HUMAN BODY COMPOSITION MODELS

AND METHODOLOGY: THEORY AND EXPERIMENT

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Theorems

1. Science consists in grouping facts so that general laws or conclusions may be drawn from them. (C. Darwin)

2. My business is to teach my aspirations to conform themselves to fact, not to try to make facts harmonize with my aspirations. (Thomas Huxley)

3. He who has once in his life experienced this joy of scientific creation will never forget it. (Prince Kropotkin)

4. Science without organization is blind. (this thesis)

5. Knowledge without thought is useless; and thought without knowledge is dangerous. (Confucius)

6. There is no limit to knowledge. (Confucius)

7. Know the hows is important, but know the whys is more important. (this thesis)

8. Research is to see what everyone alse has seen and to think no one alse has thought. (this thesis)

9. We must accept finite disappointment, but must never lose infinite hope. (Martin Luther King, Jr.)

10. The research worker remains a student all his life. (W.I.B. Beveridge, The Art of Scientific Investigation. Vintage Books, New York, 1950)

Theorems belong to the thesis of ZiMian Wang entitled "Human Body Composition Models and Methodology: Theory and Experiment".

Wageningen Agricultural University, The Netherlands, September 9, 1997.

To My Family and My Motherland

The research worker remains a student all his life.

—— W. I. B. Beveridge, *The Art of Scientific Investigation*

ABSTRACT

HUMAN BODY COMPOSITION MODELS AND METHODOLOGY: THEORY AND EXPERIMENT

Ph.D. thesis by ZiMian Wang, Department of Human Nutrition and Epidemiology, Wageningen Agricultural University, Wageningen, The Netherlands.
September 9, 1997

The study of human body composition is a branch of human biology which focuses on the *in vivo* quantification of body components, the quantitative relationships between components, and the quantitative changes in these components related to various influencing factors. Accordingly, the study of human body composition is composed of three interrelated research areas, body composition rules, body composition methodology, and body composition alterations. This thesis describes the author's recent investigations related to the first two of the three research areas.
In the rules area, this thesis begins with a comprehensive five-level model of body composition. The approximate 40 body components are systematically organized into five levels with increasing complexity: I, atomic; II, molecular; III, cellular; IV, tissue-system; and V, whole-body. Although each level and its multiple components are distinct, interconnections exist such that the model is consistent and functions as a whole. The model provides the opportunity to define clear body composition concepts and to create explicit body composition equations. As an example of a body composition rule, this thesis subsequently explores the magnitude and constancy of the proportion of adipose tissue-free body mass as skeletal muscle mass in young healthy adults.
In the methodology area, this thesis begins with a systematic organization of body composition methods. The proposed classification divides the methods into *in vitro*

and *in vivo* categories, advances to organization by measurable quantity (property and/or component), and ends with grouping of methods by mathematical function which may be statistically-derived or model-based. This classification system explains both similarities and differences between the many diverse methods, and provides a framework and a setting for all body composition methods. As an example of new body composition method development, the subsequent chapter creates a simple and safe method for predicting total body oxygen mass from body weight and total body water. The remaining four chapters deal with the evaluation of body composition methods. A six-compartment model based on *in vivo* neutron activation analysis is applied as the reference for inter-method comparisons against sixteen existing methods for estimating total body fat mass. An advanced technique, multiscan computerized axial tomography, is applied as the reference to evaluate existing skeletal muscle mass methods, including the Burkinshaw-Cohn neutron activation model, dual-energy X-ray absorptiometry, 24-hour urinary creatinine excretion, and 24-hour urinary 3-methylhistidine excretion methods.

This thesis, therefore, suggests that both theoretical and experimental investigations provide insight into human body composition.

人體組成模型和方法學：理論與實驗研究

(摘要)

人體組成學是人類生物學的一個分枝，它主要研究人體組成的活體測量，組份之間的定量關系，以及在各種影響因素作用下各組份量的變化。人體組成學相應地包含三個相互聯系的研究領域。本論文集匯集了作者在其中兩個領域(人體組成規律與方法學) 的新近研究成果。

在人體組成規律領域內，本論文集首 先提出了總括性的人體組成五層次模型。大約四十種人體組份可以組織在五個漸趨復雜的層次中，1. 原子 層次，2. 分子 層次，3. 細胞層次，4. 組織-系統層次，以及 5. 整體層次。每個層次及其組份既是顯著不同的，又是相互聯系的，由此構成了一個整體。根據 這個模型，研究者得以清晰地定義人體組成的概念，和建立正确的人體組成方程。接著，作者以青年男性骨骼肌占非脂肪組織體重的比例及其穩定性為例，探討了人體組成的規律。

在方法學領域內，本論文集首先提出了人體組成測量方法的系統分類。所有的人體組成測量方法可以分為離體和在體兩類，然後再根據測定量 (性質和/或組份) 和方法的數學式 (由統計學導出或由模型推導) 來區分。這為人體組成測量 方法的分類提供了框架，解釋了方法之間的相似性和區別。本論文集以全身氧含量的測定為例，提出了由體重和全身水含量來計算全身氧含量的簡便，安全方法，以此來說明如何發展新的人體組成測量方法。本論文集接著探討了人體組成測量方法的檢驗問題。對于全身脂肪含量的測定，作者提出了基于中子活化分析的六組份模型，並以此為基 準檢驗了十六種現有的方法。對于全身骨骼肌重量的測定，作者以新近發展的多剖面 CT技術為基準，檢驗了現有的骨骼肌測量方法，包括Burkinshaw-Cohn 中子活化模型，雙能量 X射線吸收法，尿肌酐日排泄量法，以及尿三甲基組氨酸日排泄量方法等。

本論文集由此指出，不論是理論研究還是實驗研究，對于深入了解人體組成都是必需的。

Where there's a will there's a way.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Knowledge without thought is useless; and thought without knowledge is dangerous.

—— Confucius

In 1992, Wang, Pierson, and Heymsfield proposed that the study of human body composition can be defined as a branch of human biology which mainly focuses on the *in vivo* quantification of body components, the quantitative relationships between components, and component alterations related to various influencing factors. Accordingly, the study of human body composition is composed of three research areas (**Figure 1**) (Wang et al. 1992). The first research area describes the rules of human body composition (e. g., the relatively constant relationships between body components and between components and their measurable properties). The second research area studies *in vivo* methods of measuring various body components. The third research area studies the effects of various *ab inter* and *ab extra* influencing factors on body composition.

This Ph.D. thesis develops a comprehensive framework for the study of human body composition and a systematic organization of the accumulated knowledge is proposed. Under the direction of the theoretical research, this thesis studies some practical problems such as exploration of body composition models and the development and evaluation of body composition methods.

This thesis focuses on two of the three research areas, body composition rules and methodology. Because this thesis involves considerable accumulated information from the discipline, it is useful to overview briefly the history of the study of human body composition (for further detail see the Appendix: Body Composition Study Chronological Table).

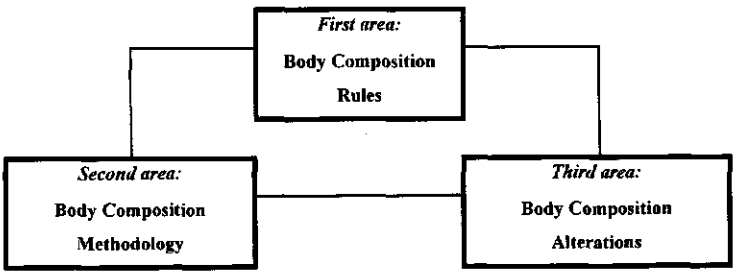

Figure 1. The study of human body composition: three research areas.

THE HISTORY OF THE STUDY OF HUMAN BODY COMPOSITION: A BRIEF REVIEW

Conjecture on human body composition can be traced back to antiquity. *Circa* 440 BC, Hippocrates, the father of medicine, proposed the idea that as a whole organism, the human body is composed of four "constituents", i.e., blood, phlegm, black bile, and yellow bile. A similar hypothesis was also proposed by ancient Chinese scholars, who suggested that there were five "elements" in the human body including metal, wood, water, fire, and earth. Good health was achieved through a balance of the five elements, and any imbalance resulted in disease. However, these ancient ideas were not based on experiments. Since that time, human beings have come a long way toward understanding the composition of their own bodies. As a branch of modern science, the study of human body composition has a history of about 150 years which can be roughly divided into two periods, the early and recent stages.

The Early Stage of the Study of Human Body Composition (1850's – 1950's)

The enlightenment of human body composition research depended upon development of other branches of science such as chemistry, anatomy, and nutrition. It was a great German chemist, Justus von Liebig (1803 – 1873), who first found that many substances in food were also present in the human body. He also found that body fluids contained more sodium and less potassium than tissues. Liebig's work, based on chemical analysis, marked the beginning of the modern study of human body composition. Reviewing the early stage of the study of human body composition, three separate but related research areas can be distinguished.

The first area of early study was concerned with collecting quantitative information on body components. Because there were no *in vivo* methods for measuring various components in the last century, cadaver autopsy was the only way to obtain quantitative data on body composition. In 1843 Schwann measured several cadaver organs. Twenty years later Bischoff (1863) analyzed several adult human cadavers for their water content; and Fehling analyzed fetuses and newborns for their water content in 1877. In 1900 Camerer and Söldner estimated the chemical composition of fetuses including water, fat, nitrogen, and major minerals. In 1938 Iob and Swanson completed whole body assays including trace minerals for fetuses and newborns.

As the original method of quantitative body composition research, cadaver study has had great importance even to the present. The largest scale cadaver dissection was the Brussels study

(Clarys et al., 1984) in which 12 male and 13 female cadavers were dissected, accumulating considerable quantitative body composition data.

The second area of early study focused on *in vivo* methods of measuring various body components. In 1909, daily urinary creatinine excretion was used by Shaffer and Coleman as an index of total body skeletal muscle mass. Skeletal muscle, the main source of urinary creatinine, was probably the first body component estimated by an *in vivo* method.

Body fluids and relevant components were measured, in the early stage, by techniques based on indicator dilution principles. Keith and his colleagues (1915) estimated blood volume using Vital Red and Congo Red as markers. In 1934, von Hevesy and Hofer used deuterium (^2H_2O) to measure total body water. Later, in 1946, Moore measured exchangeable sodium and potassium which are mainly distributed in body fluids and thus could be measured by dilution methods.

The famous principle of Archimedes (287 – 212 BC) was applied to body composition studies by Behnke et al. (1942) to estimate the relative proportions of fat and fat-free mass in the human body. In 1953 Keys and Brozek suggested the more detailed densitometric method which is still widely applied today with modifications. The ancient Archimedes' principle thus provides the possibility of measuring body fat *in vivo*.

Potassium was the first element to be measured in the human body by an *in vivo* method. Sievert (1951) demonstrated that the amount of radioactive isotope ^{40}K in the human body is large enough to be detected and quantified by radioactive techniques. In 1961, Forbes et al. estimated fat-free body mass and total body fat from total body potassium by using a whole body ^{40}K γ-ray assay. Moore et al. (1963) applied another radioactive isotope, ^{42}K, to quantify exchangeable potassium and body cell mass.

The third area of early study focused upon alterations in body composition caused by various influencing factors. Age was probably the first factor studied by the early body composition researchers. In 1857 Albert von Bezold found that growth in animals was accompanied by an increase in the proportion of ash and a decrease in the proportion of water. In 1914 Benjamin discovered that infants accumulated nitrogen during growth. Based on this finding, Moulton (1923) announced the concept of "chemical maturity" to name the life stage when the chemical composition of children approaches the adult value.

Nutrition was another factor studied during the early stage of body composition research. In 1907 Cathcart found that body nitrogen was lost during fasting. Benedict et al. (1919) further pointed out that body nitrogen was lost due to a modest reduction in food intake. Later, additional factors influencing body composition, such as exercise, race, gender, and several diseases, were investigated by early body composition researchers. The early stage of the study of human body composition lasted as long as a century (1850's – 1950's). The mark of the end of this stage might be the First Body Composition Symposium of the Society for the Study of Human Biology, held by the New York Academy of Science in 1963. At this conference, J. Brozek summarized the excellent research results achieved during the past 100 years (Brozek, 1965). The convention of the first body composition symposium also marked the beginning of the recent rapid stage of body composition research development.

The Recent Stage of the Study of Human Body Composition (1960's – present)

The recent stage of the study of human body composition, which we are still in, emerged during the 1960's. A relevant feature is that the study of human body composition has accelerated during the past 30 years. What makes the present stage unique is the recognition that the study of body composition *per se* has become a distinct branch of human biology. Advanced body composition methods including *in vivo* neutron activation analysis and imaging techniques were either introduced or fully appreciated (Forbes, 1987). It was recognized that many chronic and acute illnesses involved alteration in body composition, and that these changes might be linked with morbidity and mortality.

Recent developments can be summarized by describing published research articles, related journals and monographs, and symposia on body composition research.

Research articles Because increasing numbers of investigators are engaged in the study of human body composition, the published articles on body composition study increases very rapidly. In the 1960's, an average of 56 articles on the study of human body composition were published each year (**Table 1**). This number increased to 77 in the 1970's, and to 110 in the 1980's. During the first seven years of the 1990's, the number of yearly published articles dramatically increased to 261, growth by a factor of 4.7 compared to that in the 1960's. According to *Cumulated Index Medicus*, there were 4,255 articles published on the study of human body composition from 1960 to 1996. The total amount of published articles since 1960 meets a curve of the exponential function,

Table 1. The number of published articles on the study of human body composition (1960-1996)

Year	**Articles**	*Year*	**Articles**	*Year*	**Articles**	*Year*	**Articles**
1960	19	*1970*	91	*1980*	64	*1990*	184
1961	30	*1971*	82	*1981*	80	*1991*	199
1962	19	*1972*	65	*1982*	98	*1992*	255
1963	35	*1973*	61	*1983*	89	*1993*	266
1964	109	*1974*	87	*1984*	109	*1994*	296
1965	73	*1975*	82	*1985*	139	*1995*	301
1966	66	*1976*	90	*1986*	129	*1996*	327
1967	61	*1977*	79	*1987*	147		
1968	63	*1978*	62	*1988*	113		
1969	83	*1979*	68	*1989*	134		
1960-1969	**558**	***1970-1979***	**767**	***1980-1989***	**1,102**	***1990-1996***	**1,828**
Yearly mean	**55.8**		**76.7**		**110.2**		**261.1**
%	**100**		**137**		**197**		**468**

Information based on "*Cumulated Index Medicus*". Total amount of published articles on the study of body composition is 4,255 from 1960-1996.

$$A(Y) = -1219 + 1195.1 \times e^{[0.03786 \times (Y - 1959)]}$$

where A(Y) represents the total amount of published articles from 1960 to the Y year.

Journals During the recent stage, research reports on body composition appeared in about 50 scientific journals, which can be organized into four main categories: general science; human biology; nutrition; and medicine. This demonstrates the close relationship between body composition research and human biology, nutrition, and medicine. The following is a partial list of scientific journals that publish research articles on the study of human body composition.

Journals of general science: "*Annals of the New York Academy of Sciences*"; "*Nature*", "*Science*"; and "*Scientific American*".

Journals of human biology: "*American Journal of Human Biology*"; "*American Journal of Physical Anthropology*"; "*American Journal of Physiology*"; "*Biochemical Journal*"; "*Comparative Biochemistry and Physiology*"; "*Growth*"; "*Journal of Anatomy*"; "*Journal of Applied Physiology*"; "*Journal of Biological Chemistry*"; "*Journal of Cell Science*"; "*Journal of Gerontology*"; "*Journal of Physiology*"; "*Metabolism*"; "*Mineral Metabolism*"; "*Physiology and Behavior*"; and "*Physiological Reviews*".

Journals of nutrition: "*American Journal of Clinical Nutrition*"; "*Annual Review of Nutrition*"; "*Asia and Pacific Journal of Clinical Nutrition*"; "*British Journal of Nutrition*"; "*European Journal of Clinical Nutrition*"; "*Journal of Nutrition*"; "*Journal of Parental and Enteral Nutrition*" and "*Nutrition*".

Journals of medicine: "*American Journal of Diseases of Children*"; "*American Journal of Medicine*"; "*American Journal of Obstetrics and Gynecology*"; "*Aviation Space and Environmental Medicine*"; "*British Medical Journal*"; "*Clinical Science*"; "*European Journal of Clinical Investigation*"; "*European Journal of Pediatrics*"; "*International Journal of Obesity and Related Metabolic Disorders*"; "*Journal of the American Dietetic Association*"; "*Journal of Chronic Diseases*"; "*Journal of Clinical Investigation*"; "*Journal of Laboratory and Clinical Medicine*"; "*Journal of Nuclear Medicine*"; "*Journal of Pediatrics*"; "*Lancet*"; "*Medical Physics*"; "*Medicine and Science in Sports*"; "*Medicine and Science in Sports and Exercise*"; "*New England Journal of Medicine*"; "*Pediatric Research*"; "*Physics in Medicine and Biology*"; and "*Surgery*".

Monographs Research monographs describing body composition methodology and body composition alteration appeared at a rapid rate. To my knowledge, at least 19 monographs have been published since 1985.

1. *Human Body Composition and Fat Distribution.* Norgan NG (ed.); EUR-NUT Report 8; 1985, 250 pages.
2. *Body-Composition Assessments in Youth and Adults.* Roche AF (ed.); Ross Laboratory, Columbus, Ohio; 1985, 109 pages.
3. *Human Body Composition: Growth, Aging, Nutrition and Activity.* Forbes GB; Springer-Verlag, New York; 1987, 350 pages.
4. *In Vivo Body Composition Studies.* Ellis KJ, Yasumura S, Morgan WD (eds); The Institute of Physical Sciences in Medicine; 1987, 476 pages.
5. *Anthropometric Standardization Reference Manual.* Lohman TG, Roche AF, Martorell R (eds.); Human Kinetics Books, Champaign, Illinois; 1988, 177 pages.
6. *In Vivo Body Composition Studies: Recent Advances.* Yasumura S, Harrison JE, McNeill KG, Woodhead AD, Dilmanian FA (eds.); Plenum Press, New York; 1990, 448 pages.
7. *Principles of Nutritional Assessment.* Gibson RS; Oxford University Press; 1990, 691 pages.
8. *Nutritional Status Assessment.* Fidanza EF; Chapman & Hall, London; 1991, 486 pages.
9. *Anthropometric Assessment of Nutrition Assessment of Nutritional Status.* Himes JH (ed.); Wiley-Liss; 1991, 431 pages.
10. *Growth, Maturation and Body Composition: the Fels Longitudinal Study 1929-1991.* Roche AF; Cambridge University Press; 1992, 282 pages.
11. *Body Composition and Physical Performance: Applications for the Military Services.* Marriott BM, Grumstrup-Scott J (eds.); National Academy Press. Washington, D. C.; 1992, 356 pages.
12. *Advances in Body Composition Assessment.* Lohman TG; Human Kinetics Publishers; 1993, 150 pages.
13. *Human Body Composition: In Vivo Methods, Models, and Assessment.* Ellis KJ, Eastman JD (eds.); Plenum Press, New York; 1993, 400 pages.
14. *Recent Development in Body Composition Analysis: Methods and Applications.* Kral JG, VanItallie TB (eds.); Smith-Gordon, London; 1993, 172 pages.

15.*The Swansea Trial: Body Composition and Metabolic Studies with a Very-Low-Calorie Diet (VLCD)*. Kreitzman SN, Howard AN (eds.); Smith-Gordon, London; 1993, 168 pages.

16.*Physical Status: the Use and Interpretation of Anthropometry*. Report of WHO Expert Committee; WHO technical report series 854; Geneva; 1995, 452 pages.

17.*Body Composition Techniques in Health and Disease*. Davies PSW, Cole TJ (eds.); Cambridge University Press; 1995, 282 pages.

18.*Applied Body Composition Assessment*. Heyward VH, Stolarczyk L; Human Kinetics; 1996, 232 pages.

19.*Human Body Composition: Methods and Findings*. Roche AF, Heymsfield SB, Lohman TG (eds.); Human Kinetics; 1996, 366 pages.

Symposia Research symposia on the study of human body composition were held at increasingly frequent intervals. Body composition problems are now considered in an increasing number of symposia of relevant branches of science such as human growth, development, aging, nutrition, exercise physiology, and clinical medicine.

Since 1986, a series of specialized meetings, the International Symposia on *In Vivo* Body Composition Studies, have been held, marking a new milestone in the history of the study of human body composition. The topics of the first to the fourth symposia are illustrated in **Table 2**, covering the three research areas of the study of human body composition. The following is a list of the years, organizers, and places of the symposia held as well as participants and presented papers:

The 1st Symposium: 1986, Brookhaven National Laboratory, New York, USA, 138 participants and 74 papers;

The 2nd Symposium: 1989, University of Toronto, Canada, 108 participants and 184 papers;

The 3rd Symposium: 1992, Baylor College of Medicine, Houston, USA, 105 papers;

The 4th Symposium: 1996, Malmö University, Malmö, Sweden, 138 participants and 185 papers.

The 5th Symposium will be held in New York area, 1999.

Table 2. Topics of International Symposia on *In Vivo* Body Composition Studies

	Body composition research area		
	Components and rules	**Methodology**	**Alterations**
The 1st Symposium (1986)	Lean and fat tissues; Water and electrolytes; Skeletal tissue; Essential and toxic trace elements.	Recent developments in instrumentation and techniques.	
The 2nd Symposium (1989)	Skeletal tissue; Lean and fat tissue;	Body composition methodology; Recent development in instrumentation.	Body composition in children and infants; Trace element toxicity.
The 3rd Symposium (1992)	Body composition assessment, models and equations.	Body composition methodology and instrumentation.	Growth: infant, children, adolescent, and pregnancy; Body composition changes in disease and with therapy; Trace metals: treatment and toxicology.
The 4th Symposium (1996)	Skeletal muscle mass; Skeletal tissue; Body composition assessment and models.	Recent developments in methodology and instrumentation; New measurement methods of muscle mass.	Trace element toxicity; Body composition changes in disease and with therapy.

THE AIM OF THIS INVESTIGATION

As every branch of human biology, the study of body composition has two linked research aspects, theoretical and experimental. Although there is a 150 year history and a great deal of information, the study of human body composition is still immature at both the theoretical and the experimental aspects.

At **the theoretical aspects**, body composition terms which were ill-defined were suggested, and inconsistent body composition models were presented in published research articles. There has been no coherent scheme for organizing the accumulated knowledge. For instance, although many body composition methods have been developed, there was no logical manner with which students and investigators could build an understanding of the field grounded on a strong scientific foundation. More importantly, some basic principles in the study of human body composition remain unclear such as whether the accumulated knowledge and information can be systematically organized in each of the three research areas. Specifically,

— How many components does the human body have? Can we organize these components systematically? Are there any body composition models or constant quantitative relationships between these components?

— Can we systematically organize the available body composition methods?

— What *ab inter* and *ab extra* factors are there which may influence human body composition? Can we systematically organize these factors and generally express their influence on the body composition?

Regards **the experimental aspects**, there are still a few components such as the body cells which cannot yet be estimated *in vivo*. Some body components (e.g., total body nitrogen and total body oxygen) can be estimated, but the currently used methods (e.g., *in vivo* neutron activation analysis) involve radiation doses which might be accepted in clinical medicine, but not in the study of healthy subjects. Therefore, new simple and safe methods are needed. On the other hand, although there are several methods to assess total body skeletal muscle mass, some have so large an error as to sometimes give negative muscle values (Burkinshaw et al. 1978). Similar problems also exist within the methods of estimating total body fat mass. An example was that the total body potassium (TBK) method of Forbes overestimated total body fat (mean 38.0 % *vs.* 30.9 % by *in vivo* neutron activation method), while bioimpedance analysis (BIA) underestimated total body fat (24.5 % *vs.* 30.9 %) for the same group of

subjects (Wang et al., 1993). These examples show that there are still unresolved problems at the experimental aspect of body composition research.

This thesis will focus attention on two research areas, body composition rules and body composition methodology. The major aim of the present investigation is to build a comprehensive framework for the study of human body composition. Within this comprehensive framework, the accumulated information and knowledge of body composition research will be systematically organized; suggested body composition models will be explored by using a modern technique; a new body composition method will be developed; and finally, some currently used *in vivo* methods will be compared and evaluated.

SYNOPSIS OF THIS THESIS

The present thesis reflects the author's effort during the recent years at both theoretical and experimental aspects within the areas of body composition rules and methodology (**Table 3**).

In the area of body composition rules, a five-level body composition model is proposed [**Chapter 2**]. Based on this model, the magnitude and constancy of relationship between two tissue-system level components, skeletal muscle and adipose tissue-free body mass, is studied [**Chapter 3**].

In the area of body composition methodology, a systematic classification of body composition methods is suggested [**Chapter 4, 5**]. Based on the theoretical approach, a simple and safe method is developed for estimating total body oxygen mass [**Chapter 6**]. The currently used 16 fat estimate methods are compared with a six-compartment model [**Chapter 7**]. Four existing methods to estimate total body skeletal muscle mass are evaluated by a multiscan CT technique [**Chapter 8-10**].

Based on these studies, **Chapter 11** generally discusses the areas of body composition rules and methodology, and the interrelations between the two areas. The characteristics of the study of human body composition are also briefly discussed.

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Table 3. Structure of this thesis at the theoretical and experimental aspects of body composition research

Research area	Theoretical aspects	Experimental aspects
Body composition rules	The five-level model: a new approach to organizing body composition research. (*American Journal of Clinical Nutrition*, 1992; 56: 19-28) **[Chapter 2]**	Proportion of adipose tissue-free body mass as skeletal muscle in men: magnitude and constancy. (*American Journal of Human Biology*, 1997; in press) **[Chapter 3]**
Body composition methodology	Systematic organization of body composition methodology: an overview with emphasis on component-based methods. (*American Journal of Clinical Nutrition*, 1995; 61: 457-465) **[Chapter 4]** Systematic organization of body composition methodology: overview with emphasis on property-based methods. **[Chapter 5]**	Total body oxygen mass: assessment from body weight and total body water (*The 4th International Symposium on In Vivo Body Composition Studies*, 1996; and *Applied Radiation and Isotopes*, 1997, in press) **[Chapter 6]** Six-compartment body composition model: inter-method comparisons of total body fat measurement. (*International Journal of Obesity*, accepted) **[Chapter 7]** Skeletal muscle mass: evaluation of neutron activation and dual energy X-ray absorptiometry methods. (*Journal of Applied Physiology*, 1996; 80: 824-831) **[Chapter 8]** Total body skeletal muscle mass: evaluation of 24-h urinary creatinine excretion by computerized axial tomography. (*American Journal of Clinical Nutrition*, 1996; 63: 863-869) **[Chapter 9]** Urinary 3-methylhistidine excretion: association with total body skeletal muscle mass by computerized axial tomography. (*Journal of Parental and Enteral Nutrition*, accepted) **[Chapter 10]**

Benedict FG, Miles WR, Roth P, Smith HM (1919) *Human Vitality and Efficiency under Prolonged Restricted Diet*. Washington: Carnegie Institute.

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CHAPTER 2

THE FIVE-LEVEL MODEL: A NEW APPROACH TO ORGANIZING BODY-COMPOSITION RESEARCH

ZiMian Wang, Richard N Pierson Jr, Steven B Heymsfield

ABSTRACT

Body-composition research is a branch of human biology that has three interconnecting areas: body-composition levels and their organizational rules, measurement techniques, and biological factors that influence body composition. In the first area, which is inadequately formulated at present, five levels of increasing complexity are proposed: I, atomic; II, molecular; III, cellular; IV, tissue-system; and V, whole body. Although each level and its multiple compartments are distinct, biochemical and physiological connections exist such that the model is consistent and functions as a whole. The model also provides the opportunity to clearly define the concept of a body composition steady state in which quantitative associations exist over a specified time interval between compartments at the same or different levels. Finally, the five-level model provides a matrix for creating explicit body-composition equations, reveals gaps in the study of human body composition, and suggests important new research areas.

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INTRODUCTION

The study of human body composition spans > 100 y and continues to be an active area of basic science and clinical research. Nearly every aspect of clinical nutrition, selected areas within many medical specialties, and components of exercise science are touched on by the study of body composition.

Information related to body composition is accumulating rapidly and is extending our knowledge of human biology. Most of this information is now categorized as technical or biological. The technical category includes the many classic and continually emerging new body-composition methods. Although no systematic classification for body-composition methodology has been proposed, informal groupings are often published, such as dilution techniques and neutron-activation analysis, which are based on a physical principle or other characteristics of the techniques involved. The biological category includes information on the study of how growth, development, pregnancy, lactation, aging, exercise, and disease influence body composition.

Although the technical and biological categories would appear to encompass most body-composition information, a recent study (1) led us to appreciate a serious limitation of the field as it is now organized. We recognized that not all of the rapidly accumulating information emerging from body-composition research could be satisfactorily included into the technical and biological categories. For example, there are many mathematical models that describe the relations between different components in healthy subjects [e.g., total body water (TBW)/fat-free body mass = 0.732] (2). This formulation indicates that some quantitative associations exist that describe the relationships among compartments that are in equilibrium. Another example is provided by the reconstruction of human chemical compartments and body weight (Bwt) from elements estimated *in vivo* by neutron-activation analysis (1). This suggests that relationships exist not only between individual components but between different levels of body composition as well.

Another problem is that investigators are frequently confronted with questions about terminology. For example: Are lipid-free body mass, fat-free body mass, and lean body mass (LBM) the same or different compartments? The lack of clear definitions for body composition components has a subtle but serious consequence: many errors are evident in

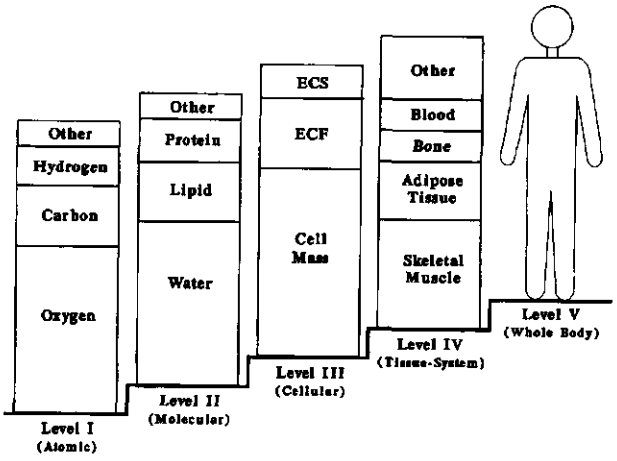

Figure 1. The five levels of human body composition. ECF and ECS, extracellular fluid and solids, respectively.

published body composition equations and models because of overlap or omission of components. In fact, we could find no clear approach to defining components and building multicompartment body-composition models in extensive reviews of previous literature.

Growing from these observations is the hypothesis that a third central category of body-composition research exists that until now has not been adequately formulated: the levels of body composition and their organizational rules. This report presents a comprehensive model of human body composition consisting of five distinct levels of increasing complexity in which each level has clearly defined components that comprise total Bwt. The five levels are I, atomic; II, molecular; III, cellular; IV, tissue-system; and V, whole body (**Figure 1**).

The following section presents a detailed description of each level and its associated components. In the next section the features or organizational rules of the model as a whole are described. Important concepts related to development of body-composition models and equations are presented in this portion of the paper, and the widely appreciated but never formally defined concept of a steady state of body composition is introduced.

FIVE-LEVEL MODEL

Atomic (I)

The fundamental building blocks of the human body are atoms or elements. Of the 106 elements, ~50 are found in the human body and their distributions in the various tissues and organs are well documented (2). Six elements (oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus) account for > 98% of Bwt, and one element, oxygen, constitutes > 60% of total body mass in the Reference Man **(Table 1)** (2). The remaining 44 elements make up < 2% of Bwt.

The equation for Bwt, as defined in the atomic level of body composition is

$$Bwt = O + C + H + N + Ca + P + S + K + Na + Cl + Mg + R \qquad [1]$$

Table 1. Body composition on the atomic level (I) for the 70-kg Reference Man

Element	Amount	Percent of body weight
	kg	*%*
Oxygen	43	61
Carbon	16	23
Hydrogen	7	10
Nitrogen	1.8	2.6
Calcium	1.0	1.4
Phosphorus	0.58	0.83
Sulfur	0.14	0.20
Potassium	0.14	0.20
Sodium	0.1	0.14
Chlorine	0.095	0.14
Magnesium	0.019	0.027
Total	69.874	99.537

Information based on reference 2 (modified).

where R is the residual mass of all elements present in amounts < 0.5% of Bwt (1).

Elemental analysis of humans is traditionally carried out in cadavers or in biopsy specimens from selected tissues and organs. In addition, the whole-body content of most major elements can now be measured directly *in vivo*: potassium by whole-body counting; sodium, chlorine, and calcium by delayed-γ neutron activation analysis (3); nitrogen by prompt-γ neutron activation analysis (1, 3); and carbon by inelastic neutron scattering (4). More than 98% of Bwt can now be reconstructed from elements that can be estimated *in vivo*, largely by neutron activation techniques. The atomic level is the foundation of body-composition analysis and is the starting point for the five levels we propose.

Molecular (II)

The 11 principal elements are incorporated into molecules that form > 100,000 chemical compounds found in the human body. These molecules range in complexity and molecular weight from water to deoxyribonucleic acid. It is neither useful nor possible to measure all of these chemical compounds individually in living humans. The alternative used in body composition research is to consider chemical compounds in categories of closely related molecular species. The major components in present use are water, or aqueous (A); lipid (L); protein (Pro); mineral (M); and glycogen (G) (**Table 2**). Because some confusion exists in these different categories, we now review the five chemical components in detail.

Water. The most abundant chemical compound in the human body is water, which comprises 60% of Bwt in the Reference Man (2).

Protein. The term protein in body-composition research usually includes almost all compounds containing nitrogen, ranging from simple amino acids to complex nucleoproteins. The most widely used representative stoichiometry for protein is $C_{100}H_{159}N_{26}O_{32}S_{0.7}$ with an average molecular weight of 2257.4 and density of 1.34 g/cm^3 at 37 °C (1, 5).

Glycogen. The primary storage form of carbohydrate is glycogen, which is found in the cytoplasm of most cells. The principal distribution is in skeletal muscle and liver, which contain ~ 1% and 2.2% of their respective wet weights in the form of glycogen (1, 2). The stoichiometry of glycogen is $(C_6H_{10}O_5)_x$, with an average density of 1.52 g/cm^3 at 37 °C (1, 2).

Table 2. Body composition on the molecular level (II) for the 70-kg Reference Man

Component	Amount	Percent of body weight
	kg	*%*
Water		
Extracellular	18	26
Intracellular	24	34
Lipid		
Nonessential (fat)	12	17
Essential	1.5	2.1
Protein	10.6	15
Mineral	3.7	5.3
Total	69.8	99.4

Glycogen, normally ≈ 400 g, is not included in the Reference Man. Information based on reference 2.

Mineral. The term mineral describes a category of inorganic compounds containing an abundance of metal elements (e.g., calcium, sodium, and potassium) and nonmetal elements (e.g., oxygen, phosphorus, and chlorine). Ash, a term similar to mineral, is the residue of a biological sample heated for a prolonged period to > 500 °C, and consists of the nonvolatile portion of mineral compounds. Total body ash is slightly lower in weight than mineral mass because of the loss of carbon dioxide from some carbonate groups and the release of tightly bound water during the heating period (1, 2). Mineral is usually divided into two subcategories: osseous and extraosseous. Osseous mineral, the largest component of which is calcium hydroxyapatite, $[Ca_3(PO_4)_2]_3Ca(OH)_2$, contains > 99% of total body calcium (TBCa) and ~86% of total body phosphorus in the Reference Man (2). Other elements, such as potassium, sodium, and chlorine. are primarily found in extraosseous mineral.

