had the highest correlation with body density. The trochanter was probably chosen as the most independent site because it had a lower correlation with the skinfolds than the femoral site and explained more of the remaining variance. The trochanteric site could also be used as a representative of proximal femoral bone density for this group.

Of the variables not in the equation, the next variable to be entered was the femoral bone breadth. The F to enter was 3.508. This suggested that bone size might also have some influence on body density in this sample.

The same type of stepwise regression analysis was performed with the 5 factors as variables and the results are presented in the following table.

Table 4.7

Stepwise Regression to Predict Body Density with the 5 Factors as Variables (n=41)

Step No. 1 Variable Entered: Factor 1, Fatness

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.734		.538	.526	.009	45.456

Step No. 2 Variable Entered: Factor 4, Thigh muscularity

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.125	.814	.662	.645	.008	37.270

(Last Step) Step No. 3 Variable Entered: Factor 2, Femoral Bone Density

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.155	.859	.738	.717	.007	34.731

Table 4.7 continued

Variables in Equation

Variable	Co-efficient	Std. Error	Std. Co-eff.	F to Remove
Intercept	1.054			
Factor 1, F	-.011	.001	-.876	97.804
Factor 4, T M	.005	.001	.413	21.550
Factor 2, F D	.003	.001	.279	10.674

The stepwise regression to predict body density with the 5 factors as variables is presented in table 4.7. The solution here began in a similar fashion to the regression equation with the individual variables. The fatness factor was entered first and was the most important. Surprisingly, the second factor entered was thigh muscularity. Although this factor did not show a large proportionate variance contribution in the factor analysis, nevertheless, the fact that it was stepped in showed that it was important to whole body density. The regression equation using the the factor scores was more illustrative of the factors influencing whole body density in this sample. The thigh muscularity factor was a stronger measure because it represented also the presence of calf and arm muscularity and the absence of thigh fatness and was correlated in a positive manner with whole body density. Another reason this factor may have been stepped in was because of the intercorrelation (negative) with the fatness factor. It was the most highly intercorrelated with the fat factor. It should also be remembered that the fatness factor included also calf muscularity. All of these indicated that muscularity also had an effect on whole body density in this sample.

The third variable entered was factor 2, the femoral bone density which again confirmed that in this sample bone density was an important component of whole body density. Again the factor score on this measure was a stronger and more generalizable variable because it represented the bone density at all the proximal femoral sites, as well as that proportion of the lumbar spine bone density which was associated with the femoral density.

The spine bone density was then forced into this equation and a new equation was created which included all the bone density factors. Thus no information was lost and the relationship between the femoral and spine bone density in this sample could be explored further. This increased the final multiple r slightly to .866 and the adjusted r squared to .723 and reduced the F-test to 27.107. The equation was still statistically significant for this sample.

This new regression equation was used to predict body density for each subject and then percent fat was calculated with Siri's equation. After that, the mean values for the two bone density factors were used to replace the actual factor scores for those two measures. A new percent fat was calculated for each subject and it was compared with the original. This mathematical analysis was done to determine the magnitude of the effect on percent fat of changing the actual bone density values to the mean values for this sample. In other words, to see what would happen to the percent fat of these subjects if they really did have a constant, average bone density as the two component model of body composition assumed.

In the following table are presented the results of stepwise regression analyses with all the anthropometric variables measured to predict the bone density measured at the various sites and the bone density factors. These did not result in statistically valid regression equations and the number of variables used was too high for the n of 41. The final regression equations are not presented. This analysis was performed to identify any anthropometric variables which could be used as a substitute for the more costly and time consuming DPA bone density measurements and variables and relationships for further research.

Table 4.8

Stepwise Regression to Predict Bone Density with All the Anthropometric Variables Measured (6 Skinfolds, 12 Girths, 4 Bone Breadths, 4 Muscle Circumferences and Age and Height, n=41)

1. To Predict Lumbar Spine Bone Density:

(Last Step) Step No. 1 Variable Entered: Femoral Bone Breadth (cm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
.489		.239	.220	.110	12.257

2. To Predict Femoral Neck Bone Density:

Step No.1 Variable Entered: Wrist Bone Breadth (cm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
.407		.166	.145	.109	7.763

(Last Step) Step No. 2 Variable Entered: Age (yrs)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
-.337	.530	.281	.243	.103	7.418

Table 4.8 continued

3. To Predict Ward's Triangle Bone Density:

Step No. 1 Variable Entered: Wrist Bone Breadth (cm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.378		.143	.121	.127	6.489

Step No. 2 Variable Entered: Age (yrs)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.366	.527	.278	.240	.118	7.302

Step No. 3 Variable Entered: Iliac Crest Skinfold (mm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.086	.605	.366	.315	.112	7.125

Step No. 4 Variable Entered: Thigh Skinfold (mm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.236	.666	.443	.382	.106	7.170

Step No.5 Variable Entered: Umbilical Girth (cm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.213	.719	.517	.448	.100	7.506

Table 4.8 continued

Step No. 6 Variable Removed: Age (yrs)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.366	.692	.479	.422	.103	8.287

(Last Step) Step No. 7 Variable Entered: Upper Thigh Girth (cm)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
.181	.739	.546	.481	.097	8.402

4. To Predict Trochanter Bone Density:

(Last Step) Step No. 1 Variable Entered: Age (yrs)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.323		.104	.081	.102	4.53

5. To Predict Factor 2, Proximal Femoral Bone Density:

(Last Step) Step No. 1 Variable Entered: Age (yrs)

Simple R	**Multiple R**	**R-Squared**	**Adj. R-Sq**	**RMS Resid**	**F-Test**
-.316		.100	.077	1.044	4.326

Table 4.8 continued

6. To Predict Factor 5, Lumbar Spine Bone Density:

Step No. 1 Variable Entered: Arm Muscle Circumference (cm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
.410		.168	.147	.886	7.871

Step No. 2 Variable Entered: Wrist Girth (cm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
-.141	.516	.267	.228	.843	6.910

Step No. 3 Variable Entered: Thigh Skinfold (mm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
.148	.583	.340	.286	.811	6.34

(Last Step) Step No. 4 Variable Entered: Triceps Skinfold (mm)

Simple R	Multiple R	R-Squared	Adj. R-Sq	RMS Resid	F-Test
-.115	.684	.469	.409	.737	7.934

Table 4.8 presents the results of the separate stepwise regression analyses to predict the bone density measured by DPA at the 4 body sites and the bone density factors.

Stepwise Regression to Predict Bone Density at the Lumbar spine,Femoral Neck, Ward's Triangle and the Trochanter

Lumbar Spine:

In general, for the 4 bone density sites, the bone breadths were the strongest indicators of bone density. This may be because they both measured bone and both contributed to skeletal mass. Although they measured different aspects of bone they were more closely related than for instance fatness and bone density. It was surprising however, that the femoral bone breadth was associated so strongly and in a positive manner with the lumbar spine bone density, when the wrist or the humerus might be expected to be the strongest indicator. The correlation between the wrist and the lumbar spine density was also significant for this sample and was .35. In fact, of the 4 bone density sites the lumbar spine had the highest correlations with all the bone breadth sites. This relationship was believed to reflect one of general body size. That the femoral bone breadth, a lower body measure, was the strongest predictor of upper body bone density in this sample might indicate that there were whole body factors such as genetically determined size and musculature that influenced lumbar bone density. The next variable to be entered was chest girth and the F to enter was 3.945.

Femoral Neck and Trochanter:

In contrast, the femoral neck bone density, a lower body measure, was associated most strongly with an upper body measure, the wrist bone breadth. As discussed before, the wrist breadth was felt to be a possible indicator of overall body density because of its association with the femoral neck site, which may contain the greatest amount of cortical bone of the 4 sites measured and in this sample had the strongest correlation with body density. Further research with measurement of whole body bone density may show a stronger connection. On the other hand, bone size and bone density may always remain separate to a great degree as they describe different dimensions of bone.

The relationship found in this sample between the bone breadths and the bone density at the femoral neck, the lumbar spine and the Ward's triangle indicated that in general, a larger bone size was related to a higher bone density. This relationship between bone breadths and bone density could be examined further with a group of osteoporotic women. Perhaps the correlation will be stronger for this group. The stereotypic osteoporotic woman has a slight build yet there seems to be no reason why a smaller frame size should mean a lower bone density since the forces acting on bone in a smaller person should be proportionately smaller.

Age was a variable that was entered into the prediction of the femoral neck and the trochanteric bone density. The correlation between both of these and age was as expected, negative. As age increases, bone density decreases. The correlations were significant for this sample group. If the sample size were larger it might have been useful to divide the subjects into ten year age blocks to see if the correlations between variables changed according to age.

Wards Triangle:

The long stepwise regression calculated for the Ward's triangle was very surprising. Since this site was basically part of the femoral neck the relationships should have been the same. According to Mazess et al., (1987) bone loss in hip fracture patients was particularly manifested in the Ward's triangle area which was low density and critical to bone strength. This analysis supported the view that the bone density found in the Ward's triangle was different from the the other two femoral sites. Perhaps further research with older and osteoporotic populations will clarify the relationships found here between the anthropometric variables and bone density.

As with the femoral neck, the wrist bone breadth was the most important variable. In general the other variables, skinfolds and girths, were lower body variables. The direction of the correlations between the thigh skinfold and the umbilical girth suggested that as fatness increased, Ward's triangle density decreased. Age was first entered and then later removed. Why so many variables with relatively low correlations with the Ward's triangle were stepped in is unclear.

Stepwise Regression to Predict the Bone Density Factors

Proximal Femoral bone density

The stepwise regression to predict factor two, femoral bone density showed clearly that for this sample there was no anthropometric variable that could be used as a substitute for the proximal femoral bone density. The only variable entered was age.

Spine Bone Density

There were 4 variables entered for factor five, the lumbar spine bone density, three upper body measures and one lower body. Age was conspicuously absent, as it was in the regression equation to predict the lumbar site. The correlation between age and the lumbar spine density was -.07. This could have been because some of the older subjects in the sample had higher than average lumbar spine bone density or vice versa, that some of the younger subjects had lower than average bone density or both. The comparison of the measured values with the literature showed that the mean lumbar spine density for this sample was slightly lower than that of the large American studies. That upper body training might have had an effect on the lumbar spine bone density of this group was indicated by the positive association with the arm muscular circumference. It was also possible that the bone density at the lumbar spine was more affected by activity and hormonal factors than that of the proximal femur because it contained a larger proportion of high turnover trabecular bone. Although the final multiple r was higher for this factor, the regression equation was not statistically strong enough to be used to replace the DPA lumbar spine bone density measurement.

Percent Fat Differences Due to Bone Density

As seen, in table 4.7, a statistically valid stepwise regression equation was created for this sample to predict whole body density using the following factors: fatness, thigh muscularity, and lumbar and femoral bone density. This equation was created to determine the effect on percent fat of the substitution of average values for actual bone density factor measures.

First the regression equation was used to predict body density for each subject and then percent fat was calculated with Siri's equation. After this, the mean values for the two bone density factors were used to replace the actual factor scores for those two measures and and a new prediction of body density was made. Since the factor scores were Z scores, the mean values were zero and as intended, using the mean values eliminated the effect of the two bone density factors in this prediction of body density. A new percent fat was calculated for each subject and it was compared with the original.

This mathematical analysis was done to determine the magnitude of the effect on percent fat of changing the actual bone density values to the mean values for this sample. As stated before, to see what would happen to the percent fat of these subjects if they really did have a constant, average bone density as the two component model of body composition assumed. This analysis also allowed further exploration into the relationship between the lumbar and femoral bone density. Because the bone density factor scores were Z scores the bone density at these two main sites could more effectively be compared.

The analyses are presented in the following tables. First the differences between percent fat predicted by stepwise regression with 4 factors and percent fat predicted by stepwise regression with the same factors and substitution of average values for the 2 factor scores on bone density are presented in table 4.9 for all the subjects and discussed. Then the results of the 12 subjects with the greatest differences in the foregoing table are collected in table 4.10 and evaluated.

Table 4.9

Difference between Percent Fat Predicted by Stepwise Regression with 4 Factors and Percent Fat Predicted by Stepwise Regression with the Same Factors and Substitution of Average Values for the 2 Factor Scores on Bone Density (n=41)

Subject No	Fact Score Fem BD	Fact Score Spine BD	%Fat RE 4 Factors	%Fat RE Avg BD	%Fat Difference
S-01	1.5	-0.9	13.4	15.3	-1.9
S-02	2.7	-0.1	14.6	19.0	-4.4
S-03	-0.1	0.4	17.1	17.2	-0.1
S-04	-0.5	1.9	12.1	12.7	-0.5
S-05	-1.6	0.3	16.1	13.8	2.3
S-06	0.5	1.0	19.2	20.7	-1.5
S-07	-0.8	0.1	14.4	13.2	1.2
S-08	0.1	0.9	26.8	27.6	-0.8
S-09	-0.8	-0.2	16.5	15.1	1.4
S-10	-0.6	1.9	15.4	15.9	-0.4
S-12	0.8	-0.9	24.1	24.9	-0.7
S-13	-1.0	-0.7	27.7	25.5	2.2
S-14	-0.8	-0.3	18.8	17.2	1.6
S-15	1.8	-1.1	26.6	29.0	-2.4
S-16	-0.8	0.0	24.0	22.7	1.3
S-17	0.2	1.5	22.9	24.4	-1.5
S-18	-1.3	-1.1	31.4	28.3	3.1
S-19	0.1	-0.2	18.1	18.2	-0.1
S-20	0.7	-0.6	15.6	16.4	-0.8
S-21	-0.8	-1.4	15.5	13.3	2.2
S-22	-1.1	-0.3	20.4	18.4	2.0
S-24	-1.3	-0.7	18.8	16.1	2.7
S-25	1.0	-0.5	24.1	25.5	-1.4
S-26	0.8	0.0	15.5	16.8	-1.3
S-27	-0.2	0.4	23.1	23.2	0.0
S-28	-1.0	1.4	17.8	17.1	0.7
S-29	-0.2	0.4	13.6	13.5	0.1
S-30	0.0	0.4	15.6	15.8	-0.2
S-31	0.5	-0.5	14.4	14.8	-0.4
S-32	1.3	-1.3	14.5	15.7	-1.2
S-33	0.4	-1.1	16.6	16.5	0.2
S-34	-2.5	-0.6	22.3	17.5	4.7

Table 4.9 continued

Subject. No	Fact Score Fem BD	Fact Score Spine BD	%Fat RE 4 Factors	%Fat RE Ave BD	%Fat Difference
S-35	2.6	-1.7	18.2	21.3	-3.2
S-36	-0.8	1.5	29.0	28.7	0.3
S-37	-0.2	-0.9	24.2	23.1	1.0
S-38	1.4	-0.2	17.8	20.0	-2.1
S-39	-0.2	0.9	29.6	29.9	-0.3
S-41	-0.2	1.1	16.9	17.4	-0.5
S-43	0.5	-0.5	23.8	24.2	-0.4
S-44	-0.1	-0.2	24.6	24.3	0.3
S-45	-0.1	1.7	17.5	18.6	-1.0
Mean	0.0	0.0	19.7	19.7	0.0
Std. Dev.	1.1	1.0	5.1	5.0	1.8
Std. Error	.2	.1	.8	.8	.3
Range	-2.5 to 2.7	-1.7 to 1.9	12.1 to 31.4	12.7 to 29.9	-4.4 to 4.7

From the analysis in table 4.9, it can be seen that substitution of average values for the two bone density factors in the regression equation to predict body density caused changes in percent fat in both directions. The direction of the change reflected the situation which occurred in percent fat estimation by underwater weighing. With the current methods, the subjects who had high bone density had their percent fat underestimated and the subjects with low bone density had their percent fat overestimated. The magnitude of the differences were related to the magnitude of the standard deviations of the factor Z scores for bone density. Using this analysis it was calculated that a standard deviation of 1 on the femoral bone density factor translated into a percent fat error of 1.6%. One standard deviation on the lumbar spine bone density factor created an error of .6% fat. If the bone density factors were both in the same direction, that is, both were above or below the mean then, taken together, one standard deviation represented a total error of 2.2% fat.