Lipid. Among the five principal chemical components on the molecular level, lipid is the most confusing because the terms lipid and fat are used interchangeably, even though strictly speaking they refer to different compartments. The traditional definition of lipid refers to a group of chemical compounds that are insoluble in water and very soluble in organic solvents such as diethyl ether, benzene, and chloroform (6, 7). About 50 different lipids are recognized

in humans, and these are divided by organic chemists into five subcategories: *1*) simple lipids (including triglycerides and waxes); *2*) compound lipids (e.g., phospholipids and sphingolipids); *3*) steroids; *4*) fatty acids; and *5*) terpenes (6).

The simple lipid, triglycerides, contains three fatty acids esterified to glycerol. The term fat is synonymous with triglycerides and therefore fat is clearly a subcategory of total lipid (6, 7). A common error is to confuse the terms fat and lipid, which can lead to errors in constructing models of body composition. In the adult, ~90% of total body lipid is fat (2).

Lipids can also be classified physiologically into two groups: essential (Le) and nonessential (Ln) (2). Essential lipids, such as sphingomyelin and phospholipids, serve important functions such as forming cell membranes. The nonessential lipids, largely in the form of triglycerides, provide thermal insulation and a storage depot of mobilizable fuel. About 10% of total body lipid is essential and 90% is nonessential in the Reference Man (2).

Although essential and nonessential lipids are structurally and physiologically different, their solubilities in organic solvents are similar and it is difficult to clearly separate them even *in vitro* (6, 7). An approximate separation can be accomplished by careful selection of the type of tissue analyzed, the extraction time and temperature, and particularly the type of solvent used (6). Solvents such as petroleum or ethyl ether are usually used alone to extract nonessential lipids, mainly the neutral fat or triglycerides. The remaining lipids, which are primarily essential, can be extracted by using binary or ternary solvent mixtures such as 45% chloroform, 10% methanol, and 45% heptane (8).

The fatty acid profile of triglycerides varies with diet, anatomic site, and other factors, but the generally accepted representative stoichiometry found in adult humans is $C_{51}H_{98}O_6$, with an average molecular weight of 806 and a density of 0.900 g/cm^3 at 37 °C (2). The stoichiometry of total lipid in humans could not be found in a review of previous studies.

The equation for Bwt as defined by the molecular level of body composition is

$$Bwt = L + A + Pro + M + G + R \qquad [2]$$

where R represents residual chemical compounds not included in the five main categories and that occur in quantities of < 1% of total Bwt (1).

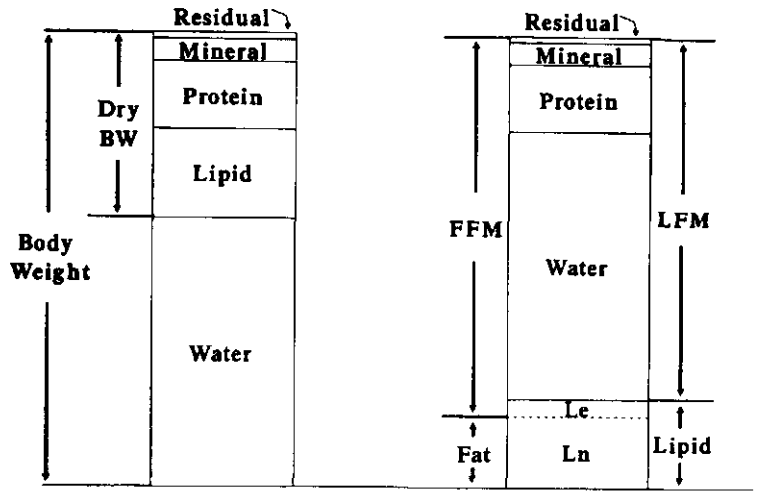

Figure 2. Body composition model on the molecular level (II). FFM, fat-free body mass; LBM, lipid-free body mass; and L_e and L_n, essential and nonessential lipids, respectively.

On the molecular level, three related equations can also be defined as follows. Dry Bwt consists of the anhydrous chemical components (**Figure 2**, left), and equation 2 can therefore be rewritten as

$$\text{Bwt} = \text{A} + \text{dry Bwt} \qquad [3]$$

Dry Bwt according to this equation is the sum of L + Pro + M + G + R.

$$\text{Bwt} = \text{L} + \text{lipid-free body mass} \qquad [4]$$

In equation 4 (**Figure 2**, right), lipid-free body mass is the material remaining after extraction of a whole-body homogenate with appropriate organic solvents and optimum conditions. Thus lipid-free body mass can be expressed as the combined weight of A + Pro + M + G + R.

As fat accounts almost entirely for total body nonessential lipid, then

$$\text{Bwt} = \text{fat} + \text{FFM} = \text{Ln} + \text{FFM} \qquad [5]$$

where FFM is fat-free body mass, which represents the combined weights of Le + A + Pro + M + G + R.

A similar term to fat-free body mass is LBM. The early definition of lean body mass suggested included at least five components: water, protein, mineral, glycogen, and an unspecified amount of essential lipid (9, 10). More recently, most investigators have used the terms LBM

and FFM interchangeably, although some debate still prevails about whether or not these are the same or different compartments. Our suggestion is that LBM and FFM henceforth be considered synonymous on the basis of the following reasoning.

In equation 4 we clearly define two fractions of Bwt, lipid and lipid-free body mass. The lipid fraction consists of two portions, essential and nonessential or fat. Accordingly,

$$\text{Body weight} = \text{Ln} + \text{LBM} = \text{fat} + \text{FFM} \qquad [6]$$

in which both LBM and FFM are the sum of essential lipid plus lipid-free body mass and the remaining Bwt is nonessential lipid or fat. All of the terms of the molecular level are consistent with each other when defined according to these guidelines and as shown in **Table 3.**

At present the main direct techniques available for estimating components on the molecular level are for water and mineral. TBW can be measured by several well-established isotope-dilution techniques (10, 11), and osseous mineral can be estimated by whole-body dual-photon absorptiometry (12). The remaining components of the model must be estimated indirectly by using measurements included in one of the other four levels. For example, protein can be determined from total body nitrogen at the atomic level by making two assumptions: that all of body nitrogen is in protein and that 16% of protein is nitrogen (1). Another example, total body fat can be calculated from body density, which is a dimension at the whole-body level, by assuming that fat and FFM have respective densities of 0.900 and 1.100 g/cm^3 (9, 10).

Table 3. Different body-composition terms on the molecular level (II)

	Lipids		Water	Protein	Mineral	Glycogen
	Ln	Le				
Body weight	×	×	×	×	×	×
Dry body weight	×	×		×	×	×
Lipid-free body mass			×	×	×	×
Fat-free body mass		×	×	×	×	×
Lean body mass		×	×	×	×	×

Ln, nonessential lipid or fat; and Le, essential lipid.

The molecular level of body composition is the conceptual foundation for the higher levels that follow. Also, the molecular level connects the study of body composition to other research areas, notably biochemistry.

Cellular (III)

Although the human body can be divided into different components at the molecular level, it is the assembly of these components into cells that creates the living organism. The coordinated functions and interactions between cells are central to the study of human physiology in health and disease. The cellular level is therefore an important area of body-composition research.

The human body is composed of three main compartments on the cellular level: cells, extracellular fluid, and extracellular solids. Each of these compartments is now described in additional detail.

Cells. The cells possess the characteristics of life including metabolism, growth, and reproduction. Although the 10^{18} cells of the adult human body share many properties in common, there are great variations in size, shape, elemental and molecular composition, metabolism, and distribution. Cells are adapted to specific functions, such as support, electrical conduction, and contraction. Based on these differences, four categories of cells can be defined: connective, epithelial, nervous, and muscular (13).

Connective cells include three groups: loose, dense, and specialized (13). Adipocytes, or fat cells, are a type of loose connective cell in which fat is stored. Bone cells, the osteoclasts and osteoblasts, and blood cells are representations of specialized connective cells.

Muscle cells include striated skeletal, smooth, and cardiac. The striated skeletal muscle cells are the foundation of human movement and account for a large fraction of body weight. Cells consist of fluid and solid components, the intracellular fluid and solids.

Extracellular fluid. The nonmetabolizing fluid surrounding cells that provides a medium for gas exchange, transfer of nutrients, and excretion of metabolic end products is referred to as the extracellular fluid.

Extracellular fluid, which is ~94% water by volume, is distributed into two main compartments: plasma in the intravascular space and interstitial fluid in the extravascular space. Plasma and interstitial fluid account for ~5% and 20% of Bwt in the Reference Man (2), respectively.

Extracellular solids. Extracellular solids are also a nonmetabolizing portion of the human body that consists of organic and inorganic chemical compounds. The organic extracellular solids include three types of fiber: collagen, reticular, and elastic (13). Both collagen and reticular fibers are composed of collagen protein whereas elastic fibers are formed from the protein elastin.

The inorganic extracellular solids represent ~65% of the dry bone matrix in the Reference Man (2). Calcium, phosphorus, and oxygen in bone are the main elements of the inorganic extracellular solids that are incorporated into calcium hydroxyapatite (1). Other inorganic components are also present in extracellular solids, including bicarbonate, citrate, magnesium, and sodium (1, 2).

From the previous discussion, the cellular level of body composition can be accurately described by the equations

$$\text{Bwt} = \text{CM} + \text{ECF} + \text{ECS} \qquad [7]$$

$$\text{CM} = \text{muscle cells} + \text{connective cells} + \text{epithelial cells} + \text{nervous cells} \qquad [8]$$

$$\text{ECF} = \text{plasma} + \text{ISF} \qquad [9]$$

$$\text{ECS} = \text{organic ECS} + \text{inorganic ECS} \qquad [10]$$

where CM is cell mass, ECF is extracellular fluid, ECS is extracellular solids, and ISF is interstitial fluid. However, because most components in equations 7-10 cannot be measured *in vivo* at present, the following equation is suggested as a practical alternative for Bwt at the cellular level

$$\text{Bwt} = \text{fat cells} + \text{BCM} + \text{ECF} + \text{ECS} \qquad [11]$$

where BCM is body cell mass. BCM is a portion of total cell mass that according to Moore et al (11) is the "working, energy-metabolizing portion of the human body in relation to its supporting structure". Hence, BCM includes the protoplasm in fat cells but does not include the stored fat, which occupies 85% to 90% of fat cell weight. Although no present method can directly measure BCM, it is a widely used term and is assumed to be represented by exchangeable or total body potassium (TBK) (11). A deviation must be noted in equation 11 in that BCM and fat cells share in common the nonfat portion of adipocytes and therefore overlap by ~1 kg in the Reference Man (2).

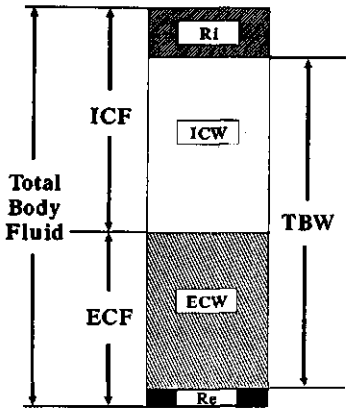

Figure 3. Relationship between body fluids. ECF, extracellular fluid; ECW, extracellular water; ICF, intracellular fluid; ICW, intracellular water; R_e and R_i, extracellular and intracellular residual; and TBW, total body water.

The fluid compartments at this level can also be related to TBW as shown in **Figure 3**. According to this model, ECW and ICW are extracellular and intracellular water, and Re and Ri are nonaqueous residual extracellular and intracellular solids.

Another relation at the cellular level is between total body mineral and inorganic solids (**Figure 4**). Each of the three components in equation 7 contribute to total mineral, inorganic cell and extracellular fluid residual, and the inorganic portion of extracellular solids.

Of the three primary compartments at the cellular level, the volume of extracellular fluid and its plasma subcompartment can be quantified directly by dilution methods (10). In contrast, no direct methods are yet available for estimating either cell mass or extracellular solids. Indirect methods of evaluating some compartments are available, such as extracellular solids estimated from TBCa measured by neutron-activation analysis (ECS = TBCa/0.177) (10). Another example is the calculation of BCM from TBK [BCM (in kg) = 0.00833 × TBK (in mmol)] (11).

Because the cellular level is the first level at which characteristics of the living organism appear, it occupies a central position in connecting the inanimate features of body composition

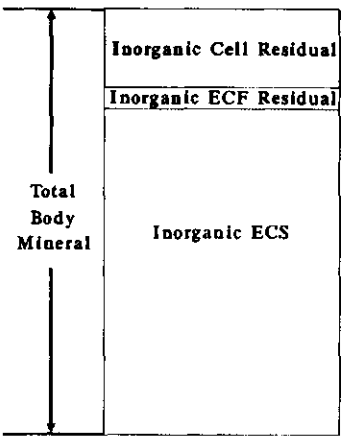

Figure 4. Relationship between total body mineral and inorganic solids. ECF, extracellular fluid; ECS, extracellular solids.

at the lower levels with those of the animate features of tissues, organs, and intact humans at the higher levels. Despite its importance in the study of human body composition, very little research has been directed at this level, perhaps because of the difficulty in quantifying some of the compartments.

Tissue-System (IV)

At the cellular level the human body is composed of cells, extracellular fluid, and extracellular solids. These three components are further organized into tissues, organs, and systems — the fourth level of body composition.

Tissues. Generally, tissues contain cells that are similar in appearance, function, and embryonic origin. All of the diverse tissues of the body can be grouped into four categories: muscular, connective, epithelial, and nervous (13).

Bwt at the tissue level of body composition is defined as

$$\text{Bwt} = \text{muscular tissue} + \text{connective tissue} + \text{epithelial tissue} + \text{nervous tissue} \qquad [12]$$

Three specific tissues are particularly important in body-composition research: bone, adipose, and muscular, which together comprise ~75% of Bwt in the Reference Man (2).

Bone is a specialized form of connective tissue that consists of bone cells surrounded by a matrix of fibers and ground substance. The distinguishing feature of bone is that the ground substance is calcified and accounts for ~65% of dry bone weight (2). The calcified ground substance is mainly hydroxyapatite, $[Ca_3(PO_4)_2]_3Ca(OH)_2$, and a small amount of calcium carbonate (14).

Adipose tissue is another type of connective tissue made up of fat cells (adipocytes) with collagenous and elastic fibers, fibroblasts, and capillaries. Adipose tissue can be divided into four types according to its distribution: subcutaneous, visceral (i.e., loosely surrounds organs and viscera), interstitial (i.e., intimately interspersed among the cells of organs), and yellow marrow (2). Muscle tissue can be subdivided into striated skeletal, smooth, and cardiac tissues (2).

Organs. The organs consist of two or more tissues combined to form large functional units such as skin, kidney, and blood vessels.

Systems. Several organs whose functions are interrelated constitute an organ system. For example, the digestive system is composed of many organs, including the esophagus, stomach, intestine, liver, and pancreas. Each organ, such as the stomach, contains several kinds of tissue (muscular, connective, epithelial, and nervous) and each tissue is made up of many cells and extracellular material.

There are nine main systems in the human body, hence Bwt at the system level of body composition can be defined as

$$\text{Bwt} = \text{musculoskeletal} + \text{skin} + \text{nervous} + \text{circulatory} + \text{respiratory} + \text{digestive} + \text{urinary} + \text{endocrine} + \text{reproductive systems} \quad [13]$$

Although Bwt can be expressed accurately on the tissue-system level, most components in equations 12 and 13 cannot be measured *in vivo* at present. The following equation is suggested as a practical alternative

$$\text{Bwt} = \text{adipose tissue} + \text{skeletal muscle} + \text{bone} + \text{viscera} + \text{blood} + \text{R} \quad [14]$$

where the five components account for 85% and R accounts for the remaining 15% of Bwt in the Reference Man (**Table 4**) (2).

Table 4. Body composition on the tissue-system level (IV) for principal tissues and organs of the 70-kg Reference Man

Tissue or organ	Amount	Percent of body weight
	kg	*%*
Skeletal muscle	28	40
Adipose tissue		
Subcutaneous	7.5	11
Visceral	5	7.1
Interstitial	1	1.4
Yellow marrow	1.5	2.1
Bone	5	7.1
Blood	5.5	7.9
Skin	2.6	3.7
Liver	1.8	2.6
Central nervous system	1.4	2
Gastrointestinal tract	1.2	1.7
Lung	1	1.4

Information based on reference 2 (modified).

The tissue-system level is complex and interfaces with several branches of human biology, including histology and histochemistry at the tissue level and anatomy and physiology at the organ and system level. Physicians, nutritionists, and exercise physiologists focus much of their interest in body composition at the tissue-system level.

Although a great deal of information is available at this level, most of it comes from cadaver studies or tissue biopsies. There are only a few *in vivo* direct methods that can be used to estimate the major compartments at the tissue-system level. An example is computerized axial tomography, which can directly determine the volume of subcutaneous and visceral adipose tissue (15). Some indirect techniques are also available at this level, such as estimation of skeletal muscle mass from 24-h urinary creatinine excretion or from TBK and nitrogen content by neutron-activation analysis (16, 17).

Whole body (V)

Both humans and some primates have similar body compositions at the atomic, molecular, cellular, and tissue-system levels. It is at the whole-body level, however, with its complex characteristics that distinguishes humans from all other primates. In addition, many biological, genetic, and pathological processes have an impact not only at the first four levels but also on the human body as a whole.

The whole-body level of body composition concerns body size, shape, and exterior and physical characteristics. There are ≥ 10 suggested dimensions at the whole-body level (18).

1) Stature: This is a major indicator of general body size and skeletal length.
2) Segment lengths: Many segment lengths are used in the study of body composition, the most common of which are lower extremity length, thigh length, calf length, shoulder-elbow length, and elbow-wrist length.
3) Body breadths: Body breadths are a measure of body shape, skeletal mass, and frame size. The sites most widely used are the wrist, elbow, ankle, knee, and biiliac.
4) Circumferences: The circumferences are useful indicators of body density, FFM, adipose tissue mass, total body protein mass, and energy stores. The most widely used circumferences are upper arm, waist (abdominal), and thigh.
5) Skinfold thickness: Skinfolds represent a double layer of adipose tissue and skin at specific anatomic locations. Triceps, subscapular, calf (medial), and abdominal are the most commonly used sites. Skinfold thickness provides a simple method of estimating fatness and the distribution of subcutaneous adipose tissue. Numerous equations for the prediction of body fat have been developed that make use of skinfold thickness.
6) Body surface area (BSA): The total BSA is an exterior characteristic that is often used to estimate basal metabolic rate and FFM.
7) Body volume: The total body volume is an important indicator of body size and is used to calculate body density.
8) Bwt: One of the simplest and most important morphologic indicators, Bwt is used in screening for growth rate, obesity, and undernutrition. The Bwt equation that defines the whole-body level is

$$\text{Bwt} = \text{head weight} + \text{neck weight} + \text{trunk weight} + \text{lower extremity weights} + \text{upper extremity weights} \quad [15]$$

9) Body mass index: Bwt and stature can be combined to form indices that correlate with total body fat. The best known of the indices is body mass index (body weight/stature^2, in kg/m^2), which is often used in obesity studies as a measure of fatness (19). However, more complex and population-specific indices, such as the Fels index (Bwt$^{1.2}$/stature$^{3.3}$), often correlate better with total body fat (18).

10) Body density: The density of the human body, derived from Bwt and volume, is widely used to indirectly estimate total body fat and FFM (9, 10) and is defined at the molecular level as

$$1/D_b = f_{fat}/D_{fat} + f_{FFM}/D_{FFM} \qquad [16]$$

where D_b, D_{fat}, and D_{FFM} are the densities (in g/cm^3) of the total body, fat, and fat-free body, respectively, and f represents the fractions of Bwt as fat and FFM, respectively (20). Similar equations for total body density based on individual components at the cellular, tissue-system, and whole-body levels can also be written.

It is clear that any major changes in body composition on the other four levels will manifest themselves on the whole-body level. Conversely, most differences at the whole-body level are related to changes in composition on the other four levels. This latter relation is the foundation for estimating the components of the other four levels by using measurements at the whole-body level. Most indicators at the whole-body level are simpler and easier to perform than are measurements at the other four levels, thus the techniques at this level are often well suited for large-scale studies or for field work.

FEATURES OF THE MODEL

The five-level model provides a structural framework for studying human body composition that goes beyond an individual compartment or level. In this section we describe some of the features of the five-level model as a whole.

Distinctions and Connections Between Different Levels

An essential aspect of the model is that the levels themselves are distinct and have unique properties that should not be confused with one another.

1) On the atomic level, there are no special elements or any fundamental differences between the human body and the inorganic world, although the ratio of elements to each other varies.
2) On the molecular level, the human body is differentiated from the inorganic world because of the appearance of complex organic compounds such as lipid and protein.
3) On the cellular level, the human body is distinct from the nonliving world because of the appearance of cells that have the characteristics of living organisms.
4) On the tissue-system level, the human body is different from the lower animal world because of the appearance of tissues, organs, and systems having complex structures and functions.
5) On the whole-body level, the human body is differentiated from all other primates because of the presence of distinct morphological features.

Although these distinct properties exist for each of the five levels, linkages are also present that are clearly recognizable in the context of the five-level model. An example is that cells that appear first on the cellular level have many of the characteristics of living organisms such as membrane transport, energy metabolism, and enzymatic processes. These characteristics of the cell are still maintained at the tissue-system and whole-body levels. Each higher level is thus unique but maintains some of the characteristics of the level below it.

Recognition of distinct levels and their connections can reveal gaps in present body composition information and suggest a direction for future research efforts. For example, it is known that many biological factors including growth, development, senescence, race, sex, nutritional status, exercise level, and the presence of disease all have important effects on body composition. However, most studies of body composition in these areas are limited in scope, focusing on only a few components at one or two levels and thereby failing to appreciate the connections between levels. For example, most previous obesity studies were limited to anthropometric changes (at the whole-body level) and alterations in fat mass (at the molecular level). Very few studies have investigated how obesity influences the other levels of body composition or more importantly the coordinated changes that occur throughout all five levels with increasing Bwt.

Table 5. Some related but distinct components on different levels

Atomic level	Molecular level	Cellular level	Tissue-system level	
			Tissue level	Organ level
Total body calcium and phosphorus	Mineral	Extracellular solids	Bone	Skeleton
Total body carbon	Lipid and fat	Fat cells	Adipose tissue	
		Skeletal muscle cells	Skeletal muscle tissue	Skeletal muscle

Distinctions and Connections Between Different Components

An important feature of the model is that every major component has a clear definition and can be included in one of the five levels. Each of these components has unique properties and yet maintains relationships with other components at the same and different levels.

It was not unusual in earlier studies for related components to be confused with each other, particularly if they were on different levels. An example of three sets of commonly confused components is presented in **Table 5**. In the first set, TBCa and phosphorus, mineral, extracellular solids, bone tissue, and skeleton, are related compartments but belong to different levels and have distinct differences from each other:

1) Calcium and phosphorus, and mineral: Most of TBCa and phosphorus exist in mineral although there is some phosphorus in protein and lipid (e.g., DNA, RNA, and phospholipids). On the other hand, in addition to calcium and phosphorus, mineral contains other elements (e.g., carbon, oxygen, hydrogen, magnesium, and sodium).
2) Mineral and extracellular solids: Most of total body mineral is in extracellular solids although there still is a small amount of mineral in cells and extracellular fluid. On the other hand, in addition to the mineral in the form of inorganic material, extracellular solids contain organic solids such as collagen, reticular fibers, and elastic fibers.
3) Extracellular solids and bone tissue: Most of total body extracellular solids are in the form of bone tissue although there still is a small amount of extracellular solids in other tissues

(e.g., in skeletal muscle). On the other hand, in addition to extracellular solids, bone tissue contains bone cells and extracellular fluid.

4) Bone tissue and skeleton: Bone tissue constitutes the majority of the skeleton although the latter also includes skeletal cartilage, periarticular tissue adhering to joints, and red and yellow marrow.

Another example of related but distinct components is total body carbon (level I), lipid and fat (level II), fat cells (level III), and adipose tissue (level IV) (**Table 5**). These terms are often confused with each other, a problem that the five-level model helps to resolve.

The third and final example in the table is the distinctly different but related components skeletal muscle cells, skeletal muscle tissue, and intact whole skeletal muscles. The model thus demonstrates that differences and relations exist between components on each of the five levels. It is therefore advisable to develop equations for body weight, volume, or density that include components from the same level in order to avoid overlap or omission of some components.

Steady-State of Body Composition

The concept of a steady-state is important not only in biochemistry, physiology, and other classic scientific disciplines but also in body-composition research. The meaning of a steady state of body composition can be defined in the context of the five-level model: A steady-state or dynamic homeostasis exists during a specified time period if Bwt and the mass of various components on different levels is maintained relatively constant.

The important implication of a steady-state is that there are stable proportions among the different components on the same level. For example, on the molecular level the average ratio of total body water content to FFM is relatively constant in healthy subjects (i.e., total body water/FFM = 0.732) (2). On the atomic level the correlation between TBK and TBCa is reproducible for males [i.e., TBK(g) = 0.1383 × TBCa(g) − 17.1] (21). On the whole-body level the relation between BSA (BSA, in m^2) and Bwt (kg) and stature (in m) is also relatively constant such that BSA = 0.007184 × $\text{stature}^{0.725}$ × $\text{body weight}^{0.425}$ (22).

There are also relatively constant proportions among the relevant components on different levels when body composition is in a steady-state. For example, total body protein/total body

nitrogen = 6.25 (1); BCM (kg)/TBK (mmol) = 0.00833 (11), and fat (kg) = [(4.95/Db) − 4.50] × Bwt (20).

The steady state of body composition indicates that although there are so many components in the human body, and all of these components differ from each other, they are well organized according to definable quantitative relations.

Quantitative Body Composition Relations

A primary aim of body-composition research is to estimate the size of each compartment, although there are numerous individual compartments of clinical relevance that have not been measured directly. An alternative is to estimate the unknown components by establishing relationships to measurable components. Body composition is relatively stable in healthy adults, and it is this property that enables investigators to establish these reproducible relations or rules. The five-level model of body composition affords a logical matrix within which to establish the quantitative steady-state relations between known measurable components and presently unmeasurable compartments.

Present research in developing body-composition equations primarily involves estimating one unknown component from a measurable component. The five-level model suggests the possibility of reconstructing Bwt and volume by writing simultaneous equations that exploit steady-state relations between several measurable and unknown components. An example is the calculation of the five major chemical components and Bwt at the molecular level from six elements (carbon, nitrogen, sodium, potassium, chlorine, and calcium) measured by *in vivo* neutron activation analysis (1). Until recently the concept of reconstructing whole levels of body composition from multiple components was limited and the studies were fragmentary. The five-level model defines explicitly the equations for Bwt at each level and presents the challenge of developing more complex and comprehensive body-composition equations.

Relation to Methodology

At present, body-composition methods are primarily categorized into technique-specific groupings such as dilution methods and neutron-activation analysis. According to the five-level model, however, the methods can be organized in a more systematic fashion.

Direct measurement methods. There are some direct methods, such as anthropometric, biochemical, and radioisotopic techniques that can be used to estimate components of body composition. Direct methods can be organized according to the five-level model as follows:

1) On the atomic level, TBK can be directly determined by whole-body ^{40}K counting (12); total body sodium, chlorine, phosphorus, and calcium by delayed-γ neutron activation (3); total body nitrogen by prompt-γ neutron activation (23); and total body carbon by inelastic neutron scattering (4).
2) On the molecular level, TBW can be directly estimated by several isotope-dilution techniques (10), and osseous mineral can be quantified by dual-photon absorptiometry (24).
3) On the cellular level, extracellular fluid (and plasma volume) can be directly determined by several isotope-dilution techniques (25).
4) On the tissue-system level, the volumes of subcutaneous and visceral adipose tissue can be directly determined by computerized axial tomography and by magnetic resonance imaging techniques (26).
5) On the whole-body level, anthropometric indices such as Bwt, body volume, stature, circumferences, and skinfold thickness can be estimated directly (18).

According to this analysis there are not many direct methods used in the study of human body composition. Moreover, most of the direct methods are concentrated on the atomic and whole-body levels. There are only a few direct techniques on the molecular, cellular, and tissue-system levels.

Indirect measurement methods. These estimate unknown components of body composition by combining direct measurement techniques with the established steady-state relationship between the directly measurable and unknown components. Indirect methods greatly expand the number of body compartments that can be evaluated. At present, some important compartments can be assessed only by indirect methods. For example, although total body fat is a major compartment of interest, there are no practical methods of directly evaluating the fat compartment *in vivo*. All of the presently used methods are indirect and based on direct measurements at different levels as follows:

1) from direct method on the atomic level (10), fat = Bwt – TBK (mmol)/68.1;
2) from direct method on the molecular level (2, 10), fat = Bwt – TBW/0.732;

Table 6. The relation between direct and indirect body-composition measurements organized by the five-level model

Direct	Indirect				
	Atomic level	**Molecular level**	**Cellular level**	**Tissue-system level**	**Whole-body level**
Atomic level TBO, TBC, TBH, TBN, TBK, TBNa, TBP, TBCl, Na_e, K_e	TBP = (0.456 × TBCa) + (0.555 × TBK)	Pro = 6.25 × TBN FFM = TBK/68.1	BCM = 0.00833 × TBK ECS = TBCa/0.177 ECF = (0.9 × TBCl)/Plasma Cl	SM = 0.0196 × TBK − 0.0261 × TBN	Bwt = O + C + H + N + Ca + P + K + Na + Cl + R
Molecular level TBW, mineral, creatinine, 3-MH		FFM = TBW/0.732 FFM = 24.1 × Cr + 20.7		SM = 11.8 × Cr + 10.1	Bwt = L + A + Pro + M + G + R
Cellular level ECF, plasma volume					Bwt = CM + ECF + ECS
Tissue-system level Volume of visceral and subcutaneous adipose tissue					Bwt = adipose tissue + skeletal muscle + bone + viscera + blood + R
Whole-body level Bwt, circumference, S, BV, skinfold	TBK = (27.3 × Bwt) + (11.5 × S) − (21.9 × Age) + 77.8	fat % = (4.95 × BV/Bwt − 4.5) × 100	ECF = 0.135 × Bwt + 7.35	SM = S × (0.0553 × CTG^2 + 0.0987 × FG^2 + 0.0331 × CCG^2) − 2445	Body surface = $0.007184 \times S^{0.725} \times BW^{0.425}$

A, water (kg); BCM, body cell mass (kg); BV, body volume (l); Bwt, body weight (kg); CCG, corrected medial calf girth (cm); CM, cell mass (kg); Cr, 24-h urine creatinine (g); CTG, corrected thigh girth (cm); ECF, extracellular fluid (kg); ECS, extracellular solids (kg); FFM, fat-free body mass (kg); FG, forearm girth (cm); G, glycogen (kg); K_e, exchangeable potassium; L, lipid (kg); M, mineral (kg); 3-MH, 24-h urine 3-methylhistidine; Na_e, exchangeable sodium; plasma Cl, plasma concentration of chlorine (mmol/l); Pro, protein (kg); R, residual (kg); S, stature (cm); SM, skeletal muscle (kg); TB, total body element (kg); and TBW, total body water (kg).

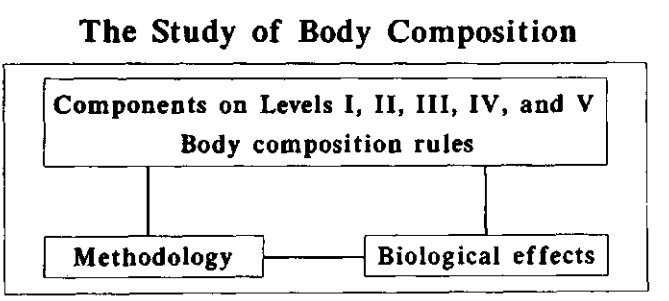

Figure 5. The three areas of body composition research.

3) from direct method on the molecular and whole-body levels, fat = 2.057 × BV − 0.786 × TBW −1.286 × Bwt, where BV is body volume in liters (20); and

4) from direct methods on the whole-body level, fat = 4.95 × BV − 4.50 × Bwt, and fat = 0.715 × Bwt − 12.1 × stature^2 (in m) (19, 20).

Thus it can be seen that indirect methods are not only based on the direct methods, but also are dependent on the steady-state proportions between known and unknown components as determined in sample populations.

Direct and indirect body-composition methodology can be outlined according to the five-level model as shown by the examples presented in **Table 6**. The table demonstrates that most of the principal elements and anthropometric indices can be directly measured and that many of the indirect methods have been developed from the direct methods on the atomic and whole-body levels, respectively (10, 17, 22, 25, 27-29). Conversely, the table shows that there are only a few direct methods on the cellular and tissue-system levels, so the relevant indirect methods are also very limited. This is one of the weak areas in body-composition methodology and could constitute an important topic for future research.

Definition of Body Composition Research

The study of body composition spans > 100 years, and the term body composition is widely used. However, it is unclear what this branch of science represents and what exactly is meant

by the term body composition. The five-level model presented in this paper not only builds an appropriate structure for body-composition research, but is conducive to clearly define human body composition as a branch of human biology that studies various body compartments and their quantitative steady-state relations or rules. Body-composition research includes three interconnecting areas: studying the proportions of various components and their steady-state associations among the atomic, molecular, cellular, tissue-system, and whole-body levels; studying the methods of measuring various components *in vivo*; and studying the influences of biological factors on various levels and components (**Figure 5**).

Conclusion

The five-level model grows from a need to organize both the rapidly developing methodologies and physiological concepts that relate to the study of human body composition. The model is intended to be a foundation on which future studies can refine or expand selected definitions or equations. The five-level model serves in this organizational capacity and also stimulates a broader view of body-composition research as a whole.

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CHAPTER 3

PROPORTION OF ADIPOSE TISSUE-FREE BODY MASS AS SKELETAL MUSCLE IN MEN: MAGNITUDE AND CONSTANCY

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ABSTRACT

Although it is often assumed that skeletal muscle (SM) is a fixed proportion of adipose tissue-free body mass (ATFM), there is little data in living humans that supports this hypothesis. Limited data from elderly cadavers suggests a relatively constant proportion of ATFM as SM in men (mean ± SD, 0.540 ± 0.046) and women (0.489 ± 0.049). The present study tested the hypothesis that SM is a relatively constant proportion of ATFM in healthy men. Whole body SM and ATFM were measured using multiscan computerized axial tomography. The SM/ATFM ratio in the men (n = 24) was 0.563 ± 0.036 which was not significantly different from the value found in the elderly male cadavers. Multiple regression analysis indicated that the SM/ATFM ratio was significantly related to 1/ATFM (P = 0.036) and body mass index (P = 0.024) and not with age. Further study is needed to find other possible factors such as physical activity, gender, race, and disease state, that may effect the relation between SM and ATFM.

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INTRODUCTION

The study of quantitative relationships between body components is an important task in body composition research (Wang et al., 1992). Several relatively stable relationships are now known to exist between components at the atomic, molecular, and cellular body composition levels. For example, the ratio of nitrogen to total body protein is relatively constant (0.161); the proportions of fat-free body mass as water and potassium are 0.732 and 0.00266 (kg/kg), respectively; and the ratio of potassium to body cell mass is stable at 0.00469 (kg/kg). These constant ratios and proportions provide not only an understanding of quantitative human body composition rules, but also provide the possibility of developing new methods of assessing an unknown component from a known component (Wang et al., 1995).

As the largest component at the tissue-system level of body composition in adults, skeletal muscle plays a central role in many biochemical and physiological processes. However, there are few studies that examine the quantitative relationships between skeletal muscle and other components. Although previous investigations expressed total body skeletal muscle mass (SM) as a proportion of body weight (BW), it is known that the SM/BW ratio is highly variable due to different degrees of adiposity between subjects.

In order to eliminate the confounding effect of adiposity on the SM/BW ratio, Clarys and his colleagues (1984, 1985) first introduced the concept of adipose tissue-free body mass (ATFM) defined as "total body mass less all dissectible adipose tissue". Based on 6 male and 7 female unembalmed cadavers in the Belgian Study, Clarys and colleagues (1984) calculated the SM/ATFM ratios (mean ± SD) as 0.540 ± 0.046 (range 0.49 - 0.59) for men and 0.489 ± 0.049 (range 0.42 - 0.55) for women, respectively. The corresponding SM/BW ratios were 0.388 ± 0.064 (range 0.27 - 0.46) for men and 0.277 ± 0.048 (range 0.19 - 0.33) for women. The variation of the SM/ATFM ratio is much smaller than that of the SM/BW ratio in both men and women. A comparable ratio, SM/fat-free body mass (0.49), was suggested by Forbes (1987) as sufficiently stable to offer a means of predicting SM from measured fat-free body mass.

Although the concept of adipose tissue-free body mass effectively eliminates the confounding effect of adiposity, fundamental questions remain unanswered. First, the proportion of ATFM as SM was only determined on a few elderly cadavers (age, 68 ± 10 yr for men, and 80 ± 7 yr for women). The magnitude of the SM/ATFM ratio in healthy adults is still unknown.

Moreover, studies in living humans by Cohn et al. (1980) suggested that SM is reduced in elderly subjects to a greater relative extent than overall fat-free body mass. An age-related selected loss of skeletal muscle is clinically important and of basic research interest, although the results of Cohn and colleagues remain uncertain due to methodological limitations (Wang et al., 1996).

Second, the proportion of ATFM as SM observed in the Belgian Study's elderly cadavers is moderately variable [coefficient of variation (CV, SD/mean) = 8.5% for the men, and CV = 10% for the women]. It is unclear if there is similar between-subject variation in the SM/ATFM ratio in healthy adults. The information of magnitude and constancy of the SM/ATFM ratio is necessary because of it's central role in SM prediction from measured ATFM.

The present study tested the hypothesis that SM is a relatively constant proportion of ATFM. Specifically, the present investigation studied the magnitude and constancy of the proportion of ATFM as SM in healthy male adults. Until recently, autopsy was the only means of studying the relationship between skeletal muscle mass and adipose tissue-free body mass in humans. Development of computerized axial tomography (CT) in the past decade provides an opportunity to accurately measure total body skeletal muscle, adipose tissue, and ATFM *in vivo* (Sjöström, 1991; Chowdhury et al., 1994). The availability of the multiscan CT technique prompted the present study that measured SM and ATFM in healthy humans. The published data from male cadavers in the Belgian Study allowed us to compare and contrast our results in healthy male adults to those obtained in a previously described elderly cohort (Clarys et al., 1984).

METHODS

Subjects

Twenty four healthy male adults were recruited from hospital staff and local university students. Routine blood analysis, including liver tests, hematological indices, and blood electrolytes confirmed the health status of each subject prior to entry into the protocol. During the study none of the subjects were on any medications or engaged in exercise training programs. The procedures were fully explained to the subject and an informed consent was

signed. The project was approved by the Institutional Review Board of St. Luke's-Roosevelt Hospital Center.

Some subjects in the present study participated in earlier reported studies of body composition (Wang et al., 1996). The observations described in the present paper were not included in earlier reports.

Multiscan Computerized Axial Tomography

The full procedure included 22 cross-sectional images produced by a Somatom DRH CT scanner (Siemens Corporation, Erlangen, Germany) at anatomic sites described by Sjöström (1991). The distances between images were obtained from the CT frontal plane scanogram to the nearest millimeter. Subjects were placed on the CT scanner bed with their arms extended above their heads. Images were made at 125 kVp, a scan time of 4 secs at 170 mA, and a slice thickness of 4 mm. The total effective CT dose was 2 - 4 mSv.

An observer (ZMW) familiar with cross-sectional anatomy evaluated scans as previously reported (Wang et al., 1996). Skeletal muscle mass were estimated with CT as both anatomic SM and adipose tissue-free SM. The difference between the two SM measures is that the former includes small amounts of intramuscular adipose tissue. In an early investigation the cross-sectional SM area was measured using the attenuation range between −29 and +120 Hounsfield units (HU), which includes mainly adipose tissue-free SM (Wang et al., 1996). In order to maintain consistency with anatomic SM as measured in the Belgian Cadaver Study, the present investigation measured the cross-sectional SM area by using the attenuation range between −190 and +120 HU. Measured anatomic skeletal muscle area therefore included the intramuscular adipose tissue pixels that range between −190 and −30 HU. Total body anatomic skeletal muscle mass was calculated using previously reported formulas (Sjöström, 1991; Chowdhury et al., 1994).

The cross-sectional area of adipose tissue was measured using the pixels that ranged between −190 and −30 HU; and total body adipose tissue mass (AT, in kg) was measured as previously reported (Sjöström, 1991; Chowdhury et al., 1994). Adipose tissue-free body mass was calculated as body weight minus total body adipose tissue mass.

Statistical Analysis

Group results are presented as mean, standard deviation (SD), and coefficient of variation (CV, SD/mean). Differences between young adults and elderly cadavers were tested by Student's T-test. A P value of < 0.05 was considered statistically significant.

Simple and multiple regression analysis was used with the SM/ATFM ratio as the dependent variable and ATFM, age, body weight, height, and body mass index (BMI) as independent variables. Differences in slope and intercept between regression lines were tested according to Kleinbaum and Kupper (1978). The Student's T-test was taken for the partial correlation coefficients of the multiple regression equation.

RESULTS

The baseline characteristics of the 24 subjects are presented in **Table 1**. The data for the 6 unembalmed male cadavers from the Belgian Study are also included in **Table 1**. There was a significant between-group difference in age ($P < 0.001$), but there were no significant differences in body weight, height, and BMI between the two groups.

Body Composition Analysis

Total body skeletal muscle mass, adipose tissue, and adipose tissue-free body mass is presented in **Table 1**. The young men had a mean of 12.0 ± 7.9 kg adipose tissue which was significantly less than that of 19.9 ± 5.9 kg in the elderly men ($P = 0.012$). Adipose tissue-free skeletal muscle mass was 33.7 ± 5.9 kg (range 23.2 - 46.6 kg) in the young subjects. Anatomic skeletal muscle mass was 35.4 ± 6.4 kg (range 24.2 - 48.3 kg) in the young subjects and 27.9 ± 9.2 kg (range 15.8 - 40.4 kg) in the elderly cadavers, respectively ($P = 0.11$).

Although the elderly male cadavers weighed on average only 3.9 kg less than the young men, the difference in average ATFM between the two groups is large (11.8 kg). Moreover, the 7.5 kg difference in skeletal muscle between the two groups accounted for a large portion (64 %) of the between-group difference in ATFM.

Relationship Between Skeletal Muscle Mass and ATFM

The proportion of body weight as anatomic skeletal muscle was 0.475 ± 0.040 and 0.388 ± 0.064 for the young adults and elderly cadavers, respectively ($P = 0.010$).

Table 1. Body composition analysis of the study subjects

	Age yr	BW kg	Ht m	BMI kg/m^2	AT kg	ATFM kg	ATFSM kg	SM kg	SM/ATFM
Young adults									
1	28	86.7	1.93	23.3	19.4	67.3	36.4	38.8	0.577
2	26	106.7	1.87	30.5	29.0	77.7	42.2	46.1	0.593
3	20	75.9	1.81	23.2	5.9	70.0	36.4	37.7	0.539
4	41	82.3	1.82	24.8	13.1	69.2	37.3	39.1	0.565
5	33	89.8	1.82	27.1	20.8	69.0	38.3	40.6	0.588
6	33	53.8	1.62	20.5	6.4	47.4	27.0	28.1	0.593
7	30	58.1	1.63	21.9	5.0	53.1	24.3	24.9	0.512
8	22	70.7	1.72	23.9	16.7	54.0	28.7	31.1	0.576
9	48	91.1	1.90	25.2	7.3	83.8	46.6	48.3	0.576
10	32	80.0	1.74	26.4	11.8	68.2	36.8	38.8	0.569
11	26	78.2	1.82	23.6	9.1	69.1	36.9	38.5	0.557
12	37	61.0	1.69	21.4	7.9	53.1	27.9	29.2	0.550
13	28	93.7	1.80	28.9	33.3	60.4	33.5	37.0	0.613
14	23	61.0	1.71	20.9	9.5	51.5	27.0	28.7	0.557
15	34	79.9	1.77	25.5	11.9	68.0	37.0	38.8	0.571
16	20	61.5	1.75	20.1	9.0	52.5	27.0	27.4	0.522
17	39	64.3	1.83	19.2	4.5	59.8	32.3	33.4	0.560
18	38	72.7	1.70	25.2	7.6	65.1	36.5	37.6	0.635
19	26	57.0	1.77	18.2	2.7	54.3	31.4	31.8	0.586
20	19	65.4	1.70	22.6	9.3	56.1	30.0	31.3	0.558
21	45	93.6	1.81	28.6	22.3	71.3	41.0	43.3	0.607
22	33	77.2	1.77	24.6	12.1	65.1	36.1	37.9	0.582
23	47	55.9	1.70	19.3	4.2	51.7	23.2	24.2	0.468
24	32	73.8	1.85	21.6	8.9	64.9	34.1	35.8	0.550
mean	31.7	74.6	1.77	23.6	12.0	62.6	33.7	35.4	0.567
± SD	8.4	14.2	0.08	3.2	7.9	9.3	5.9	6.4	0.035
Elderly cadavers									
1	65	54.8	1.66	19.9	9.7	45.1		23.3	0.517
2	59	76.8	1.73	25.7	20.8	56.0		31.2	0.557
3	81	61.0	1.77	19.5	17.0	44.0		21.8	0.495
4	73	85.1	1.72	28.8	25.7	59.4		34.8	0.586
5	73	57.7	1.64	21.5	25.3	32.4		15.8	0.488
6	55	88.9	1.87	25.4	20.8	68.1		40.4	0.593
mean	67.7**	70.7	1.73	23.5	19.9*	50.8		27.9	0.540
± SD	9.7	14.8	0.08	3.7	5.9	12.8		9.2	0.046

Student T-test between the young adults and the elderly cadavers, * $P < 0.05$; ** $P < 0.001$.
AT, adipose tissue; ATFM, adipose tissue-free body mass; ATFSM, adipose tissue-free skeletal muscle; SM, anatomic skeletal muscle.

The proportion of ATFM as anatomic SM for the young adults was 0.563 ± 0.036 (range 0.468 - 0.613) with a CV of 6.4%. This proportion was 0.540 ± 0.046 (range 0.488 - 0.593) with a CV of 8.5% for the elderly male cadavers. There was no significant difference in the SM/ATFM ratio between the two groups (P = 0.29). The mean proportion of ATFM as SM for the pooled group was 0.558 ± 0.038 (CV = 6.8%). Because anatomic SM is slightly larger than adipose tissue-free SM, the anatomic SM/ATFM ratio observed in the present study (0.563 ± 0.036) is slightly larger than that reported in our previous study (0.534 ± 0.031) (Wang et al., 1996).

Anatomic SM (in kg) and ATFM (in kg) were highly correlated in the both groups (**Figure 1**),

SM = 0.657 × ATFM – 5.8; r = 0.958, $P < 0.0001$; SEE = 1.9 kg;

n = 24 young adults. *[1]*

and SM = 0.713 × ATFM – 8.4; r = 0.996, $P < 0.0001$; SEE = 1.0 kg;

n = 6 elderly cadavers. *[2]*

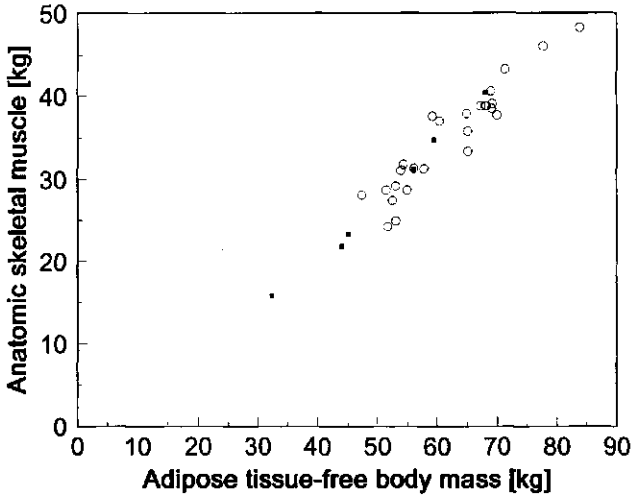

Figure 1. Total body anatomic skeletal muscle measured by CT (SM, in kg) on the ordinate; adipose tissue-free body mass measured by CT (ATFM, in kg) on the abscissa [SM = 0.657 × ATFM – 5.8; r = 0.958, $P < 0.0001$; SEE = 1.9 kg, n = 24 for young adults (○); SM = 0.713 × ATFM – 8.4; r = 0.996, $P < 0.0001$; SEE = 1.0 kg, n = 6 for elderly cadavers (■); and SM = 0.666 × ATFM – 6.3; r = 0.974, $P < 0.0001$; SEE = 1.7 kg; n = 30 for the combined group].

Because slope and intercept of equations 1 and 2 did not differ (P = 0.97), the data for the two group were combined,

SM = 0.666 × ATFM – 6.3; r = 0.974, P < 0.0001; SEE = 1.7 kg; n = 30. *[3]*

The slope and intercept of equation 3 is significantly different from one and zero, respectively (both P <0.01).

As equation 3 has a non-zero intercept, this implies that the SM/ATFM ratio is not constant. Simple regression analysis indicates that the proportion of ATFM as SM was highly correlated with the reciprocal of ATFM,

SM/ATFM = 0.665 – 6.20/ATFM; r = 0.61, P < 0.001; n = 30. *[4]*

In equation 4, both the intercept and slope are significantly different from zero and one, respectively. Equation 4 indicates that the SM/ATFM ratio is a reciprocal function of adipose tissue-free body mass. When ATFM increases within the normal range (e.g., 40 - 80 kg), the proportion of ATFM as SM increases from 0.51 to 0.59 (**Figure 2**).

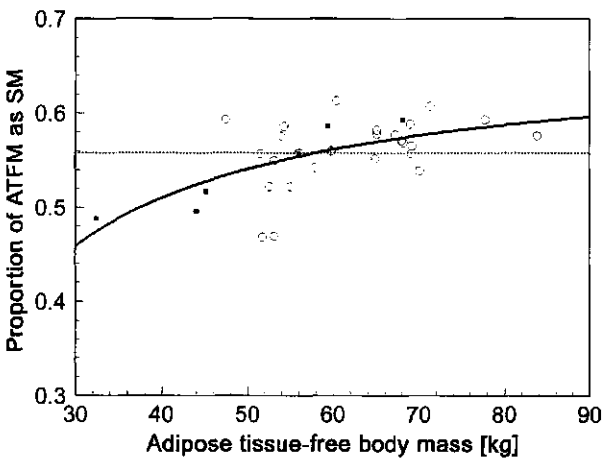

Figure 2. Relationships between proportion of ATFM as anatomic SM and adipose tissue-free body mass (kg). The horizontal line (-----) is the assumed constant SM/ATFM ratio of 0.558; and the reciprocal curve (—) is based on equation 4 (SM/ATFM = 0.665 – 6.20/ATFM); O represents young adults, and ■, elderly cadavers.

Multiple regression analysis further indicates that BMI also influences the proportion of ATFM as SM,

$$SM/ATFM = 0.512 + 0.00473 \times BMI - 3.84/ATFM;\ r = 0.69,\ P < 0.001;\ n = 30. \quad [5]$$

In equation 5 the P values of all partial correlation coefficients are < 0.05. Age, body weight, and height did not significantly influence the SM/ATFM ratio.

DISSCUSION

The current study results indicate that skeletal muscle is not a constant proportion of adipose tissue free-body mass. Specifically, we found in both young adults and elderly cadavers that a plot of SM versus ATFM had a non-zero intercept and that the SM/ATFM ratio was related to both ATFM and BMI. Other factors such as physical activity, gender, race, and diseases may also influence the relationship between SM and ATFM and these are important concerns for future studies.

Equation 4 is a reciprocal function, rather than a linear equation. Assuming ATFM is 40 kg, equation 4 indicates that the SM/ATFM ratio is 0.51. When ATFM increases to 80 kg, the SM/ATFM ratio increases to 0.59 (**Figure 2**). This indicates that if ATFM is higher for some reason, for example in obesity, SM/ATFM ratio would also be higher. Conversely, when ATFM is lower for some reason, as for example with starvation, the SM/ATFM ratio would be lower. This suggests that skeletal muscle mass can be altered easier than other lean tissues and organs.

As multiple regression analysis showed, BMI also contributed significantly to the SM/ATFM ratio after controlling first for 1/ATFM. From equation 5, one can find that when ATFM is held constant and BMI increases from 25 to 30 kg/m^2, the proportion of ATFM as anatomic SM would increase by 2.1%. This might be explained by an expansion of intramuscular adipose tissue as whole body adiposity increases.

The relationship between age and SM/ATFM ratio was also examined in the present study. Cohn et al. (1980) observed that the proportion of lean body mass as SM, a comparable ratio with SM/ATFM, was significantly lower in the elderly (0.38 for 20 - 30 years *vs.* 0.24 for 70 - 79 years in men; and 0.22 for 20 - 30 years *vs.* 0.16 for 70 - 79 years in women). As observed

in our earlier reported investigation (Wang et al., 1996), the Burkinshaw-Cohn model substantially underestimates total body SM. Therefore, it is questionable if skeletal muscle mass estimates in Cohn's study were accurate. Our results indicate that there are no significant differences in the SM/ATFM ratios between young and older men ($P = 0.29$). Regression analysis ($r = -0.29$, $P > 0.05$) also indicates that age does not significantly influence the SM/ATFM ratio in the present group.

Conclusion

Until recently autopsy was the only method available to accurately study the quantitative relationships between skeletal muscle and other tissue-system level components. Based on multiscan CT, the present investigation explored the proportion of ATFM as anatomic skeletal muscle. We found that the proportion of ATFM as SM is related to both ATFM and BMI. However, this proportion is not significantly related to age and does not differ between young and elderly men. Further study is needed to establish the influence of other possible factors such as physical activity, gender, race, and diseases which may effect the magnitude and constancy of the proportion of ATFM as SM.

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Science without organization is blind

CHAPTER 4

SYSTEMATIC ORGANIZATION OF BODY COMPOSITION METHODOLOGY: OVERVIEW WITH EMPHASIS ON COMPONENT-BASED METHODS

ZiMian Wang, Stanley Heshka, Richard N. Pierson Jr., Steven B. Heymsfield

ABSTRACT

The field of body composition research currently lacks a systematic organization of methods used to quantitate components at the atomic, molecular, cellular, tissue-system, and whole-body levels of body composition. In this report we propose a classification system for the area of body composition methodology that proceeds in steps beginning with division of methods into *in vitro* and *in vivo* categories, advances to organization by measurable quantity (property, component, or combined), and ends with grouping of methods by mathematical function (types I and II). Important characteristics of component-based methods are then developed, including a classification of component relationship types, the role of ratios and proportions in type II component-based methods, and the basis of simultaneous equations in multicomponent methods. This classification system, the first founded on a conceptual basis, explains similarities and differences between the many diverse methods, provides a framework for teaching body composition methodology theories to students, and suggests future research opportunities.

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INTRODUCTION

A rapid expansion is now taking place in the study of human body composition. In an earlier review we described how the accumulating information related to body composition could be organized into three interconnected areas (1). The first of these areas involves a characterization of the 30 or more major components that comprise the human body. Our report demonstrated that the many prevailing and sometimes disparate body composition models could all be organized systematically into a comprehensive "five-level model". The second area is *biological effects* on body composition.

The present report concerns the third area, body composition *methodology*. Our intent is to demonstrate that the various available body composition methods can all be organized according to a comprehensive classification system. The stimulus for this effort was the appearance of varied attempts at organizing body composition methods. Most authors organized methods as simple lists (2), into groups such as "direct", "indirect", and "doubly indirect" (3), by one or another component (4), or according to their own particular research experience (5). All of these attempts are not based on a fundamental concept, thus making it difficult to appreciate the similarities and differences between various methods.

In this report we demonstrate that only a few simple concepts form the framework of all body composition methods. These concepts not only allow students to rapidly learn the main principles related to body composition methodology, but they reveal unforeseen similarities and differences between available methods. Another fruit of organizing body composition methods is that gaps in methods are revealed and areas for future research are clearly identified. Most importantly, the classification can guide development of new body composition methods.

Our review is divided into two parts. In the first part we provide an overview of our proposed organization of body composition methodology. Most methods can be organized into *property-based* or *component-based*. We then greatly expand our discussion of component-based methods. The second part involves a detailed discussion of property-based methods and this will be published in a separate report.

As a prelude to our suggested organization of body composition methodology, we give a brief overview of the five-level model (1).

FIVE-LEVEL MODEL

Body composition research involves the study of *components* that are organized into five levels of increasing complexity: atomic, molecular, cellular, tissue-system, and whole-body (1). There are ~40 main components of the first four levels of body composition. Each of these components and each of these levels is distinct. This important concept allows preparation of mathematical expressions that link components to each other at the same or different level in the form of models.

If all components at each level are summed, the total is equivalent to body weight. The models which relate body weight with components at each level are summarized in **Table 1**. These body weight models are usually named in terms of the number of components on the right-hand side of the equation. A two-component model separates the human body into two parts, a three-component model into three parts, and so on.

Another type of model relates components at different levels to each other. An example is the relationship between total body hydrogen (TBH, kg) at the atomic level and it's molecular level determinants: TBH = 0.122 × fat + 0.111 × water + 0.070 × protein, where the coefficients are the proportions of the molecular level components as hydrogen (6).

Table 1. Body weight model at different body composition levels

Level	Equation	Model
Atomic	BW = O + C + H + N + Ca + P + K + S + Na + Cl + Mg	11 component
Molecular	BW = F + A + Pro + Ms + Mo + G	6 component
	BW = F + A + Pro + M	4 component
	BW = F + A + solids	3 component
	BW = F + Mo + residual	3 component
	BW = F + FFM	2 component
Cellular	BW = CM + ECF + ECS	
	BW = F + BCM + ECF + ECS	
Tissue-system	BW = AT + SM + bone + other tissues	

A, water; AT, adipose tissue; BCM, body cell mass; CM, cell mass; ECF, extracellular fluid; ECS, extracellular solids; F, fat; FFM, fat-free body mass; G, glycogen; M, mineral; Mo, bone mineral; Ms, soft tissue mineral; Pro, protein; SM, skeletal muscle. Models from references 1, 6, and 7.

These quantitative expressions of body composition provide great versatility in characterizing various levels and components, and their application is fundamental to the development of body composition methods.

CLASSIFICATION OF METHODOLOGY

Body composition *methodology* is an area of investigation dedicated to the study and application of methods used to quantify components at the five body composition levels (1). A summary of our proposed organization of body composition methods is presented in **Figure 1**. Two broad areas of body composition methodology can be defined first, *in vitro* and *in vivo* methods. *In vitro* refers to the measurement of components in either whole cadavers or in excised tissues. The analytical procedures used *in vitro* are numerous and are beyond the scope of the present report. Attention will be directed specifically towards the *in vivo* methods that are used to quantify components in humans.

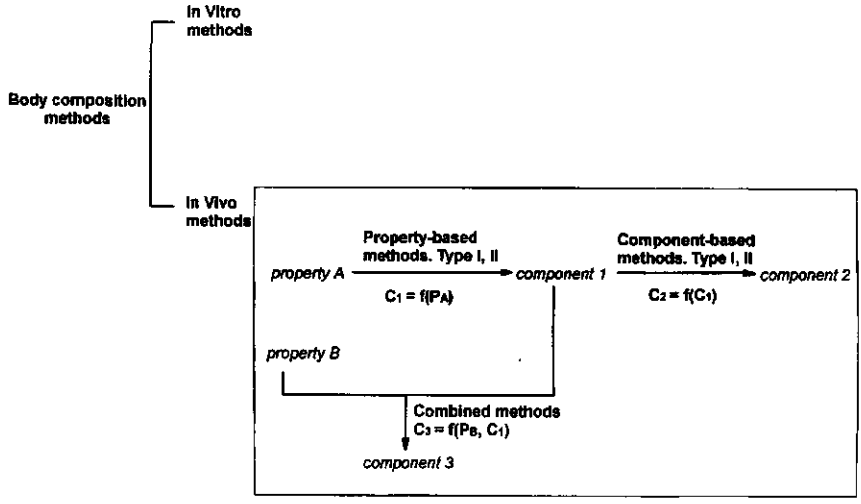


Figure 1. Suggested classification of body composition methods. C, component; *f*, mathematical function; P, measurable property.

The fundamental concepts of *in vivo* body composition methods can be summarized as the basic formula,

$$C = f(Q) \qquad [1]$$

where C represents an unknown component, Q a measurable quantity, and f the mathematical function relating Q with C. This formula shows that quantification of an unknown component depends on two distinct but closely connected parts, a measurable quantity and a mathematical function. Our analysis revealed that two main types of quantities are used in body composition methods, properties and components. These two types of quantity are the foundation of property-based and component-based methods, respectively. In addition, "combined" methods were found which include as the measurable quantities both properties and components. Our analysis also showed that two types of mathematical function are used in body composition methods.

The three types of "measurable quantity" and two types of "mathematical function" result in six categories of methods. The dozens of available *in vivo* methods can all be organized into one of these six categories. As we will show in later sections, these categories have distinguishing features such that methods within each of the six categories share many characteristics in common.

Organization by Measurable Quantity

We now expand the description of our classification by measurable quantity. All *in vivo* methods can be organized according to measurable quantity into one of three groups, property-based, component-based, and combined, each of which is characterized by the features summarized in **Table 2**.

Property-based methods Property-based methods allow an unknown component to be quantified from a measurable property. The purpose of measuring a property is to distinguish between components of interest. That is, the component to be quantified must differ from other components with respect to the measured property.

All property-based methods share in common the use of a measurable property as the "quantity" in **formula 1**. The decay profile of radioactive isotopes, impedance, X-ray attenuation, and skinfold thickness are examples of measurable properties. Two properties are sometimes used together to discriminate between components. An example is the

Table 2. Distinguishing characteristics of property-based, component-based, and combined body composition methods

	Methods		
	Property-based	**Component-based**	**Combined**
Definition	Allows unknown component to be quantified from measurable property.	Allows unknown component to be quantified from known component.	Allows unknown component to be quantified from both measurable property and known component.
How established	$C_1 = f(P_A)$	$C_2 = f(C_1)$	$C_3 = f(P_B, C_1)$
Basic formula	On the quantitative relation between measurable property and unknown component.	On the quantitative relation between known and unknown component.	On the quantitative relation between measurable property, known component, and unknown component.
Role in methodology	Foundation of all *in vivo* methods.	Expansion of property-based methods.	Expansion of property-based method.

C, component; *f*, mathematical function; P, measurable property.

hydrodensitometry method in which body volume and weight, both of which are measurable properties, are used to measure two unknown components, fat and fat-free body mass (7). Of the 30 or more well-defined major components, only about one-half can now be quantified by presently utilized properties.

Component-based methods Component-based methods allow an unknown component to be quantified from known property-derived components. Thus, an essential feature of component-based methods is that a property-based method must first be used to estimate the "known" component. The underlying concept of component-based methods is that a stable or relatively stable quantitative relationship exists between unknown and known components. For example, fat-free body mass can be derived from total body water by assuming that for healthy adults 73% of fat-free body mass is water (8, 9).

Combined methods Most *in vivo* methods can be classified as property-based or component-based. However, a third category of *combined* methods also exists **(Figure 1)** in which the quantities used to estimate unknown components include both measurable properties and known components. An example is Siri's method in which total body fat is quantified from two

measurable properties (body weight and volume) and a known component (total body water) (10).

Organization by Mathematical Function

Returning to **formula 1**, our analysis indicates that there are two types of mathematical function used in *in vivo* methods. Although it was easy to recognize these two types of mathematical function, we found it more difficult to find a pair of terms that fully characterized and distinguished between them. As a result we identify the two types of mathematical functions as either *type I* or *type II*, each of which is characterized by different features.

Type I methods The first group of methods share in common a type of mathematical function derived by statistical analysis of quantitative measurements. Typically a reference method is necessary to quantify the unknown component. Statistical methods, usually regression analysis, are then used to develop an equation for predicting the unknown component from a property and/or property-derived component. Complex type I methods may include additional variables such as age and sex.

An example of a type I method is the prediction of total body water from two measurable properties, electrical resistance and height (11). These two properties were measured in healthy subjects by bioelectrical impedance analysis and a stadiometer, respectively. A property-based method, deuterium dilution, was used as the reference for measuring total body water. The investigators then used simple linear regression analysis to derive the prediction equation for total body water (TBW, kg) from height^2/resistance (H^2/R, cm^2/ohm), $TBW = 0.63 \times H^2/R + 2.03$; $r = 0.95$, $P < 0.001$.

Type I methods are population and condition-specific. In the previous example the prediction equation for total body water may not be valid across different ethnic groups or in disease states. The same is true that a skinfold-fat prediction equation developed in healthy normal weight adults may not apply in obese subjects. Type I methods therefore need to be revalidated when used in new populations or under different conditions from which they were originally developed.

Type II methods The second group of methods share in common a type of mathematical function that incorporates constant or relatively constant ratios or proportions. These ratios

and proportions are sometimes referred to as "models" and type II methods are frequently described as "model methods".

Type II methods are based on a well-established model in the form of ratios or proportions that relate the unknown component to the measurable property or known component. Investigators characteristically make necessary assumptions when establishing these models. The validity of type II methods to a great extent rests on these assumptions.

A classic example will help to clarify the nature of type II methods. The model that relates total body water to fat-free body mass is well established (8). Although there is a growing challenge to this model (12), within reason there is a constant ratio of total body water to fat-free body mass, not only in adult humans, but in many other animal species (8). This stable relationship between water and fat-free body mass allows development of a type II method in which fat-free body mass is equal to 1.37 × total body water.

Type II methods may not be static in their design but evolve over time. An example is the measurement of total body fat based on underwater weighing. Behnke and colleagues proposed in 1942 a method of separating body weight into fat and "lean" body mass (13). Behnke's method had two measurable properties, underwater weighing-derived body volume (BV, L) and body weight (BW, kg). This type II method assumed that fat and fat-free (the term now used in place of lean) body mass densities are constant at 0.900 g/cm^3 and 1.100 g/cm^3, respectively. Stable proportions of fat-free body mass as water, protein, and mineral are also assumed in Behnke's model. A type II method was then derived (13), fat (kg) = 4.95 × BV − 4.50 × BW.

Siri next suggested in 1961 expanding Behnke's method to a three-component model (**Table 1**) (10). In Siri's type II method (fat [kg] = 2.118 × BV − 0.78 × TBW − 1.354 × BW) there are three measurable quantities, body volume, total body water (TBW, kg), and body weight. The measurement of total body water eliminates errors in Behnke's method related to individual differences in hydration. However, Siri's method still assumes that the ratio of protein to mineral remains constant within and between subjects.

Later investigators subsequently expanded on Siri's method by adding bone mineral measurements to eliminate errors related to individual differences in bone mineral content (14). In this type II method (fat [kg] = 2.747 × BV − 0.714 × TBW + 1.146 × Mo − 2.0503 × BW)

four quantities are measured, body volume, total body water, bone mineral (Mo, kg), and body weight. Assumptions, although relatively minor, were also needed in developing this method. From this example we see that type II methods may not be fully perfected when first developed but can evolve as new models and measurement techniques become available.

An important consideration is that type I methods can be established between an unknown component and any measurable quantity provided there is a statistically significant correlation between the component and measurable quantity. However, the requirements for type II methods are much more stringent in that the models from which the methods are derived must incorporate constant or relatively constant ratios or proportions. Accordingly, there are relatively few type II methods compared to the large number of type I methods in current use.

Representative Examples of Classification

A useful step in understanding this methodological classification is to analyze the classic "total body water approach" for measuring total body fat mass (**Figure 2**). The first stage of this approach is to administer a dose of tritium-labeled water which emits a 0.018 MeV β-ray.

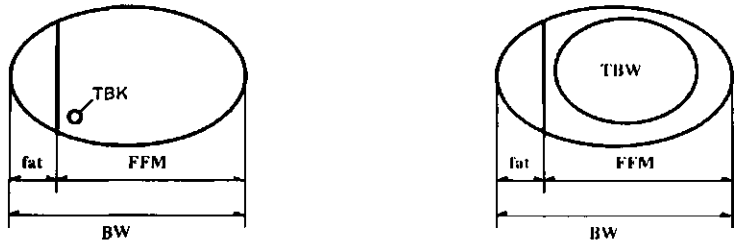


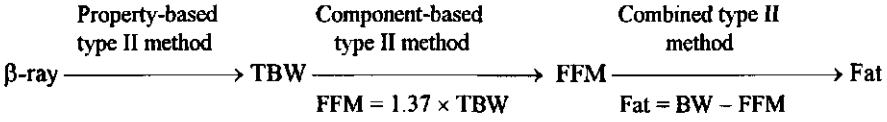
Figure 2. Total body fat quantified by total body water (TBW) and total body potassium (TBK) approaches. BW, body weight; FFM, fat-free body mass.

Because there is a known constant ratio between the β-ray counts and tritium, the tritium dilution volume can be measured based on the "dilution model". Total body water can then be calculated from tritium dilution volume (2). Total body water measurement according to this strategy is thus a property-based type II method. Here the measurable property is the tritium decay profile and the mathematical function is based on the dilution model. Total body water, a body composition component, is now known.

The next stage is based on the long-standing observation that the ratio of total body water to fat-free body mass is relatively constant (0.73) (2). This ratio can be used as the model to estimate fat-free body mass from total body water. This is a component-based type II method in which water is the measurable quantity and the assumed constant ratio of fat-free body mass to water (1.37) is used to create the mathematical function.

Finally, a combined type II method which involves a measurable property (body weight) and a measurable component (fat-free body mass) is used to solve for total body fat. The equation (fat = body weight – fat-free body mass) is derived from a two-component body weight model (**Table 1**).