The factor score for the femoral bone density had a greater effect on the final value because of its greater correlation with body density. In most cases, a subject with higher than average femoral bone density had a higher percent fat when average values were used in the regression equation and the reverse was true for subjects with low femoral bone density. There were 8 subjects however, with slightly lower than average factor scores for the proximal femur and much higher scores at the spine and these subjects showed the same direction of fat difference as those with higher than average femoral scores. But the percent fat differences for these subjects were low, 1 percent or less. Subjects 04, 10, 41, and 45 were good examples of this. The others were 03, 27, 30 and 39. Subject 33 was the only one with the reverse situation i.e. slightly higher than average femoral bone density and lower than average spine density and therefore a fat difference in the same direction as someone with low femoral density. Again the percent fat difference was very low.

The diversity of the bone density combinations of just these two sites was well illustrated and the independence of the sites, that is, the degree of bone density observed at the femoral site did not necessarily correspond to the degree observed at the spine. There was a greater range of variation at the proximal femur than the spine. High or low factor scores on the femoral bone density factor caused the greatest differences in percent fat but in this analysis these could be tempered, augmented, or reversed by the score on the spine bone density factor.

This analysis showed clearly that there were errors in percent fat prediction due to bone density in this group of normal young women. In general, the percent fat differences in this sample were small but they did range from -4.4 to 4.7% fat. The magnitude of the error was quantified using the factor score deviations. One standard deviation above or below the mean on both the femoral bone density factor and the lumbar spine factor caused an error of fat prediction of 2.2%. The largest differences were for the subjects who scored almost 3 standard deviations from the mean on the femoral bone density factor. This meant that if the distribution of bone density in this sample were the same as the normal bell shaped curve, approximately 30% of the sample would have errors due to bone density which were higher than errors due to measurement.

The magnitude of the error should also be discussed with reference to the percent fat of the individuals. For example, the percent fat difference for subject 2 was -4.4. This difference represented 30% of the predicted percent fat of 14.6. On the other hand, subject 34 had a larger difference in the opposite direction of 4.7% fat but for this subject, this difference represented only 21% of the predicted percent fat of 22.3. Thus, the error is a function of the initial fatness of the subject and is greater in lean individuals.

Table 4.10

Subjects with the Greatest Differences (1.9-4.7 % Fat) between Percent Fat Predicted by Stepwise Regression with 4 Factors and Percent Fat Predicted by Stepwise Regression with the Same Factors and Substitution of Average Values for the 2 Factor Scores on Bone Density (n=12)

Subject No	Fact Score Fem BD	Fact Score Spine BD	%Fat RE 4 Factors	%Fat RE Avg BD	%Fat Difference
Subjects Fatter with Average Bone Density Regression Equation (n=5)					
S-02	2.7	-0.1	14.6	19.0	-4.4
S-35	2.6	-1.7	18.2	21.3	-3.2
S-15	1.8	-1.1	26.6	29.0	-2.4
S-38	1.4	-0.2	17.8	20.0	-2.1
S-01	1.5	-0.9	13.4	15.3	-1.9
Mean	2.0	-.8	18.1	20.9	-2.8
Std. Dev.	.6	.7	5.2	5.0	1.0
Std. Error	.3	.3	2.3	2.3	.5
Range	1.4 to 2.7	-1.7 to -.1	13.4 to 26.6	15.3 to 29.0	-4.4 to -1.9
Subjects Leaner with Average Bone Density Regression Equation (n=7)					
S-34	-2.5	-0.6	22.3	17.5	4.7
S-18	-1.3	-1.1	31.4	28.3	3.1
S-24	-1.3	-0.7	18.8	16.1	2.7
S-05	-1.6	0.3	16.1	13.8	2.3
S-13	-1.0	-0.7	27.7	25.5	2.2
S-21	-0.8	-1.4	15.5	13.3	2.2
S-22	-1.1	-0.3	20.4	18.4	2.0
Mean	-1.4	-.6	21.7	19.0	2.8
Std. Dev.	.6	.5	5.9	5.8	.9
Std. Error	.2	.2	2.2	2.2	.4
Range	-2.5 to -.8	-1.4 to .3	15.5 to 31.4	13.3 to 28.3	2.0 to 4.7
All (n=12)					
Mean	0.0	-.7	20.2	19.8	.4
Std. Dev.	1.8	.6	5.7	5.3	3.0
Std. Error	.5	.2	1.6	1.5	.9
Range	-2.5 to 2.7	-1.7 to .3	13.4 to 31.4	13.3 to 29.0	-4.4 to 4.7

Table 4.10 presented the twelve subjects with the greatest differences (1.9 to 4.7% fat). They represented 29% of the total n of 41, which indicated a basically normal distribution of bone density in this sample. They were divided into two groups, those who were fatter with average bone density in the regression equation and generally had high overall bone density (n=5) and those who were leaner with average bone density in the regression equation and who had low overall bone density (n=7). In general, these were the ones with the highest hip factor scores in both directions. Both groups had the same mean percent fat difference (2.8%), but in this analysis there were more subjects with low overall bone density. This showed that this sample deviated somewhat from the ideal normal distribution of bone density.

The 2 subjects with the greatest differences (4.4 to 4.7% fat) had combined standard deviations on the bone density factors of greater than 2. These subjects were 4.8% of my sample and again this percentage was close to the ideal curve. For the normal curve, 5% of the subjects will have standard deviations greater than 2. Based on the bone density distributions in this sample, which approximated the normal curve, the error due to bone density in percent fat prediction for this sample was quantified as follows: 29% of the subjects had errors greater than 1.9% fat and greater than the error of measurement in this study. These scored greater than one standard deviation on the combined bone density factors. Of these, 24% had errors between approximately 2 and 4% fat and 5% had errors between 4 and 5%. The last group scored greater than two standard deviations on the bone density factor scores.

Looking at the subjects who were fatter with average bone density it was surprising to find that they did not have high positive factor scores at both the femur and the spine. In fact, all the subjects with high femoral bone density had lower than average bone density at the spine. This might have been a function of the mean age of the sample which was 31. If the sample had included more younger subjects perhaps there would have been a greater group with high density at both sites. It was also possible that this distribution was activity related, that is, for the subjects in this study the type of activity that promoted high hip density eg. jogging did not improve the spine or overactivity caused hormonal changes and promoted bone loss. It was apparent from this and the preceding table that the subjects who scored high on the spine bone density factor formed a different group who had moderate hip bone density and small percent fat differences. It was possible that this group engaged in activities to promote the spine and not the hip such as weight training or rowing. This again confirmed the independence of the sites and suggested that the spine might be more sensitive to activity and hormonal factors.

It was also surprising to find that the subjects with low femoral bone density more consistently had low spine bone density. If this was activity related perhaps this group did no exercise at all, or were older and more subject to age related bone loss. To learn more about the 12 subjects who were most at risk for errors due to percent fat calculation due to bone density, an analysis of variance was performed, and the two separate groups outlined in table 4.10 and were compared with the rest of the subjects. It was also done to identify any anthropometric variables that could be used replace bone density measurements.

To learn as much as possible about the relationship between the femoral neck and spine bone density an analysis of variance was also performed with the 7 subjects who had spine bone density factor scores greater than 1. These were also compared with the rest of the group. The results of these analyses are presented in the following tables.

Table 4.11

Comparison of Physical Characteristics of Subjects who were Fatter with Average Bone Density in The Regression Equation based on 4 Factors (n=5) with the Rest of the Group (n=36)

Variable	P	Measure	Subj Fatter	Rest of Group
Height (cm)	.10	Mean	171.4	165.8
		Std. Dev.	3.9	7.1
		Std. Error	1.8	1.2
		Mean Diff.	5.6	
Calf girth (cm)	.10	Mean	36.5	35.3
		Std. Dev.	1.6	1.6
		Std. Error	.7	.3
		Mean Diff.	1.2	
Lum BD. (g/cm^2)	.37	Mean	1.280	1.226
		Std. Dev.	.058	.130
		Std. Error	.026	.022
		Mean Diff.	.054	
Fem BD (g/cm^2)	.0001	Mean	1.241	1.007
		Std. Dev.	.077	.092
		Std. Error	.034	.015
		Mean Diff.	.234	
Ward's BD (g/cm^2)	.0001	Mean	1.175	.917
		Std. Dev.	.105	.106
		Std. Error	.047	.018
		Mean Diff.	.258	

Table 4.11 continued

Variable	**P**	**Measure**	**Subj Fatter**	**Rest of Group**
Troch BD (g/cm^2)	.002	Mean	.991	.843
		Std. Dev.	.074	.097
		Std. Error	.033	.016
		Mean Diff.	.148	
Age (yrs)	.12	Mean	25.4	31.4
		Std. Dev.	6.2	8.2
		Std. Error	2.3	1.4
		Mean Diff.	-6.0	
Factor 2 fem BD	.0001	Mean	2.0	-.3
		Std. Dev.	.6	.8
		Std. Error	.3	.1
		Mean Diff.	2.3	
Factor 5 spine BD	.047	Mean	-.8	.1
		Std. Dev.	.7	.9
		Std. Error	.3	.2
		Mean Diff.	-.9	

Table 4.11 shows the results of the comparison of physical characteristics of the 5 subjects who were fatter with average bone density in the regression equation based on 4 factors with the rest of the group. This group of subjects was one of two most at risk for error due to their bone density in percent fat calculation with underwater weighing. These subjects had significantly higher bone density at the femoral neck ($p \leq .0001$), Ward's triangle ($p \leq .0001$), and the trochanter ($p \leq .002$). On the other hand, they had higher but not significantly higher lumbar bone density. They had higher factor 2, femoral bone density ($p \leq .0001$) but

significantly lower factor 5 spine bone density ($p \leq .047$). This showed that the factor scores were more illustrative of general trends because they were composite scores.

There were no significant differences with the rest of the group in skinfolds, girths, muscle circumferences, bone breadths, and percent fat by under water weighing.

The following trends (close significance) were observed. These subjects were taller ($p \leq .10$), had a greater calf girth ($p \leq .10$) and were younger ($p \leq .12$) than the rest.

These subjects were impossible to identify unless one had their bone density measurements. It was surprising that this group did not have significantly higher lumbar bone density than the rest. That this might be activity related, was indicated by the greater calf girth of these subjects. The preferred form of activity may have been running which contributed greatly to femoral density and calf muscle but not as greatly to spine density. The higher femoral density of these subjects may also have been associated strongly with their younger age. Perhaps a larger and more diverse sample would have shown age as the best indicator of high hip density. There was also the suggestion that greater height which might have represented greater weight in this sample was associated with greater femoral bone density.

Table 4.12

Comparison of Physical Characteristics of Subjects who were Leaner with Average Bone Density in The Regression Equation based on 4 Factors (n=7) with the Rest of the Group (n=34)

Variable	P	Measure	Subj Leaner	Rest of Group
Chest girth (cm)	.04	Mean	82.4	85.3
		Std. Dev.	2.4	3.4
		Std. Error	.9	.6
		Mean Diff.	-2.9	
Arm MC (cm)	.06	Mean	21.3	22.4
		Std. Dev.	1.6	1.4
		Std. Error	.6	.2
		Mean Diff.	-1.2	
Lumbar BD (g/cm^2)	.0001	Mean	1.069	1.267
		Std. Dev.	.074	.105
		Std. Error	.028	.018
		Mean Diff.	-.198	
Fem BD (g/cm^2)	.0001	Mean	.879	1.067
		Std. Dev.	.084	.097
		Std. Error	.032	.017
		Mean Diff.	-.188	
Ward's BD (g/cm^2)	.0001	Mean	.776	.984
		Std. Dev.	.082	.115
		Std. Error	.031	.020
		Mean Diff.	-.208	
Troch BD (g/cm^2)	.0001	Mean	.692	.896
		Std. Dev.	.074	.073
		Std. Error	.028	.013
		Mean Diff.	-.204	

Table 4.12 continued

Variable	P	Measure	Subj Leaner	Rest of Group
Age (yrs)	.055	Mean	36.0	29.6
		Std. Dev.	8.7	7.7
		Std. Error	3.3	1.4
		Mean Diff.	6.4	
Factor 2 fem BD	.0001	Mean	-1.4	.3
		Std. Dev.	.6	.9
		Std. Error	.2	.2
		Mean Diff.	-1.6	
Factor 5 spine BD	.049	Mean	-.6	.1
		Std. Dev.	.6	1.0
		Std. Error	.2	.2
		Mean Diff.	-.8	

The results of the comparison of the physical characteristics of the 7 subjects who were leaner with average bone density in the regression equation based on 4 factors with the rest of the group is presented in table 4.12. These subjects had significantly lower bone density at the lumbar spine and at the 3 proximal femoral sites ($p \leq .0001$ for all sites). They also had lower factor 2, hip density ($p \leq .0001$) and factor 5, spine density ($p \leq .049$) and a significantly smaller chest girth ($p \leq .04$).

There were no significant differences with the rest in height, skinfolds, bone breadths and percent fat by underwater weighing. There were two trends. They were older ($p \leq .055$), and had a smaller arm muscle circumference ($p \leq .06$).

Unlike the subjects with high femoral density, these subjects had low bone density at all the sites. Given the diversity of bone density of the two main sites for most of the subjects why this occurred is not clear. It could have been due to choice of sample which was not random, and/or was related to the greater age of this group. There may be different forces working in the femur and spine. The spine may be more sensitive to activity and hormonal changes because of a greater proportion of trabecular bone and the femur to heredity factors and age related changes in bone density. These subjects may have been not only older, but also inactive.

These subjects were also impossible to identify without bone density measurements. The smaller chest girth and arm muscle circumference identified their lower spine density but there was no physical characteristic clearly associated with their low femoral density - the most important factor. Research with a larger sample of women with both low density at the hip and spine may establish how strongly chest girth and arm muscle circumference is associated with bone density at the femur. Further research with women with spinal osteoporosis could establish these two anthropometric variables as important indicators of spine density.