This example reveals that measuring total body fat by tritiated water dilution is not a simple approach. It includes three separate methods: property-based, component-based, and combined. All three stages are type II methods which are summarized by the pathway:



Another classic approach for measuring total body fat, the "total body potassium method", also includes three type II methods (**Figure 2**). The first stage is to measure the γ-ray decay (1.46 MeV) of natural ^{40}K found in human tissues. Because there are known constant ratios between the γ-ray counts and ^{40}K, and between ^{40}K and total body potassium, the mass of total body potassium can be measured by using a property-based type II method. The next stage is a component-based type II method which calculates fat-free body mass from total body potassium (TBK, kg), FFM [kg] = 376 × TBK. This equation is based on the assumption that the TBK/FFM ratio is relatively constant at 0.00266 kg/kg (2). The final step is the same

as for the total body water approach. The three stages in the estimation of total body fat are summarized as follows:

$$\gamma\text{-ray} \xrightarrow{\text{Property-based type II method}} \text{TBK} \xrightarrow[\text{FFM} = 376 \times \text{TBK}]{\text{Component-based type II method}} \text{FFM} \xrightarrow[\text{Fat} = \text{BW} - \text{FFM}]{\text{Combined type II method}} \text{Fat}$$

This systematic analysis of methods may also have a potential use in studying methodological errors. Sources of error, both measurement and assumption, can be clearly defined. In these two examples, both methods involve two measurements (body weight and either the 0.018 MeV β-ray counts of tritium or the 1.46 MeV γ-ray counts of ^{40}K) and a series of clearly defined assumptions, each with quantifiable error terms.

Features of the Classification

Organizational framework Our suggested organization of body composition methodology begins with a division of methods into *in vitro* and *in vivo* (**Figure 1**). *In Vivo* methods can then be organized according to both measurable quantity and mathematical function. Three categories of quantity and two types of mathematical function were identified. According to this approach, all *in vivo* methods can be classified as one of six categories: property-based type I and type II methods, component-based type I and type II methods, and combined type I and type II methods. Each *in vivo* body composition method thus occupies one and only one position in the classification.

Similarities and differences between methods This classification system reveals similarities and differences between *in vivo* methods, including the requirement for or lack of underlying assumptions, sources of potential error, and population specificity. An example is given in **Table 3** which demonstrates the methods for quantifying total body fat and fat-free body mass which are organized according to the suggested classification. There are some strong similarities between methods within each of the classification categories. For example, anthropometry, bioimpedance analysis, infrared interactance, total body electrical conductivity, and 24 hour urinary creatinine excretion are all used in property-based type I methods which share characteristics in common. All of the methods are based on measured properties; all of

Table 3. Organization of methods for quantifying total body fat and fat-free body mass according to the proposed classification

	Property-based methods	Component-based methods	Combined methods
Type I methods	***Anthropometry***: fat = 0.65 × BW − 0.21 × H + 14.1 (2) ***Bioimpedance***: FFM = 0.85 × H^2/Z + 3.04 (5) ***Infrared interactance*** ***TOBEC*** ***Urinary creatinine***: FFM = 0.0241 × Cr + 20.7 (2) ***Oxygen consumption***: FFM = 0.2929 × O − 7.36 (23)		
Type II methods	***UWW***: fat = 4.95 × BV − 4.50 × BW; FFM = 5.50 × BW − 4.95 × BV (13) ***DXA*** ***Gas uptake***	***TBK***: FFM = 376 × TBK (2) ***TBW***: FFM = 1.37 × TBW ***TBC, TBN, TBCa***: fat = 1.30 × TBC − 4.45 × TBN − 0.06 × TBCa (18)	***BW, FFM***: fat = BW − FFM ***BW, BV, TBW***: fat = 2.118 × BV − 0.78 × TBW − 1.354 × BW (10) ***BW, BV, TBW, Mo***: fat = 2.747× BV − 0.714 × TBW +1.146 × Mo − 2.0503 × BW (14)

BV, body volume (L); BW, body weight (kg); Cr, urinary creatinine excretion (g/day); DXA, dual energy X-ray absorptiometry; H, height (cm); Mo, bone mineral (kg); O, basal oxygen consumption; TBC, total body carbon (kg); TBCa, total body calcium (kg); TBK, total body potassium (kg); TBN, total body nitrogen (kg); TBW, total body water (kg); TOBEC, total body electrical conductivity; UWW, underwater weighing; Z, impedance (ohm).

the methods are based on a reference method to estimate fat or fat-free body mass; all of the prediction equations are statistically-derived; and all of the methods are population specific. Similarities can also be observed between the methods within the other categories of **Table 3**.

Methodology rules We now summarize some general "methodology rules" that emerge from the proposed classification:

♦ Property-based methods are the foundation of *in vivo* methods. Component-based and combined methods are an expansion of property-based methods. Therefore, all *in vivo* methods are based on measurement of quantifiable properties.

♦ Type I methods are developed using a reference method and regression analysis to derive the mathematical function. Type II methods are developed based on ratios or proportions between unknown components and measurable quantities that are stable or relatively stable within and between subjects. Therefore, all *in vivo* methods are based on the quantitative relationships between unknown components and measurable quantities.

COMPONENT-BASED BODY COMPOSITION METHODS

Component-based methods can be expressed as the general formula

$$C_u = f(C_k) \qquad [2]$$

where C_u is an unknown component, C_k a known component, and f the mathematical function relating C_k with C_u. Component-based methods can be divided into type I and type II according to mathematical function.

Component-Based Type I Methods

Component-based type I methods allow quantification of an unknown component from a known component and a statistically-derived equation. All type I component-based methods require a reference method to quantify the unknown component. Regression analysis is then used to develop an equation linking the known component to the unknown component.

An example is prediction of total body protein from total body water. Healthy subjects (n = 41) were evaluated by neutron activation analysis and tritium dilution to quantify total body nitrogen and total body water, respectively (15). Total body nitrogen was used as the reference for measuring total body protein. The investigators then used linear regression analysis to derive the total body protein (TBPro, kg) prediction equation from total body water (TBW, kg), TBPro = 0.335 × TBW – 2.53; r = 0.92, $P < 0.001$.

Component-based type I methods are population and condition-specific. In the previous example, 56 surgical patients were also studied (15). The prediction equation was TBPro = 0.308 × TBW – 2.01; r = 0.87, $P < 0.001$. Surgery patients, according to this equation, have less protein for the same amount of total body water compared to healthy subjects. This observation is anticipated as surgical patients ordinarily have a relative increase in extracellular fluid and total body water (15). Clearly, the prediction equation developed in healthy adults should not be used in surgical patients. The application of type I methods outside of the original development subjects or conditions therefore requires cross-validation.

The development of component-based type I methods is a rapidly evolving field. Important considerations include subject selection, sample size, choice of reference method, magnitude of correlation's observed, and cross-validations.

Component-Based Type II Methods

All type II component-based methods require a well-established ratio or proportion model which relates the known component to the unknown component. The models from which type II component-based methods are derived can be expressed as the general formula

$$C_A = \Sigma (R_n \times C_n) \qquad [3]$$

where R represents the constant or relatively constant ratio (when n = 1) or proportions (when $n \geq 2$) of C_n as C_A. When n = 1, **formula 3** takes the simplest form, $C_A = R_1 \times C_1$. An example is the model relating total body nitrogen (TBN) to protein (TBN/TBPro = 0.161) that will be discussed in detail below. When $n \geq 2$, **formula 3** takes the form $C_A = R_1 \times C_1 + R_2 \times C_2 + \ldots + R_n \times C_n$. For example, total body oxygen (TBO, kg) can be determined from molecular level components as TBO = 0.119 × fat + 0.889 × water + 0.227 × protein + 0.400 × bone mineral.

The ratio and proportion models used in type II methods are often based on assumptions. A classic example is the model that relates total body nitrogen with total body protein, TBN/TBPro = 0.161. This model is founded on two assumptions: that all of body nitrogen is in the form of protein and that nitrogen averages 16.1% of protein mass. Although assumed constant, there is of course variation in this ratio over time within and between subjects. For this reason the accuracy of a type II component-based method, to a great extent, is dependent

upon the model's "stability". The smaller the model's variation within and between subjects, the more accurate the derived type II method, and *vice versa*.

As component pairs were listed, we found that many component pairs fail to form stable ratios, as for example total body carbon and water. A reasonable question thus arises as to why some component pairs are more suitable for model development than others. One approach to answering this question is to examine the characteristics of each component pair. However, because over 30 components exist at the five levels of body composition, there are over 400 possible pairs of components (1). Thus it is impractical to carry out a case-by-case analysis of component pairs.

Another strategy is to divide the > 400 component pairs into several relationship types based on a few principles and then to see which types are suitable for establishing body composition models. Applying this approach, we found that all component pairs can be divided into three categories according to *dependency relationships* that indicate the quantitative association between components. If one component completely exists within the other, we term this *subordinate* ($A \subset B$, a notation used in Boolean algebra, implies that A is a subordinate of B); if two components share part of their mass in common, we define these as *overlapping* ($\cup$); and if there is no common mass between components, they are defined as *separate* (16).

We also found that components pairs could be divided into two categories according to *combination characteristics* which describes the nature of the association between components. Some components are joined chemically, and these are termed *chemical* combinations. A characteristic of chemical combinations is that component ratios are very stable. For example, H_2O, is made up of 11.1% hydrogen and 88.9% oxygen; and the main constituent of bone mineral, calcium hydroxyapatite, $[Ca_3(PO_4)_2]_3Ca(OH)_2$, is 39.8% calcium (9). The remaining components are linked by *non-chemical* combinations. The three dependency relationships and two combination characteristics allow organization of the >400 component pairs into six relationship types (**Table 4**). We now review the main features of each relationship type.

Subordinate A pair of components in which one exists completely within another is defined as a subordinate relationship (**Table 4**). There are very few pure subordinate relationships between components; although almost all nitrogen exists as protein, there is still a small

Table 4. The six relationship types between two body composition components and some examples

Combination characteristics	Dependency relationships		
	Subordinate	**Separate**	**Overlapping**
Chemical	TBN ⊂ protein TBS ⊂ protein TBCa ⊂ Mo	TBN, TBS	TBO ∪ TBW TBC ∪ fat TBP ∪ Mo TBH ∪ protein
Nonchemical	TBK ⊂ FFM TBW ⊂ FFM ASM ⊂ SM SM ⊂ ATFM Mo ⊂ FFM bone ⊂ skeleton	TBW, protein ECF, ECS SM, bone TBC, TBO fat, TBW AT, SM	TBK ∪ BCM TBN ∪ SM TBC ∪ AT fat ∪ AT TBW ∪ SM TBC ∪ SM

⊂ represents a subordinate relationship in which > 98% of component A is within component B; ∪ represents an overlapping relationship; ASM, appendicular skeletal muscle; AT, adipose tissue; ATFM, adipose tissue-free body mass; BCM, body cell mass; ECF and ECS, extracellular fluid and solids; FFM, fat-free body mass; Mo, bone mineral; SM, total body skeletal muscle; TB, total body elements C, Ca, H, K, N, O, P, and S; TBW, total body water.

amount of nitrogen in creatine and phospholipids. Similarly, although 99% of calcium exists as bone mineral, there is still 1% of calcium in soft tissue (17). The subordinate as understood in body composition research therefore differs somewhat from the pure subordinate as defined in mathematics (16). If >98% of a component exists within another, we assume that they exist in a subordinate relationship.

Chemical subordinate. Chemical subordinate relationships exist between a few atomic level components and related molecular level components. In this relationship, two components are expressed as a chemical formula, and these associations are very stable, even in disease states (**Table 5**). This is because most chemical components are discrete molecules with well-defined elemental make-up. For example, both total body nitrogen and total body sulfur form chemical subordinate relationships with protein, $C_{100}H_{159}N_{26}O_{32}S_{0.7}$. The proportions of nitrogen and sulfur in protein are stable at 16.1% and 1.0% which are similar across all ages and both sexes in humans.

Table 5. Chemical combinations between components at the atomic and molecular levels of body composition

Component	Chemical formula	Elemental proportion						
		C	O	H	N	Ca	S	P
Water	H_2O		88.9	11.1				
Fat	$C_{51}H_{98}O_6$	75.9	11.9	12.2				
Protein	$C_{100}H_{159}N_{26}O_{32}S_{0.7}$	53.2	22.7	7.0	16.1		1.0	
Glycogen	$(C_6H_{10}O_5)_x$	44.4	49.4	6.2				
Calcium hydroxy-apatite	$[Ca_3(PO_4)_2]_3Ca(OH)_2$		41.4	0.2		39.8		18.5

Information modified from ref. 9.

Two classic type II methods which are formulated on chemical subordinate relationships are the measurement of total body protein and bone mineral from total body nitrogen (kg) and total body calcium (TBCa, kg), respectively (9, 17):

$$\text{TBPro (kg)} = \text{TBN}/0.161 = 6.21 \times \text{TBN}$$

$$\text{Bone mineral (kg)} = 2.94 \times \text{TBCa}$$

Both of the methods are considered "gold standards" for measuring protein and bone mineral, respectively. Moreover, as chemical combinations are thought to be constant across all living human subjects, they are usually considered population-general.

Non-chemical subordinate. This is a type of subordinate relationship in which two components cannot be expressed as a chemical formula. Therefore, the associations between the components are not as stable as those between chemical subordinate components. However, ratio models can still be developed based on relatively stable relationships that exist between a few non-chemical subordinate components which are physiologically linked. For example, the ratio of total body water to fat-free body mass is about 0.73 (range, 0.70 - 0.76) in humans and most mammalian species analyzed (8). This is because the constituents of fat-free body mass (water, protein, glycogen, and minerals) occur in relatively constant proportions. One can also develop a hypothesis that explains the stability of this relationship: a relatively constant proportion of water is needed to maintain osmolality stable at ~300 mOsm for the soluble fat-free solids consisting of proteins, glycogen, and minerals/electrolytes.

Classic type II methods were derived from non-chemical subordinate relationships between total body water (TBW, kg) and fat-free body mass (FFM, kg),

$$FFM = 1.37 \times TBW,$$

and between total body potassium (TBK, kg) and fat-free body mass,

$$FFM = 376 \times TBK.$$

Appendicular skeletal muscle (ASM) and total body skeletal muscle also form a non-chemical subordinate relationship. The ratio between these two components is relatively constant (0.75) (17). A type II component-based method was thus derived to measure total body skeletal muscle (SM) (18),

$$SM = ASM/0.75 = 1.33 \times ASM.$$

Appendicular skeletal muscle can be measured by a property-based method, dual energy X-ray absorptiometry.

Because non-chemical combinations are not as stable as chemical combinations, the ratios between non-chemical subordinate components vary in health and disease and thus tend to be population-specific.

Separate In both subordinate and overlapping relationships the two components have a common mass. However, many pairs of components have no common mass (**Table 4**). A component model therefore cannot be directly established from separate relationships. However, an indirect approach can be used to develop type II component-based methods from a few component pairs that exist in separate relationships.

Chemical separate. In this type of separate relationship, two separate components existing only at the atomic level form chemical subordinate relationships with a molecular level component. For example, total body sulfur (TBS) and nitrogen exist as a chemical separate relationship. Both components form chemical subordinate relationships with total body protein (TBS $\subset$ TBPro and TBN $\subset$ TBPro) and the ratios of total body sulfur to protein (0.010) and total body nitrogen to protein (0.161) are highly stable (**Table 5**). Thus, a reasonable model that relates total body sulfur (kg) to total body nitrogen (kg) can be derived and the ratio between the two components is also stable:

$$TBS/TBN = (TBS/TBPro)/(TBN/TBPro) = 0.010/0.161 = 0.062$$

A type II method for estimating total body sulfur can then be derived (9),

$$TBS = 0.062 \times TBN.$$

Non-chemical separate. This is a type of separate relationship in which two components form non-chemical subordinate relationships with a large component such as fat-free body mass. An example is total body water and protein, both of which form non-chemical subordinate relationships with fat-free body mass. The ratios between protein and fat-free body mass (0.187) and between water and fat-free body mass (0.73) can be used to derive the ratio of protein to water,

$$TBPro/TBW = (TBPro/FFM)/(TBW/FFM) = 0.187/0.73 = 0.255.$$

Although a type II-like method can thus be derived from the non-chemical separate relationship between protein and water ($TBPro = 0.255 \times TBW$), this is not a very accurate method. In general, we observed that ratios between non-chemical separate components are moderately variable within and between subjects and might be useful only as rough predictors. Type I component-based methods are therefore usually suitable for non-chemical separate components. As we described above, total body protein can be predicted from total body water by using population-specific type I methods (15).

Overlapping A pair of components where part of each exists within the other is defined as an overlapping relationship (**Table 4**). Overlapping relationships always involve more than two components at different body composition levels. For example, total body carbon and fat form an overlapping relationship. A portion of the carbon exists as fat, but there is some carbon in protein, glycogen, and bone mineral. However, fat contains oxygen and hydrogen in addition to carbon (**Table 5**).

As overlapping relationships involve more than two components, multicomponent models must be established ($n \geq 2$ in **formula 3**). An important point is that it is possible to express a higher level component as a function of components at a lower level, although these multicomponent equations are not used in practice. For example,

$$Fat = R_1 \times TBC + R_2 \times TBH + R_3 \times TBO,$$

where TBC is total body carbon. In this equation the between-subject variation in proportions (R_1 - R_3) is very large. For example, the proportion of total body carbon as fat varies with degree of adiposity. This expression therefore cannot be used in type II component-based methods.

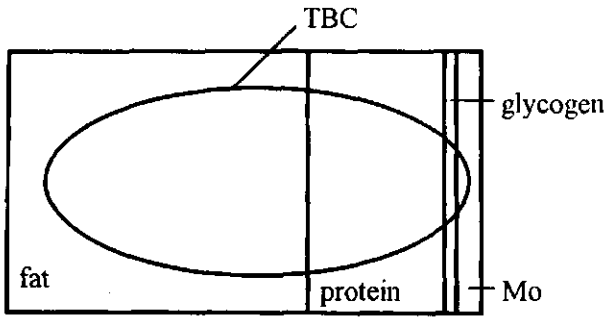


Figure 3. The overlapping chemical relationships between the atomic level component total body carbon (TBC) and the related molecular level components fat, protein, glycogen, and bone mineral (Mo).

Chemical overlapping. Chemical overlapping relationships exist between an atomic level component and related molecular level components, and can be expressed as a stable proportion model. For example (**Figure 3**) (6),

$$TBC = 0.759 \times fat + 0.532 \times protein + 0.444 \times glycogen + 0.180 \times bone\ mineral$$

The most important characteristic of this equation is that the proportions of the molecular level components are not known to be influenced appreciably by age, sex, and disease because they are based on chemical formulae (**Table 5**) (9). Therefore, the overall stability of this multicomponent model is very high.

Chemical overlapping models are used in simultaneous equations to derive type II component-based methods. For example, total body fat can be quantified from the four simultaneous equations,

$$TBC = 0.759 \times fat + 0.532 \times protein + 0.444 \times glycogen + carbon\ in\ bone\ mineral$$

$$protein = 6.25 \times TBN$$

$$glycogen = 0.044 \times protein$$

$$carbon\ in\ bone\ mineral = 0.05 \times TBCa$$

A multicomponent method for measuring total body fat can thus be derived (19),

$$fat = 1.30 \times TBC - 4.45 \times TBN - 0.06 \times TBCa$$

where all units are in kg. All of these elements can be quantified by property-based neutron activation analysis methods.

Non-chemical overlapping. Non-chemical overlapping relationships exist between lower level components and related components at higher levels (i.e., the cellular and tissue-system levels). Unlike chemical overlapping, non-chemical overlapping relationships cannot be expressed as very stable proportions. For example,

$$TBC = 0.640 \times AT + 0.107 \times SM + 0.148 \times bone + 0.121 \times other\ tissues$$

The coefficients of this equation are calculated from the Reference Man data (17). They vary within a range due to the influences of age, sex, and disease both within and between subjects. Caution must therefore be used when developing type II component-based methods formulated on non-chemical overlapping relationships.

However, a few non-chemical overlapping relationships are relatively stable and can be developed into useful methods. For example, total body potassium forms non-chemical overlapping relationships with both body cell mass (BCM, kg) and extracellular fluid (ECF, kg). Their relationships can be expressed as a model,

$$TBK\ (mmol) = R_1 \times BCM + R_2 \times ECF$$

where R_1 and R_2 are the potassium concentrations in body cell mass and extracellular fluid, respectively. Potassium mass in extracellular fluid only accounts for ~2% of total body potassium and thus can be neglected (17). A simplified type II component-based method for estimating body cell mass was therefore derived as (20)

$$BCM = 0.00833 \times TBK$$

Another example is the estimation of extracellular fluid from total body chlorine. Total body chlorine (TBCl) forms non-chemical overlapping relationships with both extracellular fluid (ECF) and intracellular fluid (ICF). The model is as follows,

$$TBCl = R_1 \times ECF + R_2 \times ICF$$

where R_1 and R_2 are the chlorine concentrations in extracellular and intracellular fluid, respectively. Assuming that chlorine in intracellular fluid accounts for ~10% of total body chlorine, a simplified type II component-based method for estimating extracellular fluid was derived from total body chlorine (mmol) and plasma chlorine concentration [Cl^-, mmol/L], which represents the chlorine concentration in extracellular fluid (21),

ECF (L) = 0.9 × TBCl / plasma [Cl^-].

Relationship Types and Component-Based Methods

The more than 400 component pairs were grouped into six relationship types. Type I component-based methods could be developed within any of the six relationship types if a statistically significant correlation exists between two components. The requirements for developing type II component-based methods are much more stringent than they are for type I methods. Type II methods can be developed from chemical subordinate, chemical separate, and a few non-chemical subordinate relationships. We also found that chemical overlapping and a few non-chemical overlapping components were suitable for use in type II multicomponent methods. Component-based methods and their equations, to a very large extent, can thus be understood in terms of well-defined component pair interactions.

SUMMARY AND CONCLUSIONS

It is said that science without taxonomy is blind. Our aim in this report was to suggest for the first time a systematic classification of body composition methods founded on a conceptual basis and thereby to provide new and previously unforeseen research opportunities. Many generalizations and simplifications are made in this overview. Nevertheless, we have set down a framework upon which investigators can modify, build upon, or start anew.

Methods used to quantify components are first divided into two groups, *in vitro* and *in vivo*. A basic formula, $C = f(Q)$, can be used to define the two parts that are characteristic of all *in vivo* body composition methods. The first part is the measurable quantity (Q), and we show that there are two main categories (property and component) and a third combined category. The second part of the basic formula is a mathematical function (f), and we found two types, referred to as type I and type II. All *in vivo* body composition methods can be organized into one of six categories according to this classification.

We then expand our analysis of component-based methods based on six relationship types between components. Relationship types reveal a great deal about type II component-based methods, including insight into why some are more accurate than others and they help to explain which equation types are appropriate.

This classification of body composition methods should allow investigators to systematically organize and communicate their research findings. The approach should also allow students to base their learning of body composition methodology on concepts rather than on simple listings devoid of an underlying theme. Finally, our attempts at organizing body composition methods uncovered several research questions worthy of future investigation.

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CHAPTER 5

SYSTEMATIC ORGANIZATION OF BODY COMPOSITION METHODOLOGY: OVERVIEW WITH EMPHASIS ON PROPERTY-BASED METHODS

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ABSTRACT

All *in vivo* body composition methods can be summarized by the general formula, $C = f(Q)$ in which computation of an unknown component (C) is mathematically expressed as a function (*f*) of measurable quantities (Q). Measurable quantities can be properties (e.g., the emission of specific γ-rays from ^{40}K), known components (e.g., total body water), or a combination of properties and components. Mathematical prediction equations are statistically-derived (type I) or model-based (type II). In the present study we describe the main features of property-based and combined methods. Type I property-based methods can be developed from any measurable property if a statistically significant correlation exists between the property and the unknown component. In contrast, type II methods can only be derived from well-known stable body composition models. All *in vivo* body composition methods, without exception, are ultimately based on measurement of various body properties.

INTRODUCTION

Over 3,900 papers were published since 1960 in the area of body composition research (1). A substantial proportion (~25%) of these papers concern methods which were developed to assess the ~ 40 major body components.

The increasing information related to body composition methodology recently led us to propose a systematic approach to classifying available methods. The main concept we advanced is that all *in vivo* body composition methods can be summarized into

$$C = f(Q) \qquad [1]$$

This fundamental formula shows that quantification of an unknown component (C) depends on two distinct but closely connected parts, a measurable quantity (Q) and a mathematical function (f) relating Q with C. Two main categories of measurable quantities were identified, property and component. Accordingly, most body composition methods can be organized as property-based and component-based methods (2). In addition, combined methods also exist in which both properties and components are used as the measurable quantities.

Two main types of mathematical function (f) were also identified in *in vivo* methods. Type I methods share in common mathematical functions derived by statistical analysis of experimental observations. In contrast, type II methods share in common mathematical functions which are developed based on well-established models within and between individuals (2). Accordingly, all *in vivo* body composition methods can be divided into six categories as shown in **Table 1**.

Our initial report included an expanded discussion of component-based methods which allow an unknown component (e.g., fat-free body mass) to be quantified from known components (e.g., total body water) (2). The general formula of component-based methods is

$$C_u = f(C_k), \qquad [2]$$

where C_u represents an unknown component mass, C_k is a known component mass, and f is a mathematical function relating C_k with C_u.

In the present study our attention is directed towards the remaining method categories, property-based and combined body composition methods. The following sections present the definitions, principles, and sub-classification of property-based and combined methods.

Table 1. Six categories of *in vivo* body composition methods

Measurable quantity (Q)	Mathematical function (*f*)	
	Type I	**Type II**
Q = P	***Type I property-based methods***	***Type II property-based methods***
	e.g., $TBW = 0.63 \times H^2/R + 2.03$ (3)	e.g., $fat = 4.95 \times BV - 4.50 \times BW$ (4)
$Q = C_k$	***Type I component-based methods***	***Type II component-based methods***
	e.g., $protein = 0.335 \times TBW - 2.53$ (5)	e.g., $fat = 1.30 \times TBC - 4.45 \times TBN - 0.06 \times TBCa$ (6)
$Q = P, C_k$	***Type I combined methods***	***Type II combined methods***
	e.g., $ECW = 0.52 \times TBW \times Z_{100}/Z_1 - 0.50$ (7)	e.g., $fat = 2.057 \times BV - 0.786 \times TBW - 1.286 \times BW$ (8)

BV, body volume; BW, body weight; C_k, known component; ECW, extracellular water; H, height; P, measurable property; R, electrical resistance; TBC, total body carbon; TBCa, total body calcium; TBN, total body nitrogen; TBW, total body water; Z_{100} and Z_1, electrical impedance at 100 and 1 kHz, respectively.

PROPERTY-BASED METHODS

Overview

Property-based methods allow an unknown component to be quantified from a relevant measurable property. The following is the general formula of property-based methods,

$$\mathbf{C} = f(\mathbf{P}), \qquad [3]$$

where C represents an unknown component mass, P is a measurable property, and *f* is a mathematical function relating P with C. Of the ~40 major components at the five body composition levels (9), about half can be quantified at present by property-based methods. Property-based methods can be further classified according to mathematical function derivation. Type I property-based methods are derived from measured properties and subsequent statistical analysis. An example is the estimation of total body water (TBW, in kg) from electrical resistance (R, in ohms) and height (H, in cm) (3). Resistance and height were measured in a group of healthy subjects and deuterium dilution was used as the reference for

measuring TBW. A type I property-based method was derived by simple linear regression analysis in which TBW (the predicted unknown component) was the dependent variable and R and H the independent variables ($TBW = 0.63 \times H^2/R + 2.03$; $r = 0.95$, $P < 0.0001$).

In contrast, type II property-based methods are derived from well-established property-component models. These models assume that a stable quantitative relationship exists between the component and related property. An example is total body fat and fat-free mass (FFM) measured by the underwater weighing method (4). This type II method is based on a body weight model (BW = fat + FFM) and body volume model ($BV = fat/D_{fat} + FFM/D_{FFM}$). The coefficients (e.g., fat and FFM densities, D_{fat} and D_{FFM}) are assumed constant within and between individuals.

Currently Applied Properties

We now provide a brief overview of properties that are used in body composition methods. Similar to all materials in the nature, the human body and its ~40 major components have various properties including physical, chemical, and biological. Of the properties, not all are suitable for development of body composition methods. For example, body temperature, heart rate, and respiratory rate are important biological properties, but they are of no value for body composition measurements. Only the properties whose measure has quantitative associations with component mass are possibly applied in body composition measurement. This is the basis upon which all property-based methods are developed. Some properties, ranging from radioactivity to physiological indices, that are applied in body composition measurement are listed in **Table 2**.

There are two features related to currently applied properties. First, most properties applied in *in vivo* body composition measurement are inherent to the human body. On the other hand, subjects can be endowed with an exogenous property in order to estimate a specified component. For example, there is almost no ^3H_2O in the human body. After a dose of ^3H_2O is added, 0.018 MeV β-ray, a radioactive property of ^3H, exists in the subject's body. When ^3H_2O homogeneously distributes in the subject's water space, the measurable radiation of blood sample can be used to estimate total body water (10). Exploring new exogenous properties is an important future direction of body composition research. The relative exogenous compounds, of course, must not be hazardous and only exist for a short time in the human body.

Table 2. Properties currently applied in body composition methodology

Property	Examples	Applied method
Radioactive	0.018 MeV β-ray (^3H_2O)	Radioactive isotope dilution
	3.10 MeV γ-ray (^{49}Ca)	INVA
	4.44 MeV γ-ray (^{14}C)	INVA
	10.83 MeV γ-ray (^{15}N)	IVNA
Dilute	Br^-	Special tracer dilution
	^2H (^2H_2O)	Stable isotope dilution
	^{18}O ($H_2{}^{18}O$)	Stable isotope dilution
Electro-magnetic	Electrical conductivity;	TOBEC
	Impedance; Resistance; Reactance	BIA
	Magnetic resonance	MRI
Absorptive	X-ray attenuation;	CT, DXA
	Photon attenuation;	DPA
	Ultrasound attenuation	Ultrasound
Physiological	Urinary creatinine excretion;	24 hr urine creatinine method
	Urinary 3-methylhistidine excretion	24 hr urine 3-methylhitidine method
Morphological	Height; Thickness; Distance; Circumference	Anthropometry
Weight	Body weight	UWW
Volume	Body volume	UWW

BIA, bioimpedance analysis; CT, multiscan computerized axial tomography; DXA, dual energy X-ray absorptiometry; DPA, dual photon absorptiometry; IVNA, *in vivo* neutron activation analysis; MRI, magnetic resonance imaging; TBW, total body water; TOBEC, total body electric conductivity; UWW, underwater weighing.

Second, not every property in **Table 2** can be used to establish a stable property-component model. As mentioned above, the necessary condition for type II methods is to establish a stable model linking the component with a corresponding property. Therefore, only a portion of the applied properties can be used in type II property-based methods, although all are suitable for type I property-based methods.

The most widely used property, body weight, can be expressed as models at the five body composition levels, atomic, molecular, cellular, tissue-system, and whole-body. All coefficients in these models are always equal to one (9):

$$BW = O + C + H + N + Ca + P + S + K + Na + Cl,$$

$$BW = \text{lipid} + \text{water} + \text{protein} + \text{mineral} + \text{glycogen},$$

$$BW = \text{cell mass} + \text{extracellular fluid} + \text{extracellular solids},$$

$$BW = \text{adipose tissue} + \text{skeletal muscle} + \text{bone} + \text{other tissues},$$

$$BW = \text{head} + \text{neck} + \text{trunk} + \text{arms} + \text{legs}.$$

Another widely applied property is body volume which forms two- or multi- component models at the molecular level,

$$BV = \text{fat}/0.900 + \text{FFM}/1.100,$$

$$BV = \text{fat}/0.900 + \text{water}/0.994 + \text{protein}/1.34 + \text{mineral}/3.04 + \text{glycogen}/1.52.$$

The molecular level components in these models are linked with body volume by assumed constant coefficients (i.e., component densities).

Type I Property-Based Methods

Type I property-based methods allow quantification of an unknown component based on a measurable property and a statistically derived prediction equation. All type I property-based methods share three characteristics in common: evaluation of a well-defined subject population; use of a reference method for estimating the unknown component; and application of statistical analysis to develop the prediction equation. Examples include anthropometry (10, 11), bioelectrical impedance analysis (3), total body electrical conductivity (12), and 24 hour urinary creatinine and 3-methylhistidine excretion (10, 13) (**Table 3**).

In order to derive a prediction equation, one needs an established method as the reference to measure the unknown component. It is obvious that the error of the reference method will propagate to the derived type I method. Therefore, only methods with high accuracy and precision are suitable as reference methods.

Table 3. Examples of type I property-based methods

Method (ref.)	Unknown component	Measurable property	Prediction equation
Anthropometry (10, 11)	Skeleton	Height	skeleton = $0.0326 \times H^{2.46}$
	FFM	AC	FFM = $1.03 \times 10^{-5} \times H^3$
			FFM = $0.514 \times H + 0.0178 \times AC - 49.7$
BIA (3)	TBW	Resistance	TBW = $0.63 \times H^2/R + 2.03$
	TBK	Height	TBK = $2.56 \times H^2/R - 23.1$
24-h urine Cr and 3MH (10, 13)	SM	Cr	SM = $11.8 \times Cr + 10.1$
		3MH	SM = $0.118 \times 3MH - 3.45$

AC, arm circumference (cm); BIA, bioelectrical impedance analysis; Cr, 24 hour urinary creatinine excretion (g); FFM, fat-free body mass (kg); H, height (cm); 3MH, 24 hour urinary 3-methylhistidine excretion (μmol); R, electrical resistance (ohm); TBK, total body potassium (g); TBW, total body water (kg).

A statistical analysis is necessary to quantify the relationship between measurable properties and an unknown component mass. In theory, type I property-based methods can be developed from a single property provided a statistically significant correlation exists between the property and the unknown component. However, type I property-based methods often include several properties as independent variables in order to establish a higher correlation coefficient. For example, FFM (kg) = $0.344 \times BW + 0.328 \times H^2/R + 0.576 \times SW - 9.63$; $r = 0.955$, SEE = 1.12 kg; where BW is body weight (kg), H height (cm), R electrical resistance (ohm), and SW shoulder width (cm) (14).

Some type I property-based methods include additional characters such as age, sex, and ethnicity. For example, fat/BW (%) = $1.20 \times BW/H^2 + 0.23 \times age - 10.8 \times sex - 5.4$; $r^2 = 0.79$, SEE = 4.1%; where BW is body weight (kg), H height (m), and sex equals 1 (males) or 0 (females) (15). Type I property-based methods are often population and condition-specific. For example, a prediction equation for total body water which includes height and electrical resistance (TBW = $0.63 \times H^2/R + 2.03$) may not be valid across different ethnic groups or in some diseased states (3). Type I property-based methods therefore need to be cross-validated when used in other populations or under different conditions.

Developing new type I property-based methods is a rapidly growing research area. Important considerations include selection of reference method, choice of measurable properties and other influencing characters, population selection, sample size, magnitude of observed correlation, and cross-validation of prediction equations.

Type II Property-Based Methods

Type II property-based methods allow unknown components to be quantified by using a prediction equation which is derived from a well-established property-component model. Type II methods are therefore sometimes referred to as "model methods". The requirements for type II property-based methods are more stringent than that for type I methods because a stable property-component model is necessary. Accordingly, there are relatively few type II property-based methods compared to the large number of type I property-based methods in current use.

Several type II property-based methods are referred to as "geometric methods" as they quantify an unknown component based on a well-known geometric formula. With all geometric methods the whole body is divided into a series of segments. Some geometric methods assume that the unknown component in each segment has a regular geometric configuration such as truncated cone or cylinder. For example, a truncated cone volume formula was used to estimate leg muscle mass (10), leg muscle mass = $d \times \sum [\pi \times (r_1^2 + r_1r_2 + r_2^2) \times L/3]$; where bone volume is ignored, d is muscle density, r_1 and r_2 the muscle circular radii, and L the distance between two cross-sections. Radius can be estimated from muscle shadow on a standard radiograph or by using caliper or ultrasonic techniques.