Table 4.13

Comparison of Physical Characteristics of Subjects who had Factor 5 (Spine Bone Density) Scores Greater than 1.0 (n=7) with the Rest of the Group (n=34)

Variable	P	Measure	Subj Hi Spine	Rest of Group
Height (cm)	.049	Mean	171.2	165.5
		Std. Dev.	5.6	6.9
		Std. Error	2.1	1.1
		Mean Diff.	5.7	
Forearm girth (cm)	.07	Mean	24.1	23.4
		Std. Dev.	.7	.9
		Std. Error	.3	.2
		Mean Diff.	.7	
Chest girth (cm)	.02	Mean	87.5	84.2
		Std. Dev.	4.5	2.9
		Std. Error	1.7	.5
		Mean Diff.	3.3	
Arm MC (cm)	.02	Mean	23.4	22.0
		Std. Dev.	1.6	1.4
		Std. Error	.6	.2
		Mean Diff.	1.4	
Lumbar BD (g/cm^2)	.0001	Mean	1.385	1.202
		Std. Dev.	.060	.111
		Std. Error	.023	.019
		Mean Diff.	.183	
Fem BD (g/cm^2)	.71	Mean	1.020	1.038
		Std. Dev.	.060	.127
		Std. Error	.023	.022
		Mean Diff.	-.019	

Table 4.13 continued

Variable	P	Measure	Subj Hi Spine	Rest of Group
Ward's BD (g/cm^2)	.97	Mean	.945	.949
		Std. Dev.	.094	.143
		Std. Error	.035	.025
		Mean Diff.	-.005	
Troch BD (g/cm^2)	.26	Mean	.903	.853
		Std. Dev.	.069	.111
		Std. Error	.026	.019
		Mean Diff.	.050	
Age (yrs)	.94	Mean	30.4	30.7
		Std. Dev.	8.0	8.3
		Std. Error	3.0	1.4
		Mean Diff.	-.3	
Factor 4 thigh MC	.08	Mean	-.6	.1
		Std. Dev.	.8	1.0
		Std. Error	.3	.2
		Mean Diff.	-.7	
Factor 5 spine BD	.0001	Mean	1.6	-.3
		Std. Dev.	.3	.7
		Std. Error	.1	.1
		Mean Diff.	1.9	

The results of the analysis of the comparison of the 7 subjects who had the highest spine bone density factor scores with the rest of the group is presented as table 4.13. These subjects had significantly higher lumbar bone density ($p \leq .0001$), higher factor 5, spine density ($p \leq .0001$), greater chest girth ($p \leq .02$), greater arm muscle circumference ($p \leq .02$), and they were taller than the rest ($p \leq .05$).

There were no significant differences with the rest in skinfolds, age, femoral neck, Ward's triangle and trochanter bone density and percent fat by underwater weighing.

The trends (close significance) for this group were greater forearm girth ($p \leq .07$) and less factor 4, thigh muscle circumference ($p \leq .08$), greater humerus bone breadth ($p \leq .11$), and greater weight ($p \leq .12$).

These subjects had the most physical variables describing them but some of the smallest errors of fat prediction due to the effect of bone density. As with the last group of subjects, chest girth and arm muscle circumference seemed to be strong indicators of spine bone density. For these subjects greater chest girth and arm muscle circumference was associated with higher spine density. Therefore in upper body characteristics this group was the reverse of the subjects with both low spine and femoral density. What was conspicuously different here was that age was not related to high spine density. Again physical activity seemed to be a factor. These subjects had a higher arm muscular circumference and lower thigh muscularity as measured by factor 4. This suggested specific upper body training which did not promote the lower body muscle.

This group was taller than the rest and had a greater humerus bone breadth and also a greater weight. All these might be related to greater femoral bone density. Although these subjects did not necessarily have high bone density at the femoral neck and the Ward's triangle they did have higher density at the trochanteric site. The 5 subjects with high femoral bone density were taller as well. In general, the greater height seemed to be associated with a greater weight for both groups and a higher femoral bone density. This relationship could be an artifact of the DPA areal bone density measurement which is two rather than three dimensional and which does not completely correct for size.

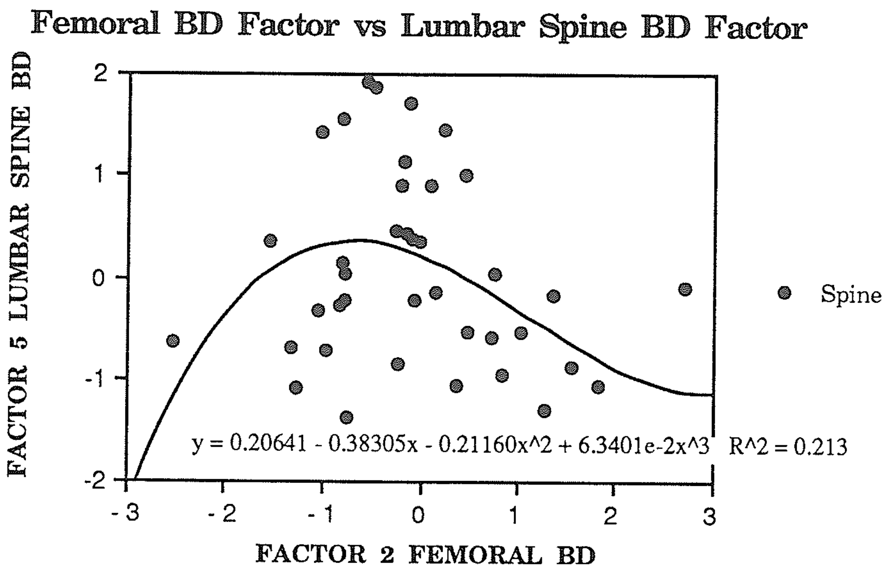


Figure 4.2

Femoral Bone Density Factor Versus Lumbar Spine Bone Density Factor

The relationship between the femoral bone density factor and the lumbar spine bone density factor is graphed in figure 4.2. The factor scores presented a different picture because they were a composite of all the variables entered. The femoral bone density factor included all the femoral sites as well as that portion of the spine density that was correlated with femoral bone density. Spine density also appeared as a separate factor and was closely associated with the muscle circumference of the arm.

A curve was fitted to this graph. This took on the appearance of a normal curve that was positively skewed. It was speculated that initially, as femoral bone density increased spine density also increased. This continued to a maximum and then began to level off and eventually decreased. The rate of decrease was more gradual than the rate of increase. It appeared that the highest levels of spine bone density were associated with moderate levels of femoral bone density and the highest levels of femoral bone density were associated with moderate or moderately low spine bone density.

This graph suggested the relationships found with the preceding analysis of variance of the two groups most at risk for error in percent fat due to their bone density and the subjects with the highest scores on the spine bone density factor with the rest of the group. The positive skewness may be caused by the effect of physical activity on bone density and may be peculiar to this sample. It was surmised that the type of activity which promoted high femoral bone density did not promote high lumbar bone density and at extreme levels possibly contributed to a reduction of the the density at the spine through hormonal factors. Conversely, the activity which promoted the the highest lumbar bone density may have affected only that site and did not greatly increase the bone density at the femur.

Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass

The previous analysis which used the stepwise regression to predict body density and determined the percent fat error with substitution of average values for the bone density factors in the regression equation was one way of determining if there were errors in percent fat estimation due to bone density in this group and suggested the magnitude of the error. The dilemma was that one could not determine the true percent fat of the subjects in this study without dissection and weighing of fat and therefore all fat calculations must be based on theory. Given the inconstancy of the fat-free mass, which was illustrated clearly in this sample by the diversity of femoral and spine bone density combinations, a model which acknowledged and included normal variation was an improvement over the two component model which assumed a constant fat and fat-free mass. The three component model of the fat-free mass was presented in the literature review as a way to analyze mathematically, the effect on whole body fat-free density of variation in the fat-free body components. Since the bone density measurements taken at the four sites provided a good measure of individual bone density variation for the subjects in this study and an extensive literature search had determined a coefficient of variation for whole body bone density for this sample of normal young women, the three component model was used to calculate for each subject a new value of percent fat estimation which incorporated individual bone density variation.

It was hypothesized that there would be errors greater than 5% fat prediction due to differences in bone density in this sample. The following analysis with the three component model provided mathematical confirmation of this hypothesis as well as more accurate values of percent fat for each subject.

The three component model of the fat-free mass assumes that the mean bone density of the body is 1.43 g/ml. The literature search for this study determined that the coefficient of variation for the areal bone density of the whole body as measured by DPA and SPA for this sample of young women was 7%. (This was the same as the variation cited in the literature for measurement of total body bone density by DPA and close to the variation found for the bone density at the radius site by SPA which was 8%). It was assumed that real bone density expressed as g/ml would vary in this population by the same amount. Since the bone density at the proximal femur was the most highly correlated with overall body density it was felt to be the best indicator of the bone density of the body for this study.

Using a coefficient of variation of 7% and the mean value 1.43 g/ml gave a standard deviation of .1001 g/ml for whole body bone density in this sample. This was then multiplied by each subject's femoral density factor score (which was a standard score) to give an individual standard deviation. This value was added to 1.43 g/ml and a new fat-free density was calculated for each subject using the equation for the fat-free density generated by the three component model of the fat-free mass. Only the bone density component of the equation was altered. The mean values for muscle and residual and the percentage composition for muscle, bone and residual were unaltered. The values and fractions given in table 2.4 of the literature review were used. This new fat-free density was different from the constant

1.1 g/ml used in the two component model and Siri's equation and varied according to each individual's femoral density factor score.

The new fat-free density was then combined with the whole body density calculated by underwater weighing and a constant fat density of .9 g/ml to determine a new percent fat with the following formula:

$$mf = \frac{\dfrac{dff \ \mathrm{x} \ df}{dff - df}}{D} - \frac{df}{dff - df}$$

For example, for subject no 1 the femoral bone density factor score was 1.5 (given in table 4.9). The overall standard deviation of .1001 g/ml was multipled by this factor score to give an individual standard deviation of .1502 g/ml. This standard deviation was then added to the overall mean value for bone density of 1.43 g/ml and a new individual value of 1.58 g/ml was calculated for this subject. Using the equation for the fat-free density generated by the three component model of the fat-free mass and subsitution of 1.58 g/ml for the mean value of 1.43 g/ml for the bone density component in this equation, an individual fat-free density was determined which was 1.113 g/ml for this subject (given in table 4.14). This fat-free density was combined with the whole body density determined by underwater weighing and a constant density for fat of .9 g/ml and a new prediction of percent fat for this subject which was 18.5% (given in table 4.14) was calculated. This percent fat estimation incorporated individual bone density variation.

Finally, this percent fat was compared with the percent fat calculated with a constant fat-free density of 1.1 g/ml and a constant fat density of .9 g/ml and the whole body density determined by underwater weighing according to Siri's equation (14.2%, table 4.14) and the percentage difference noted (-4.3%, table 4.14). The results of this analysis for all the subjects are summarized in the following tables.

Table 4.14

Comparison of Percent Fat by Underwater Weighing with Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass (n=41)

Subject No	Underwater Weighing %Fat	Three Comp Model for FFM		% Fat Difference
		FFD	% Fat	
S-01	14.2	1.113	18.5	-4.3
S-02	17.0	1.121	23.6	-6.6
S-03	19.5	1.099	19.2	0.3
S-04	09.0	1.095	07.2	1.8
S-05	16.6	1.084	10.7	5.9
S-06	18.5	1.104	19.8	-1.4
S-07	12.1	1.092	09.2	2.9
S-08	29.0	1.101	29.3	-0.2
S-09	11.9	1.092	09.1	2.8
S-10	18.9	1.095	17.1	1.8
S-12	26.1	1.107	28.2	-2.1
S-13	30.5	1.090	27.7	2.9
S-14	15.5	1.092	12.5	3.0
S-15	25.3	1.115	29.7	-4.3
S-16	23.4	1.093	21.0	2.4
S-17	22.1	1.102	22.8	-0.7
S-18	23.9	1.087	19.7	4.2
S-19	15.2	1.101	15.6	-0.5
S-20	18.0	1.107	20.2	-2.1
S-21	12.7	1.093	10.0	2.7
S-22	24.2	1.089	20.8	3.4
S-24	18.1	1.087	13.3	4.8
S-25	27.5	1.109	30.1	-2.5
S-26	14.6	1.107	16.9	-2.3
S-27	23.8	1.099	23.3	0.5
S-28	19.9	1.090	16.4	3.5

Table 4.14 continued

Subject No	Underwater Weighing %Fat	Three Comp Model for FFM		% Fat Difference
		FFD	% Fat	
S-29	12.7	1.098	11.8	0.8
S-30	14.8	1.100	14.8	2.0E-2
S-31	12.9	1.104	14.4	-1.5
S-32	15.7	1.111	19.3	-3.6
S-33	19.5	1.103	20.5	-1.1
S-34	31.2	1.072	22.1	9.1
S-35	16.0	1.121	22.5	-6.5
S-36	24.9	1.092	22.4	2.5
S-37	25.2	1.098	24.5	0.7
S-38	19.3	1.112	23.0	-3.6
S-39	32.4	1.098	31.9	0.5
S-41	14.0	1.098	13.4	0.6
S-43	21.5	1.104	22.9	-1.4
S-44	23.1	1.100	23.0	0.1
S-45	18.3	1.099	18.0	0.3
Mean	19.7	1.099	19.4	0.3
Std. Dev	5.8	.010	6.2	3.2
Std. Err	0.9	.002	1.0	0.5
Range	9.0 to 32.4	1.072 to 1.121	7.2 to 31.9	-6.6 to 9.1

Table 4.14 shows the comparison of percent fat by underwater weighing with the percent fat adjusted for bone density with the three component model of the fat-free mass. It was apparent again, in this different type of mathematical analysis, that there were differences in percent fat due to bone density that were greater than the error of measurement in this sample. The intraclass correlation for the reliability estimate was .99 and the mean percent fat difference for the retest subjects in this study was -.7% fat. The differences here were larger than the analysis using the regression equation to predict body density and ranged from -6.6 to 9.1% fat. In the previous analysis, they ranged from -4.4 to 4.7% fat.

Just by varying the bone density component in the three component model of the fat-free mass, the fat-free density of almost all the subjects changed from the assumed constant 1.1 g/ml. In this analysis the fat-free densities varied from 1.072 to 1.121 g/ml. The subjects who had a higher percent fat with the three component model had higher than average scores on the femoral bone density factor. Their higher than average bone density caused their percent fat by underwater weighing to be underestimated. Their percent fat, more correctly calculated via the three component model of the fat-free mass, was higher. The reverse was true for the subjects with low femoral bone density. Their percent fat via the three component model of the fat-free mass was lower than the standard method of percent fat calculation and thus correctly took into consideration their lower bone density.

As in the previous analysis (table 4.9) the magnitude of the error was related to the fatness of the subjects and was larger in the leaner individuals. In table 4.14 the difference for subject 2 is -6.6% which is 39% of the percent fat calculated by underwater weighing. The difference for subject 34 is greater (9.1%) but only represents 29% of the percent fat calculated by underwater weighing (31.2%).

Table 4.15

Subjects with Differences Greater than 3 Percent Fat Between Percent Fat Predicted by Underwater Weighing and Percent Fat Adjusted for Bone Density via the Three Component Model of the Fat-Free Mass (n=12)

Subject No	**Underwater Weighing %Fat**	**Three Comp Model for FFM**		**% Fat Difference**
		FFD	**% Fat**	
S-01	14.2	1.113	18.5	-4.3
S-02	17.0	1.121	23.6	-6.6
S-05	16.6	1.084	10.7	5.9
S-15	25.3	1.115	29.7	-4.3
S-18	23.9	1.087	19.7	4.2
S-22	24.2	1.089	20.8	3.4
S-24	18.1	1.087	13.3	4.8
S-28	19.9	1.090	16.4	3.5
S 32	15.7	1.111	19.3	-3.6
S-34	31.2	1.072	22.1	9.1
S-35	16.0	1.121	22.5	-6.5
S-38	19.3	1.112	23.0	-3.6
Mean	19.5	1.100	20.0	0.2
Std. Dev	6.0	.017	5.0	5.5
Std. Err	1.5	.005	1.4	1.6
Range	11.9 to 31.2	1.072 to 1.121	10.7 to 29.7	-6.6 to 9.1

The subjects with differences greater than 3% fat from the last analysis have been collected in table 4.15. Ten out of these twelve are the same subjects who had the greatest percent fat differences on the analysis using the stepwise regression to predict body density (table 4.10). There are two different subjects in this analysis because the earlier analysis used the bone density factor scores for both the spine and femur and this one used only the femur. The 2 subjects who were different (S-28 and S-32) in table 4.15 as compared to table 4.10 were ones with low differences in percent fat.