The above geometric methods assume that the unknown component in each segment has a regular geometric shape or configuration. This assumption is obviously too simplified, and will thus inevitably introduce varying degrees of model error. The application of computerized axial tomography (multiscan CT) and magnetic resonance imaging (multiscan MRI) in recent decades enable investigators to pursue measurements of tissue-system level components such as adipose tissue and skeletal muscle (16, 17). Although CT and MRI are fundamentally different, both methods measure the area of an arbitrary cross-section and are based on the same geometric formula (**Table 4**). If the number of cross-sections is large enough, the model error should be small. Multiscan CT and MRI methods are therefore used as a reference for evaluating other *in vivo* methods (18, 19).

Table 4. Examples of type II property-based methods

Method (ref.)	Unknown component	Measurable property	Property-component model	Prediction equation
Geometric method (CT and MRI) (16, 17)	AT, SM	S, L	$C = d \times \sum_{i=1}^{n} [0.5 \times (S_1 + S_2) \times L]$	$C = d \times \sum_{i=1}^{n} [0.5 \times (S_1 + S_2) \times L]$
Whole body ^{40}K counting (10)	TBK	γ-ray (1.40 MeV)	γ dpm = 198 × TBK	TBK = γ dpm/198
***In Vivo* neutron activation** (20)	TBCa	γ-ray (3.10 MeV)	γ cpm = a_1 × TBCa	TBCa = γ cpm/a_1
	TBC	γ-ray (4.44 MeV)	γ cpm = a_2 × TBC	TBC = γ cpm/a_2
	TBN	γ-ray (10.83 MeV)	γ cpm = a_3 × TBN	TBN = γ cpm/a_3
Dilution (10)	TBW	β-ray (0.018 MeV)	T = c × V	V = T/c
Underwater weighing (4)	fat, FFM	BW	BW = fat + FFM	fat = 4.95 × BV − 4.50 × BW
		BV	BV = fat/0.900 + FFM/1.10	FFM = 5.50 × BW − 4.95 ×BV
Gas uptake (21)	fat	BW	BW = fat + FFM	fat = (G − a_2 × BW)/(a_1 − a_2)
		G	G = a_1 × fat + a_2 × FFM	FFM = (a_1 × BW − G)/(a_1 − a_2)
Dual energy X-ray absorptiometry (DXA) (22)	Mo, fat, fat-free soft tissue	R	$R = [f_A \times (\mu_A)_{40} + f_B \times (\mu_B)_{40}]/[f_A \times (\mu_A)_{70} + f_B \times (\mu_B)_{70}]$ $f_A + f_B = 1$	

Geometric method (CT and MRI): AT, adipose tissue; C, unknown component mass; d, assumed constant density of the unknown component; L length between two cross-sections; *n*, the number of cross-sections; S, cross-sectional area; SM, skeletal muscle.

Whole body ^{40}K counting method: dpm, disintegration per minute; TBK, total body potassium (g).

***In Vivo* neutron activation method**: cpm, counts per minute; TBC, TBCa, and TBN, total body carbon, calcium, and nitrogen; *a*, the constant determined by phantom test.

Dilution method: c, tracer concentration in sample of unknown component at equilibrium; T, known tracer amount at equilibrium; TBW, total body water; V, volume of unknown component.

Underwater weighing method: BV, body volume (L); BW, body weight (kg); 0.900 and 1.100, the assumed constant density (kg/L) of fat and fat-free body mass, respectively.

Gas uptake method: G, the measurable gas amount taken up by the body; a_1 and a_2, the known gas solubility in fat and fat-free body mass, respectively.

DXA method: f_A, and f_B, the fractions of mixture (A + B) as component A and B, respectively; Mo, bone mineral; μ_A and μ_B, the known mass attenuation coefficient for component A and B, respectively; R, the measurable ratio of attenuation for the mixture (A + B) at 40 KeV and 70 KeV.

Besides geometric methods, most type II property-based methods share in common a property-component model,

$$\mathbf{P = \Sigma(R_n \times C_n)} \qquad [4]$$

where P represents the whole body value of the property, C total body component mass, R the assumed constant coefficient relating C with P, and n the component number.

When $n = 1$, model 4 takes a simple form and a type II property-based method can be derived,

$$\mathbf{P = R \times C}, \text{ and } \mathbf{C = P / R} \qquad [5]$$

A classic example is the decay property of naturally occurring ^{40}K, which is radioactive and occupies a constant proportion (0.0118%) of total body potassium (TBK) (10). When ^{40}K decays, it emits a 1.40 MeV γ-ray and a constant relationship exists between the two such that γ dpm (1.40 MeV) = 198 × TBK (g). This relationship allows estimation of TBK by using whole-body counting, although technical issues must be considered in regard to body size, shape, and configuration. Other examples include *in vivo* neutron activation analysis (20) and dilution methods (10) (**Table 4**). These type II property-based methods share in common the characteristic that one property is involved with one component at a given body composition level. Thus only one property-component model is needed to derive the prediction equation.

When $n = 2$, model 4 takes following form at a given body composition level,

$$\mathbf{P = R_1 \times C_1 + R_2 \times C_2} \qquad [6]$$

These type II property-based methods share in common the characteristic that one property (e.g., body volume) is involved with two components (e.g., fat and fat-free body mass) at a given body composition level. Two simultaneous property-component models are therefore necessary to derive the prediction equation (**Table 4**). A classic example is the underwater weighing method for measuring total body fat and FFM (4). Two property-component models, body weight (BW) and body volume (BV), are needed. Resolving the simultaneous models, one can derive equations for predicting total body fat and fat-free body mass. Other examples are the gas uptake method (21) and dual energy X-ray absorptiometry (22). Generally, if there are n unknown components at a given body composition level, n simultaneous property-component models are necessary to develop a type II property-based method.

COMBINED METHODS

Combined methods are the third and final category of *in vivo* body composition methods which allow an unknown component to be quantified from both measurable properties and known components. Combined methods can be expressed as the general formula,

$$C_u = f(P, C_k), \qquad [7]$$

where C_u represents an unknown component mass, P the measurable property, C_k the known component mass, and f the mathematical function relating P and C_k with C_u.

Combined methods can also be sub-classified into type I and II according to mathematical function derivation. In order to develop a type I combined method, a reference method is necessary to measure the unknown component. A prediction equation is then established from both measurable properties and known components by statistical analysis (e.g., multiple regression analysis). To our knowledge there are very few examples of type I combined methods in current use. An example is the assessment of extracellular water (ECW) by both total body water (TBW) and electrical impedance at 100 kHz (Z_{100}) and 1 kHz (Z_1), ECW = $0.52 \times TBW \times Z_{100}/Z_1 - 0.50$; $r^2 = 0.82$, SEE = 1.19 kg (7).

Type II combined methods are derived from simultaneous property-component models and component-component models. **Table 5** illustrates a few type II combined methods in current use which measure total body fat mass.

In 1942 Behnke suggested a type II property-based method for measuring total body fat (fat = $4.95 \times BV - 4.50 \times BW$) which included two measurable properties, body weight and body volume (4). This method assumes that the proportions of fat-free body mass as water, protein, and mineral are constant. Because there are individual differences in these proportions, model errors are known to exist.

During the decades following publication of Behnke's method several type II combined methods were suggested to improve total body fat measurement (8, 24-26). Despite differences in the prediction equations, these methods share two characteristics in common. First, these type II combined methods expand the two-compartment body weight model and body volume model used in Behnke's method into four- or more compartment models (**Table 5**). Second, all of these type II combined methods measure two properties (body weight and body volume) and one or two components (total body water and/or mineral). These

Table 5. Type II combined methods of measuring total body fat: fat = $f(P, C_k)$

Author (ref.)	Measurable property	Known component	Property-component model and component-component model	Prediction equation
Siri (8)	BW BV	water	BW = fat + water + protein + mineral BV = fat/0.9007 + water/0.994 + protein/1.34 + mineral/3.04 protein = 2.40 × mineral	fat = 2.057 × BV − 0.786 × water − 1.286 × BW
Lohman (24)	BW BV	mineral	BW = fat + water + protein + mineral BV = fat/0.9007 + water/0.994 + protein/1.34 + mineral/3.04 water = 4.00 × protein	fat = 6.386 × BV + 3.961 × mineral − 6.09 × BW
Baumgartner et al. (25)	BW BV	water mineral	BW = fat + water + protein + Mo + Ms BV = fat/0.9007 + water/0.994 + protein/1.34 + Mo/2.982 + Ms/3.317 Ms = 0.235 × Mo	fat = 2.75 × BV − 0.714 × water + 1.148 × mineral − 2.05 × BW
Selinger (26)	BW BV	water Mo	BW = fat + water + protein + Mo + Ms BV = fat/0.9007 + water/0.994 + protein/1.34 + Mo/2.982 + Ms/3.317 Ms = 0.0105 × BW	fat = 2.75 × BV − 0.714 × water + 1.129 × Mo − 2.037 × BW

BV, body volume (L); BW, body weight (kg); C_k, known component; *f*, mathematical function; Mo, bone mineral (kg); Ms, soft tissue mineral (kg); P, measurable property. Modified from reference 23.

approaches eliminate model errors related to individual variation in hydration and/or mineral proportions.

However, these type II combined methods still assume some constant ratios between components (**Table 5**). Siri assumed that the ratio of mineral to protein is constant at 5:12 (8). Lohman assumed a constant protein/water ratio of 1: 4 (24) and Baumgartner et al. assumed a soft tissue mineral/bone mineral ratio of 0.235: 1 (25). Selinger made the assumption that soft tissue mineral is constant at 1.05% of body weight (26). Because these ratios actually vary within and between individuals, model error still exists in the type II combined methods of measuring total body fat, although they are smaller than the error of Behnke's two-compartment model.

CONCLUSION

Our previous report (2) and present overview suggest for the first time a systematic organization of body composition methods founded on a conceptual basis. This approach should facilitate scientific organization and communication regarding body composition research findings. Our suggested approach also indicates that four body composition methodology rules exist as follows:

♦ Body composition methodology contains two broad areas, *in vitro* and *in vivo*.

♦ Any *in vivo* body composition method can be summarized by the general formula:

$\mathbf{C = f(Q)}$. All *in vivo* methods are distinguished according to measurable quantity (property and/or component) and mathematical function derivation (type I or II).

♦ Property-based methods are the basis of component-based and combined methods; and component-based and combined methods are the expansion of property-based methods,

♦ *In Vivo* body composition methods are ultimately based on measurement of body properties.

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Research is to see what everyone alse has seen
and to think no one alse has thought.

CHAPTER 6

TOTAL BODY OXYGEN MASS: ASSESSMENT FROM BODY WEIGHT AND TOTAL BODY WATER

ZiMian Wang, Paul Deurenberg, Ruimei Ma, Donald Kotler, Steven B. Heymsfield

ABSTRACT

Although oxygen is the most abundant element of the human body, at present there are very few reports concerning the measurement of total body oxygen mass (TBO). The purpose of the present study was to develop a simple method to assess TBO *in vivo*. Based on the known stoichiometries and proportions of relevant components at the molecular level, a TBO (in kg) prediction equation was derived from body weight (BW, in kg) and total body water mass (TBW, in kg): TBO = 0.113 × BW + 0.845 × TBW. Total body mass of 8 elements (C, H, N, Ca, P, K, Na, and Cl) was measured in 19 healthy male subjects and 18 male AIDS patients using *in vivo* neutron activation and ^{40}K whole-body counting. The difference between BW and the sum of the 8 elements was used to estimate TBO and thereby to evaluate the proposed TBO model. The mean oxygen mass for the pooled subjects estimated by the proposed model (TBO_M) agreed within 0.2% of oxygen mass measured by neutron activation (TBO_{NA}). The two TBO measures were highly correlated: $TBO_M = 0.956 \times TBO_{NA} + 1.9$; r = 0.975, $P < 0.001$, SEE = 1.6 kg, n = 37. The mean difference (± SD) between TBO_M and TBO_{NA} was –0.02 ± 0.46 kg for the combined group. These results confirm the adequacy of the proposed model which provides a simple, low cost, and safe method of assessing TBO *in vivo*.

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INTRODUCTION

Elements are fundamental building blocks of the human body. At the atomic level, the human body is composed of 10 main elements, O, C, H, N, Ca, P, S, K, Na, and Cl (Wang et al., 1992). Most of these elements can now be measured *in vivo* using traditional and new neutron activation analysis-whole body counting methods (Ma et al., 1993).

Oxygen is the most abundant element in the human body which, for example, accounts for 61% of body weight in Reference Man (Snyder et al., 1975). Measurement of total body oxygen (TBO) mass and its change with age, race, sex, nutrition, and diseases might be useful in understanding human body composition. At present, the methodology for measuring TBO is complex and not widely available. There are few investigations of oxygen mass in humans and it's association with other body composition components. The first available method of measuring oxygen mass is to quantify relevant chemical components (i.e., fat, water, protein, and mineral). Protein is measured with neutron activation analysis, and then the amount of oxygen in these components is calculated.

In the second available method total body oxygen and carbon are simultaneously measured (Kehayias and Zhang, 1993; Mitra et al., 1995). The third method of estimating TBO is to calculate the difference between body weight and the sum of other main elements (i.e., C, H, N, Ca, P, S, K, Na, and Cl). All three methods require *in vivo* neutron activation facilities which are very expensive and also expose subjects to radiation.

The purpose of the present study was to derive a TBO prediction equation from total body water (TBW) and body weight (BW). As methods for estimating TBW are widely available, the proposed method potentially provides a simple, low cost, and safe method of assessing the mass of TBO *in vivo*.

METHODS

Model Derivation

The chemical composition of human body can be expressed as (Wang et al., 1992)

$$BW = \text{fat} + \text{water} + \text{protein} + \text{glycogen} + \text{mineral}. \qquad [1]$$

As an initial phase this proposal developed an equation relating total body oxygen with the components at the molecular level. The chemical stoichiometry of relevant components are

$C_{55}H_{102}O_6$ for fat, H_2O for water, $C_{100}H_{159}N_{26}O_{32}S_{0.7}$ for protein, $(C_6H_{10}O_5)_x$ for glycogen, and $[Ca_3(PO_4)_2]_3Ca(OH)_2$ for calcium hydroxyapatite, the main compound of bone mineral (Heymsfield et al., 1991). The proportions of these components as oxygen are: 11.3% of fat, 88.9% of water, 22.7% of protein, 49.4% of glycogen, and 41.4% of calcium hydroxyapatite, respectively. Hence, the following TBO equation is derived,

$$\begin{aligned} TBO &= 0.113 \times \text{fat} + 0.889 \times \text{water} + 0.227 \times \text{protein} + 0.494 \times \text{glycogen} \\ &\quad + 0.414 \times \text{bone mineral} \\ &= 0.113 \times (\text{BW} - \text{fat-free body mass}) + 0.889 \times \text{water} + 0.227 \times \text{protein} \\ &\quad + 0.494 \times \text{glycogen} + 0.414 \times \text{bone mineral}. \end{aligned} \qquad [2]$$

Whole body *in vitro* chemical analysis of six cadavers revealed that the proportions of fat-free body mass as water, protein and mineral are relatively constant at 0.724 ± 0.034, 0.205 ± 0.028 and 0.071 ± 0.013, respectively (Garrow, 1993). Glycogen accounts for ~ 1% of fat-free body mass in weight-stable adults (Lentner, 1963). Thus equation 2 can be simplified as

$$TBO = 0.113 \times BW + 0.845 \times TBW. \qquad [3]$$

Subjects

The subject pool of the present study consisted of both healthy men and men with AIDS. The rational for the inclusion of patients with weight loss was twofold: it provided the opportunity to exam total body oxygen mass under actual clinical conditions; and weight loss might reveal possible limitations of the proposed model that are not evident in healthy subjects.

The subjects pool consisted of 19 healthy male adults and 18 men with AIDS. Healthy subjects were recruited from the staff of the hospital and from the students of the local university. Each subject completed a medical history, physical examination, and routine screening blood studies to exclude the presence of underlying diseases. The men with AIDS were recruited from among patients cared for at the hospital. All were ambulatory, afebrile, and met the medical center for disease control criteria for AIDS.

Subjects were first screened to ensure that they met the study entry criteria. All subjects signed an informed consent that was approved by the hospital's Institutional Review Board. They then completed tritium dilution for total body water, and *in vivo* neutron activation analysis and ^{40}K whole-body counting for total body element contents. Body weight was obtained to the nearest 0.1 kg just before neutron activation analysis.

Body Composition Measurement

Tritium space (^3H_2O, in liter) was measured at the Body Composition Unit of St. Luke's-Roosevelt Hospital with the precision of 1.7% (C.V.) (Ma et al., 1996). The tritium space was then converted into total body water (in kg) by multiplying with the correction factor and water density at body temperature (TBW = $^3H_2O \times 0.96 \times 0.994$).

Total body potassium was determined using the St. Luke's 4 π whole body counter with the precision of 3.2% (Pierson et al., 1982). Total body content of 7 elements was quantified using the *in vivo* neutron activation facility at Brookhaven National Laboratory (Ma et al., 1993). Total body carbon was measured using inelastic neutron scattering system with the precision of 11.0% (Ma et al., 1996). Total body calcium, phosphorus, sodium, and chlorine were measured using delayed- γ neutron activation with the precision of 1.6%, 4.5%, 1.8%, and 1.2%, respectively (Ma et al., 1993). Total body nitrogen and hydrogen were determined by prompt- γ neutron activation with the precision of 1.8% (Ma et al., 1993).

Total body oxygen content was calculated as the difference between 99.3% of body weight and the sum of the 8 main elements:

$$TBO = 0.993 \times BW - \sum(C, H, N, Ca, P, K, Na, Cl) \qquad [4]$$

In the equation the coefficient 0.993 is based on the consideration that sulfur and other trace elements account for 0.7% of body weight in the Reference Man (Snyder et al., 1975).

Statistical Analysis

Results are expressed as the group mean and standard deviation. Simple linear regression analysis was used to describe the relationship between TBO derived from the proposed model and TBO calculated from 8 elements by neutron activation. Student T-test was used to test the significant difference between the two TBO estimates. The Bland and Altman method (1986) was applied to analyze the relationship between difference and mean value of the two TBO estimates.

Data were analyzed using SAS version 5 (SAS Institute, Cary, NC).

Table 1. Results of body composition studies

	Healthy males (n = 19)			Male AIDS (n = 18)			*P*
	mean	**SD**	**range**	**mean**	**SD**	**range**	
Age, yr	36.7	13.2	19-72	38.9	10.3	22-62	0.58
BW, kg	76.6	13.3	55.8-104.5	64.0	7.9	48.5-77.1	0.0015
Ht, m	1.76	0.08	1.62-1.93	1.73	0.07	1.57-1.82	0.18
BMI, kg/m^2	24.5	2.9	20.1-30.5	21.3	2.3	17.0-26.0	0.00067
TBC, kg	16.2	4.0	9.5-24.2	12.1	3.7	6.8-21.5	0.0033
TBH, kg	7.7	1.4	5.5-10.7	6.4	0.8	4.9-7.9	0.0018
TBN, kg	2.07	0.24	1.68-2.53	1.70	0.21	1.33-2.13	0.00001
TBCa, kg	0.954	0.139	0.708-1.289	0.839	0.101	0.62-1.057	0.0067
TBP, kg	0.573	0.081	0.452-0.727	0.523	0.074	0.349-0.64	0.057
TBK, kg	0.164	0.031	0.105-0.219	0.127	0.018	0.085-0.158	0.0001
TBNa, kg	0.085	0.010	0.068-0.110	0.078	0.010	0.058-0.101	0.051
TBCl, kg	0.089	0.012	0.072-0.125	0.084	0.011	0.064-0.109	0.25
TBW, kg	47.0	7.4	34.5-59.4	40.6	5.3	32.6-50.4	0.0047
TBO_{NA}, kg	48.2	8.1	37.0-64.5	41.7	5.3	31.5-50.0	0.0062
TBO_{M}, kg	48.2	7.7	35.6-61.8	41.4	5.2	33.0-50.1	0.0033

BMI, body mass index; BW, body weight; Ht, height; SD, standard deviation; TB, total body mass of element; TBO_{NA}, total body oxygen mass by *in vivo* neutron activation analysis; TBO_{M}, total body oxygen mass calculated with proposed model; TBW, total body water mass.

RESULTS

Some characteristics and the body composition analysis of the healthy males (n = 19) and the males with AIDS (n = 18) are presented in **Table 1**. Among the 8 measured elements, there were significant differences in 5 elements (C, H, N, Ca, and K) between the healthy and the AIDS groups. Total body water mass of the healthy males was also higher than that in the

males with AIDS. However, there was no significant difference in the proportion of body weight as oxygen between the healthy group (0.631 ± 0.025) and the AIDS group (0.641 ± 0.043).

The mean total body oxygen mass for the pooled subjects estimated by the proposed model (equation 3) (TBO_M = 44.9 ± 7.4 kg) agreed within 0.2% of total body oxygen mass measured by NA (TBO_{NA} = 45.0 ± 7.5 kg). The two measures of total body oxygen were highly correlated,

$$TBO_M = 0.938 \times TBO_{NA} + 3.0;\ r = 0.982,\ P < 0.001;$$
$$n = 19 \text{ healthy males} \qquad [5]$$

$$TBO_M = 0.925 \times TBO_{NA} + 2.9;\ r = 0.939,\ P < 0.001;$$
$$n = 18 \text{ males with AIDS} \qquad [6]$$

Because the slopes and intercepts of equations 5 and 6 did not different ($P > 0.05$), the data for the two groups were combined (**Figure 1**),

$$TBO_M = 0.956 \times TBO_{NA} + 1.9;\ r = 0.975,\ P < 0.001;$$
$$SEE = 1.6 \text{ kg};\ n = 37 \qquad [7]$$

Agreement between model-calculated and NA-measured TBO values was evaluated using the analysis of Bland and Altman (1986) in which the difference between the values is plotted against their mean of the two methods (**Figure 2**). The mean difference of the two methods was 0.11 ± 0.34 kg for the healthy males, −0.16 ± 0.54 kg for the males with AIDS, and −0.02 ± 0.46 kg for the combined groups. These results confirm the adequacy of the proposed model of predicting TBO from body weight and total body water.

DISCUSSION

Based on the theoretical considerations, a simple model was derived relating total body oxygen with body weight and total body water. The TBO mass calculated from the model was confirmed by TBO mass using *in vivo* neutron activation analysis as reference both in healthy males and males with AIDS. This indicated that although AIDS is a wasting disease, it had no influence on the validity of the proposed model.

The present method has two main error sources that may contribute to TBO measurement, i.e., error from water measurement, and error from model derivation. The error associated

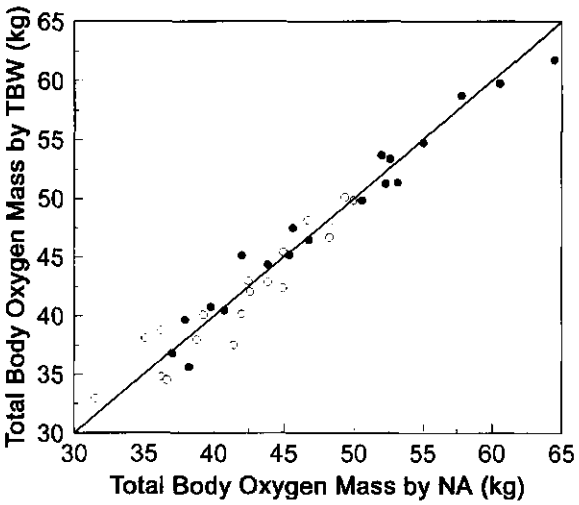

Figure 1. Total body oxygen mass calculated from the proposed model (TBO_M, in kg) on the ordinate; and total body oxygen mass based on *in vivo* neutron activation analysis (TBO_{NA}, in kg) on the abscissa [$TBO_M = 0.938 \times TBO_{NA} + 3.0$; r = 0.982, $P < 0.001$; n = 19 for the healthy males (●); $TBO_M = 0.925 \times TBO_{NA} + 2.9$; r = 0.939, $P < 0.001$; n = 18 for the males with AIDS (O); and $TBO_M = 0.956 \times TBO_{NA} + 1.9$; r = 0.975, $P < 0.001$, SEE = 1.6 kg; n = 37 for the combined group]. The line of identity is shown in the figure.

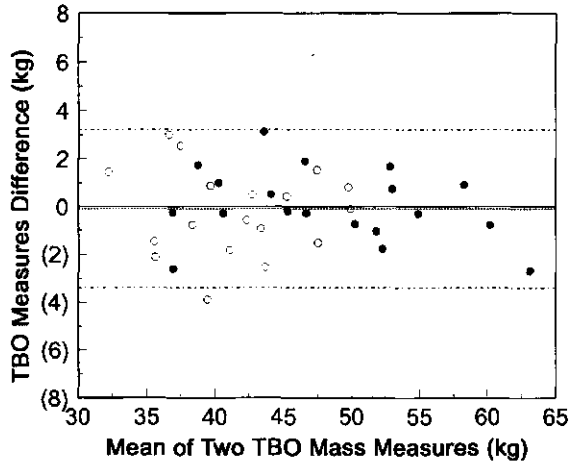

Figure 2. Assessment of agreement between total body oxygen masses derived from the proposed model (TBO_M) and *in vivo* neutron activation analysis (TBO_{NA}). The difference between the two TBO measures on the ordinate; and the mean of the two TBO measures on the abscissa. The lines of mean difference (–0.1 kg), mean + 2SD (3.2 kg) and mean – 2SD (–3.4 kg) are indicated; n = 37, ●: healthy subjects, O: males with AIDS.

with body weight measurement is very small. Total body water mass is measured with a precision of 1.7% by ^3H_2O dilution method in our group (Ma et al., 1996). For the Reference Man of 70 kg body weight and 42 kg body water, the method's error from water measurement is 0.60 kg of oxygen.

The method's error from model derivation is concerned with the assumptions which were used in the model. Based on the stoichiometry of relevant components at the molecular level, we calculated the proportions of these components as oxygen, resulting in TBO equation (equation 2). The actual proportions may slightly differ from the assumed ones, and may vary within and between individuals. However, the main source of oxygen in human body is water, and the proportion of water as oxygen is constant (0.889). Therefore, the variation of proportions of fat and protein as oxygen may only lead to a minor model error. Another assumption is that the proportions of fat-free body mass as water, protein, and mineral are constant. It is known that these proportions vary somewhat within and between individuals. This should be the major error source from the suggested model. For the Reference Man, the method's error from the model assumptions is 2.13 kg of oxygen.

Total error of the suggested TBO method is 2.2 kg or 5.1% for the Reference Man with 43 kg oxygen. Comparatively, the TBO measurement error in the NA method (equation 4) is smaller (1.54 kg or 3.6% for the Reference Man) than that of our prediction equation. However, the NA method is unable be widely applied because of the high cost and the radiation exposure.

At present, total body potassium can be measured by whole body ^{40}K counting (Pierson et al., 1982); and total body hydrogen can be estimated from body weight (TBH = 0.101 × BW) (Vartsky et al., 1984). The present study provided a model which estimates total body oxygen mass. Thus, the elemental composition of about 75% body weight (O, H, and K) could be determined by using simple, safe and relatively low cost methods. Along with the progress of understanding relationships between various body components, it should be possible to develop new models which could simply estimate other main elements.

Conclusion

Although there are different possibilities for estimating total body oxygen *in vivo*, these methods are expensive and radiation-involved. The proposed TBO equation in the present study is only involved with measurement of body weight and total body water; and the prediction result is relatively accurate.

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Science consists in grouping facts so that general laws or conclusions may be drawn from them.

—— C. Darwin

CHAPTER 7

SIX-COMPARTMENT BODY COMPOSITION MODEL: INTER-METHOD COMPARISONS OF TOTAL BODY FAT MEASUREMENT

ZiMian Wang, Paul Deurenberg, Shumei S. Guo, Angelo Pietrobelli, Jack Wang, Richard N. Pierson Jr, Steven B. Heymsfield

ABSTRACT

OBJECTIVE: To compare 16 currently used total body fat methods to a 6-compartment criterion model based on *in vivo* neutron activation analysis.

DESIGN: Observational, inter-method comparison study.

SUBJECTS: Twenty-three healthy subjects (17 males and 6 females).

MEASUREMENTS: Total body water (TBW) was measured by tritium dilution; body volume by underwater weighing (UWW); total body fat and bone mineral by dual-energy X-ray absorptiometry; total body potassium by whole-body ^{40}K counting; total body carbon, nitrogen, calcium, phosphorus, sodium, and chlorine by *in vivo* neutron activation analysis; skinfolds/circumferences by anthropometry (Anth); and resistance by single-frequency bioimpedance analysis (BIA).

RESULTS: The average of total body fat mass measurements by the 6-compartment neutron activation model was 19.7 ± 10.2 kg (mean ± SD) and comparable estimates by other methods ranged from 17.4 to 24.3 kg. Although all 16 methods were highly correlated with the 6-compartment criterion model, three groups emerged based on their comparative characteristics (technical error, coefficient of reliability, Bland-Altman analysis) relative to criterion fat estimates: in decreasing order of agreement, 1. multi-compartment model methods of Baumgartner (19.5 ± 9.9 kg), Heymsfield (19.6 ± 9.9 kg), Selinger (19.7 ± 10.2 kg), and Siri (19.6 ± 9.9 kg); 2. DXA (20.0 ± 10.8 kg), Pace-TBW (18.8 ± 10.1 kg), Behnke-UWW (20.0 ± 9.9 kg), and Brozek-UWW (19.4 ± 9.2 kg) methods; and 3. Segal-BIA (17.4 ± 7.2 kg), Forbes-TBN (21.8 ± 10.5 kg), Durnin-Anth (22.1 ± 9.5 kg), Forbes-TBK (22.9 ± 11.9 kg), and Steinkamp-Anth (24.3 ± 9.5 kg) methods.

CONCLUSION: Relative to criterion fat estimates, body composition methods can be organized into three groups based on inter-method comparisons including technical error, coefficient of reliability, and Bland-Altman analysis. These initial groupings may prove useful in establishing the clinical and research role of the many available fat estimation methods.

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INTRODUCTION

At the present time there are more than ten methods of estimating total body fat *in vivo*. These methods can be generally organized into two groups (1). The first group, statistically-derived or "type I" fat estimation methods such as anthropometry and bioimpedance analysis (BIA), share two features in common: they depend on a reference method and subsequent statistical data analysis for the development of a prediction formula. Methods of the second group are model-dependent or "type II" and are derived from well-known body composition models based on stable component relationships. Examples of model-dependent methods include the total body potassium (TBK) method of Forbes (2, 3), the total body water (TBW) method of Pace and Rathbun (4), the densitometry methods introduced and extended by Behnke et al. (5) and Brozek et al. (6), the newly developed dual energy X-ray absorptiometry (DXA) approach (7), and multi-compartment models (8).

Although numerous statistically-derived and model-based methods are available, fat estimates in individuals often differ, sometimes substantially, from each other. An example is that mean total body fat predicted by the Segal-BIA method provided fat results far lower than that provided by the Forbes-TBK method (24.5% *vs.* 38.0% of body weight) in the same subjects (9). These discrepancies between methods suggests the need for an inter-method comparison study of total body fat measurement methods. Although these issues have been previously examined, two major problems exist in earlier reports.

The first problem with earlier studies involves the criterion for total body fat measurement. As the "true" value of total body fat mass is unmeasurable in living humans, a criterion with high accuracy is necessary to evaluate other methods. Although several traditional methods (e.g., Forbes-TBK and Pace-TBW methods) are often used as criteria, they are not optimum for evaluating other methods because they are formulated on assumptions of uncertain validity (8). The Cohn-neutron activation and DXA methods are also applied as criteria (7, 10, 11), although these methods have inherent error sources that could bias or cause large within-individual errors in fat estimates. For example, the Cohn-neutron activation model fails to consider the glycogen and soft tissue mineral components. There are also several unresolved issues related to the DXA method such as hydration effects and underlying geometric models (12). A more accurate criterion is thus needed for evaluation of total body fat.

Another problem with early studies is that up to the present a complete inter-method comparison of currently used total body fat estimation methods is lacking. Previous studies usually compared two or three methods and in a few studies seven or eight methods were compared (9, 13, 14). Such incomplete inter-method comparisons can not provide a comprehensive picture of total body fat estimation methods. Ideally, total body fat should be estimated in the same subject group using all currently available methods. However, this is not practical for most body composition laboratories due to the limited number of available techniques at any one center.

The purpose of the present investigation was to carry out an inter-method comparison between a newly derived criterion based on *in vivo* neutron activation analysis and other currently used total body fat estimation methods. Thirteen model-based methods and three statistically-derived methods were compared to the six-compartment *in vivo* neutron activation analysis model.

METHODS

Subjects

The subject group consisted of 23 healthy adults (17 males and 6 females; 12 White, 3 African American, and 8 Puerto Ricans) who ranged in age from 19 to 69 years and in body weight from 54 to 106 kg. The subjects in the present investigation participated in other large studies of body composition. Healthy subjects were recruited from hospital staff and local residents. All subjects signed an informed consent that was approved by the Hospital's Institutional Review Board. Each subject completed a medical history, physical examination, and routine blood studies to exclude the presence of underlying diseases.

All subjects completed tritium dilution, underwater weighing, anthropometry, DXA, BIA, ^{40}K whole-body counting, and *in vivo* neutron activation analysis studies.

Six-Compartment Model for Total Body Fat Measurement

Cohn and his colleagues suggested a model which treats the whole body as a four compartment system composed of fat, water, protein, and bone minerals (Mo) (10). Total body fat was then determined as the difference between body weight (BW) and the sum of the other three components, fat = BW – (water + protein + Mo). Two components in fat-free body mass (FFM), soft tissue mineral (Ms) and glycogen (G), are ignored in the Cohn model.

Although Ms and G are much smaller than the other components, this simplification inevitably introduces a model error and leads to an over-estimation of total body fat mass.

A six-compartment model was proposed by Heymsfield and his colleagues (15),

$$\text{fat} = \text{BW} - (\text{water} + \text{protein} + \text{Mo} + \text{Ms} + \text{G}) \quad [1]$$

where water is measured by tritium dilution; protein is measured based on total body nitrogen (TBN), protein = 6.25 × TBN; Mo is calculated from total body calcium (TBCa), Mo = TBCa/0.364; Ms is calculated from total body potassium, sodium, chlorine, and calcium, Ms = 2.76 × TBK + TBNa + 1.43 × TBCl − 0.038 × TBCa (16); and G is estimated based on an assumption, G = 0.044 × protein = 0.275 × TBN (15). In the present study a corresponding model is applied as the criterion to compare with the 16 other total body fat estimation methods,

$$\text{fat} = \text{BW} - (\text{water} + 6.525 \times \text{TBN} + 2.709 \times \text{TBCa} + 2.76 \times \text{TBK} + \text{TBNa} + 1.43 \times \text{TBCl}). \quad [2]$$

This new equation was developed by combining the equations for protein, bone mineral, soft tissue mineral, and glycogen along with water to solve for fat mass. The model error in equation 2 is small, although there still exists measurement errors for the various components.

Body Composition Measurements

Consenting subjects were studied after an overnight fast. Body weight was measured to the nearest 0.1 kg and height to 0.5 cm.