The three component model gave a stronger weighting to the subjects who had low femoral bone density and this was reflected in the mean difference of +.2% fat. Comparison of the subjects with the highest and lowest differences, S-02 and S-34 showed that although they had almost the same factor score on the femoral density factor (2.7 for S-02 and -2.5 for S-34) the subject with the lowest femoral bone density showed the largest difference (9.1% fat for S-34 compared with -6.6% fat for S-02). Why this occurred is unclear. In table 4.15 therefore, 1 standard deviation on the femoral bone density factor score below the mean (low bone density) represented a mean error in percent fat estimation by conventional underwater weighing of 3.5%, and one standard deviation on the femoral bone density factor score above the mean (high bone density) represented an mean error of 2.6% fat. If all twelve subjects are grouped together, the mean error in percent fat estimation for 1 standard deviation on the femoral bone density factor score in either direction is 3%. This overall mean error of 3% due to bone density is almost the same as the one determined in the last analysis which was 2.2% per standard deviation of bone density. They are different because the methods used to determine them are different.

The distributions for the subjects in table 4.15 are similar to the normal distributions outlined for those in table 4.10. The 12 subjects in table 4.15 were the subjects with femoral bone density factor scores of greater than 1 standard deviation in both directions and represented 29% of the sample. They had errors greater than 3% fat prediction. There were three subjects with femoral bone density factor scores greater than 2 standard deviations and they comprised 7% of the sample. They had errors in fat prediction from -6.5 to 9.1%.

This analysis and the previous one showed that there were serious errors in percent fat estimation by the conventional two component model of underwater weighing, that were due to bone density, in this sample of normal young women. Both analyses showed that the errors due to bone density affected an important proportion of the sample (29%) and that they were larger than the error due to measurement. The true magnitude of the errors could not be verified without dissection, which was impossible. The overall error was estimated to be between 1.6 and 3% fat for every standard deviation in femoral bone density above or below the mean. If these overall errors are applied to a normal sample with varying amounts of fatness, the errors are proportionately greater in lean individuals.

The two component model, as proposed by Keys and Brozek in 1953, was based on a reference man. Since that time, new techniques to measure bone density have shown that bone mass at all sites and ages is greater for men than women. (Wasserman and Barzel, 1987). This fact alone will lead to an overestimation of percent fat in a normal group of women by the conventional method of underwater weighing. This error can become even greater when added to the normal variability in bone density as reported in the literature review and the subjects of this study. These errors due to bone density make Siri's equation to predict percent fat from whole body density clearly unsuitable for a normal group of women.

This study dealt with only a limited sample of young women. The errors in fat prediction due to bone density might be greater in female populations that are younger and older than the subjects in this study or extremely muscular.

Chapter 5

SUMMARY AND CONCLUSIONS

Summary

The purpose of this study was to assess the amount of variability in bone mineral density in young adult women and to determine the effect of this variability on whole body density and body fat as estimated by hydrostatic weighing. It was hypothesized that whole body density as determined by hydrostatic weighing would be positively and significantly correlated with the bone mineral density measured at the lumbar spine and proximal femur and that the variation in bone density found in the subjects of this study would result in changes of greater than five percent fat estimation as assessed by hydrostatic weighing.

Forty one normal, healthy, premenopausal female adults aged 19 to 48 years, engaged in varying levels of activity were recruited. The bone density of the subjects at the lumbar spine and the proximal femur was measured by dual photon absorptiometry, whole body density and percent fat by underwater weighing, and 6 skinfolds, 12 girths, 4 muscle circumferences and 4 bone breadths, as well as height and weight were determined by anthropometry. The mean values and coefficients of variation for these variables were calculated and analyzed by correlation, factor analysis and stepwise regression to determine the relationships between them and their relationship to whole body density and percent fat. Subsequent separate mathematical analyses with stepwise regression and a three component model of the fat-free mass quantified the extent of the effect of bone density on percent fat by underwater weighing in this sample.

These analyses were performed with the ultimate aim of improving the prediction of fat from whole body density for the population examined. This research was part of a larger study which was investigating bone density and percent fat in young, middle aged, and old, adult males and females, as well as very lean muscular young adults with the intention of devising for these populations modified densitometric equations for percent fat estimation which included bone density.

The review of the literature showed that despite widespread use of many techniques, none of the methods for fat estimation had been validated in humans. Since the only truly direct method of determining body fat was by dissection and weighing of fat, all methods of fat prediction in humans must be indirect. Hydrostatic or underwater weighing was considered to be the the "criterion" method of fat determination and had proven to be reliable for over forty years. This method used Archimedes' principle of water displacement to determine the volume of the body. Using body weight as mass and the volume measurement, whole body density was determined from the formula density = mass / volume.

This relationship was also used to derive percent fat from whole body density. The body was divided into a two component model consisting of the fat portion and the fat-free portion. These components were further subdivided into their relative masses and densities and rearranged mathematically to isolate fat as a percentage of the total mass in the body.

However, to get percent fat from whole body density important assumptions had to be made - the principal ones being that the density of the fat and the fat-free mass were known and had constant values. For the latter to be true, it was also necessary to assume that all individuals had the same proportions of muscle, bone and water. Given the normal human biological variability the above assumptions were unlikely.

Several researchers reported problems with the use of body density to determine fat and some found extremely low values and even "negative" fat in lean, highly muscular individuals. These values were deemed impossible since an essential amount of fat was needed to sustain life and the measured subjects showed obvious skinfold fat. In general, the literature showed that the density of the fat-free component was not necessarily constant and varied according to age, sex and race. The largest source of variation was probably bone density. However, the extent of the effect of variation in bone density on percent fat by underwater weighing was unclear.

A mathematical analysis using a three component model of the fat-free mass composed of muscle, bone, and residual, proposed by Martin and Drinkwater (1990, unpublished), was presented and explained. This indicated that normal variation in bone density in relation to similar variation in muscle and bone fraction could cause a wide range of fat-free densities and also large errors in percent fat prediction.

An extensive literature search was also done to determine the coefficient of variation and thus the variability in normal young women in the bone mineral content and the bone mineral density of the lumbar spine, the proximal femur, the radius and ulna and total body bone mineral and density as measured by single and dual photon absorptiometry and total body calcium as measured by neutron activation analysis. The populations examined were Canadian, American and European.

Conclusions

A number of conclusions were reached in this study. The major ones are listed below:

1. The literature search to determine the variability of the total body bone density in normal young Canadian women showed that all the bone density sites examined showed different amounts of variation and that the measurements at one site did not necessarily represent that of another site or the skeleton as a whole. The radius shaft showed the highest correlation with total body bone mineral content probably because it contained the greatest proportion of cortical bone (>90%). Based on the coefficient of variation observed for the studies which measured total body bone mineral density by DPA and the bone mineral density of the radius shaft by SPA, a value of 7% was estimated for the coefficient of variation for whole body bone density in a normal young Canadian female population.

2. The subjects in the study represented a normal group of young women with a typical range of body density and bone density. The bone densities measured in this study at the lumbar spine and the proximal femur were comparable to the American values reported in the literature as were the coefficients of variation for these sites. The coefficients of variation were as follows: lumbar spine, 10.1%; femoral neck, 11.4%; Ward's triangle, 14.2%; and trochanter 12.2%.

3. All the bone density sites measured were positively correlated with the whole body density determined by hydrostatic weighing, but only one site, the femoral neck was significantly correlated with whole body density (r=.34). This may be because this site had the greatest proportion of cortical bone of the sites measured (approximately 75%), and because this

proportion was similiar to the proportion of cortical bone in the whole body (approximately 80%). It was thus the best representative in this sample of whole body bone density.

4. A factor analysis showed that the bone density and the anthropometric measurements described factors that had physiological counterparts. Although there was some intercorrelation, in general, the skinfolds suggested fatness, the DPA bone density measurements, bone density, the muscle circumferences, muscularity, and the bone breadths, bone size. The bone density at the femur and the spine appeared as separate bone density factors which illustrated the independence of the sites in this sample.

5. Stepwise regression to predict whole body density showed that there were two significant contributors to body density in this group; fatness, which was primary and bone density, which was secondary. There was also an indication that muscularity contributed to body density in this sample.

6. Stepwise regression to predict bone density indicated that the anthropometric variables most closely associated with the bone density measurements in this sample were the bone breadths. This was probably because bone breadths and DPA measurements both measured bone, and/or were related to the same factor, bone mass. Although one was an indicator of the other they both measured different dimensions of bone, namely size and density and so might always remain separate to a great degree. There were no anthropometric variables or combinations of them which could be used to replace the costly DPA bone density measurements

for this sample. Age was related to femoral density and not to lumbar spine density in this group. This was believed to be activity related.

7. Two separate mathematical analyses showed that there were errors in percent fat prediction due to bone density in this group of normal young women. The errors greater than the error of measurement in this study, affected 29% of the sample. They ranged from -4.4 to 4.7% fat for the first analysis, and from -6.6 to 9.1% fat for the second. This 29% represented the subjects who were more than one standard deviation, above or below the mean, on femoral bone density. The true magnitude of the errors could not be verified without dissection and weighing of fat which is impossible in living individuals. The overall error was estimated to be between 1.6 and 3% fat for every standard deviation in femoral bone density above or below the mean. If this overall error is applied to a normal sample of women with varying amounts of fatness, the errors are proportionately larger in lean individuals. The highest errors occurred in the subjects who varied more than 2 standard deviations from the mean and depending on the analysis they represented between 5 and 7% of the sample.

8. The subjects most at risk for error in percent fat prediction due to bone density were impossible to identify without their bone density measurements. Analysis of variance with the rest of the subjects indicated that the subjects most at risk for an underestimation of percent fat due to their high bone density in this sample, were taller, ($p \leq .10$) younger (mean age 25 yrs,.$p \leq .10$) and had significantly higher bone density at all the femoral sites ($p \leq .0001$ for all sites) but not the spine. On the other hand, the subjects at risk for overestimation of fat due to low bone density were older (mean age 36 yrs, $p \leq .055$) and had significantly lower bone density at both

the femoral and spine sites ($p \leq .0001$ for all sites). The low spine bone density of the latter group was also associated significantly with a lower chest girth ($p \leq .04$) and with close significance to a lower arm muscle circumference ($p \leq .06$).

Recommendations

The following general recommendations are made on the basis of the findings of the current study:

1. The errors due to bone density make Siri's equation as it presently stands with a constant value of 1.1 g/ml for the density of the fat-free mass, unsuitable for the prediction of percent fat from whole body density for a normal group of young women. It is impossible, however, at the present time, to determine the bone density profile of young women without costly and invasive measurements like dual photon absorptiometry. Future research with diverse populations may establish some anthropometric indicators of bone density for select groups such as thigh or chest girth perhaps in combination with bone breadths. In the meantime, the possibility of error, and the probable magnitude, due to bone density should be acknowledged when estimating percent fat by underwater weighing.

2. Continued research with bone density measurements, particularly whole body bone density will clarify the bone density status of diverse groups of men and women. It is also recommended that research be directed towards exploring further, the relationship between muscularity and fatness, and muscularity and bone density and whole body density.

3. Most important of all, new equations for fat prediction from whole body density should be established for women which reflect gender differences in bone density and which allow for normal variability.

BIBLIOGRAPHY

Adams, J., Mottola, M., Bagnall, K. M., & McFadden, K. D. (1982). Total body fat content in a group of professional football players. Canadian Journal of Applied Sports and Sciences, 7, 36-44.

Aloia, J. F., Vaswani, A., Ellis, K., Yuen, K., & Cohn, S. H. (1985). A model for involutional bone loss. The Journal of Laboratory and Clinical Medicine, 106(6), 630-637.

Bakker, H. K., & Struikenkamp, R. S. (1977). Biological variability and lean body mass estimates. Human Biology, 49, 187-202.

Bates, D. V., Macklem, P.T., & Christie, R. V. (1971). Respiratory function in disease. Philadelphia: W. B. Saunders.

Behnke, A. R., & Wilmore, J. H. (1974). Evaluation and regulation of body build and composition. New Jersey: Prentice-Hall.

Behnke, A. R., Feen, B. G., & Welham, W. C. (1942). Specific gravity of healthy men. The Journal of the American Medical Association, 118, 495-498.

Bennett, K. A., & Osborne, R. H. (1986). Interobserver measurement reliability in anthropometry. Human Biology, 58(5), 751-759.

Boyd, R. M., Cameron, E. C., McIntosh, H. W., & Walker, V. R. (1974). Measurement of bone mineral content in vivo using photon absorptiometry. Canadian Medical Association Journal, 7(3), 1201-1205.

Brodie, D. A. (1988a). Techniques of measurement of body composition. Part I. Sports Medicine, 5, 11-40.

Brodie, D. A. (1988b). Techniques of measurement of body composition. Part II. Sports Medicine, 5, 74-98.

Brozek, J., Grande, F., Anderson, J. T., & Keys, A. (1963). Densitometric analysis of body composition: revision of some quantitative assumptions. Annals New York Academy of Sciences, 110, 113-140.

Cohn, S. H., Vaswani, A., Zanzi, I., & Ellis, K. J. (1976). Effect of aging on bone mass in adult women. American Journal of Physiology, 230, 143-148.

Drinkwater, D., Plato, C., Lakatta, L., Goldberg, A., & Andres, R. (1987). Significance of bone mineral content to determination of body density in men over 60 years of age. (Abstract, unpublished).

Durnin, J. V. G. A., & Satwanti. (1982). Variations in the assessment of the fat content of the human body due to experimental technique in measuring body density. Annals of Human Biology, 9(3), 221-225.

Ellis, K. J., & Cohn, S. H. (1975). Correlation between skeletal calcium mass and muscle mass in man. Journal of Applied Physiology, 38(3), 455-460.

Eriksson, S., Isberg, B., & Lindgren, U. (1988). Vertebral bone mineral measurement using dual photon absorptiometry and computed tomography. Acta Radiologica, 29(1), 89-94.

Fidanza, F., Keys, A., & Anderson, J. T. (1953). Density of body fat in man and other animals. Journal of Applied Physiology, 6, 252-256. (Cited in Behnke & Wilmore, 1974).

Forbes, R. M., Cooper, A. R., & Mitchell, H. H. (1953). The composition of the adult human body as determined by chemical analysis. Journal of Biology and Chemistry, 203, 359-366. (Cited in Keys & Brozek, 1953).

Genant, H. K. (1987). Quantitative computed tomography: Update 1987. Calcified Tissue International, 41, 179-186.

Genant, H. K., Block, J. E., Steiger, P., Gluer, C. C., & Smith, R. (1987). Quantitative computed tomography in assessment of osteoporosis. Seminars in Nuclear Medicine, 17(4), 316-333.

Geusens, P., Dequeker, J., Verstraeten, A., & Nijs, J. (1986). Age -, sex -, and menopause - related changes of vetebral and peripheral bone: population study using dual and single photon absorptiometry and radiogrammetry. The Journal of Nuclear Medicine, 27(10), 1540-1549.

Gluer, C. C., Steiger, P., & Genant, H. K. (1988). Validity of dual-photon absorptiometry. Radiology, 166, 574-575.

Goodwin, P. N. (1987). Methodologies for the measurement of bone density and their precision and accuracy. Seminars in Nuclear Medicine, 17(4), 293-304.

Gorsuch, R. L. (1983) Factor analysis. Hillsdale: Lawrence Erlbaum Associates.

Gotfredsen, A., Podenphant, J., Norgaard, H., Nilas, L., Nielsen, V. H., & Christiansen, C. (1988). Accuracy of lumbar spine bone mineral content by dual photon absorptiometry. The Journal of Nuclear Medicine, 29(2), 248-254.

Gotfredsen, A., Hadberg, A., Nilas, L., & Christiansen, C. (1987). Total body bone mineral in healthy adults. Journal of Laboratory and Clinical Medicine, 110(3), 362-368.

Hansson, T., & Roos, B. (1986). Age changes in the bone mineral of the lumbar spine in normal women. Calcified Tissue International, 38, 249-251.

Harrison, J. E., McNeill, K. G., Hitchman, A. J., & Britt, B. A. (1979). Bone mineral measurements of the central skeleton by in vivo neutron activation analysis for routine investigation of osteopenia. Investigative Radiology, 14, 27-34.

Jackson, A. S., & Pollock, M. L. (1985). Practical assessment of body composition. The Physician and Sports Medicine, 13(5), 76-90.

Jackson, A. S. (1984). Research design and analysis of data procedures for predicting body density. Medicine and Science in Sports and Exercise, 16(6), 616-620.

Jackson, A. S., Pollock, M. L., & Ward, A. (1980). Generalized equations for predicting body density of women. Medicine and Science in Sports and Exercise, 12(3), 175-182.

Kaplan, F. S. (1987). Osteoporosis. Clinical Symposia, 4, 1-32.

Keys, A., & Brozek, J. (1953). Body fat in adult man. Physiological Reviews, 33(3), 245-325.

Krolner, B. (1982). Osteoporosis and normality: how to express the bone mineral content of lumbar vertebrae. Clinical Physiology, 2, 139-146.

Lesnick Smith, L. (1990). Precision of dual photon absorptiometry. (Unpublished).

Lindquist, O., Bengtsson, C., Hansson, T., & Roos, B. (1981). Bone mineral content in relation to age and menopause in middle aged women. Scandinavian Journal of Clinical and Laboratory Investigation, 41, 215-223.

Lohman, T. G., Roche, A. F., & Martorell, R. (1988). Anthropometric standardization reference manual. Champaign: Human Kinetics.

Lohman, T. G. (1984). Research progress in validation of laboratory methods of assessing body composition. Medicine and Science in Sports and Exercise, 16(6), 596-603.

Lohman, T. G., Slaughter, M. H., Boileau, R. A., Bunt, J., & Lussier, L. (1984). Bone mineral measurements and their relation to body density in children, youth and adults. Human Biology, 56(4), 667-679.

Lukaski, H. C. (1987). Methods for the assessment of human body composition: traditional and new. American Journal of Clinical Nutrition, 46, 537-556.

Martin, A. D., & Drinkwater, D. T. (1990). Variability in the measures of body fat: assumptions or technique? (Unpublished).

Martin, A. D. (1984). An anatomical basis for assessing human body composition: evidence from 25 dissections. Ph.D. thesis, Simon Fraser University, Burnaby.

Mazess, R. B., Barden, H. C., & Ettinger, M. (1988). Radial and spinal bone mineral density in a patient population. Arthritis and Rheumatism, 31(7), 891-897.

Mazess, R. B., Barden, H. S., Ettinger, M., Johnston, C., Dawson-Hughes, B., Baron, D., Powell, M., & Notelovitz, M. (1987). Spine and femur density using dual-photon absorptiometry in U.S. white women. Bone and Mineral, 2, 211-219.

Mazess, R. B., & Christiansen, C. (1982). A comparison of bone mineral results from Denmark and the U.S. Human Biology, 54(2), 343-354.

Mazess, R. B., Peppler, W. W., Harrison, J. E., & McNeill, K. G. (1981). Total body bone mineral and lean body mass by dual photon absorptiometry. III. Comparison with trunk calcium by neutron activation analysis. Calcified Tissue International, 33, 365-368.

Mendez, J., & Lukaski, C. (1981). Variability of body density in ambulatory subjects measured at different days. American Journal of Clinical Nutrition, 34, 78-81.

Mitchell, H. H., Hamilton, T.S., Steggerda, F. R., & Bean, H. W. (1945). Chemical composition of the adult human body and its bearing on the biochemistry of growth. Journal of Biology and Chemistry, 158, 625-637. (Cited in Keys & Brozek, 1953).

Murby, B., & Fogelman, I. (1987). Bone mineral measurements in clinical practice. British Journal of Hospital Medicine, 37(5), 453-458.

Nelson, M. E., & Evans, W. J. (1987). H.W. in amenorrheic women [Letter to the Editor]. Medicine and Science in Sports and Exercise, 19(6), 621.

Nilas, L., Gotfredsen, A., Hadberg A., & Christiansen, C. (1988). Age-related bone loss in women evaluated by the single and dual photon technique. Bone and Mineral, 4, 95-103.

Nilas, L., Gotfredsen, A., & Christiansen, C. (1986). Total and local bone mass before and after normalization for indices of bone and body size. Scandinavian Journal of Clinical and Laboratory Investigation, 46, 53-57.

Nordin, B. F. C., Chatterton, B. E., Steuer, T. A., & Walker, C. J. (1985). Forearm bone mineral content does not decline with age in premenopausal women. Clinical Orthopaedics and Related Research, 211, 252-256.

Oppliger, R. A., Looney, M. A., & Tipton, C. M. (1987). Reliability of hydrostatic weighing and skinfold measurements of body composition using a generalization study. Human Biology, 59(1), 77-96.

Ott, S. M., Kilcoyne, R. F., & Chesnut III, C. H. (1988). Comparisons among methods of measuring bone mass and relationship to severity of vertibral fractures in osteoporosis Journal of Clinical Endocrinology and Metabolism, 66(3), 501-507.

Ott, S. M., Murano, R., Zewellen, T. K., Nelp, W. B., & Chestnut III, C. H. (1983). Total body calcium by neutron activation analysis in normals and osteoporotic populations: a discrimination of significant bone mass loss. Journal of Laboratory and Clinical Medicine, 102(4), 637-645.

Pollock, M. L., & Jackson, A. S. (1984). Research progress in validation of clinical methods of assessing body composition. Medicine and Science in Sports and Exercise, 16(6), 606-613.

Pollock, M. L., Gettman, L. R., Jackson, A., Ayres, J., Ward, A., & Linnerud, A. C. (1977). Body composition of elite distance runners. New York Academy of Sciences, 301, 361-370.

Rathbun, E. N., & Pace, N. (1945). Studies on body composition: I. Determination of body fat by means of body specific gravity. Journal of Biology and Chemistry, 158, 667-676. (Cited in Keys & Brozek, 1953).

Riggs, B. L., Wahner, H. W., Seeman, E., Offord, K. P., & Dunn, W. L. (1982). Changes in bone mineral density of the proximal femur and spine with aging. Journal of Clinical Investigation, 70, 716-723.

Riggs, B. L., Wahner, H. W., Dunn, W. L., Mazess, R. B., & Offord, K. P. (1980). Differential changes in bone mineral density of the appendicular and axial skeleton with aging. Journal of Clinical Investigation, 67, 328-335.

Roche, A. F. (1987). Some aspects of the criterion methods for the measurement of body composition. Human Biology, 59(2), 209-220.

Sandborn, C. F., & Wagner, W. W. (1987). H.W. in amenorrheic women. [Letter to the editor]. Medicine and Science in Sports and Exercise, 19(6), 621-622.

Schutte, I. E., Townsend, E. J., Hugg, J., Stoup, R. F., Malina, R. M., & Blomquist, C. G. (1984). Density of lean body mass is greater in blacks than in whites. Journal of Applied Physiology, 56(6), 1647-1649.

Seidell, J. C., Deurenberg, P., & Hautvast, J. G. A. J. (1987). Obesity and fat distribution in relation to health - current insights and recommendations. World Review of Nutrition and Dietetics, 50, 57-91.

Seldin, D. W., Esser, P. D., & Alderson, P. O. (1988). Comparison of bone density measurements from different skeletal sites. The Journal of Nuclear Medicine, 29(2), 168-173.

Siri, W. E. (1956). The gross composition of the body. Advances in Biological and Medical Physics. New York: Academic Press. (Cited in Brodie, 1988).

Tothill, P., Smith, M. A., & Sutton, D. (1983). Dual photon absorptiometry of the spine with a low activity source of gadolinium 153. The British Journal of Radiology, 56, 829-835.

Wahner, H. W., Dunn, W. L., & Riggs, B. L. (1984). Assessment of bone mineral. Part 2. Adjunctive Medical Knowledge, 25(11), 1241-1253.

Wahner, H. W., Dunn, W. L., & Riggs, B. L (1983). Noninvasive bone mineral measurements. Seminars in Nuclear Medicine, 17(3), 282-289.

Wasserman, S. H. S., & Barzel, U. S. (1987). Osteoporosis: The State of the Art in 1987: A review. Seminars in Nuclear Medicine, 17(4), 283-292.

Werdein, F. J., & Kyle, L. H. (1960). Estimation of the constancy of density of the fat free body. Journal of Clinical Investigation, 39, 626-629.

Widdowson, E. M., McCance, R. A., & Spray, C. M. (1951). Chemical composition of the human body. Clinical Science, 10, 113-125. (Cited in Keys & Brozek, 1953).

Willmon, T. L., & Behnke, A. R. (1948). Residual lung volume determinations by the methods of helium substitution and volume expansion. American Journal of Physiology, 138-142.

Wilmore, J. H. (1983). Body composition in sport and exercise: directions for future research. Medicine and Science in Sports and Exercise, 15(1), 21-31.

Wilmore, J. H., & Pollock, M. L. (1974). Body composition and physiological characteristics of active endurance athletes in their eighth decade of life. Medicine and Science in Sports, 6(1), 44-48.

Yasumura, S., Aloia, J. F., Gundberg, C. M., Yeh, J., Vaswani, A. N., Yuen, K., LaMonte, A. F., Ellis, K. J., & Cohn, S. H. (1987). Serum osteocalcin and total body calcium in normal pre and postmenopausal women and postmenopausal osteoporotic patients. Journal of Clinical Endocrinology and Metabolism, 64(4), 681-685.

Appendix A

Numerical Estimation of Fat from Density

Numerical Estimation of Fat

From Density

(Deduced from Keys and Brozek, 1953)

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}} = \frac{g}{ml}$$

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

Two Component Model

Fat	Fat Free
mf	mff
df	dff

In a two component system of:

1) different densities df (density of fat)
+ dff (density of fat free)

2. different masses mf (mass of fat)
+ mff (mass of fat free)

A. the total mass is $M = mf + mff$

B. the total density if $D = df + dff$

C. the volume is $V = \frac{M}{D} = \frac{mf}{df} + \frac{mff}{dff}$

$$= \frac{mff}{dff} + \frac{mf}{df}$$

A) Rearranging C we get a new equation for D.

$$\boxed{D = \frac{mff + mf}{\frac{mff}{dff} + \frac{mf}{df}} = \frac{M}{V}}$$

B) If the total mass is 1 = mf + mff then mff = 1 - mf

then by substituting in the above equation we get

$$D = \frac{(1 - mf) + mf}{\frac{(1 - mf)}{dff} + \frac{mf}{df}}$$

$$= \frac{1}{\frac{(1 - mf)}{dff} + \frac{mf}{df}} \quad * \text{ (see below)}$$

$$\boxed{\frac{1}{D} = \frac{(1 - mf)}{dff} + \frac{mf}{df}}$$

*Let $\frac{(1 - mf)}{dff} + \frac{mf}{df} = X$ $\qquad D = \frac{1}{X}$

$X.D = 1$ $\qquad X = \frac{1}{D}$

C) From the previous formula we can rearrange as follows, to estimate the proportional mass of the fat component - mf.

$$\frac{1 - mf}{dff} + \frac{mf}{df} = \frac{1}{D}$$

$$\frac{1}{dff} - \frac{mf}{dff} + \frac{mf}{df} = \frac{1}{D}$$

$$\frac{mf}{df} - \frac{mf}{dff} = \frac{1}{D} - \frac{1}{dff}$$

$$mf \left(\frac{1}{df} - \frac{1}{dff}\right) = \frac{1}{D} - \frac{1}{dff}$$

$$\frac{mf \left(\frac{1}{df} - \frac{1}{dff}\right)}{\left(\frac{1}{df} - \frac{1}{dff}\right)} = \frac{\frac{1}{D} - \frac{1}{dff}}{\left(\frac{1}{df} - \frac{1}{dff}\right)}$$

$$mf = \frac{dff \left(\frac{1}{D} - \frac{1}{dff}\right)}{dff \left(\frac{1}{df} - \frac{1}{dff}\right)}$$

$$\boxed{mf = \frac{\frac{dff}{D} - 1}{\frac{dff}{df} - 1}}$$

) The previous formula can also be written as follows:

$$mf = \frac{\frac{dff}{D} - \frac{D}{D}}{\frac{dff}{df} - \frac{df}{df}} = \frac{\frac{dff - 1}{D}}{\frac{dff - df}{df}} = \frac{\frac{A}{B}}{\frac{C}{D}}$$

$$= \frac{\frac{A}{B} \times \frac{D}{C}}{\frac{C}{D} \times \frac{D}{C}} = \frac{AD}{BC}$$

$$mf = \left(\frac{dff - 1}{D}\right)\left(\frac{df}{dff - df}\right)$$

$$= \frac{1}{D}\ (dff - 1)\ \left(\frac{df}{dff - df}\right)$$

$$= \frac{1}{D}\left(\frac{dff.df - df}{dff - df}\right)$$

$$= \frac{1}{D}\left(\frac{dff.df}{dff-df}\right) - \left(\frac{df}{dff - df}\right)$$

$$\boxed{mf = \frac{\frac{dff.df}{dff-df}}{D} - \frac{df}{dff-df}}$$

E) As noted, the fraction of fat in the whole body is given by

$$mf = \frac{\frac{dff \cdot df}{dff - df}}{D} - \frac{df}{dff - df} = \frac{a}{D} - b$$