Tritium space (^3H_2O, in L) was measured at the Body Composition Unit of St. Luke's-Roosevelt Hospital with a precision (CV) of 1.5% (17). The tritium space was then converted into total body water (TBW, in kg) by multiplying with the correction factor for nonaqueous hydrogen exchange and water density at 36 °C (TBW = $^3H_2O \times 0.96 \times 0.994$).

Body density was measured by underwater weighing in a stainless steel water tank using a standard method with a technical error of 0.0020 g/cm^3 (18). Residual lung volume was estimated after immersion in a sitting position by means of the closed-circuit O_2 dilution method (19).

A Lunar DXA scanner (Lunar DPX with software version 3.6; Madison, WI) was used which has a precision of 1.28% for bone mineral (15) and in the range of 3% - 4% for body fat (20).

Table 1. Thirteen model-based methods for estimating body fat mass

Method (ref.)	Prediction equation	Main assumption
Baumgartner (24)	fat = 2.75 × BV – 0.714 × TBW + 1.148 × M – 2.05 × BW	M = 1.235 × Mo
Behnke (5)	fat = 4.95 × BV – 4.50 × BW	densities of fat and FFM are 0.90 and 1.10 g/cm^3
Brozek (6)	fat = 4.570 × BV – 4.142 × BW	constant proportions of water, protein, and mineral in FFM
Cohn (10)	fat = BW – TBW – 6.25 × TBN – 2.747 × TBCa	1. ignore Mo and G; 2. protein = 6.25 × TBN; 3. Mo = TBCa/0.364
DXA (7, 25)		constant hydration in fat-free soft tissues
Forbes-TBK (2, 3)	fat = BW – 375.6 × TBK (males); fat = BW – 398.4 × TBK (females)	TBK/FFM = 0.00266 (males) and 0.00251 (females)
Forbes-TBN (26)	fat = BW – 30.30 × TBN	TBN/FFM = 0.033
Heymsfield (8)	fat = 2.513 ×BV – 0.739 × TBW + 0.947 × Mo – 1.79 × BW	density of mixture (protein + Ms + G) is 1.404
Kehayias (27)	fat = 1.30 × TBC – 4.45 × TBN – 0.065 × TBCa	1. protein = 6.25 × TBN; 2. G = 0.044 × protein; 3. C/Ca = 0.05 g/g in bone
Lohman (28)	fat = 6.386 × BV + 3.961 × M – 6.09 × BW	1. TBW/protein = 3.80; 2. M = 1.235 × Mo
Pace (4)	fat = BW – 1.3661 × TBW	TBW/FFM = 0.732
Selinger (29)	fat = 2.75 × BV – 0.714 × TBW + 1.129 × Mo – 2.037 ×BW	Ms = 0.0105 × BW
Siri (30)	fat = 2.118 × BV – 0.78 × TBW – 1.351 × BW	mineral/protein = 0.351

BV, body volume (in liter); BW, body weight (kg); FFM, fat-free body mass; G, glycogen; M, mineral (kg); Mo, bone mineral (kg); Ms, soft tissue mineral (kg); TB; total body; TBW, total body water (kg).

The method uses X-rays of two distinct energy levels that are differently attenuated by bone mineral, fat, and fat-free soft tissues. The relative attenuation of the two X-ray energies is used to estimate the proportion of soft tissue as fat. The bone mineral content (BMC) measured by DXA represents ashed bone (21). One gram of bone mineral yields 0.9582 g of ash because labile components such as bound water and CO_2 are lost during heating (22). The BMC therefore needs to be converted to bone mineral as Mo = BMC × 1.0436 (i.e., 1/0.9582).

TBK was determined using the St. Luke's 4 π whole body counter with a precision of 3.2% (17). Total body content of 7 elements was quantified using the *in vivo* neutron activation facilities at Brookhaven National Laboratory (22). TBN was determined by prompt- γ neutron activation with a precision of 1.8% (23). Total body calcium, phosphorus, sodium, and chlorine were measured using delayed- γ neutron activation with precisions of 1.6%, 4.5%, 1.8%, and 1.2%, respectively (23). TBC was measured using inelastic neutron scattering with a precision of 11.0% (23).

Table 1 shows the calculation formulas of the 13 model-based methods as well as their main assumptions.

The anthropometric equations of Durnin and Womersley were used to predict body density with logarithmic transformation of the sum of four skinfold thickness, triceps, biceps, subscapula, and iliac crest. The equations are age and sex specific. Body fat was then calculated from body density. The precision of this method is about 3.5% for percentage body fat (31).

Steinkamp's anthropometric method was based on assessment of seven circumferences, two limb lengths, five diameters and four skinfold thickness. Different prediction equations were applied to estimate body fat according to age, sex, and race. The standard error of estimate (SEE) of total body fat was 2.0 - 3.8 kg (32).

BIA was used to estimate total body fat using a standard protocol and an RJL instrument (model 101, RJL Systems, Mt Clemens, MI). An equation developed by Segal et al. was used in the present study to predict body density from resistance values. The Behnke densitometry equation was then applied to calculate percentage fat from body density (33).

Statistical Analysis

Results are expressed as group mean and standard deviation (mean ± SD). Simple linear regression analysis was applied to describe the relationship between total body fat measured by

the six-compartment model and that estimated by the 16 other methods. The difference in total body fat estimates between the six-compartment model and methods under examination was related to the six-compartment model, as described by Bland and Altman (34).

Inter-method differences were compared using statistical methods described by Fleiss (35). These analyses include calculation of mean absolute difference, technical error (TE), and intraclass coefficient of reliability (CR) to account for the degree of partitioned variance between the participants and the methods (35). The values for TE were calculated as

$$TE = \sqrt{\frac{\sum_{i=1}^{n}(y_{i1} - y_{i2})^2}{2n}}$$

where y_{i1} and y_{i2} are total body fat, and n is the total number of subjects. The CR was computed as

$$CR\% = \frac{\sigma_s^2}{\sigma_s^2 + \sigma^2}\%$$

where $\sigma_s^2 + \sigma^2$ is total variation and σ_s^2 is between subject variation.

RESULTS

Physical characteristics and body composition analysis of the subjects are presented in **Table 2**. The mean total body fat measured by the six-compartment model was 19.7 ± 10.2 kg or 25.4 ± 11.6% of body weight for the subject group (**Table 3**). Values estimated by the 16 methods varied from 17.4 kg (the Segal-BIA method) to 24.3 kg (the Steinkamp anthropometric method). There were no significant differences (< ± 0.3 kg, paired t-test, all $P > 0.05$) between total body fat measured by the six-compartment model and the values estimated by the Selinger, Baumgartner, Heymsfield, Siri, Brozek, Behnke, and DXA methods. In contrast, the Pace, Lohman, and Kehayias methods under-estimated total body fat by 0.9 kg, 1.1 kg, and 2.2 kg (all $P < 0.05$), and Forbes-TBN and TBK methods over-estimated total body fat by 2.1 kg and 3.2 kg (all $P < 0.001$), respectively. All three statistically-derived methods (the Segal, Durnin, and Steinkamp) substantially under- or over-estimated total body fat ($P < 0.01$).

There were strong correlations ($r^2 > 0.80$) between the six-compartment model and all 16 methods. Very high correlations ($r^2 > 0.97$) also existed between the six-compartment model

Table 2. Characteristics and body composition analysis of subject group (n = 23)

	Mean	SD	Range
Age (y)	44.5	16.3	19 - 69
Body weight (kg)	76.7	12.7	53.9 - 105.9
Height (m)	1.70	0.11	1.51 - 1.89
BMI (kg/m^2)	26.6	4.0	19.3 - 33.7
Body density (g/cm^3)	1.0406	0.0244	1.0006 - 1.0830
Atomic Level			
TBC (kg)	19.7	6.9	9.3 - 33.9
TBN (kg)	1.81	0.37	1.19 - 2.53
TBCa (kg)	0.834	0.188	0.552 - 1.289
TBP (kg)	0.554	0.119	0.367 - 0.800
TBK (kg)	0.142	0.041	0.085 - 0.219
TBNa (kg)	0.077	0.013	0.055 - 0.110
TBCl (kg)	0.062	0.011	0.046 - 0.093
Molecular Level			
TBW (kg)	42.39	9.24	28.50 - 59.09
Mo (kg)	2.860	0.673	1.960 - 4.515

BMI, body mass index; Mo, bone mineral; TB, total body; TBW, total body water.

and the Selinger, Baumgartner, Heymsfield, Cohn, Siri, Pace, and DXA methods. Regression equations were developed that relate total body fat estimated by the 16 methods to total body fat derived by the six-compartment model (**Table 3**).

The results of inter-method difference analysis comparing each method with the six-compartment model are summarized in **Table 4**. The methods of Siri, Heymsfield, Selinger, Baumgartner, and Cohn gave results consistent with the six-compartment model. The technical errors were all less than 0.75 kg with coefficients of reliability above 99.5 %. Bland-Altman analysis indicated that the standard deviations of difference between the six-compartment model and these methods were less than 1.1 kg.

Table 3. Total body fat assessed by the six-compartment model and 16 methods

Method (ref.)	Total body fat (kg)			Regression equation			
	Mean	SD	Range	*a*	*b*	r^2	SEE (kg)
Segal (33)	**17.4**	7.2	6.8 - 32.0	1.36	−3.94	0.923	2.88
Kehayias (27)	**17.5**	9.7	2.6 - 37.8	0.94	1.012	0.938	2.59
Lohman (28)	**18.6**	9.8	5.4 - 36.8	1.02	0.77	0.952	2.27
Pace (4)	**18.8**	10.1	5.2 - 38.7	0.99	1.006	0.992	0.95
Brozek (6)	**19.4**	9.2	7.1 - 39.1	1.08	−1.25	0.956	2.18
Baumgartner (24)	**19.5**	9.9	7.0 - 39.2	1.02	−0.40	0.989	1.07
Heymsfield (8)	**19.6**	9.9	6.9 - 39.5	1.03	−0.41	0.990	1.02
Siri (30)	**19.6**	9.9	6.7 - 40.0	1.02	−0.24	0.996	0.97
6-C criterion model	**19.7**	10.2	6.0 - 40.0	-	-	-	-
Selinger (29)	**19.7**	10.2	6.9 - 39.9	1.01	−0.22	0.989	1.08
DXA (7, 25)	**20.0**	10.8	4.9 - 39.4	0.93	1.17	0.972	1.73
Behnke (5)	**20.0**	9.9	6.5 - 41.2	1.00	−0.35	0.955	2.21
Cohn (10)	**20.7**	10.1	7.2 - 40.9	1.01	−1.20	0.999	0.22
Forbes-TBN (26)	**21.8**	10.5	7.2 - 42.8	0.94	−0.75	0.937	2.60
Durnin (31)	**22.1**	9.5	9.7 - 44.0	0.98	−1.84	0.837	4.19
Forbes-TBK (2, 3)	**22.9**	11.9	6.2 - 44.5	0.82	0.85	0.926	2.82
Steinkamp (32)	**24.3**	9.5	10.5 - 44.8	1.02	−5.07	0.908	3.14

6-C criterion model, the six-compartment neutron activation model; *a* and *b*, the slope and intercept of linear regression equation ($FAT_{6\text{-C model}} = b + a \times$ FAT by other method); r, correlation coefficient; SD, standard deviation; SEE, standard error of estimate.

Table 4. Inter-method comparison between the six-compartment model and 16 methods

Method (ref.)	Absolute difference		CV	TE	CR	Bland-Altman analysis		
	mean	SD			%	mean Δ	SD	r
Siri (30)	0.78	0.56	0.0343	0.67	99.6	0.12	0.96	0.28
Heymsfield (8)	0.84	0.56	0.0362	0.71	99.5	0.09	1.02	0.34
Selinger (29)	0.86	0.58	0.0372	0.73	99.5	− 0.06	1.06	0.18
Baumgartner (24)	0.88	0.60	0.0380	0.75	99.5	0.08	1.07	0.32
Cohn (10)	1.02	0.23	0.0367	0.74	99.5	− 1.02	0.23	0.39
Pace (4)	1.04	0.75	0.0466	0.90	99.2	0.89	0.92	0.15
DXA (7, 25)	1.51	1.11	0.0661	1.31	98.4	− 0.30	1.87	− 0.27
Behnke (5)	1.75	1.25	0.0760	1.51	97.7	− 0.34	2.15	0.22
Lohman (28)	1.78	1.69	0.0897	1.72	97.0	1.08	2.22	0.29
Brozek (6)	1.81	1.30	0.0799	1.56	97.4	0.24	2.24	0.51
Forbes-TBN (26)	2.76	1.94	0.1143	2.37	94.8	− 2.20	2.68	− 0.04
Kehayias (27)	2.87	1.66	0.1256	2.33	94.6	2.18	2.53	0.29
Segal (33)	3.30	2.95	0.1671	3.10	87.8	2.29	3.82	0.85
Forbes-TBK (2,3)	4.08	2.42	0.1565	3.33	91.1	− 3.26	3.48	− 0.37
Durnin (31)	4.13	2.25	0.1584	3.31	88.9	− 2.40	4.11	0.35
Steinkamp (32)	4.75	2.82	0.1768	3.89	85.2	− 4.60	3.07	0.36

CR, coefficient of reliability; CV, coefficient of variation; mean Δ, mean difference between the standard 6-C model and the other methods (in kg); r, correlation coefficient; SD, standard deviation; TE: technical error (in kg).

DISCUSSION

Total Body Fat Reference Method

As the "true" value of total body fat is unmeasurable, a reference with high accuracy is necessary to evaluate other less accurate methods. The reference should meet two main criteria: it should avoid major assumptions and have maximal precision.

Several methods over the span of many years were applied as criteria for total body fat estimation, and these include Forbes-TBK, Pace, Brozek, and Behnke methods. Each of these methods is dependent on a major assumption such as potassium or water constancy in FFM or a constant proportion of main FFM components. These assumptions have been challenged over the years and the aforementioned methods may therefore not be ideal as criteria.

The DXA method was recently used as the criterion in some body fat comparison studies (7, 11, 22). DXA is based on the differential X-ray attenuation of fat, bone mineral, and fat-free soft tissues. This method is methodologically orthogonal to other fat estimation methods. However, the DXA method is dependent on geometric models and the assumption of constant hydration in fat-free soft tissues (12). There are also now many animal studies which show small but statistically significant discrepancies between DXA fat estimates and carcass lipid content. The DXA method may therefore not be an ideal fat measurement criterion.

The Cohn neutron activation model was also applied as a criterion in earlier studies (9). In this model FFM is estimated by summing the major chemical components including water, protein and bone mineral. However, Cohn's model ignored two components, soft tissue mineral and glycogen. This results in a small but systematic over-estimation of total body fat.

A six-compartment body composition model was applied in the present study that extends the earlier approach suggested by Cohn and his colleagues (10). The error caused by measurement of model components (σ_{fat}) can be estimated in the present study subjects by assuming a mean body composition as shown in **Table 2** and measurement precision as stated in the Methods section. Accordingly,

$$(\sigma_{fat})^2 = (0.1)^2 + (42.39 \times 0.015)^2 + (6.525 \times 1.81 \times 0.018)^2 + (2.709 \times 0.834 \times 0.016)^2 + (2.76 \times 0.142 \times 0.032)^2 + (0.077 \times 0.018)^2 + (1.43 \times 0.062 \times 0.012)^2$$

Solving this equation, $(\sigma_{fat})^2 = 0.461$. The resulting propagated measurement error of the six-compartment model is 0.68 kg of body fat for the present study subjects. Additionally, this model has minimal model error and has no assumptions that are known to be influenced by

age, sex, gender, and disease. Although model and measurement errors are likely small, the six-compartment model, as with all *in vivo* methods, ultimately requires evaluation in human cadavers and animals. Moreover, as the methods used in developing the six-compartment model (prompt and delayed-γ *in vivo* neutron activation analysis) are not widely available and include radiation exposure, there is little role for the model in routine subject testing. The importance of the six-compartment model is that it can be used to evaluate other body composition methods.

Inter-Method Comparison

A method designed to quantify total body fat mass should be equally accurate in health and disease, and across all age, gender, and race groups. The 16 evaluated methods, however, differed widely in their estimates of total body fat in our cohort of healthy ethnically mixed subjects (**Table 3** and **Figure 1**). Each of the methods outlined in the table has two sources of

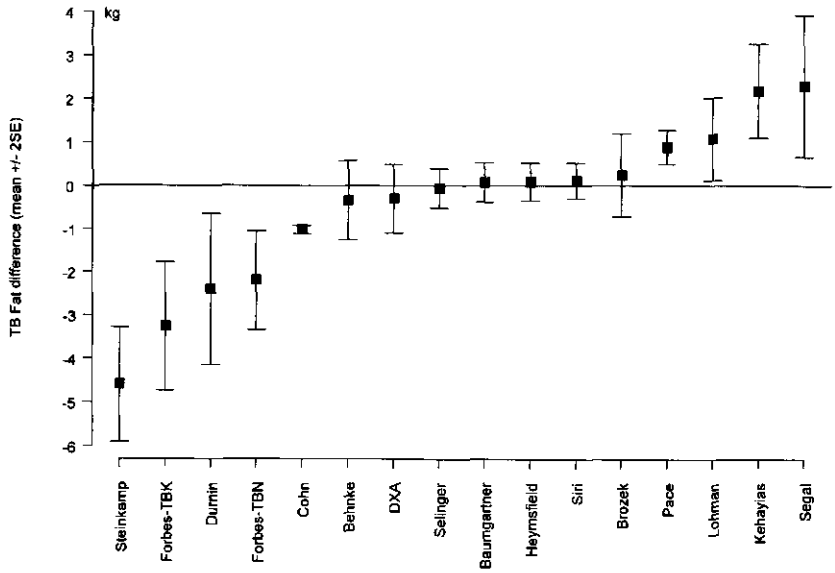

Figure 1. Inter-method comparison of total body fat mass (TB Fat) measurements. The mean and standard error of estimation (mean ± 2 SE) between total body fat measured by the six-compartment criterion model and other 16 methods are indicated.

error: 1. reference method error (for statistically-derived methods) or model assumption error (for model-based methods); and 2. measurement error. Ideally, a body composition method should have low technical error (TE) and high coefficient of reliability (CR). The 16 currently used methods varied in the results of the inter-method analysis (**Table 4**), and they can be empirically organized into three groups as follows:

Group 1 methods all have TE's less than 0.8 kg, CR's larger than 99.5 %, and SD of method difference (i.e., Bland-Altman analysis) less than 1.1 kg. These methods, Baumgartner, Heymsfield, Selinger, Siri, and Cohn all share in common their derivation from multi-compartment body composition models and a requirement for total body water estimates. Although water measurement is still required, model assumptions do not involve total body water which is the largest molecular level component. Accordingly, the model error of these methods should be small and total body fat measurements by these five methods are not known to be age, gender, race, and health status dependent. These methods form a core with the six-compartment criterion model. Because of their small TE's and high CR's, these methods are well suited for accurate measurement of body fat. The Cohn neutron activation model systematically over-estimated total body fat by about 1.0 kg or 5%, presumably because the model ignores two small components, soft tissue mineral and glycogen.

There are other published models in this group that we did not evaluate, such as Fuller et al's three and four compartment models (36). These methods were very similar to those in this first group of multicompartment models and their results as analyzed in this report would be indistinguishable from those presented in **Tables 3** and **4**.

As neutron activation facilities are limited and the six compartment model cannot be applied in most research laboratories, the present findings suggest that the simpler and reduced radiation methods proposed by Baumgartner, Heymsfield, Selinger, and Siri may serve as alternative practical reference methods. However, it should be noted that the present study subjects were relatively young, and comparative analyses such as those set forth in the present study should be extended to other populations such as the elderly.

Group 2 includes methods with TE's between 1 kg and 2 kg, CR's between 97% and 99%, and SD's of method difference (i.e., Bland-Altman analysis) less than 2.5 kg (Pace method's TE is 0.90 kg). These include the DXA, Pace, Behnke, Brozek, and Lohman methods. The DXA, Pace, Behnke, and Brozek methods share in common an assumption of constant

hydration at 0.73, making these methods more appropriate in normal subjects than in disease states in which hydration may be abnormal. Lohman's method also assumes a constant ratio of total body water to protein (28). This group of methods are therefore suitable for use in subjects with normal hydration and may not applicable across all age, gender, and disease groups. There might be an error if these methods are applied to subjects with abnormal hydration such as AIDS and obese patients.

Among the 16 fat methods, the DXA method uniquely provides both whole body and regional body composition measurements. Use of DXA for assessing body fat is supported by several studies (7, 11, 22). **Tables 3** and 4 establish the accuracy of the DXA method in healthy subjects. However, the errors of DXA method are still of concern if it were to be used as the criterion. This is because the DXA method is dependent on the assumption of uniform hydration (25). Recently, Stall et al. pointed out that DXA is suited for use in well-dialyzed peritoneal dialysis patients (37). The question still exists if DXA can accurately estimate total body fat for other patients in whom hydration is abnormal. Finally, in a recent study DXA failed to accurately measure the composition of packets containing various materials placed over selected anatomic sites in human subjects (38). Although these experiments have been criticized (39), there remain important questions related to underlying DXA geometric reconstruction models (12).

Group 3 includes methods with TE's larger than 2 kg, CR's less than 95%, and Bland-Altman analysis SD's of larger than 2.5 kg. These include the Forbes-TBN, TBK, Segal-BIA, and two anthropometric methods. Both Segal-BIA and anthropometric methods are statistically derived and are referenced against traditional two-compartment methods. For example, the Steinkamp method used both the Brozek and Forbes-TBK methods as the reference (32). The error of these traditional methods is thus propagated into the anthropometric method. Moreover, the BIA and anthropometric methods have potential technical errors such as incorrect gel electrode position and skinfold estimation. Therefore, it is not surprising that the two anthropometric methods have the largest TE (> 3 kg) and the lowest CR (< 90%) of the 16 methods. This group of methods is therefore not suitable for situations in which accuracy is required, although they may still be applied in field studies where low cost and ease of performance are important.

Of the 16 methods, the Kehayias total body carbon method (27) is virtually orthogonal to other fat estimation methods. It is derived from the proportions of carbon in the carbon-containing components including fat, protein, glycogen, and mineral. This method is not effected by hydration state and results are not dependent on age, gender, and disease status. Moreover, the Kehayias method has only a small model error because the quantitative proportions of carbon in the carbon-containing components is determined by rigorous chemical relationships. As **Tables 3** and **4** show, however, there is a considerable difference in total body fat mass as estimated by the Kehayias method and the six-compartment criterion model. This discrepancy could be explained by a measurement error of TBC in the present investigation. Previous studies on phantoms reported a 3 % (CV) precision of TBC estimation by inelastic neutron scattering (27). However, our group recently reported a poor precision (CV = 11.0 %) when TBC was estimated in humans (23). We also calculated expected carbon content of the present study subjects using total body fat measured by the six-compartment model (21.4 ± 7.4 kg) and calculated TBC was significantly lager than that estimated by inelastic neutron scattering (19.5 ± 6.7 kg, $P = 0.0005$), although the two estimates are highly correlated (r = 0.97). This indicates that our inelastic neutron scattering facility may now underestimate TBC by 1.9 kg or 8.9%. If calibration of our inelastic scattering system for measuring TBC can be improved, the Kehayias method would then be accurate relative to the six-compartment model for total body fat estimation.

Conclusion

The present study reports a comprehensive comparison of fat measurement methods using a six-compartment model based on *in vivo* neutron activation analysis as the criterion. Results clearly show between-method agreement differences with criterion fat estimates, although empirically three groups emerged. Fat estimates by the first group of methods were strongly associated (e.g., by technical error, coefficient of reliability, and Bland-Altman analysis) with criterion fat estimates, an observation which suggests they are applicable as reference methods in future studies. Methods in the second group showed less agreement with criterion fat estimates, opening questions as to their specific roles in clinical evaluations and in research studies. The third and least-associated group of methods showed relatively poor associations with criterion fat estimates and may be of value only for group estimates in field studies.

Finally, while the present investigation was comprehensive in terms of evaluated methods, future similar studies are needed in more varied and larger cohorts.

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CHAPTER 8

SKELETAL MUSCLE MASS: EVALUATION OF NEUTRON ACTIVATION AND DUAL ENERGY X-RAY ABSORPTIOMETRY METHODS

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ABSTRACT

Although skeletal muscle (SM) is a major body composition component, whole body measurement methods remain limited and inadequately investigated. The aim of the present study was to evaluate the Burkinshaw *in vivo* neutron-activation analysis (IVNA)-whole body ^{40}K counting and dual energy X-ray absorptiometry (DXA) methods of estimating SM by comparison to adipose tissue-free SM measured using multiscan computerized axial tomography (CT). In the Burkinshaw method the potassium-to-nitrogen ratios of SM and non-SM lean tissue are assumed constant; in the DXA method the ratio of appendicular SM to total SM is assumed constant at 0.75. Seventeen healthy men [77.5 ± 13.8 (SD) kg body wt] and eight men with acquired immunodeficiency syndrome (AIDS; 65.5 ± 7.6 kg) completed CT, IVNA, and DXA studies. SM measured by CT was 34.4 ± 6.2 kg for the healthy subjects and 27.2 ± 4.0 kg for the AIDS patients. Compared with CT, the Burkinshaw method underestimated SM by an average of 6.9 kg (20.1%, $P = 0.0001$) and 6.3 kg (23.2%, $P = 0.01$) in the healthy men and the men with AIDS, respectively. The DXA method minimally overestimated SM in both groups (2.0 kg and 5.8% in healthy men, $P = 0.001$; 1.4 kg and 5.1% in men with AIDS, $P = 0.16$). This overestimate could be explained by a higher actual ratio of DXA-measured appendicular SM to total body SM (actual = 0.79 ± 0.05, assumed = 0.75). The current study results reveal that large errors are present in the Burkinshaw SM method and that substantial refinements in the models that form the basis of this IVNA approach are needed. The model upon which the DXA-SM method is based also needs further minor refinements, but this is a promising *in vivo* approach because of less radiation exposure and lower cost than the IVNA and CT.

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INTRODUCTION

Skeletal muscle is the largest non-adipose tissue component at the tissue-system level of body composition in adult humans (18). Despite its central role in many physiological processes, no accurate validated *in vivo* techniques for measuring total body skeletal muscle mass were available until recently. The presently known, but rather limited information on skeletal muscle is based largely on inadequately validated techniques for estimating the muscle compartment *in vivo* (2, 5, 9).

Studies carried out by Sjöström and his colleagues over the past several years indicate that multiscan computerized axial tomography (CT) is an accurate and reproducible method of measuring adipose tissue and skeletal muscle mass *in vivo* (3, 11, 12, 15). The technique is based on deriving total body volume by integrating information obtained from 22 contiguous cross-sectional CT images. Limitations of CT as a routine body composition method are its expense and associated radiation exposure.

An alternative to CT for estimating skeletal muscle is based on *in vivo* neutron activation analysis (IVNA) combined with ^{40}K whole-body counting (2, 5). The method, suggested originally by Burkinshaw et al. (2), has as its foundation the difference in the potassium-to-nitrogen ratio found between skeletal muscle (3.03 mmol/g or 0.118 g/g) and non-skeletal muscle-lean mass (1.33 mmol/g or 0.052 g/g). An equation derived using these two ratios can be applied to predict a subject's skeletal muscle if total body potassium (TBK) and total body nitrogen (TBN) are known from ^{40}K whole-body counting and prompt-γ neutron activation analysis, respectively. Much of our present knowledge regarding skeletal muscle mass in living humans, and more specifically its relation to aging, has been derived using the Burkinshaw method (2, 5). However, there have been no validation studies of this method, even though skeletal muscle estimates based on the Burkinshaw method are much lower than those observed in limited human autopsy studies such as the Belgian cadaver project (4). This method of estimating skeletal muscle mass is even more expensive than CT and is also associated with moderate levels of radiation exposure (~0.8 mSv).

The recent introduction of dual energy X-ray absorptiometry (DXA) provides another opportunity to measure skeletal muscle *in vivo* with lower cost, substantially less radiation exposure (< 0.05 mSv) (10), and wide availability of instrumentation (9). The DXA approach first allows separation of the total body into two compartments, bone mineral and soft tissue.

Soft tissue can be further separated into fat and fat-free soft tissue by use of the ratio of X-ray attenuations at 40 and 70 KeV (R). This is because the measured R is related to the proportion of soft tissue as fat and fat-free soft tissue. Most whole-body DXA systems also allow regional measurements, and this permits separation of the extremity from trunk measurements. Because the fat-free soft tissue of the extremities is almost entirely skeletal muscle, except for a small amount of skin and connective tissues, DXA provides the opportunity to measure appendicular skeletal muscle. In an earlier report, we demonstrated that appendicular skeletal muscle mass measured by DXA was highly correlated with other indexes of skeletal muscle such as TBK (9).

DXA can also be used to estimate total body skeletal muscle by assuming that appendicular skeletal muscle represents 75% of total body skeletal muscle (16). This approach is thus promising, but the relations between total body skeletal muscle quantified by DXA and actual total body skeletal muscle mass remains unstudied.

The availability of a method of measuring total body skeletal muscle mass by CT prompted the present investigation. Specifically, we compared total body skeletal muscle estimated by Burkinshaw's IVNA method with adipose tissue-free skeletal muscle measured by CT, and we examined the relations between appendicular and total body skeletal muscle estimated by the DXA approach and total body skeletal muscle measured by CT. The study was carried out in healthy men and men with varying degrees of weight loss secondary to acquired immunodeficiency syndrome (AIDS).

METHODS

Protocol

Subjects were first screened to ensure that they met the study entry criteria. They subsequently completed four studies within 2 wk: CT for total body skeletal muscle and adipose tissue mass, IVNA for TBN, whole-body ^{40}K counting for TBK, and DXA for appendicular skeletal muscle mass. The healthy men also completed underwater weighing for measurement of body volume.

Subjects

The subject pool consisted of 17 healthy men and 8 men with AIDS. The rational for inclusion of patients with weight loss was twofold: *1*) it provided the opportunity to examine skeletal

muscle mass measurement methods under actual clinical conditions; and *2*) weight loss might reveal limitations of the various body composition models that are not evident in healthy normal-weight subjects.

Healthy subjects were recruited from local sources including flyers posted in the medical center and newspaper advertisements. Each healthy subject completed a medical history, physical examination, and routine screening blood studies to confirm the absence of underlying disease. All men participated in recreational physical activities and none were actively engaged in a sports training program.

The men with AIDS were recruited from among patients with nutritional disorders and weight loss cared for at the medical center. All were ambulatory, afebrile, and met the Center for Disease Control Criteria for AIDS. All AIDS patients were weight stable (± 1 kg) during the body composition study period.

All study participants signed an informed consent that was approved by the hospital's Institutional Review Board.

Multiscan CT

The full procedure required 22 cross-sectional images at anatomic locations described by Sjöström et al (3, 11, 12, 15). A Somatom DRH scanner (Siemens, Erlangen, Germany) was used in the study. Subjects were placed on the scanner platform with their arms extended above their heads to minimize beam hardening artifacts. Each CT image was completed at 125 kVp with a scanning time of 4 s at 170 mA. Slice thickness was set at 4 mm. The effective dose equivalent of a 22-slice CT is 2 – 4 mSv (12)

An observer who was familiar with cross-sectional CT anatomy encircled the skeletal muscle region in each scan with a light pen. Our preliminary study showed that the attenuation of soft tissue ranged from –190 to +120 Hounsfield units in most regions. On the basis of the early determinations of Sjöström and colleagues (3, 11, 12, 15), adipose tissue area was measured using the pixels that range from –190 and –30 Hounsfield units. We completed a similar analysis by examining histograms and found distributions almost identical to those reported by Sjöström et al. Accordingly, in the present study the range of –29 to +120 Hounsfield units was used to measure adipose tissue-free skeletal muscle area in each slice. The distances between cross-sectional images were obtained from the CT frontal scanogram to the nearest millimeter.

Total body adipose tissue-free skeletal muscle mass was calculated as follows

$$SM_{CT} = 0.00104 \times \sum_{i=1}^{n} [A_i \times (B_i + B_{i+1}) / 2], \quad [1]$$

where SM_{CT} is the total body adipose tissue-free skeletal muscle mass (kg) measured by CT; 0.00104 is the assumed constant density (kg/cm^3) of adipose tissue-free skeletal muscle (16); A_i, the distance (cm) between scans; and B_i and B_{i+1} are the adipose tissue-free skeletal muscle areas (cm^2) in adjacent scans.

Similarly, total body adipose tissue mass (AT, in kg) was calculated from CT as follows

$$AT = 0.00092 \times \sum_{i=1}^{n} [A_i \times (C_i + C_{i+1}) / 2] \quad [2]$$

where 0.00092 is the assumed constant density (kg/cm^3) of adipose tissue (16); A_i is the distance (cm) between scans; and C_i and C_{i+1} are the adipose tissue areas (cm^2) in adjacent scans that are measured by selecting the attenuation values between −190 and −30 Hounsfield units.

Only small variation in the assumed densities of skeletal muscle and adipose tissue, even under clinical conditions, can be anticipated. Our preference was therefore to present skeletal muscle and adipose tissue results in mass rather than volume units for uniformity and ease in interpretation.

Adipose tissue-free body mass was calculated as the difference between body weight and adipose tissue mass measured by CT.

Using the attenuation values between −900 and +3000 Hounsfield units, we evaluated the 22 slice integration procedure by calculating whole body volume from the total slice areas. This derived body volume was then compared with body volume measured by underwater weighing in healthy subjects. The underwater weighing procedure, including residual lung volume analysis, is described in an earlier report (19).

IVNA-^{40}K Whole-Body Counting

TBN and TBK were measured by IVNA analysis and ^{40}K whole-body counting, respectively (13, 14). TBN was measured using the prompt-γ neutron activation analysis system at Brookhaven National Laboratory. Nitrogen mass was calculated by using hydrogen counts as

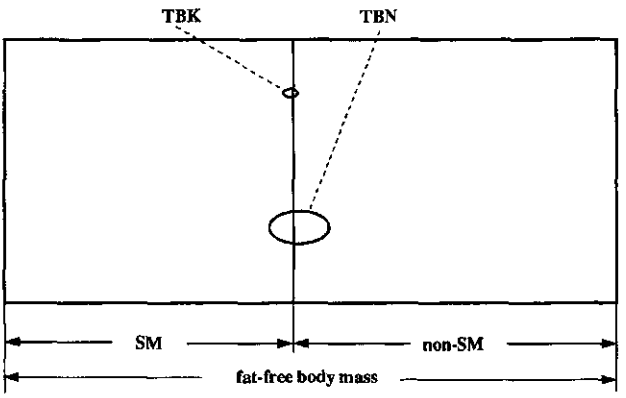

Figure 1. Relationships between fat-free body mass components. TBK, total body potassium; TBN, total body nitrogen; SM, total body skeletal muscle; non-SM, nonskeletal muscle lean component.

an internal standard. The TBN measurement has a precision of ± 3.6%. TBK was evaluated using the St. Luke's 4 π ^{40}K whole-body counter. This system has a reproducibility of ± 3.2%. Detailed descriptions of the two systems are provided in earlier reports (13, 14).