If we assume that

$$dff = 1.10 \quad \text{and} \quad df = 0.90$$

We get the following values for a + b:

$$mf = \frac{\frac{(1.1 \text{ x } .9)}{(1.1 - .9)}}{D} - \frac{.9}{(1.1 - .9)}$$

$$= \frac{\frac{.99}{.2}}{D} - \frac{.9}{.2}$$

$$mf = \frac{4.95}{D} - 4.50$$

F) From the above, percentage fat would be:

$$\% \, mf = 100 \left(\frac{4.95}{D} - 4.50\right) = \frac{495}{D} - 450$$

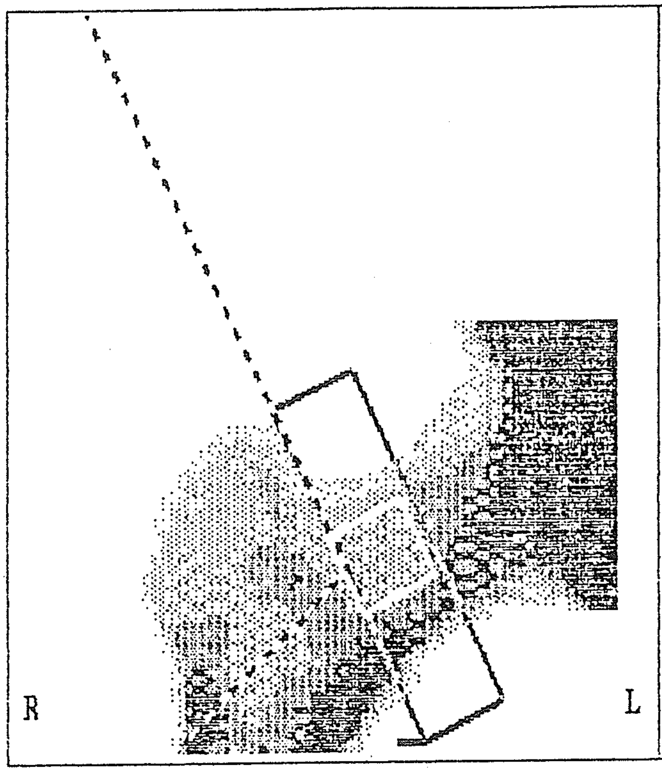
Appendix B

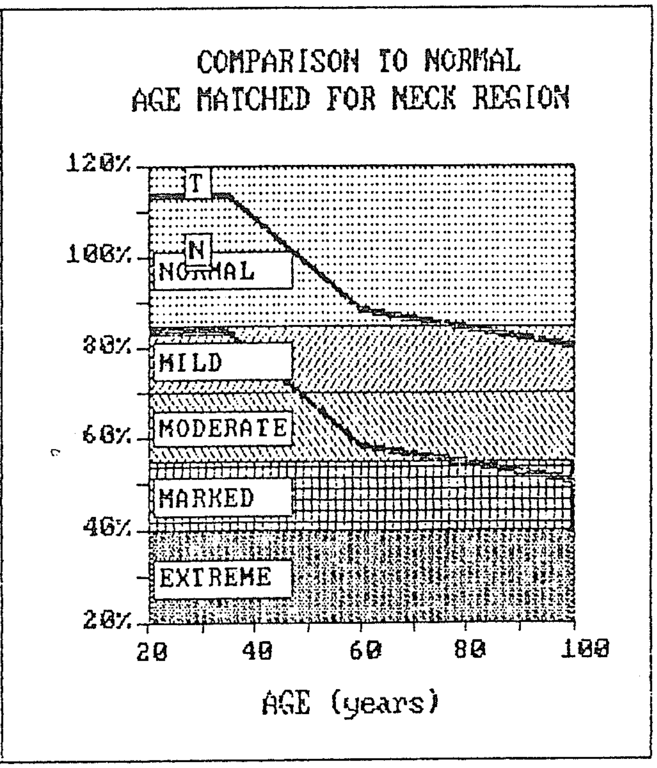
Example of DPA Scan Results

DP3 SPINE/FEMUR SCAN RESULTS
ST. BONIFACE GENERAL HOSPITAL

409 Tache Avenue, Winnipeg, Manitoba, Canada

ID:		SCAN:	2.2 3/18/89
NAME:		ANALYSIS:	2.2 03/18/89





Age (years).........	29	Large Standard......	18.47	Scan Speed (mm/s)...	2.5
Sex.................	Female	Medium Standard.....	13.76	Step Distance (mm)..	2.5
Weight (Kg).........	53.6	Small Standard......	9.81	Collimation (mm)....	13
Height (cm).........	167	Corrected R value...	1.42	Region height (cm)..	6.00
Ethnic..............	White	44 KeV Air Value....	58319	Region width (cm)...	1.50
Side................	Right	100 Kev air value...	48099	Region angle (deg)..	64

Femoral neck : BMC (grams) = 4.65 AREA (cm^2) = 4.58
Ward's Triangle : BMC (grams) = 1.85 AREA (cm^2) = 1.98
Trochanteric : BMC (grams) = 10.32 AREA (cm^2) = 10.50

REGION	BMD g/cm^2	% Young Normal	% Age* Matched	Fracture Risk
FEMORAL NECK	1.01	101.4	102.9	NORMAL
WARD'S TRIANGLE	0.94	99.8	101.3	NORMAL
TROCHANTERIC	0.98	121.4	123.2	NORMAL

* Age matched adjusted for Sex, Age, Ethnic, Weight.

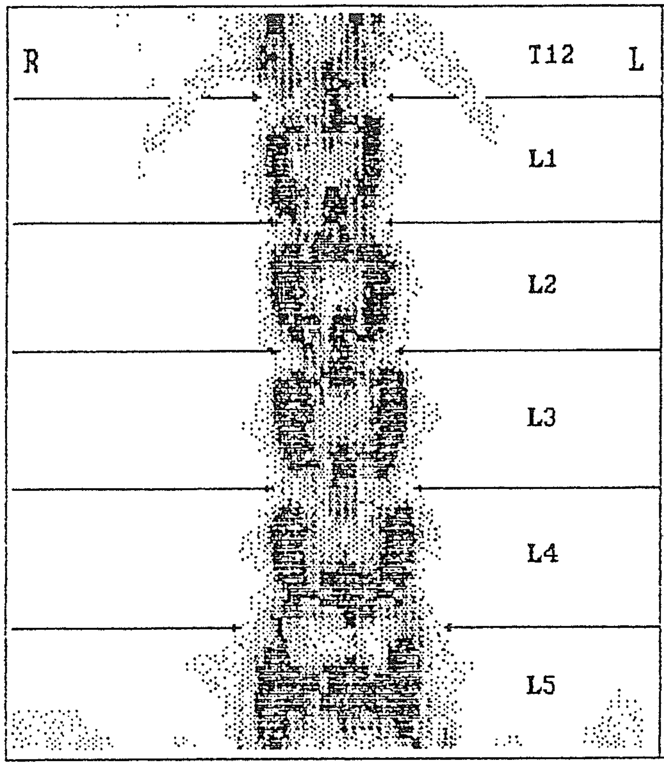
LUNAR

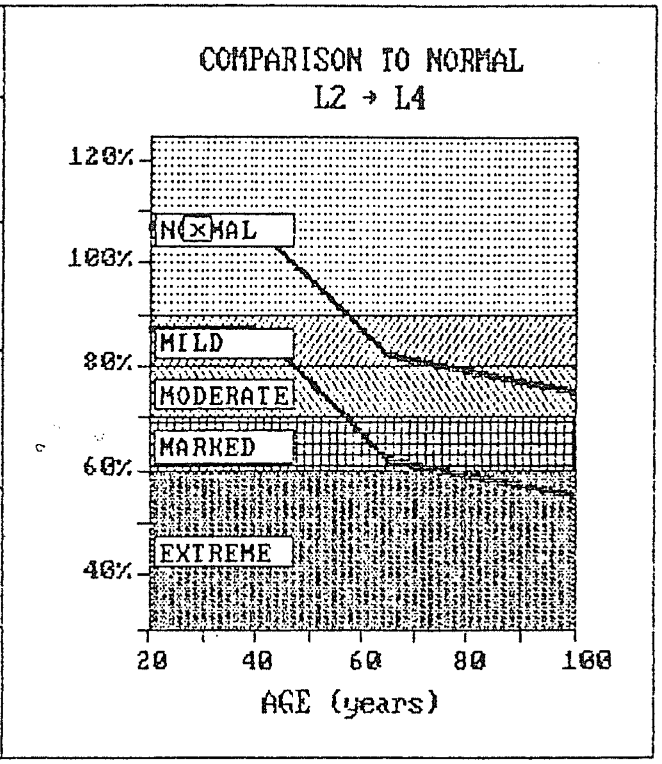
DP3 SPINE/FEMUR SCAN RESULTS
ST. BONIFACE GENERAL HOSPITAL

409 Tache Avenue, Winnipeg, Manitoba, Canada

ID:
NAME:

SCAN: 2.2 3/18/89
ANALYSIS: 2.2 3/18/89





Age (years)........	29	Large Standard......	18.47	Scan Speed (mm/s)...	2.5
Sex................	Female	Medium Standard.....	13.76	Step Distance (mm)..	4.5
Weight (Kg)........	53.6	Small Standard......	9.81	Collimation (mm)....	13
Height (cm)........	167	44 KeV Air Value....	58319	Corrected R value...	1.45
Ethnic.............	White	100 KeV Air Value...	48099		

REGION	BMD g/cm²	% Young Normal	% Age* Matched	Fracture Risk
L1	1.260	106.9	110.4	NORMAL
L2	1.376	109.4	112.7	NORMAL
L3	1.342	106.7	109.9	NORMAL
L4	1.305	103.8	106.9	NORMAL
L1 -> L2	1.319	109.2	112.6	NORMAL
L1 -> L3	1.328	108.1	111.5	NORMAL
L1 -> L4	1.321	106.7	110.0	NORMAL
L2 -> L3	1.358	107.9	111.2	NORMAL
L2 -> L4	1.338	106.3	109.6	NORMAL
L3 -> L4	1.322	105.1	108.3	NORMAL

---

* Age matched adjusted for sex, age, ethnic, weight.

DP3 SPINE/FEMUR SCAN RESULTS
ST. BONIFACE GENERAL HOSPITAL

409 Tache Avenue, Winnipeg, Manitoba, Canada

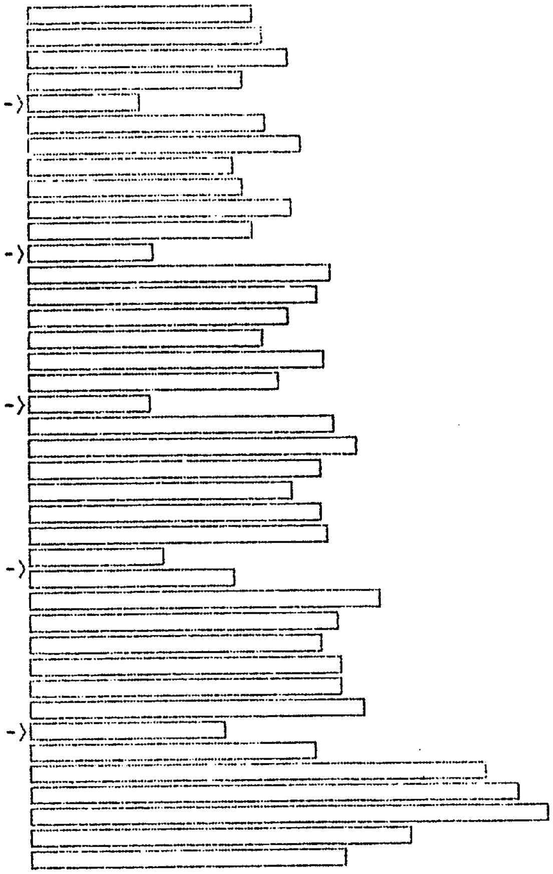
ANCILLARY SPINE INFORMATION FOR

SCAN ON: 2.2 3/18/89 ANALYSIS ON: 2.2 3/18/89

Region of Interest	BMC (grams)	Area (cm²)	Width (cm)	BMC/W (g/cm)	Central Density (g/cm²)	Corpus Density (mg/cm³)	Trabecular Density (mg/cm³)
L1	13.76	10.92	3.55	3.88	1.17	276	206
L2	15.73	11.43	3.76	4.18	1.26	288	236
L3	17.74	13.22	4.12	4.31	1.27	261	227
L4	19.82	15.18	4.51	4.39	1.27	221	218
L1 -> L2	29.49	22.36	3.58	8.25	1.21	283	221
L1 -> L3	47.23	35.58	3.70	12.76	1.23	275	224
L1 -> L4	67.05	50.76	3.86	17.36	1.24	258	222
L2 -> L3	33.48	24.65	3.86	8.68	1.26	273	231
L2 -> L4	53.29	39.83	4.01	13.29	1.26	253	226
L3 -> L4	37.56	28.40	4.20	8.94	1.27	239	222

Line #	BM	Base	Edge1	Edge2
40	17.53	4.43	50	86
39	18.31	4.43	51	89
38	20.44	4.43	51	83
37	16.94	4.43	57	84
36	8.84	4.44	58	84
35	18.65	4.44	59	85
34	21.45	4.44	56	86
33	16.06	4.44	56	87
32	16.77	4.44	55	87
31	20.53	4.44	56	85
30	17.56	4.44	57	85
29	9.93	4.43	62	84
28	23.59	4.42	59	88
27	22.70	4.42	57	90
26	20.44	4.42	57	90
25	18.35	4.42	58	89
24	23.10	4.40	58	88
23	19.61	4.38	58	88
22	9.71	4.40	62	86
21	23.78	4.41	60	91
20	25.68	4.42	58	92
19	23.00	4.43	57	95
18	20.71	4.41	57	92
17	23.02	4.41	59	91
16	23.38	4.42	59	92
15	10.69	4.43	62	88
14	16.06	4.42	60	92
13	27.45	4.42	57	95
12	24.06	4.42	55	96
11	22.82	4.41	57	94
10	24.51	4.40	59	92
9	24.36	4.40	59	92
8	26.25	4.40	55	95
7	15.32	4.40	54	97
6	22.39	4.38	51	98
5	35.34	4.38	47	101
4	37.98	4.37	48	97
3	40.04	4.40	50	100
2	29.70	4.44	50	101
1	24.66	4.45	51	101

Histogram



Appendix C

Example of Self Report Subject Questionnaire

The University of Manitoba

SUBJECT STATUS QUESTIONNAIRE

Bone Density and Fat Estimation Study in Young Women

DATE: ________ / ________ / ________
day month year

NAME: ______________________ SIGNATURE: ______________________

DATE OF BIRTH: ________ / ________ / ________ AGE: __________

Please answer the following questions on your general health, medical history and menstrual status.

Please circle the correct response YES or NO (Y or N) and fill in the blanks where appropriate.

A. GENERAL HEALTH AND MEDICAL HISTORY

1. Have you ever been told by your doctor that you have heart trouble ? Y N

   If so, what type ? ______________________________

2. Do you have high blood pressure ? Y N

3. Do you often feel faint or have spells of severe dizziness ? Y N

4. Are you afraid of submersion under water ? Y N

5. Do you have any respiratory disorders such as asthma or difficulty with breathing ? Y N

6. Do you smoke ? Y N

   If no, have you ever smoked ? Y N

   If so, for how many years ? ______________________________

   What year did you quit ? ______________________________

7. Do you take any medications on a regular basis, such as the following drugs, which may affect calcium metabolism ?

a) anticonvulsants Y N

b) corticosteroids Y N

c) estrogens (other than birth control pills) Y N

d) other medications Y N

If so, what type ? ____

8. Have you had or do you now have any of the following diseases ?

a) diabetes Y N

b) alcoholism Y N

c) osteoporosis or other bone disease Y N

d) renal disease Y N

9. Do you have limb or joint disorders ? Y N

If so, what type ? ____

10. Have you recently been immobilized (prolonged bed rest) for a month or more ? Y N

11. Do you have any chronic disorders which might worsen with the testing to be conducted in this study ? Y N

If so, what type ? ____

B. MENSTRUAL STATUS

1. Are you pregnant ? Y N

2. Are you post menopausal ? Y N

3. Have you had a hysterectomy ? Y N

....Thanks...

Appendix D

Example of Description of Research Project Information to Participant and Consent Form

The University of Manitoba

Description of Research Project

Information to Participant and Consent Form

The effect of bone density on the
estimation of body fat in young women

Investigator: A. M. Jacobsen

Introduction

The determination of body composition is an important aspect of adult fitness and health and accurate measurements of body fat are needed to develop sound weight reduction and exercise programs. Despite the widespread use of many techniques none of the methods for fat estimation have been validated in humans. Since the only truly direct method of determining body fat is by dissection and weighing of fat, all methods of fat prediction in humans must be indirect.

Hydrostatic (or underwater) weighing (HW) is considered to be the "criterion" method of fat determination. It has proven to be reliable over forty years of use and uses Archimedes' principle of water displacement to determine the volume of the body. Using body weight as mass and the volume measurement, whole body density is determined from the formula $\text{density} = \frac{\text{mass}}{\text{volume}}$.

This relationship is also used to derive percent fat from whole body density. The body is divided into a two component model consisting of the fat portion and the fat free portion. These components are further subdivided into their relative masses and densities and rearranged mathematically to isolate fat as as percentage of the total mass of the body.

However, to get percent fat from whole body density important assumptions have to be made - the principal ones being that the density of the fat and fat free mass are known and have constant values. For the latter to be true it is also necessary to assume that all individuals have the same proportions of muscle, bone and water. Given the normal human biological variability, the above assumptions are unlikely.

Several researchers have reported problems with the use of body density to determine fat and have found extremely low values and even "negative" fat in lean, highly muscular individuals. These values must be impossible since an essential amount of fat is needed to sustain life and the measured subjects showed obvious skinfold fat. In general, it appears that the density of the fat free component is not necessarily constant and may vary according to age, sex and race. The largest source of variation is probably bone density. However, the extent of the effect of variation in bone density on percent fat by HW is unclear.

Purpose of the Study

The purpose of this study is to assess the variability in bone mineral density in young women and to determine the effect of this variability on whole body density and body fat as estimated by hydrostatic weighing. This study is part of a larger study which will investigate young, middle aged and old, adult males and females as well as very lean, muscular, young adults, with the intention to devise a model of fat estimation which incorporates bone mineral density.

Methods

Thirty normal female women aged 20-45 years will be recruited from the local community for this study.

The following measurements will be made on all participants. With the exception of bone density measurements which will be done at St. Boniface Hospital, all measurements will be made at the Exercise Physiology Laboratory, Max Bell Centre, Fort Garry Campus, University of Manitoba.

1. Anthropometry

Height, weight, several skinfold measurements, girths, and breadths, will be done. These measurements will allow us to estimate the volumes of fat, muscle and bone in your body. Measurements will be made in triplicate and size-adjusted sum of skinfolds will be used as an index of fatness. The procedure will take about 1 hour. Subjects should wear a 2 piece bathing suit or loose fitting running shorts and T-shirt or halter top.

2. Residual Lung Volume Measurement

During the underwater weighing procedure you will be asked to "blow out" as much air as you can, that is, exhale maximally. After you've done this, some air will remain in your lungs and we need to determine how much. To do this, we will ask you to breathe into a spirometer. A small known quantity of helium will be added to the air in the spirometer. You will then be asked to breathe in as much as possible (maximal inhalation) then breathe out as much as possible (maximal exhalation) 2 or 3 times. This procedure will take about 15 minutes and will be done just prior to the underwater-weighing procedure.

3. Underwater Weighing Procedure

To determine your % fat we must first determine your body density. To do this, you will change into a swim suit, then be weighed. Next, you will then be asked to climb a ladder to the top of a tank filled with warm water, have a harness placed around your chest, then climb into the tank and stand with the water up to your neck. The harness is attached to an overhead weight scale. You will then be asked to exhale maximally, lean forward and lift your legs such that you are suspended in the tank with your head completely under water. You will need to stay in this position for about 10 seconds wile your "under-water" weight is measured. This procedure will be repeated 5 to 7 times. Participants should wear a tight-fitting SPEEDO style spandex swim suit. Including changing into your swim suit and getting re-dressed, this procedure will take about 30 minutes.

4. Bone Density Measurements

Bone density measurements will be made in the department of Nuclear Medicine at St. Boniface Hospital. This procedure requires that you be exposed to a small amount of radiation (less than a chest x-ray). Measurements will be made of the vertebrae in your lower back (vertebrae L1-L4), and of the hip region (head and neck of the femur) on your right side. These bone density measurements will allow us to estimate the bone mineral content of your body and allow us to adjust results from the underwater-weighing procedure to get a more accurate estimate of your % fat. For the spine scan you will be asked to lie on your back with your legs raised on a support; this procedure will take about 20 minutes. For the hip scan, you will also lie on your back with your right foot in a support; this procedure takes about 30 minutes. Wear loose fitting, comfortable clothing without metal buttons or zippers.

5. Questionnaire

A brief medical history, general health, and physical activity questionnaire will also be administered to all participants to allow us screen for possible risks and assess bone normality.

Repeat Measurements

To assess and assume methodological reliability in our study, we will be asking a randomly selected subset of participants to undergo a repeat series of measurements. Thus you should consider that you may be asked to undergo a second set of measurements.

Risks

It is highly unlikely that injury or illness will result from the assessments outlined. All tests will be conducted by qualified individuals. Potential risks could be unknown effects due to gamma radiation exposure during the density measurements, and any abnormality you might have which would cause loss of consciousness under water during hydrostatic weighing. Others could be dizziness, fainting, nausea or chest discomfort. Should you feel any discomfort or pain during the tests you must immediately inform the tester so the test can be stopped.

CONSENT FORM

Bone Density and Fat Estimation Study in Young Women

I have read the description of the study and understand the measurement procedures involved.

I also understand that my participation in this study is voluntary and that I may withdraw from it at any time without prejudice.

All information will be kept confidential.

____________________ Date

__________________________________ Participant

__________________________________ Parent or Guardian

(required if participant is less than 18 years old)

__________________________________ Witness

Appendix E

Example of Residual Lung Volume Worksheet

University of Manitoba

RESIDUAL LUNG VOLUME WORKSHEET

Bone Density and Fat Estimation Study in Young Women

NAME: ______________________________ DATE: ______ / ______ / ______

AGE: ______________ SEX: ____________ MEASURER: ________________

VC, Vital Capacity (L)	______________	______________
	______________	______________

RV, Residual Lung Volume (L) Using Age Factor x VC

Age 16 - 34, RV = 0.25 x VC = 0.25 x __________ = __________

Age 35 - 49, RV = 0.305 x VC = 0.305 x __________ = __________

Using Helium Dilution

RVATPS Residual Lung Volume Ambient Temperature Pressure Saturated (L)

Hi, Initial Fraction of He		__________	__________	__________
Vi, Initial Volume of Bag (L)		__________	__________	__________
Hf, Final Fraction of He	p	__________	__________	__________
	3	__________	__________	__________
	2	__________	__________	__________
	1	__________	__________	__________
	0	__________	__________	__________
		__________	__________	__________
DS, Dead Space (L)		__________	__________	__________

Name: ____________________________________ DATE: ______/______/______

$$RVATPS = \frac{Hi \times Vi}{Hf} - Vi - DS$$

1. RVATPS = $\frac{\qquad \times \qquad}{\rule{4cm}{0.4pt}}$ - __________ - ________ = __________

2. RVATPS = $\frac{\qquad \times \qquad}{\rule{4cm}{0.4pt}}$ - __________ - ________ = __________

3. RVATPS = $\frac{\qquad \times \qquad}{\rule{4cm}{0.4pt}}$ - __________ - ________ = __________

Correction to Residual Volume Body Temperature Pressure Saturated (RVBTPS)

TS, Temperature of Spirometer (C) ______________________

P, Room Barometric Pressure (mmHg) ______________________

PH20, Pressure of Water at Room Temperature (mmHg) ______________________

Temp	PH20
18	15.477
19	16.477
20	17.535
21	18.650
22	19.827
23	21.068
24	22.377

$$RVBTPS\ (L) = RVATPS \left(\frac{310}{273 + TS}\right) \left(\frac{P - PH20}{P - 47}\right)$$

$$= \rule{3cm}{0.4pt} \left(\frac{\qquad}{\qquad + \qquad}\right) \left(\frac{\qquad - \qquad}{\qquad - \qquad}\right)$$

$$= \rule{3cm}{0.4pt} \left(\rule{4cm}{0.4pt}\right) \left(\rule{4cm}{0.4pt}\right)$$

$$= \rule{3cm}{0.4pt}$$

Appendix F

Example of Underwater Weighing Worksheet

The University of Manitoba

UNDERWATER WEIGHING WORKSHEET

Bone Density and Fat Estimation Study in Young Women

NAME: ________________________________ DATE: ______ / ______ / ______

BIRTH DATE: ______ / ______ / ______ MEASURER: ____________________

WA, Weight in Air (kg)		
WW, Weight in Water (kg)		
WH, Wet Wt. of Harness (kg)		
RV, Residual Lung Vol. (L)		
Water Temperature (C)	36C = 0.994	

DW, Density of Water (kg/L)

25 - 27C = 0.997	34 - 36C = 0.994
28 - 30C = 0.996	37 - 39C = 0.993
31 - 33C = 0.995	40 - 42C = 0.992

IG, Intestinal Gas (L) constant = 0.1

D, Body Density (kg/L) =

$$\frac{WA}{\frac{(WA - WW + WH)}{DW} - (RV + IG)} = \frac{\qquad\qquad}{\frac{(\qquad - \qquad + \qquad)}{\qquad} - (\qquad + .1)}$$

$$= \underline{\qquad\qquad} = \underline{\qquad\qquad}$$

Body Fat (%) =

$$\frac{495}{D} - 450 = \frac{495}{\underline{\qquad}} - 450 = \underline{\qquad\qquad}$$

Appendix G

Example of Anthropometric Worksheet

Sport & Exercise Sciences Research Institute
University of Manitoba

Anthropometric Proforma

Name____ Sex - M / F
(last) (first & initial) (circle one)

Birth Date ____/______/____ Measurement Date ____/______/____
(day / month / year) (day / month / year)

Body Size:

Height [stature] (cm)	______•__	______•__	______•__	______•__
Weight (kg)	______•__	______•__	______•__	______•__

Skinfolds (mm):

triceps	______•__	______•__	______•__	______•__
subscapular	______•__	______•__	______•__	______•__
iliac crest	______•__	______•__	______•__	______•__
abdominal (umbilical)	______•__	______•__	______•__	______•__
front thigh	______•__	______•__	______•__	______•__
medial calf	______•__	______•__	______•__	______•__

Girths (cm):

arm |_____._|_____._|_____._|_____._|

forearm |_____._|_____._|_____._|_____._|

wrist |_____._|_____._|_____._|_____._|

chest |_____._|_____._|_____._|_____._|

waist |_____._|_____._|_____._|_____._|

abdominal (umbilical) |_____._|_____._|_____._|_____._|

gluteal |_____._|_____._|_____._|_____._|

upper thigh |_____._|_____._|_____._|_____._|

mid thigh |_____._|_____._|_____._|_____._|

calf |_____._|_____._|_____._|_____._|

head |_____._|_____._|_____._|_____._|

neck |_____._|_____._|_____._|_____._|

Breadths (cm):

humerus |_____._|_____._|_____._|_____._|

wrist |_____._|_____._|_____._|_____._|

femur |_____._|_____._|_____._|_____._|

ankle |_____._|_____._|_____._|_____._|

Appendix H

Example of Helium Dilution Procedure for Subjects

Helium Dilution Procedure for Subjects

Preliminaries

Introduce yourself. On the worksheet, record the subject's name, age, and sex, the date, and your name. Find out if the subject has a background in physiology and has taken this test before. Give an overview of the procedures and explain TV, VC, RV, and TLC (lung volumes) by means of a simple diagram.

Vital Capacity

Using a separate spirometer, take 3 measurements of the subject's vital capacity. Explain briefly how the spirometer works. The subject should be seated with nose clips on. Ask the subject to inspire as deeply as possible and immediately afterwards to expire forcefully and for as long as possible into the spirometer hose. Encourage the subject to make a maximum effort. Record 3 trials. Do more trials if the subject is still improving. Calculate the RV using the age factor times the VC from the highest trial.

Helium Dilution Dry Run

With the He dilution apparatus,take the subject through a dry run first. The subject's individual VC volume is used to determine how much air to put in the large bag. For most female subjects, 3 L is adequate. However, if the VC is 3L or less, use 2.5L and if the VC is 4.5L or more use 3.5L. These are not absolute values. After the dry run, add or subtract .1 or .2L or more as necessary for the first trial. The subject should have enough air to complete the procedure comfortably, yet be able to take in the whole contents of the large bag with each breath.

For the dry run, the subject goes through the whole procedure with only room air. Allow the subject to try the mouthpiece and nose clips. Explain that when the He is added to the large bag there will be no bad taste, smell, or side effects. They will not sound like Donald Duck when it is over. Explain that they will be consuming the oxygen in the large bag as they rebreathe the air and He mixture.

Explain briefly how He is used to calculate RV. The subject will be connected to the bag via the mouthpiece. She will be asked to exhale to RV and then to breathe the contents of the bag in and out 8-10 times. The bag contains about 13% He and the rest is room air. As the mixture is being rebreathed some of the He leaves the bag and mixes with the air left in the lungs. We use the He analyzer to determine exactly how much He is in the bag at the start and at the end of the procedure. From these two measurements and the initial volume of the bag we can calculate mathematically the volume of air in the subject's lungs at the start of rebreathing.

Go through the whole procedure as follows first: With the mouthpiece and noseclips on, take a few breaths of room air and get comfortable. When you are ready, inspire deeply and immediately afterwards blow out all the air you can. Keep blowing until you can't blow any more. A maximum effort is important each time. When you are at RV, signal me by tapping the table. I will put the stopper in and close the valve as quickly as possible and tell you to breathe in. You will take from 8-10 breaths, breathing in and out deeply from the big bag. I will help you count the rhythm. You can watch the spirometer or listen to my voice. As you breathe in the spirometer will go up to 0,and as you exhale you should try to get back to your RV, i.e. you should exhale everything you breathed in. If you are able to exhale more air into the bag than we put in, the trial is invalid. You were not down to RV when you gave me the signal.

Do not be concerned if you cannot get the spirometer exactly to 0. Keep a steady rhythm and inhale and exhale as fully as you can. Breathe in slowly and use your abdominal muscles to exhale. Be aware of air leaking in or out your nose or mouth. This will also invalidate the test. At the end of the 8 breaths, I will close the valve again on an exhalation and tell you to come off. If you come off too soon room air will be sucked into the bag and the test will be invalid. You come off by releasing the nose clips or taking the mouthpiece out. Take a kleenex. If you swallow while rebreathing your ears will feel blocked. Any questions?

Explain the main points again briefly and then do the dry run.

Helium Dilution Trials

Set up the apparatus for the first trial, and change the volume in the bag to suit the subject if necessary. Do a second trial after the tank.If the two are 100ml apart do a third trial and use the mean of the two closest. Tell the subject a third trial may be necessary.

Check the level of water in the spirometer and add more if necessary. Be aware of leaks from the big bag during the dry run. Open the stopcock, clamp off the He line, and flush the large bag 10 times. Pull up on the bell and push down slowly to flush.

Collapse the large bag using the spirometer to create pressure. Take off the hose and pull up on the bell. Reattach the hose and push on the bell with gentle pressure while closing the valve. Zero out the spirometer by removing the hose. Reattach the hose.

On the analyzer set the O2 setting to 18% and the display to 0. Attach the analyzer, remove the clamp and bleed the dead space for 1 minute. In the meantime, record the room barometric pressure and the temperature. Change the Co2 scrubber (soda lime) with each trial and the moisture scrubber (drierite) after 6 trials. Tape the vials.

Clamp off the large bag and remove the analyzer hose. Open the He tank and attach the line to the bag. Flush the lines with He. Remove and return small stopper as He is filling hose. Remove the clamp. Fill the bag with 700ml He and replace the clamp.

Change the pressure in the box by pulling up gently on the bell of the spirometer. Open the stopcock and suck in room air quickly in one smooth motion until the total volume is about 5.5L. Close the valve quickly.

Collapse the small bag by rolling it up tightly. Attach the small bag to the stopcock, open the valve and move the spirometer bell up and down slowly 5 times to mix the contents of the large bag. Collapse the small bag by rolling it up again, close the valve and remove the bag.

Attach the He analyzer and remove the clamp. Wait until the reading is stable and record the reading on the worksheet as initial fraction of He. Wait and clamp off at the appropriate volume for the subject and record this as the initial volume. Add .040L to this volume to account for the dead space volume which does not appear on the spirometer. Detach and remove the analyzer hose. Attach the mouthpiece and tape all connections.