The Burkinshaw method (2) modified by Cohn and colleagues (5) was used to predict total body skeletal muscle mass, as outlined in **Figure 1**. This figure shows fat-free body mass as two components: skeletal muscle and nonskeletal muscle lean. Potassium is distributed mainly in skeletal muscle with overlap into the nonskeletal muscle lean component. Nitrogen, on the other hand, is distributed mainly in the nonskeletal muscle lean (non-SM) component with overlap into the skeletal muscle (SM) component. Two simultaneous equations can be used to characterize the relationships shown in **Figure 1**,

$$TBK = 3.56 \times SM + 1.88 \times \text{non-SM, and} \quad [3]$$

$$TBN = 30 \times SM + 36 \times \text{non-SM.} \quad [4]$$

where the coefficients suggested by Burkinshaw and colleagues represent the assumed constant concentrations (g/kg) of potassium and nitrogen in SM and nonskeletal muscle lean component. Accordingly, equations 3 and 4 were solved for SM

$$SM_B = 0.503 \times TBK - 0.0263 \times TBN \quad [5]$$

where SM_B is total body skeletal muscle mass (kg) estimated by the Burkinshaw method and TBK and TBN are total body potassium and nitrogen mass (g), respectively.

DXA

Subjects were scanned using a DXA (model DP-4, Lunar Radiation, Madison, WI) whole-body scanner. The Lunar system uses a filtered X-ray source to provide peak energies at 40 and 70 keV. The system software divides pixels first into bone mineral and soft tissue compartments. The soft tissue is then further separated by the system software into fat and fat-free soft tissue. After completion of each subject's scan, the whole body was divided into six regions by the DXA system's software as described previously (9). The appendicular fat-free soft tissue derived from these regional measurements was then assumed to represent appendicular skeletal muscle mass.

According to data compiled for the Reference Man (16), appendicular skeletal muscle represents ~75% of total body skeletal muscle mass. Total body skeletal muscle mass was therefore calculated as follows

$$SM_{DXA} = ASM/0.75 = 1.33 \times ASM \qquad [6]$$

where SM_{DXA} is total body skeletal muscle mass (kg) measured by the DXA method and ASM is appendicular skeletal muscle mass (kg).

Statistical Analysis

Group results are presented as the mean ± SD. Differences between the healthy subjects and the AIDS patients were tested by Student's t-test. $P < 0.05$ was considered statistically significant.

Simple linear regression analysis was used to describe the relationship between total body skeletal muscle quantified by CT and other body composition components.

The relationship between skeletal muscle measured by CT, Burkinshaw, and DXA methods were examined by simple linear regression analysis. Mean differences between skeletal muscle mass measured by CT and the other two methods were then tested for statistical significance by paired t-tests. The difference in skeletal muscle estimates between CT and the method under examination was then related to the mean value of the two methods, as described by Bland and Altman (1).

Data were analyzed using SAS version 5 (SAS Institute, Cary, NC).

Table 1. Baseline characteristics of the healthy subjects and AIDS patients

Subject/ Patient No	Age yr	BW kg	Ht m	BMI kg/m²	AT kg	AT BW%	ATFM kg
Healthy subjects							
1	37	61.0	1.69	21.4	7.9	13.0	53.1
2	33	77.2	1.77	24.6	12.1	15.7	65.1
3	26	57.0	1.77	18.2	2.7	4.7	54.3
4	72	75.3	1.70	26.1	20.3	27.0	55.0
5	32	80.2	1.74	26.5	11.8	14.7	68.4
6	30	58.1	1.63	21.9	5.0	8.6	53.1
7	34	81.8	1.78	25.8	11.9	14.5	69.9
8	42	93.6	1.81	28.6	22.3	23.8	71.3
9	48	91.1	1.90	25.2	7.3	8.0	83.8
10	26	106.6	1.87	30.5	29.0	27.2	77.6
11	23	61.0	1.71	20.9	9.5	15.6	51.5
12	19	65.4	1.70	22.6	9.3	14.2	56.1
13	26	78.2	1.82	23.6	9.1	11.6	69.1
14	41	82.1	1.81	25.1	13.1	16.0	69.0
15	50	73.7	1.82	22.2	15.9	21.6	57.8
16	28	86.2	1.93	23.1	19.4	22.5	66.8
17	33	89.2	1.82	26.9	20.8	23.3	68.4
Mean	35.3	77.5	1.78	24.3	13.4	16.6	64.1
± SD	12.7	13.8	0.08	3.0	7.0	6.7	9.5
AIDS patients							
18	30	76.4	1.73	25.5	11.9	15.6	64.5
19	41	61.9	1.65	22.7	9.6	15.5	52.3
20	31	69.5	1.83	20.8	15.3	22.0	54.2
21	33	60.9	1.75	19.9	4.6	7.6	56.3
22	33	70.5	1.80	21.8	4.3	6.1	66.2
23	44	71.8	1.73	24.0	23.8	33.1	48.0
24	34	56.4	1.70	19.5	10.8	19.1	45.6
25	34	56.4	1.68	20.0	11.6	20.6	44.8
Mean	35.0	65.5	1.73	21.8	11.5	17.5	54.1
± SD	4.9	7.6	0.06	2.2	6.2	8.5	8.1

AIDS, acquired immunodeficiency syndrome; AT, adipose tissue mass measured by computed axial tomography; ATFM, adipose tissue-free body mass (ATFM = BW – AT); BMI, body mass index; BW, body weight; Ht, height.

RESULTS

Subjects

The baseline characteristics of the 17 healthy male subjects are presented in **Table 1**. The group had a mean age of 35 ± 13 yr, body weight of 77.5 ± 13.8 kg, and body mass index of 24.3 ± 3.0 kg/m^2. Adipose tissue represented 16.6 ± 6.7% of the subject's body weight.

The baseline characteristics of the eight male AIDS patients are also presented in **Table 1**. All of the patients had lost weight since the onset of their illness, although none were acutely ill or had significant electrolyte abnormalities at the time of study. The group had a mean age of 35 ± 5 yrs, body weight of 65.5 ± 7.6 kg, and body mass index of 21.8 ± 2.2 kg/m^2. Adipose tissue was 17.5 ± 8.5% of body weight for the group as a whole.

The AIDS patients weighed on average 12.0 kg less than the healthy control group; 2.0 kg of this difference was composed of adipose tissue and the remainder (10.0 kg) was adipose tissue-free mass.

Body Volume Measurements

Body volume derived by CT (BV_{CT}, in liters) was highly correlated with body volume measured by underwater weighing [BV_{UWW}, in liters; $BV_{CT} = 1.002 \times BV_{UWW} + 1.1$; $r = 0.99$, $P = 0.0001$, standard error of the estimate (SEE) = 1.9 liters, $n = 17$]. The slope and intercept of this regression line were not significantly different from 1.0 and 0, respectively.

Mean BV_{CT} (74.8 ± 13.9 liters) agreed closely with mean BV_{UWW} (73.6 ± 13.7 liters) for the 17 healthy subjects (**Table 2**), although there was a significant difference between the two results ($P = 0.02$). The difference between BV_{CT} and BV_{UWW} was not significantly related to the mean of the two methods ($P = 0.75$).

Total Body Skeletal Muscle Measured by CT

Total body skeletal muscle measured by CT was 34.4 ± 6.2 kg for the healthy subjects and 27.2 ± 4.0 kg for the AIDS patients (**Table 2**). The 7.2 kg difference in skeletal muscle between the two groups accounted for a large portion of the between-group difference in body weight (12.0 kg) and adipose tissue-free body mass (ATFM; 10.0 kg).

The proportion of ATFM as skeletal muscle measured by CT (SM_{CT}/ATFM) was significantly higher ($P = 0.002$) in the healthy subjects (53.4 ± 3.1%) than the AIDS patients (50.4 ± 1.2%). There was a good correlation between total body skeletal muscle mass and ATFM in

Table 2. Total body skeletal muscle mass and other body composition components in healthy subjects and AIDS patients

Subject/ patients No.	SM_{CT} kg	TBN g	TBK g	SM_B kg	ASM kg	SM_{DXA} kg	BV_{UWW} L	BV_{CT} L
Healthy subjects								
1	27.9	1880	140.4	21.4	23.2	30.9	57.8	56.7
2	36.1	2010	154.6	25.2	29.7	39.6	72.4	72.1
3	31.4	1920	138.7	19.5	22.5	30.0	52.6	58.5
4	26.4	1710	124.9	18.1	21.7	29.0	72.8	71.7
5	36.8	2380	183.9	30.2	30.6	40.8	75.4	76.2
6	24.3	1800	120.8	13.7	20.8	27.7	54.3	52.4
7	37.0	2360	169.7	23.6	29.5	39.4	76.9	77.2
8	41.0	2140	184.3	36.7	31.5	42.0	91.5	92.5
9	46.6	2540	212.9	40.6	37.2	49.5	86.1	87.3
10	42.2	2520	208.2	38.8	33.2	44.2	102.6	104.2
11	27.0	1620	131.7	23.8	22.9	30.5	57.6	60.3
12	30.0	1800	145.5	26.1	25.5	34.0	61.3	64.0
13	36.9	2130	173.7	31.6	26.8	35.7	73.4	74.1
14	37.3	2110	169.6	30.1	27.8	37.1	77.4	77.8
15	29.0	2020	135.3	15.2	24.3	32.4	72.1	73.2
16	36.4	1870	166.3	34.7	27.0	36.0	81.9	85.3
17	38.3	2210	190.0	37.7	29.8	39.7	85.2	88.7
Mean	34.4	2060	161.8	27.5	27.3	36.4	73.6	74.8
± SD	6.2	275	28.3	8.4	4.5	6.0	13.7	13.9
AIDS patients								
18	32.6	2130	157.6	23.5	25.9	34.5		
19	26.9	1720	131.9	21.3	21.1	28.2		
20	26.9	1620	126.1	21.0	24.9	33.2		
21	29.5	1790	137.1	22.1	20.6	27.5		
22	32.4	2000	137.4	16.8	26.4	35.2		
23	23.6	1600	118.9	17.9	18.0	24.0		
24	22.8	1490	113.5	18.1	16.8	22.3		
25	22.9	1400	124.7	26.1	17.7	23.6		
Mean	27.2	1719	130.9	20.9	21.4	28.6		
± SD	4.0	248	13.6	3.1	3.9	5.2		

ASM, appendicular skeletal muscle mass measured by dual-energy X-ray absorptiometry (DXA); BV_{CT}, body volume measured by computerized axial tomography (CT); BV_{UWW}, body volume measured by underwater weighing; SM_B, total body skeletal muscle mass estimated by Burkinshaw method; SM_{CT}, total body skeletal muscle mass measured by CT; SM_{DXA}, total body skeletal muscle mass measured by DXA; TBK, total body potassium; TBN, total body nitrogen.

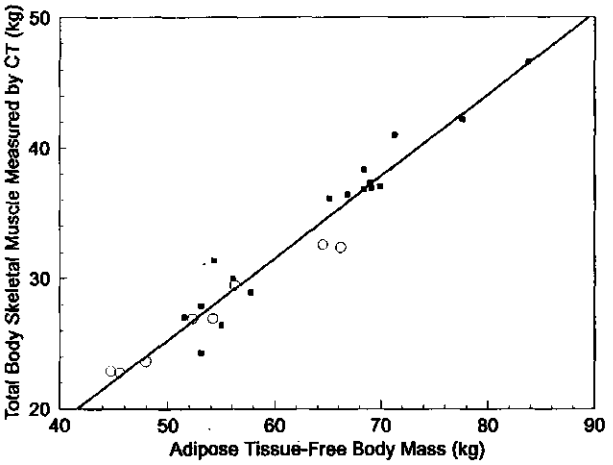

Figure 2. Total body skeletal muscle measured by computerized axial tomography (CT; SM_{CT}) *vs.* adipose tissue-free body mass (ATFM). SM_{CT} = 0.627 × ATFM − 6.1; (r = 0.97, P = 0.0001, standard error of estimate = 1.5 kg, n = 25). ■, Healthy subjects; O, patients with acquired immunodeficiency syndrome. Solid line, regression line.

each subject group (SM_{CT} = 0.639 × ATFM − 6.6; r = 0.97, P = 0.0001, SEE = 1.6 kg, n = 17 for healthy subjects and SM_{CT} = 0.489 × ATFM + 0.8; r = 0.99, P = 0.0001, SEE = 0.7 kg, n = 8 for AIDS patients; **Figure 2**).

Total Body Skeletal Muscle Estimated by Burkinshaw Method

Total body skeletal muscle estimated by the Burkinshaw method was 27.5 ± 8.4 kg for the healthy subjects and 20.9 ± 3.1 kg for the AIDS patients (**Table 2**). This skeletal muscle estimates was significantly lower than that by CT: 34.4 ± 6.2 kg for the healthy subjects (mean difference 6.9 kg or 20.1%, P = 0.0001) and 27.2 ± 4.0 kg for the AIDS patients (6.3 kg or 23.2%, P = 0.01).

The proportion of ATFM as skeletal muscle estimated by the Burkinshaw method was 42.2 ± 8.8% in the healthy subjects and 39.5 ± 9.0% in the AIDS patients. These proportions were substantially lower than those measured by CT: 53.4 ± 3.1% for the healthy subjects (P = 0.0001) and 50.4 ± 1.2% for the AIDS patients (P = 0.0082).

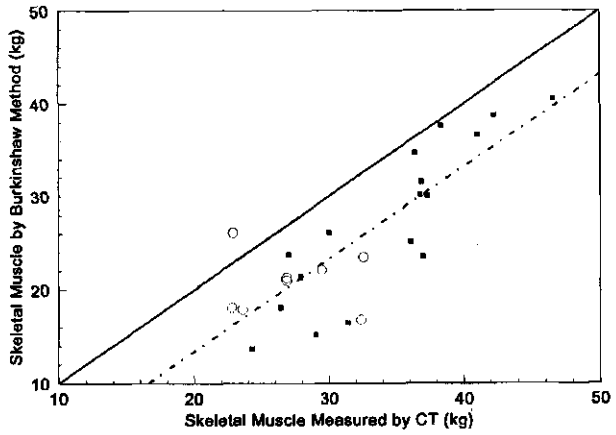

Figure 3. Total body skeletal muscle measured by Burkinshaw method (SM_B) *vs.* SM_{CT}. $SM_B = 0.990 \times SM_{CT} - 6.4$; r = 0.83, $P = 0.0001$, standard error estimate = 4.4 kg, n = 25). ■, Healthy subjects, O: patients with acquired immunodeficiency syndrome. Dashed line, regression line, solid line, line of identity.

There was a significant correlation between total body skeletal muscle quantified by the Burkinshaw method (SM_B) and SM_{CT} ($SM_B = 0.990 \times SM_{CT} - 6.4$; $r = 0.83$, $P = 0.0001$, SEE = 4.4 kg, n = 25; **Figure 3**). The intercept of this regression line was not significantly different from zero ($P = 0.17$). The difference between SM_B and SM_{CT} was not significantly associated with the mean of the two methods ($P = 0.14$).

Empirical equations were developed that link TBK and TBN to SM_{CT}. The first equation was based on multiple linear regression analysis in which the intercept term was set to 0: $SM_{CT} = 0.188 \times TBK + 0.00183 \times TBN$ (r = 0.96; $P = 0.001$; P for TBK and TBN coefficients = 1.17×10^{-6} and 0.154, respectively; SEE = 1.8 kg; n = 25). The second equation was developed with intercept $\neq 0$: $SM_{CT} = 0.182 \times TBK + 0.00395 \times TBN - 3.33$ (r = 0.97; $P = 0.001$; P for TBK and TBN coefficients = 0.182×10^{-6} and 0.154, respectively; SEE = 1.7 kg; n = 25).

Total Body Skeletal Muscle Measured by DXA

Appendicular skeletal muscle. Appendicular skeletal muscle measured by DXA was 27.3 ± 4.5 kg for the healthy subjects and 21.4 ± 3.9 kg for the AIDS patients (**Table 2**). Of the 7.2 kg

mean difference in total skeletal muscle between the two groups, 5.9 kg were accounted for by appendicular skeletal muscle, as suggested by DXA.

There was a good correlation between DXA-measured appendicular skeletal muscle and SM_{CT} for the pooled group (appendicular skeletal muscle = 0.743 × SM_{CT} + 1.6; r = 0.95, P = 0.0001, SEE = 1.6 kg, n = 25).

The ratio of appendicular skeletal muscle to SM_{CT} was 0.80 ± 0.05 for the healthy men and 0.79 ± 0.07 for the AIDS patients. Because there was no significant difference between mean appendicular skeletal muscle-to-skeletal muscle ratios for the healthy subjects and AIDS patients (P = 0.62), the results were pooled to provide a mean for all men combined (0.79 ± 0.05).

Total body skeletal muscle. SM_{DXA} calculated using equation 6 was 36.4 ± 6.0 kg and 28.6 ± 5.2 kg for the healthy subjects and AIDS patients, respectively. This measurement of skeletal muscle was significantly larger than SM_{CT} in the healthy subjects (2.0 kg or 5.8%, P = 0.001) but not significantly different from SM_{CT} in the AIDS patients (1.4 kg or 5.1%, P = 0.16).

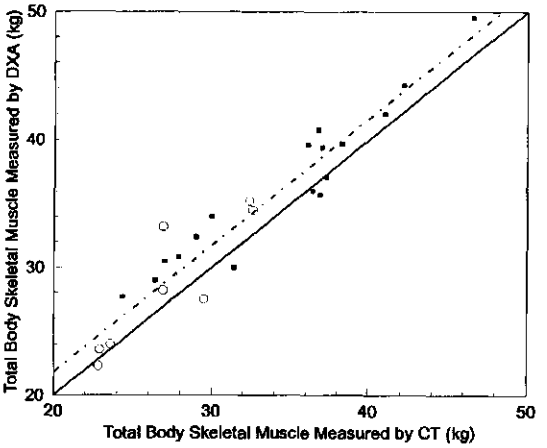

Figure 4. Total body skeletal muscle measured by dual-energy X-ray absorptiometry (DXA; SM_{DXA}) method *vs.* SM_{CT}. SM_{DXA} = 0.989 × SM_{CT} + 2.1 (r = 0.95, P = 0.0001, standard error of estimate = 2.1 kg, n = 25). ■, Healthy subjects; **O**, patients with acquired immunodeficiency syndrome. Dashed line, regression line; solid line, line of identity.

The overall correlation between SM_{DXA} and SM_{CT} is shown in **Figure 4** for all subjects combined ($SM_{DXA} = 0.989 \times SM_{CT} + 2.1$; $r = 0.95$, $P = 0.0001$, SEE = 2.1 kg; n = 25). The difference between SM_{DXA} and SM_{CT} was not significantly related to the mean value of the two methods ($P = 0.57$).

DISCUSSION

Skeletal muscle, despite its major contribution to body weight, remains a component under investigation in the study of human body composition because of previous methodological limitations. The relevance of studying muscle in disease processes is evident in the patients with AIDS evaluated in the present study. More than one-half (60%) of the weight difference between the men with AIDS and the healthy men of the similar average age and stature was accounted for by skeletal muscle.

Multiscan CT Method for Measuring Skeletal Muscle

There is substantial evidence that CT and another imaging method, magnetic resonance imaging, are the most accurate methods of measuring skeletal muscle. Earlier studies of phantoms or excised cadaver organs indicate a good agreement (± 5%) between CT-derived weights and actual phantom or organ weights (6-8).

In our study the observed ratios of skeletal muscle to ATFM derived by CT measurements (53.4 ± 3.1% and 50.4 ± 1.2% in the healthy subjects and AIDS patients, respectively) are in close agreement with those reported for the Reference Man (50.9%) and Belgian unembalmed male cadavers (54.0 ± 4.6%, n = 6) (4, 16). These collective observations provide strong support for the validity of the SM_{CT} method.

Furthermore, Sjöström (15) demonstrated that the CT-measured mass of individual components at the tissue-system level can be summed to closely approximate absolute body weight. We confirmed in the present study that BV_{CT} agrees closely with that measured by underwater weighing, although BV_{CT} is slightly larger (1.2 liters, 1.6%) than BV_{UWW}. Our hypothesis that attempts to explain this small but statistically significant difference is that with underwater weighing residual lung volume can be measured and body volume can be corrected for air trapped in the pulmonary system (19). In contrast, air is difficult to fully correct for in analysis of chest CT images. First, subjects are not asked to exhale maximally during the CT

scan as they are with underwater weighing. Therefore an unknown and variable amount of air is present in the lung during each scan sequence. Second, the mean attenuation of lung, including air, is about −700 Hounsfield units. We assume that air-free lung has an attenuation similar to other lean tissues of about −29 to +120 Hounsfield units. The very large surface area of pulmonary alveoli creates a major source of "partial volume" effect in which it is extremely difficult to quantitatively separate air and air-free lung tissue. We therefore suggest that CT-measured lung volume is larger than lung volume measured by underwater weighing and that this may be the main reason for the larger body volume observed with CT.

Burkinshaw Method for Estimating Skeletal Muscle

Our findings strongly support a long-standing observation that the Burkinshaw-Cohn neutron activation-whole body counting method underestimates total body skeletal muscle mass. Except for one outlier (*subject 25*), the Burkinshaw method gave muscle estimates that were on average 20.1% and 23.2% lower than that observed by CT for the healthy subjects and the AIDS patients, respectively. There are several possible explanations for these large differences. First, the errors in measuring TBK and TBN are ~3.5%. As shown in **Figure 1**, TBK and TBN account for small proportions of fat-free body mass (e.g., for the Reference Man, 0.25 and 3.2% of fat-free body mass is potassium and nitrogen, respectively) (16). Therefore, even small errors in measuring TBK and TBN may lead to large errors in skeletal muscle calculated using equation 5.

Second, TBK forms an overlapping relationship with two components, skeletal muscle and nonskeletal muscle lean. The assumed constant potassium content of the nonskeletal muscle lean component (1.88 g/kg) is actually a function of the proportional potassium contributions of each organ and tissue. Because each organ varies in potassium content, any change in the relative makeup of the nonskeletal muscle lean component will cause variation in the component's potassium content.

Third, an assumed constant skeletal muscle potassium content (3.56 g/kg) is used in equation 3. Burkinshaw et al. (2) arrived at the value of 3.56 g of potassium per kilogram of fat-free wet skeletal muscle by using a weighted average of skeletal muscle potassium found in 11 published reports. These potassium contents of skeletal muscle, however, were 76.7 – 107 mmol/kg (i.e., 3.00 – 4.18 g/kg). This wide variation may be due to such factors as the specific muscle group analyzed and the age, gender, physical condition, and other similar

demographic factors of study subjects. The assumption of 3.56 g/kg as the constant potassium content of skeletal muscle is most likely inappropriate and may lead to large errors in calculated muscle mass. Similar concerns also arise for the assumed nitrogen contents used in equation 4. These various issues related to the assumed constant potassium and nitrogen contents of body composition components should be explored in future studies.

The Burkinshaw method is based on a model with two independent variables: TBK and TBN. An alternative approach to skeletal muscle mass prediction is derivation of empirical regression models based on actual subject data. As part of our data analysis, using multiple linear regression analysis with TBK and TBN as independent variables, we developed SM_{CT} equations with intercepts set at 0 or $\neq 0$. In both of these equations the TBK term was highly significant whereas the TBN term was not statistically significant. The question therefore arises whether or not TBN measurement are needed for estimating skeletal muscle mass by use of empirically derived prediction equations. Our subjects were relatively young males, and to satisfactorily answer this question requires a larger and more diverse subject population.

DXA Method for Estimating Skeletal Muscle

The DXA approach gave mean total body skeletal muscle estimates that agreed closely with muscle measured by CT, although DXA tended to systematically overestimate total body muscle by ~5%. This extends our previous observation that DXA appendicular skeletal muscle estimates correlate well with other muscle indexes such as TBK (9).

The good agreement between CT and DXA can be explained in part by the observation that appendicular skeletal muscle accounts for a very large proportion of total body skeletal muscle mass (e.g., 75% in the Reference Man) (16). Small errors in measuring appendicular muscle mass may therefore not lead to large errors in calculated total body skeletal muscle mass.

Appendicular skeletal muscle is entirely within the total muscle component. This subordinate relationship between appendicular skeletal muscle and total body skeletal muscle is ideally suited for developing ratio models (i.e., equation 6) for predicting total muscle mass from appendicular muscle. Many other analogous body composition methods in current use are based on subordinate component relationships (17). For example, total body water is found entirely within fat-free body mass, and the stable total body water-to-fat-free body mass ratio (i.e., 0.73 kg/kg) is widely used in body composition methodology.

The present study showed that the DXA approach gave muscle estimates that were on average 5.8 and 5.1% higher than those observed by CT for the healthy subjects and AIDS patients, respectively. There are two possible explanations for the small overestimation of total body skeletal muscle by the DXA method. First, the ratio of appendicular to total body skeletal muscle by use of the DXA approach (0.79) was larger than that assumed (0.75) on the basis of the Reference Man (16). A likely factor contributing to the high actual ratio is the DXA cut-points used to estimate the appendicular skeletal muscle component. It is probable that some gluteal and other muscles were included that anatomically are not considered appendicular muscle. In the present study we were unable to directly compare DXA with CT-measured appendicular skeletal muscle, because accurate measurements of appendicular skeletal muscle are not possible with the 22-slice CT method. We are now working with other investigators to develop standardized DXA appendicular skeletal muscle measurement protocols in an attempt to improve the reproducibility and accuracy of this component's measurement.

Second, within- and between-subject variation in the relationship between appendicular muscle and total body skeletal muscle is possible. Of concern is that the appendicular-to-total body skeletal muscle mass ratio may vary between groups. We did not observe significant differences in this ratio between healthy men and men with AIDS, although this question needs to be explored in future studies before models such as equations 6 are appropriate for use across all age, gender, and ethnic groups.

Conclusion

In conclusion, we use three *in vivo* methods to examine total body skeletal muscle mass in healthy men and men with AIDS. On the basis of extensive earlier observations, we assumed that the most accurate representation of total body skeletal muscle mass was provided by the multiscan CT method. In this study we added support to earlier observations (6 - 8, 15) by demonstrating extremely close agreement between BV_{CT} and BV_{UWW}. Moreover, the proportion of ATFM as muscle observed in our subjects was in good agreement with earlier cadaver studies (4). We therefore assume that CT provided us with an accurate measurement of total body skeletal muscle mass in our subjects.

Compared with measurement of skeletal muscle by CT, the Burkinshaw method substantially underestimated muscle and the DXA method slightly overestimated total body skeletal muscle mass. These findings prompted us to suggest a number of possible explanations for these

discrepant results, and we suggest future studies that might confirm the validity of these hypotheses. Finally, because of substantially less radiation exposure, low cost, and availability at many research and clinical centers, our findings suggest that the DXA method may be a practical alternative to CT as a means of quantifying skeletal muscle mass *in vivo*.

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My business is to teach my aspirations to conform themselves to fact, not to try to make facts harmonize with my aspirations.

—— Thomas Huxley

CHAPTER 9

TOTAL BODY SKELETAL MUSCLE MASS: EVALUATION OF 24-HOUR URINARY CREATININE EXCRETION BY COMPUTERIZED AXIAL TOMOGRAPGY

ZiMian Wang, Dympna Gallagher, Miriam E. Nelson, Dwight E. Matthews, Steven B. Heymsfield

ABSTRACT

A classic body composition method is estimation of total body skeletal muscle mass (SM, in kg) from 24-h urinary creatinine excretion (in g). Two approaches of unknown validity have been used to calculate SM from creatinine: one assumes a constant SM to creatinine ratio, the so-called creatinine equivalence (k) and that SM = $k \times$ creatinine; and the other suggests a highly variable ratio of SM to creatinine and is based on regression equations of the form SM = $b + a \times$ creatinine. We explored these two extreme possibilities by measuring SM with whole-body computerized axial tomography and collecting urinary creatinine during meat-free dietary conditions in 12 healthy adult men. Prediction equations were developed in the men that fit these two models, SM = 21.8 $\times$ creatinine (SD and CV of the ratio of SM to creatinine: 1.3 kg and 6.0%, respectively) and SM = 18.9 $\times$ creatinine + 4.1 (r = 0.92, $P = 2.55 \times 10^{-5}$, and SEE = 1.89 kg). The validity of each model is reviewed in the context of theoretical aspects of creatine-creatinine metabolism. This first investigation of the method of measuring urinary creatinine excretion to determine SM by using modern techniques raises important practical and basic questions related to SM prediction.

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INTRODUCTION

There is growing interest in quantifying total body skeletal muscle mass (SM) *in vivo*. Until recently, however, available methods were of limited accuracy or lacked adequate validation. Recent advances in body composition methods now make it possible to accurately measure total body SM with both computerized axial tomography (CT) (1) and magnetic resonance imaging (MRI) (2). The validity of these two methods in measuring components at the tissue-system level of body composition, including SM, is supported by a large and growing body of literature reporting excellent results in phantoms, excised organs (3, 4), and in cadavers (5). Both multiscan CT and MRI, however, are costly and labor intensive methods. The CT method has the additional disadvantage of exposing subjects to radiation, thus precluding the study of children and pre-menopausal women.

An important alternative to CT and MRI in assessing total body SM is the 24-h urinary creatinine excretion method (6, 7). Creatinine in healthy adults is produced by nonenzymatic dehydration of intracellular creatine and phosphocreatine. Creatinine, a metabolic end-product, is then released into extracellular fluid and excreted unchanged in urine and to a small extent is disposed of through non-renal mechanisms (6). SM is the main source of urinary creatinine, although a small and inadequately characterized amount (< 20% of total) is produced in smooth muscles, brain, and other organs (6, 8).

When examining previous publications we found that investigators reported two main types of urinary creatinine-SM methods. The first type is formulated on the assumption that 24-h urinary creatinine (in g) is directly proportional to total body SM (in kg). According to this approach the ratio of total body SM to 24-h urinary creatinine excretion (SM:creatinine) approximates a constant. The term creatinine equivalence describes this classically applied assumed constant ratio.

Many attempts over this century were made to estimate the creatinine equivalence. Burger (9) in 1919 proposed that 1 g urinary creatinine output/d was equivalent to 22.9 kg of wet SM. Using additional information and correcting some mathematical errors in Burger's report, Talbot (10) in 1938 suggested that 1 g urinary creatinine/d corresponds to 17.9 kg SM on a general diet. Graystone (11) in 1968 subsequently suggested that 20 kg fat-free wet SM produces 1 g urinary creatinine/d in subjects on a low creatine diet. Based on [^{14}C]-creatine dilution studies in a small cohort of adults, Kriesberg et al. (12) in 1970 reported that 1 g

urinary creatinine was equivalent to 16.2 kg wet SM in subjects ingesting a general diet. Carrying out similar studies in children, Picou et al. (13) in 1976 suggested that 1 g urinary creatinine was equivalent to 18.6 kg wet SM in subjects ingesting a meat-free diet.

All of these studies describe the mathematical relation between total body SM and 24-h urinary creatinine excretion as

$$SM = k \times Cr \qquad [1]$$

SM:creatinine can be expressed as

$$SM/Cr = k, \qquad [2]$$

where k is the creatinine (Cr) equivalence. Although it is assumed to be a constant, k estimates are influenced by at least two independent factors, diet and SM-measurement methods. Multiple dietary factors influence urinary creatinine excretion, the most important of which is ingestion of creatine, mainly from meat (6). In the aforementioned studies k ranged from 16.2 to 17.9 kg/g on *ad libitum* diets and from 18.6 to 20.0 kg/g on meat-free diets. Methods of measuring SM mass also differed between studies and this might account for variation in SM:creatinine values reported previously. Because the introduction of CT and MRI, SM-measurement methods were based on numerous models and assumptions of unknown validity which might explain some of the variability in k even after previous studies are organized into *ad libitum* and meat-free diet categories.

The second type of prediction method is based on the observation that the SM:creatinine in healthy adults is not a constant even under controlled experimental conditions. An alternative SM prediction equation can be written for these methods that takes the general form

$$SM = b \times Cr + a, \qquad [3]$$

where b is the slope and a is the intercept for the experimentally determined SM-creatinine regression line. SM:creatinine can be expressed as

$$SM/Cr = b + a/Cr \qquad [4]$$

Equation 4 indicates that when $b \neq 0$, SM:creatinine is not a constant but changes as a reciprocal function of urinary creatinine excretion. SM prediction methods based on equation 3 were suggested by Forbes et al. (7, 14). Because no validated methods for measuring total body SM were available, Forbes first developed two equations relating 24-h urinary creatinine excretion to fat-free body mass (FFM, kg) measured by ^{40}K-whole body counting

$FFM = 29.1 \times Cr + 7.38$; $r^2 = 0.97$, SEE = 2.57 kg;
n = 34, *ad libitum* diet *[5]*

$FFM = 24.1 \times Cr + 20.7$, $r^2 = 0.91$, SEE not reported,
n = 57, meat-free diet *[6]*

Forbes next converted FFM to SM by assuming that 49% of the FFM is SM. Forbes based his assumption of a constant ratio of SM to FFM on previous animal and cadaver studies (7). Prediction equations were then derived for calculating total body SM from 24-h urinary creatinine excretion on an *ad libitum* diet and on a meat-free diet, respectively,

$SM = 14.4 \times Cr + 3.6$; *ad libitum* diet *[7]*

$SM = 11.8 \times Cr + 10.1$; meat-free diet *[8]*

SM:creatinine can be expressed as

$SM/Cr = 11.8 + 10.1/Cr$ *[9]*

Equation 9 suggests that SM:creatinine is a reciprocal function of urinary creatinine. When creatinine changes within the normal range for adult men from 1.0 to 2.0 g/d, SM:creatinine decreases substantially from 21.9 to 16.9 kg/g (7).

The distinction between the two types of SM prediction equations (equations 1 and 3) is an important one. The urinary creatinine-SM method is optimum if SM:creatinine approaches a constant. There would be little influence of other creatinine sources on SM estimates. If, on the other hand, SM:creatinine is not relatively constant between subjects, then SM prediction equations such as those developed by Forbes will ultimately be required.

The present investigation was designed as an exploratory study that examined the relation between total body SM, measured by multiscan CT, and 24-h urinary creatinine excretion under carefully controlled meat-free dietary conditions. Our aim was to evaluate the magnitude and constancy of SM:creatinine in a group of healthy adult men. This study is the first attempt to investigate the urinary creatinine-SM method using an accurate total body SM measurement method.

SUBJECTS AND METHODS

The study aim was accomplished by evaluating total body SM and 24-h urinary creatinine excretion in a cohort of healthy physically active adult men. Once enrolled in the study subjects completed the CT protocol and urine collections within two weeks of each other. The urinary creatinine concentration was assayed on aliquots of samples collected over 24-h periods during the last three days of a one week meat-free diet protocol (15). Creatinine excretion was measured in each of the three daily urine samples.

Subjects

Men were recruited from among hospital staff and local university students. None of the subjects were engaged in high-levels of exercise training or were taking any medications just before and during the study. Routine blood studies, including electrolytes, liver tests, and hematological indexes confirmed the health status of each subject before entry into the protocol. The procedures were fully explained to subjects and an informed consent form was signed. The project was approved by the Institutional Review Board of St. Luke's-Roosevelt Hospital Center. The subjects in the present investigation participated in a larger study of body composition (16). The observations described in the present report were not included in our earlier investigations.

Urinary Creatinine Protocol and Measurement

During the experimental period the subjects consumed a creatine-free diet that consisted of a mixture of meat-free foods and fresh vegetables. Instructions on food-item selection were provided by a research dietitian. All subjects were weight stable (± 1 kg) during the experimental week and physical activity was maintained at habitual levels. Timed 24-h urine collections were made during the last three days of the experimental week (15). Urine volume was measured to the nearest milliliter, and aliquots stored at −10°C until analysis. The urinary creatinine concentration was determined by an autoanalyzer that used the Jaffe reaction (17). The between-measurement CV for the Jaffe method is < 2% in our laboratory. Daily urinary creatinine excretion was calculated as the product of 24-h urine volume and urinary creatinine concentration.