Go through the procedure as described in the dry run with the subject. On the last breath close the valve on the exhalation and tell the subject to come off.

Change the 02 setting to 9%. Attach the He analyzer, remove the clamp and record the peak value of the reading and the value at 2, 1, and 0L. Take the mean of the peak and 1L as the final He reading. If the He value drops more than .10 the scrubbers were not working and the test is invalid.

Calculate the RV using the formula on the worksheet. The dead space for subjects is .190L. Do a second trial after the tank and a third trial if necessary. Between trials remove the mouthpiece, stand it up and let it dry out. Turn the valve and open the large bag to air as well.

At the end of the session, detach the spirometer, open the stopcock and take off the clamp. Remove the vials from the analyzer and shut off the display. Keep the power on.

Appendix I

Validity of Helium Dilution Method to Calculate Residual Lung Volume

Table 1

Residual Lung Volume in Litres and Descriptive Statistics of Male and Female Subjects (n=7) Determined by Helium Dilution and Nitrogen Washout and Estimates Based on Percentage of Vital Capacity and Age and on Age, Sex, and Height

Id. No.	**Sex**	**Helium Dilution**		**Nitrogen Washout**		**% Vital Capac.**	**Age, Sex, Ht**
		Date	**Value**	**Date**	**Value**		
1	Male	11/07/89	1.092	11/07/89	1.602	1.476	1.935
2	Male	21/06/89	0.697	24/05/89	1.110	1.335	1.795
3	Male	19/06/89	0.824	24/05/89	1.027	1.120	1.570
4	Female	21/06/89	1.634	24/05/89	1.549	1.409	2.000
4 Retest	Female	12/07/89	1.658	11/07/89	1.454	1.385	2.000
5	Female	16/06/89	0.510	11/07/89	0.407	0.995	1.440
6	Female	11/07/89	1.364	11/07/89	1.240	1.275	1.580
7	Female	29/06/89	0.955	24/05/89	1.270	1.098	1.500
n			8		8	8	8
Mean			1.092		1.207	1.262	1.727
Std Dev			.427		.382	.172	.232

Helium Dilution (mean value of two trials), **Nitrogen Washout** (one trial only), **% Vital Capac.** (age 16-34, RV=0.25xVC; age 35-49, RV=0.305xVC), **Age, Sex, Ht** (estimate from Bates, D. V. , Macklem, P. T. , & Christie, R. V. (1971) . Respiratory function in disease. Philadelphia: W. B. Saunders.)

Table 2

Correlation Coefficients Between the Helium Dilution Method for Determining Residual Lung Volume and Nitrogen Washout, and the Estimates Using a Percentage of Vital Capacity and Age, Sex, and Height

Count	**Covariance**	**Correlation**	**R-Squared**
Helium Dilution vs Nitrogen Washout:			
8	.125	.770 *	.593
Helium Dilution vs Estimate based on Age, Sex, and Height:			
8	.068	.687 ns	.472
Helium Dilution vs Estimate based on % of Vital Capacity:			
8	.049	.663 ns	.440

KEY: * (signifigant at $p \leq .05$), ns (not signifigant at $p \leq .05$).

Table 3

Residual Lung Volume in Litres of Female Subjects (n=45) Determined by Helium Dilution and Estimated as a Percentage of Vital Capacity and Age, Sex, and Height

Helium Dilution	**% of Vital Capacity**	**Age, Sex, Height**
.849	1.020	1.580
1.893	.897	1.440
.708	1.028	1.410
1.340	1.120	1.900
.630	.930	1.410
1.229	.880	1.580
1.581	1.281	1.790
1.146	.793	1.260
1.218	.860	1.320
.875	.945	1.670
.982	1.159	1.580
1.175	1.247	1.710
1.067	1.160	1.670
1.591	1.065	1.490
1.079	.990	1.900
.892	.840	1.670
1.646	1.397	2.000
1.384	.897	1.700
1.416	.855	1.490
.991	.895	1.460
1.285	.885	1.630
1.525	1.494	2.000
1.177	1.128	1.660
1.553	1.092	1.540
.510	.995	1.440
.999	1.100	1.630
.492	.800	1.100
1.553	1.250	1.850
1.000	.875	1.320
1.157	1.040	1.670
1.364	1.275	1.580
.872	1.000	1.630
1.039	1.058	1.900
1.417	1.232	1.580
.997	1.025	1.670
1.573	.850	1.670

Table 3 continued.

Helium Dilution	% of Vital Capacity	Age, Sex, Height
.955	1.098	1.500
1.225	1.015	1.630
.895	.946	1.220
.948	1.240	1.900
1.350	.960	1.320
1.890	.868	1.410
.772	.960	1.490
1.345	1.147	1.710
1.582	1.208	1.750

Table 4

Descriptive Statistics and Correlation Coefficient Between Residual Lung Volume in Litres of Female Subjects (n=45) Determined by Helium Dilution and Estimated as a Percentage of Vital Capacity and Age, Sex, and Height

Method	Mean	Std. Dev.	Range	Coef. Variat.
Helium Dil.	1.181	.336	.492-1.893	28.413
% Vital Cap.	1.040	.164	.793-1.494	15.776
Age, Sex, Ht.	1.596	.205	1.100-2.000	12.837
	Count	**Covariance**	**Correlation**	**R-Squared**
Correlation R.V. by Helium Dilution vs R.V. by % of Vital Capacity				
	45	.015	.283 ns	.080
Correlation R.V. by Helium Dilution vs R.V. by Age, Sex, and Height				
	45	.022	.318 *	.101

KEY: ns (not signifigant at p≤05), * (signifigant at p≤.05)

Appendix J

Validity and Reliability Estimates for Helium Dilution Trials with the Bag

Validity and Reliability Estimates for Helium Dilution Trials with the Bag

Table 1

Volume Predicted using Helium Dilution with a Rubber Bag Filled with Varying Known Amounts of Air (n=48 trials)

Vol. LB (l)	**Volume in Small Bag (l)**					
	.5	**Diff.**	**1.0**	**Diff.**	**1.5**	**Diff.**
3.0	.518	-.018	1.003	-.003	1.478	.022
3.0	.517	-.017	1.016	-.016	1.521	-.021
3.0	.518	-.018	.985	.015	1.479	.021
3.0	.515	-.015	1.023	-.023	1.492	.008
3.0	.508	-.008	1.003	-.003	1.522	-.022
3.0	.494	.006	.983	.017	1.485	.015
3.0	.484	.016	1.007	-.007	1.489	.011
3.0	.513	-.013	.989	.011	1.505	-.005
3.0	.496	.004	1.005	-.005	1.491	.009
3.0	.503	-.003	.998	.002	1.498	.002
3.0	.506	-.006	.986	.014	1.520	-.020
3.0	.503	-.003	1.008	-.008	1.515	-.015
3.5			.992	.008		
3.5			1.002	-.002		
3.5			1.007	-.007		
3.5			1.019	-.019		
3.5			.993	.007		
3.5			1.004	-.004		
2.5			1.020	-.020		
2.5			1.013	-.013		
2.5			1.003	-.003		
2.5			1.008	-.008		
2.5			1.001	-.001		
2.5			.975	.025		

KEY: **Vol. LB** (volume in large bag), **Diff.** (difference between known volume of air-.5, 1.0, or 1.5 litres-and predicted volume by Helium Dilution)

Table 2

Descriptive Statistics for Volumes Predicted on Trials with the Bag

Group **(l)**	**Vol. Lge.Bag** **(l)**	**Count**	**Mean** **(l)**	**Std. Dev.** **(l)**	**Std. Error** **(l)**
.5	3.0	12	.506	.011	.003
Diff. .5	3.0	12	-.006	.011	.003
1.0	3.0	12	1.000	.013	.004
1.0	3.5	6	1.003	.010	.004
1.0	2.5	6	1.003	.016	.006
1.0 all	3.0,3.5,2.5	24	1.002	.012	.003
Diff. 1.0 all	3.0,3.5,2.5	24	-.002	.012	.003
1.5	3.0	12	1.500	.017	.005
Diff. 1.5	3.0	12	.0004	.017	.005

Table 3

Volume Predicted using Helium Dilution with a Rubber Bag Filled with Varying Known Amounts of Air (The Trials Have Been Randomly Split into Two Halves for the Reliability Estimate. n=24 trials)

Known Volume (l)	**Predicted Volume (l)**	
	Trial 1	**Trial 2**
.5	.518	.517
.5	.518	.508
.5	.518	.494
.5	.513	.484
.5	.506	.496
.5	.503	.503
1.0	1.016	1.003
1.0	.985	1.023
1.0	.983	1.003
1.0	1.005	1.007
1.0	.986	.989
1.0	1.008	.998
1.0	1.002	.992
1.0	1.007	1.019
1.0	.993	1.004
1.0	1.003	1.020
1.0	1.008	1.013
1.0	1.001	.975
1.5	1.478	1.521
1.5	1.492	1.479
1.5	1.522	1.485
1.5	1.505	1.489
1.5	1.491	1.498
1.5	1.515	1.520

Table 4

Descriptive Statistics for Volumes Predicted on Split Halves Trials with the Bag

Group (l)	**Trial**	**Count**	**Mean (l)**	**Std. Dev. (l)**	**Std. Error (l)**
All	1	24	1.003	.357	.073
All	2	24	1.002	.361	.074
All	Trial 1 vs Trial 2, **Mean difference (l)**: .002				

Table 5

Summary of Analysis of Variance for Reliability Estimate of All Predicted Volumes

Source	**df**	**SS**	**MS**	**F**	**P**
Between subjects	23	5.921703	.257465	1398.391969	.0001
Within subjects	24	.004419	.000184		
Trials	1	.000030	000030	.158759 *	.6940
Interaction	23	.004388	.000191		
Total	47	5.926122			

KEY: **df** (degrees of freedom), **SS** (sum of squares), **MS** (mean square), **F** (F test), **P** (P value), * (not signifigant at the .05 level).

Table 6

Calculation of R (Intraclass Correlation) for Reliability

Group	Statistic	Formula	Calculation	
	R	$= \frac{\text{MS subj - MS error}}{\text{MS subjects}}$		
All	MS error	$= \frac{\text{SS trials + SS interact}}{\text{df trials + df interaction}}$	$= \frac{.000030 + .004388}{1+23}$	= .000184
All	R	$= \frac{\text{MS subj - MS error}}{\text{MS subjects}}$	$= \frac{.257465 - .000184}{.257465}$	= .999

Appendix K

Intraclass Correlation for Reliability Estimate of Residual Lung Volume for Subjects

Intraclass Correlation for Reliability Estimate of Residual Lung Volume for Subjects

Table 1

Two Repeated Trials * of Residual Lung Volume, ATPS, in Litres, Determined by Helium Dilution (n=56)

Subject No.	**Residual Lung Volume (l)**	
	Trial 1	**Trial 2**
1	.807	.846
2	1.932	1.872
3	.637	.630
4	1.294	1.304
5	.598	.561
6	1.161	1.201
7	1.465	1.445
8	1.090	1.041
9	1.128	1.126
10	.836	.736
11	.921	.885
12	1.149	1.075
13	1.022	.963
14	1.486	1.427
15	.992	1.014
16	.811	.840
17	1.515	1.523
18	1.290	1.284
19	1.271	1.322
20	.858	.957
21	1.164	1.212
22	1.443	1.378
23	1.105	1.083
24	1.379	1.493
25	.479	.469
26	.947	.892
27	.484	.425

Table 1 continued.

Subject No.	Residual Lung Volume (l)	
	Trial 1	Trial 2
28	1.464	1.423
29	.879	.980
30	1.080	1.071
31	1.278	1.244
32	.727	.878
33	1.001	.959
34	1.298	1.308
35	.911	.952
36	1.471	1.439
37	.876	.890
38	1.128	1.150
39	.847	.799
40	.874	.879
41	1.222	1.290
42	1.742	1.819
43	.691	.730
44	1.208	1.337
45	1.421	1.476
1	.740	.746
2	1.628	1.609
3	.682	.681
4	1.177	1.180
6	1.144	1.067
10	.869	.791
12	1.098	1.049
17	1.540	1.528
33	.973	.942
42	1.685	1.748
44	1.213	1.243

* Trials were successive or taken immediately before and after underwater weighing.

Table 2

Descriptive Statistics for Residual Lung Volume Trials

Group	**Count**	**Mean (l)**	**Std. Dev. (l)**	**Std. Error (l)**
Trial 1	56	1.110	.322	.043
Trial 2	56	1.111	.328	.044

Trial 1 vs Trial 2 , **Mean difference (l)** : -.001

Table 3

Summary of Analysis of Variance for Reliability Estimate

Source	**df**	**SS**	**MS**	**F**	**P**
Between subjects	55	11.5455	.2099	137.2727	.0001
Within subjects	56	.0856	.0015		
Trials	1	.0001	.0001	.0377 ns	.8467
Interaction	55	.0856	.0016		
Total	111	11.6311			

KEY: **df** (degrees of freedom), **SS** (sum of squares), **MS** (mean square), **F** (F test), **P** (P value), ns (not significant at the .05 level).

Table 4

Calculation of R (Intraclass Correlation)

$$\text{R (Intracl. corr.)} = \frac{\text{MS subjects} - \text{MS error}}{\text{MS subjects}}$$

$$\text{MS error} = \frac{\text{SS trials} + \text{SS interact.}}{\text{df trials} + \text{df interaction}} = \frac{.0001 + .0856}{1 + 55} = .00153$$

$$\text{R (Intracl. corr.)} = \frac{\text{MS subjects} - \text{MS error}}{\text{MS subjects}} = \frac{.2099 - .00153}{.2099} = 0.993$$

Appendix L

Intraclass Correlation for Reliability Estimate of Percent Fat for Subjects

Intraclass Correlation for Reliability Estimate of Percent Fat for Subjects

Table 1

Percent Fat of Selected Subjects (n=10) on Two Trials

Subject No.	% Fat Trial 1	 Trial 2
1	14.22	14.16
2	16.85	17.23
3	19.66	19.29
4	7.92	9.98
6	17.95	18.99
10	18.65	19.19
12	26.42	25.74
33	18.35	20.62
42	9.42	9.86
44	22.48	23.79

Table 2

Descriptive Statistics for Percent Fat Trials

Group	Count	Mean (%)	Std. Dev. (%)	Std. Error (%)
Trial 1	10	17.2	5.6	1.8
Trial 2	10	17.9	5.3	1.7

Trial 1 vs Trial 2 , **Mean difference (%) :** - 0.7

Table 3

Summary of Analysis of Variance for Reliability Estimate

Source	df	SS	MS	F	P
Between subjects	9	524.493	58.277	86.566	.0001
Within subjects	10	6.732	.673		
Trials	1	2.405	2.405	5.003 ns	.0521
Interaction	9	4.327	.481		
Total	19	531.225			

KEY: **df** (degrees of freedom), **SS** (sum of squares), **MS** (mean square), **F** (F test), **P** (P value), ns (not significant at the .05 level).

Table 4

Calculation of R (Intraclass Correlation)

$$\text{R (Intraclass corr.)} = \frac{\text{MS subjects} - \text{MS error}}{\text{MS subjects}}$$

$$\text{MS error} = \frac{\text{SS trials} + \text{SS interaction}}{\text{df trials} + \text{df interaction}} = \frac{2.405 + 4.327}{1 + 9} = 0.6732$$

$$\text{R (Intraclass corr.)} = \frac{\text{MS subjects} - \text{MS error}}{\text{MS subjects}} = \frac{58.277 - .6732}{58.277} = 0.988$$