Multiscan Computerized Axial Tomography

Total body SM measured by CT required 22 cross-sectional images at the anatomic locations described by Sjöström (1). Subjects were placed on the Somatom DRH scanner (Siemens

Corporation, Erlangen, Germany) platform with their arms extended above their heads to reduce beam hardening artifacts. CT images were prepared at 125 kVp and 170 mA, a scan time of 4 secs, and a slice thickness of 4 mm. The CT protocol for measuring total body SM and adipose tissue was described elsewhere in detail (1, 18, 19). The attenuation ranges used to measure cross-sectional areas of SM and adipose tissue were −29 to +120 Hounsfield units (HU) and −190 to −30 HU, respectively. The values 1.04 and 0.92 g/cm^3 were used as the assumed densities of SM and adipose tissue, respectively (20). Adipose tissue-free body mass (ATFM) was calculated as the difference between body weight and CT-measured adipose tissue.

Statistical Methods

Sample size was based on the results of Forbes et al. (7, 14). The investigators showed a significant intercept term for the regression line of SM versus 24-h urinary creatinine excretion (equation 3) and a nonconstant ratio of SM to creatinine in subjects ingesting a meat-free diet. Our power calculations were based on the data provided in the publication of Forbes et al. (14) for subjects ingesting an *ad libitum* diet. This is because no raw data was provided by Forbes (7) for his equation developed in subjects ingesting a meat-free diet. The intercept term in the meat-free diet equation (equation 6) was larger than that for subjects ingesting *ad libitum* diets (equation 5) and we therefore considered our sample size estimates ample to evaluate the significance of the observed intercept term for the regression equation of SM versus creatinine. We reanalyzed the creatinine-FFM equation (equation 5) and found that the SE of the equation slope and intercept were 0.000877 and 1.08, respectively. The minimum number of subjects needed to establish a significant intercept term with a power of 0.80 and an α level of 0.05 (two tailed) was 10.

Twenty-four hour urinary creatinine excretion for individuals is expressed as the mean ± SD of the three timed 24-h urine collections. The CV for between day differences in urinary creatinine excretion was calculated as SD/mean. Body composition results are expressed as the group mean ± SD. Simple linear regression analysis was used to explore the relations between 24-h urinary creatinine excretion and body composition. Data were analyzed using SAS version 5 (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics

Twelve healthy men met the study entry criteria and their baseline results are presented in **Table 1**. The group as a whole was age 31 ± 8 yrs (range 20 - 47 yrs) and had a body mass (in kg/m^2) index of 22.7 ± 3.3 (range: 18.2 - 28.9). The subjects had 10.4 ± 8.2 kg adipose tissue measured by CT, which represented 13.9 ± 8.5% of body weight. SM measured by CT was 31.9 ± 4.5 kg and this represented 45.7 ± 5.1% of body weight. SM was 53.1 ± 3.4 % of ATFM with a range of between 44.9 % and 57.8 %.

Creatinine Excretion and Body Composition

Individual 24-h urinary creatinine excretions are presented in **Table 1**. The between-day CV for urinary creatinine excretion in the 12 men was 9.8 ± 4.6%. The mean 24-h urinary creatinine excretion in this group was 1.467 ± 0.220 g (range: 1.132 – 1.816 g).

The first creatinine-SM method is based on equation 1. The mean SM:creatinine (i.e., k) in the present study subjects was 21.8 ± 1.3 kg/g (CV: 6.0 %). The SM prediction equation based on the current results takes the form

$$SM = 21.8 \times Cr. \qquad [10]$$

This equation indicates that creatinine excretions of 1.0 and 2.0 g/d represent 21.8 and 43.6 kg of SM, respectively.

The second creatinine-SM method is based on equation 3. For the present study subjects this SM prediction equation takes the form

$$SM = 18.9 \times Cr + 4.1, \qquad [11]$$

for which r = 0.92, P = 0.0000255, and SEE = 1.89 kg (**Figure 1**). The intercept in this equation (4.1 kg) was not significantly different from zero (P = 0.31) and was lower than that observed by Forbes and Bruining (14) in subjects ingesting a meat-free diet (10.1 kg, equation 8). According to equation 11, creatinine excretion of 1.0 and 2.0 g/d represents 23.0 and 41.9 kg of SM, respectively.

When equation 11 is rearranged, SM: creatinine can be expressed as

$$SM/Cr = 18.9 + 4.1/Cr \qquad [12]$$

When creatinine increases from 1.0 to 2.0 g/d, the SM:creatinine, according to equation 12, decreases from 23.0 to 21.0 kg/g. SM:creatinine therefore changes by ~10 % across a range of urinary creatinine excretions typical of healthy adults.

Table 1. Results of experimental studies

Subject No	Age yr	BW kg	Ht m	BMI kg/m^2	AT kg	SM kg	Creatinine g/d
1	37	61.0	1.69	21.4	7.9	27.9	1.384 ± 0.281
2	28	93.7	1.80	28.9	33.3	33.5	1.518 ± 0.095
3	32	73.8	1.85	21.6	8.9	34.1	1.692 ± 0.153
4	33	77.2	1.77	24.6	12.1	36.1	1.579 ± 0.115
5	26	57.0	1.77	18.2	2.7	31.4	1.391 ± 0.042
6	47	55.9	1.70	19.3	4.2	23.2	1.132 ± 0.119
7	20	75.8	1.81	23.1	5.9	36.4	1.816 ± 0.116
8	22	70.7	1.72	23.9	16.7	28.7	1.185 ± 0.190
9	20	61.5	1.75	20.1	9.0	26.0	1.191 ± 0.114
10	39	64.3	1.83	19.2	4.5	32.3	1.436 ± 0.154
11	38	72.7	1.70	25.2	7.6	36.5	1.598 ± 0.200
12	32	80.2	1.74	26.5	11.8	36.8	1.681 ± 0.103
mean	31.2	70.3	1.76	22.7	10.4	31.9	1.467
± SD	8.4	11.0	0.05	3.3	8.2	4.5	0.220

AT adipose tissue; BMI, body mass index; BW, body weight; Cr, 24-h urinary creatinine excretion, mean ± SD of 3 day collection; Ht, height; SM, total body skeletal muscle mass measured by computerized axial tomography.

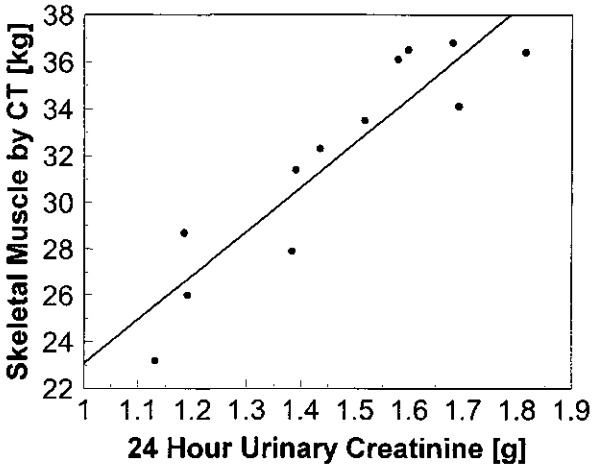

Figure 1. Relation between total body skeletal muscle mass (SM) measured by computerized axial tomography (CT) and 24-h urinary creatinine excretion (n = 12).

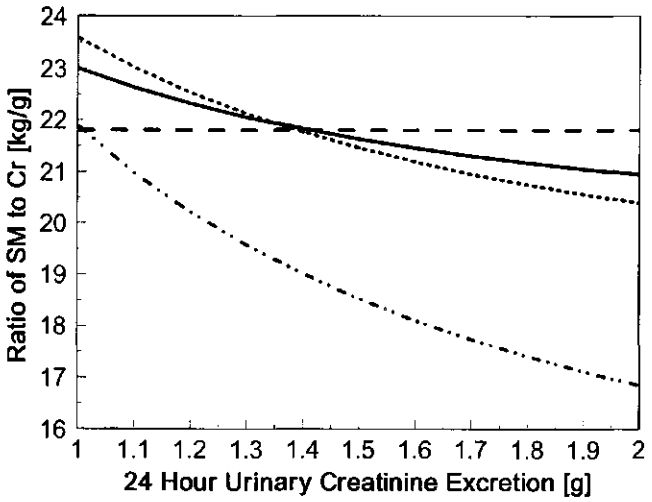

Figure 2. Relation between the ratio of skeletal muscle mass (SM) to creatinine and 24-h urinary creatinine excretion. The horizontal line (– – –) is the creatinine equivalence of 21.8 kg/g; curve (—) is actual observations based on equation 11 (SM = 18.9 × creatinine + 4.1); the curve (— · · —) is based on Forbes's equation 8 (SM = 11.8 × creatinine + 10.1); and the curve (· · · · · ·) is based on Forbes's revised equation 16 (SM = 17.2 × creatinine + 6.4).

The relation between SM:creatinine and 24-h urinary creatinine as evident in the present study is graphically summarized in **Figure 2**. SM:creatinine formulated according to equation 12 shows a small non-linear decline (from 23.0 to 21.0 kg/g) when urinary creatinine excretion increases from 1.0 to 2.0 g/d. SM:creatinine based on Forbes equation 9 shows a very large non-linear decrease (from 21.9 to 16.9 kg/g) when urinary creatinine excretion increases from 1.0 to 2.0 g/d.

DISCUSSION

Although investigators have expressed interest in the creatinine-SM method since early in the 20th century, fundamental questions remained unanswered because of limited methods. Our study focused on two closely related questions: What is the magnitude of the ratio of SM to creatinine in a group of healthy adult men ingesting a meat-free diet? and Is this ratio, classically referred to as the creatinine equivalence, constant, or more specifically, independent of urinary creatinine excretion? These questions are directly related to the application of urinary creatinine excretion as a measure of SM.

Magnitude of the Ratio of SM to Urinary Creatinine

Our findings indicate that in healthy adult men the mean ratio of SM to 24-h urinary creatinine excretion is 21.8 kg/g. This is somewhat higher than previously observed ratios of SM to creatinine in children ingesting meat-free diets (18.6 and 20 kg/g) (11, 13). One explanation for the observed differences between our study and the earlier investigations is our more accurate and possibly higher SM estimates. A second possibility is that SM and urinary creatinine have different relations in children and adults.

Third, other factors that might explain the observed between-study differences in the SM:creatinine are urinary creatinine determinants including length of time on meat-free diet, physical activity level, and the creatinine analysis method used (6). Switching a subject from a diet that contains meat to a creatine-free diet causes a curvilinear decline in urinary creatinine excretion (21). The most rapid phase of creatinine decline is in the first week, although changes in the amount excreted continue for up to six weeks. A portion of the change in urinary creatinine was due to elimination of preformed creatinine found mainly in meats. A second cause is a change in dietary creatine and phosphocreatine intake which alter creatine pool size (21). In the current study we standardized measurements by collecting urine during the last three days of a one week meat-free diet protocol. Variation in diet and timing of urine collections could thus explain some of the observed between-study differences in SM:creatinine. Extremely strenuous exercise also influences urinary creatinine, possibly through changes in renal function and alterations in the ratio of phosphocreatine to total creatine ratio (6). We standardized physical activity in all of our subjects who were asked to refrain from non-habitual strenuous tasks during the study protocol. Again, failure to adequately control physical activity could cause variation in the SM:creatinine. In addition, as we will expand on in the next section, the SM:creatinine is related to urinary creatinine excretion and this may also account for between-study differences.

Constancy of the Ratio of SM to Urinary Creatinine

The second and more critical question in relation to the creatinine-SM method was whether or not the ratio of SM to creatinine was sufficiently constant between subjects so that it could be used as an equivalence to estimate SM from 24-h urinary creatinine excretion. As we described in the Introduction, previous investigators reported two extreme possibilities for the linear regression equation of SM versus creatinine: a hypothetical zero intercept and thus a

constant ratio of SM to creatinine; and a large significant intercept term and thus a non-constant ratio of SM to creatinine. The findings of the present study distinguished between these two extreme possibilities. Our results indicate that in healthy adult men the regression equation of SM versus creatinine has a smaller intercept term (4.1 kg; equation 11) than that of the study by Forbes (10.1 kg; equation 8) (7). Based on the current data, when urinary creatinine excretion increases from 1.0 to 2.0 g/d, the ratio of SM to creatinine decreases by 8.7 % (i. e., from 23 to 21 kg/g). In addition, the ratio of SM to creatinine (21.8 ± 1.3 kg/g) based on the average of three 24-h urine collections had a between subject CV of 6.0 %, suggesting moderate variability among the 12 men. In comparison, the widely used ratio of total body water to FFM (0.732) has a CV of 2.3% in healthy young men (22).

The current results, in a small group of healthy adult men, indicate that the SM:creatinine is modestly variable and that the formula SM = 21.8 × creatinine has the potential to provide an approximate measure of total body SM in subjects ingesting a meat-free diet. The underlying issues, however, are more complex. To appreciate these complexities and to provide a background for future suggested investigations, we present an expanded analysis of urinary creatinine determinants as they apply to the present study results. In particular, we examine the conditions under which the ratio of SM to creatinine may be a constant.

Twenty-four hour urinary creatinine excretion is representative of creatinine produced by both endogenous and exogenous sources. Endogenous creatinine is produced by SM and potentially by non-SM adipose tissue-free tissues. Accordingly,

$$Cr = (R_1 \times SM) + (R_2 \times NSM) + Cr_{diet} \qquad [13]$$

where NSM is non-SM adipose tissue-free body mass; R_1 and R_2 are daily SM and NSM production rates of creatinine, respectively; and Cr_{diet} is daily exogenous urinary creatinine excretion resulting from dietary intake. Daily exogenous urinary creatinine excretion can vary greatly between subjects because of differences in meat intake. On a meat-free diet, Cr_{diet} approaches zero and thus the elimination of Cr_{diet} removes one source of potential variation in urinary creatinine excretion.

Equation 13, when applied in subjects on a meat-free diet, can be simplified and rewritten to solve for the ratio of SM to creatinine by assuming that the ratio of NSM to SM ratio is m and that NSM = $m \times$ SM,

$$SM/Cr = 1/(R_1 + m \times R_2) \qquad [14]$$

This is a general formula for SM:creatinine. This equation indicates that the SM:creatinine is constant under two different conditions. The first is SM is the only source of urinary creatinine and R_2 is thus equal to zero. In this condition SM:creatinine = $1/R_1$, and if the daily creatinine production rate of SM (R_1) is not effected by exercise and/or other intramuscular biochemical processes, the SM:creatinine will be constant within and between subjects. The second condition occurs when NSM tissues produce significant amounts of creatinine. Equation 14 indicates that even under this complex condition (i.e., $R_2 > 0$) SM:creatinine will remain constant provided both daily creatinine production rates of SM and NSM (i.e., R_1 and R_2) and the ratio of NSM to SM (i.e., m) are stable between subjects. Therefore, a zero creatinine production rate of NSM (i.e., $R_2 = 0$) is not a requirement for a constant ratio of SM to creatinine. Conversely, even when the SM:creatinine is experimentally constant, it does not mean that SM is the only source of urinary creatinine. It is clear, however, that if there is substantial production of creatinine outside of SM then the conditions required for a constant ratio of SM to creatinine become stringent.

Although in the present study we did not investigate NSM creatinine sources, there are two lines of evidence in support of creatinine production outside of SM. First, Afting et al. (8) observed creatinine in the urine of a totally paralyzed patient who had no clinically detectable SM tissue. The investigators found 0.24 g/d of urinary creatinine in the paralyzed patient and 1.38 g/d in a group of healthy control subjects. This observation led Afting et al. to hypothesize that ~15 - 20 % of daily urinary creatinine excretion is from NSM sources. The second line of evidence for NSM sources of creatinine comes from direct tissue analyses and nuclear magnetic resonance spectroscopy studies indicating measurable amounts of creatine and phosphocreatine in tissues such as heart, brain, and involuntary muscles such as those found in blood vessels and gastrointestinal tract (23). The quantitative contribution of these non-SM sources to urinary creatinine excretion is unknown.

The foregoing analysis suggests that the assumed constant ratio of SM to creatinine only occurs under limited specific conditions. Our results in a relatively small and homogeneous cohort of men show modest variability in this ratio (e.g., CV: 6%). However, the current study results need to be extended to more diverse populations that vary in age, gender, ethnicity, and health status. Moreover, additional studies are needed to examine the quantitative significance of NSM sources of urinary creatinine. Finally, the constancy of creatinine production rates for

SM and NSM components needs to be established. Exploring these issues will lead to an improved understanding of the clinical applicability of the creatinine-SM method.

An important consideration related to the constancy of the ratio of SM to creatinine is the reliability of urinary creatinine measurements. Under normal conditions in outpatients, the CV for 24-h urine creatinine analyses ranges from 4% to 8% (6). Our carefully trained subjects had a relatively large between-day urinary creatinine CV of 9.8 ± 4.6%. These observations strongly emphasize the need for evaluating appropriately educated and compliant subjects.

Reexamination of Forbes Equation

The results of the present study raised the following questions: Why did the Forbes equation (equation 8) underestimated SM by 14% for the group of 12 men (27.4 ± 2.6 kg compared with 31.9 ± 4.5 kg by CT, $P = 2.65 \times 10^{-5}$); and Why did the Forbes equation suggests such a large intercept term (10.1 kg) and thus highly variable ratio of SM to creatinine. On reexamining Forbes equations in light of the present study results, we found a small but important error in the conversion of FFM to SM. Based on his own results (7) and with support from the gross dissection data of the Belgian Cadaver Study (24), Forbes suggested that both the ratio of SM to adipose tissue-free body mass (0.54) and the ratio of SM to FFM (0.49) are constant. Forbes then used the SM/FFM ratio (0.49) to convert FFM to SM (7). We reviewed the original data of the Belgian Cadaver Study and found that the ratio of SM to ATFM for six unembalmed male cadavers actually varies from 48.8% to 59.4% (CV: 8.5%). Using a sole value of the ratio of SM to ATFM or SM to FFM may therefore produce a large error in converting ATFM or FFM to SM, respectively. We therefore explored the Belgian Cadaver data further and found that there is a very high correlation between SM and ATFM for the six unembalmed male cadavers,

$$SM = 0.713 \times ATFM - 8.4;\ r = 0.996,\ P < 0.001 \qquad [15]$$

We then combined equation 15 with Forbes equation 6 (FFM = 24.1 × Cr + 20.7). Assuming FFM approximately equal to ATFM and solving these two simultaneous equations, we rederived Forbes's equation as follows,

$$SM = 17.2 \times Cr + 6.4 \qquad [16]$$

Equation 16 is very close to our CT-based creatinine-muscle prediction (equation 11) for both slope and intercept terms. There is no significant difference in SM between the results predicted by the revised Forbes equation 16 and our SM measurements by CT (31.6 ± 3.8 and

31.9 ± 4.5 kg, respectively; $P = 0.31$). When creatinine increases from 1.0 to 2.0 g/d, SM:creatinine, according to equation 16, varied from 23.6 to 20.4 kg/g. This is remarkably similar to the results of our CT-based equation 12 (23.0 to 21.0 kg/g) **(Figure 2)**. Moreover, the relation between SM and ATFM in our subjects (SM = 0.634 × ATFM − 6.1, r = 0.93, $P < 0.001$) was very similar to those obtained in the Belgian male cadavers (equation 15). The results from the revised equation of Forbes were consistent with the results of the present study and lends further support to a non-constant, modestly variable ratio of SM to creatinine. Note that our study was carried out in adult men. The respective equations from the present study should therefore not be used in women and children without prior cross-validation. Because MRI can be used to estimate SM without radiation exposure, it is now feasible to extend analyses as reported in the present study to young women and children.

Conclusion

The value of the creatinine-SM method is that urine creatinine is simple to quantify in locations without advanced instruments such as CT or MRI. In the present study we explored the relation between 24-h urinary creatinine excretion and total body SM. Our results suggest that under carefully controlled dietary conditions a good correlation exists between SM measured by CT and creatinine excretion in adult men; and that urinary creatinine collected in adult men ingesting a meat-free diet can be used to estimate SM as either SM = 21.8 × Cr or SM = 18.9 × Cr + 4.1. Finally, we identified the need to evaluate larger and more diverse subject populations and to examine basic questions related to creatine and creatinine metabolism *in vivo*.

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We must accept finite disappointment, but
must never lose infinite hope.

—— Martin Luther King, Jr.

CHAPTER 10

URINARY 3-METHYLHISTIDINE EXCRETION: ASSOCIATION WITH TOTAL BODY SKELETAL MUSCLE MASS BY COMPUTERIZED AXIAL TOMOGRAPHY

ZiMian Wang, Paul Deurenberg, Dwight E. Matthews, Steven B. Heymsfield

ABSTRACT

Background: The urinary excretion of endogenous 3-methylhistidine (3MH) has been proposed as a predictor of skeletal muscle mass (SM). In this study, we report the relationship between 24-h urinary 3MH excretion and SM.

Methods: Total body SM was measured by multiscan computerized axial tomography (CT) in a sample of 10 healthy adult men who followed a meat-free diet for 7 days. 3MH was measured during the last 3 days of the meat-free diet protocol on consecutive 24-h urine collections.

Results: The 3MH excretion was 216.3 ± 44.7 μmol/day (mean ± SD) and was found well associated with SM (in kg), SM = 0.0887 × 3MH + 11.8; r = 0.88, $P < 0.001$. Compared with CT, the previous 3MH-SM prediction equation suggested by Lukaski et al. underestimated SM by an average of 8.9 kg in the 10 healthy men. This difference was caused by the Burkinshaw-Cohn neutron activation model, which underestimated SM and was used as the reference in the Lukaski method.

Conclusion: 24-h urinary 3MH excretion can be applied for estimating SM in healthy adult men on a meat-free diet.

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INTRODUCTION

There is a growing interest in quantifying total body skeletal muscle (SM) *in vivo*, although some of the available methods lack adequate validation. Multiscan computerized axial tomography (CT) and magnetic resonance imaging (MRI) are recent advances in body composition research and provide accurate SM measurement, as well as the possibility of evaluating other existing methods (1). Compared to the CT method, the Burkinshaw-Cohn neutron activation model, dual-energy X-ray absorptiometry, and 24-h urinary creatinine excretion methods of estimating SM were evaluated in previous reports (2, 3). In the present study, another SM estimation method, 24-h urinary 3-methylhistidine (3MH) excretion, was evaluated by the multiscan CT technique.

The 3MH-SM method is based on a series of assumptions as summarized by Lukaski (4): 1. the histidine residues in muscle-protein are methylated; and the majority of 3MH is produced by two muscle-proteins, actin found in all muscle fibers and myosin which is present in white fibers; 2. when myofibrillar protein is catabolized, released 3MH is neither metabolized nor reused in metabolism; 3. muscle-protein synthesis and catabolism are in balance in healthy adults and thus 3MH is produced at a constant daily rate; and 4. produced 3MH is completely excreted in urine. If these conditions are met, 24-h urinary 3MH excretion can be used as a marker of total body SM on a meat-free diet.

Using the Burkinshaw-Cohn neutron activation model as the reference, Lukaski and his colleagues proposed a SM prediction equation from 3MH for healthy men (5),

$$SM_L = 0.118 \times 3MH - 3.45;\ r = 0.91,\ P < 0.001;\ SEE = 2.07\ kg;\ n = 14; \qquad [1]$$

where SM_L (in kg) is total body SM predicted by the Lukaski equation; and 3MH is 24-h urinary 3-methylhistidine excretion (in μmol) on a meat-free diet. The Lukaski method, although the first 3MH-SM prediction equation, has never been adequately evaluated.

The present investigation was designed as an exploratory study that examined the relationship between 3MH and SM under carefully controlled meat-free dietary conditions. Specifically, we evaluated total body SM estimated by the Lukaski equation by using the multiscan CT technique.

METHODS

Subjects

The subject pool consisted of 10 healthy men who were recruited from local sources. None of the subjects were actively engaged in a sports training program. Each subject completed a medical history, physical examination, and routine screening blood studies to confirm their health status. All subjects signed an informed consent that was approved by the Hospital's Institutional Review Board. They subsequently completed urine collection for urinary metabolites and multiscan CT for total body SM and adipose tissue measurements. Some subjects in this study participated in a larger project related to body composition (2). However, the observations on urinary 3MH excretion described in the present report were not included in our earlier investigation.

Urinary 3MH and Creatinine Excretion Measurement

Subjects consumed a meat-free diet for over one week. Instructions were provided for food item selection and maintaining a relatively constant body weight (± 1 kg) during the meat-free diet period. Timed 24-h urine samples were collected during the last three days of the meat-free diet week. Urine volume was measured to the nearest milliliter and aliquots stored at −10 °C until analysis. The urinary 3MH concentration was determined by gas chromatography-mass spectrometry technique and the urinary creatinine concentration was determined by an autoanalyzer that used the Jaffe reaction (6, 7). Daily urinary 3MH (or creatinine) excretion was calculated as the product of 24-h urine volume and urinary 3MH (or creatinine) concentration. Results are expressed as the mean ± SD for three consecutive samples.

Multiscan Computerized Axial Tomography

Total body SM and adipose tissue masses were measured using the multiscan CT technique proposed by Sjöström and colleagues (1). The full procedure required 22 cross-sectional images at pre-defined anatomic locations. A Somatom DRH scanner (Siemens Corporation, Erlangen, Germany) was used in the study. The subject was placed on the scanner platform with arms extended above head to minimize beam hardening artifacts. CT scans were completed at 125 kVp with a scanning time of 4 sec. at 170 mA. Slice thickness was 4 mm. The attenuation ranges of −29 to +120 Hounsfield units (HU) and −190 to −30 HU were set for cross-sectional areas of SM and adipose tissue, respectively. The values of 1.04 g/cm^3 and

Table 1. Results of experimental studies

Subject No	BW kg	Ht m	Age yr	BMI kg/m^2	AT kg	SM_{CT} kg	SM_L kg	3MH μmol/d	Cr g/d
1	61.0	1.69	37	21.4	7.9	27.9	21.6	212.4 ± 9.5	1.384 ± 0.281
2	93.7	1.80	28	28.9	33.3	33.5	22.8	222.2 ± 28.3	1.518 ± 0.095
3	73.8	1.85	32	21.6	8.9	34.1	28.8	272.9 ± 14.7	1.692 ± 0.153
4	57.0	1.77	26	18.2	2.7	31.4	20.0	198.4 ± 14.0	1.391 ± 0.042
5	55.9	1.70	47	19.3	4.2	23.2	13.5	144.0 ± 17.0	1.132 ± 0.119
6	75.8	1.81	20	23.1	5.9	36.4	31.1	293.1 ± 22.1	1.816 ± 0.116
7	70.7	1.72	22	23.9	16.7	28.7	19.8	196.9 ± 48.0	1.185 ± 0.190
8	61.5	1.75	20	20.1	9.0	26.0	16.8	171.3 ± 24.2	1.191 ± 0.114
9	64.3	1.83	39	19.2	4.5	32.3	21.1	207.8 ± 76.1	1.436 ± 0.154
10	80.2	1.74	32	26.5	11.8	36.8	25.4	244.2 ± 13.9	1.681 ± 0.103
Mean	69.4	1.77	30	22.2	10.5	31.0	22.1	216.3	1.443
± SD	11.9	0.06	9	3.4	9.0	4.5	5.3	44.7	0.235

AT, adipose tissue; BMI, body mass index; BW, body weight; Cr, 24-h urinary creatinine excretion, mean ± SD of 3 day collections; Ht, height; 3MH, 24-h urinary 3-methylhistidine excretion, mean ± SD of 3 day collections; SD, standard deviation; SM_{CT}, total body skeletal muscle mass measured by multiscan CT; SM_L, total body skeletal muscle mass calculated by Lukaski equation.

0.92 g/cm^3 were used as the assumed constant densities of SM and adipose tissue, respectively. Adipose tissue-free body mass was calculated as the difference between body weight and adipose tissue mass. The CT protocol for measuring body composition at the tissue-system level is described elsewhere in detail (1).

Statistical Methods

Group results are presented as the mean and standard deviation (SD). Simple linear regression analysis was used to describe the relationship between 24-h urinary 3MH excretion and total body SM quantified by CT, and between urinary 3MH and creatinine excretion. Differences in slope and intercept between regression lines were tested according to Kleinbaum and Kupper (8). Data were analyzed using SAS Version 5 (SAS Institute, Cary, NC).

RESULTS

3MH-SM Prediction Equation

Subject characteristics are presented in **Table 1**. Total body SM by CT was 52.6 ± 3.5% of adipose tissue-free body mass with a range from 44.9% to 57.8%. Based on 3MH excretion and CT-measured SM, a regression equation was derived for the subjects **(Figure 1)**,

$$SM_{CT} = 0.0887 \times 3MH + 11.8;\ r = 0.88,\ P < 0.001;\ SEE = 2.3\ kg. \qquad [2]$$

The slope and intercept of this equation are significantly different from one ($P < 0.0005$) and zero ($P < 0.01$), respectively. The Lukaski 3MH-SM equation is also shown in **Figure 1**. The slope of our new 3MH-SM equation is not significantly different from that of the Lukaski equation ($P > 0.05$), although the intercepts in the two SM estimation equations differ significantly ($P < 0.001$).

According to the Lukaski method (equation 1), total body SM of the group was only 22.1 ± 5.3 kg. The Lukaski equation therefore underestimated SM in the healthy men by 8.9 ± 2.5 kg ($P < 0.001$). However, there is a good correlation between SM estimates by CT (SM_{CT}) and the Lukaski equation (SM_L) **(Figure 2)**,

$$SM_L = 1.04 \times SM_{CT} - 10.2;\ \ r = 0.89,\ P < 0.001. \qquad [3]$$

The slope of equation 3 is not significantly different from one ($P > 0.05$), but the intercept is significantly different from zero ($P < 0.001$).

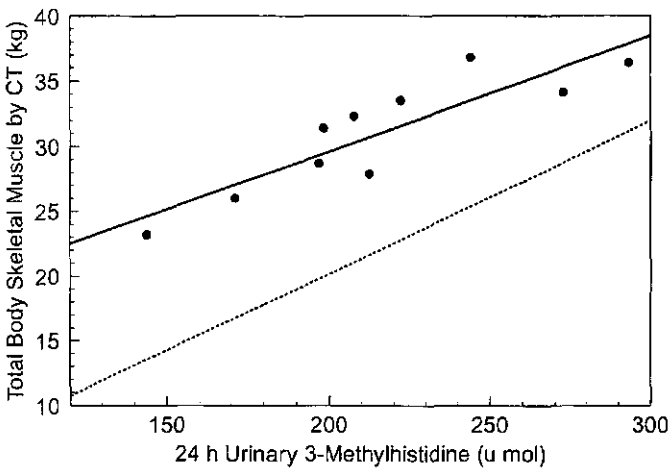

Figure 1. Total body skeletal muscle mass measured by CT (SM, in kg) on the ordinate *vs.* 24-h urinary 3-methylhistidine excretion (3MH, in μmol) on the abscissa (—, SM = 0.0887 × 3MH + 11.8; r = 0.88, $P < 0.001$; SEE = 2.3 kg). The Lukaski equation (. . . ., SM = 0.118 × 3MH – 3.45) is also shown in the figure.

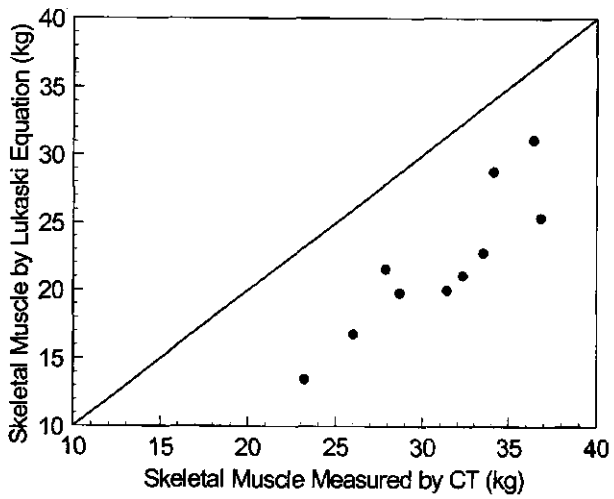

Figure 2. Total body skeletal muscle mass measured by the Lukaski equation (SM_L, in kg) on the ordinate *vs.* total body skeletal muscle mass measured by multiscan CT method (SM_{CT}, in kg) on the abscissa ($SM_L = 1.04 \times SM_{CT} - 10.2$; r = 0.89, $P < 0.001$). The line of identity is shown in the figure.

Relation Between 3MH and Creatinine Excretion

The between-day CV for urinary 3MH and creatinine (Cr) excretions were 13.0 ± 10.2% and 9.8 ± 5.1%, respectively. The mean 24-h urinary 3MH and creatinine excretions in the group were 216.3 ± 44.7 μmol (range 144.0 - 293.1 μmol) and 1.443 ± 0.235 g (range 1.132 - 1.816 g), respectively. The mean 3MH/Cr ratio in the subjects was 149.2 ± 11.6 μmol/g. There was a high correlation between 24-h urinary 3MH and creatinine excretions,

$$3MH = 181.0 \times Cr - 44.9; \quad r = 0.95, P < 0.001. \qquad [4]$$

DISCUSSION

Few previous investigations have attempted to establish the relationship between endogenous 3MH excretion and human body composition including total body SM (5, 9, 10). The use of 3MH as a marker of SM has been criticized because of the potential influence of non-SM protein turnover on 3MH excretion rate (11). However, other studies indicate that only a weak correlation (r = 0.33) between endogenous 3MH excretion and non-SM protein mass is present (5).

Prediction Equation Validity

The purpose of the present study was to establish the relationship between 3MH and total body SM under carefully controlled dietary conditions. In order to derive a reliable 3MH-SM prediction equation, the recently developed multiple CT technique was applied as the criterion for measuring total body SM. The validity of multiscan CT in measuring body components at the tissue-system level, including SM, is supported by earlier studies which report excellent results in phantoms, excised human organs, and in cadavers (12-14). Our earlier study also showed that body volume measured by multiscan CT was very close to actual body volume measured by underwater weighing (2). In addition, the ratio of SM to adipose tissue-free body mass (SM/ATFM, 0.526 ± 0.035) in the present study men was similar with that found in the Belgian Cadaver Study (0.540 ± 0.046) (15). These observations support the validity of the multiscan CT technique for measuring total body SM *in vivo*.

Another requirement for deriving a reliable 3MH-SM prediction equation is accurate measurement of 24-h urinary 3MH excretion. Under carefully controlled meat-free diet conditions, the 24-h urinary 3MH excretion of our subjects was 216.3 ± 44.7 μmol (mean ±

SD) which is close to the 225 ± 8, 230 ± 10 μmol (mean ± SE), and 237.7 ± 87.3 (mean ± SD) of healthy men reported in previous studies (5, 9, 11). Our results showed that there was a high correlation between 3MH and Cr (r = 0.95); and the 3MH/Cr ratio in the men of this study (mean ± SD, 149.2 ± 11.6 μmol/g) was close to that reported by previous authors (mean ± SE, 132.4 ± 2.9, 138.2 ± 4.1, and 151.9 ± 7.3 μmol/g) (5, 9, 16). These observations support the validity of our 3MH measurement.

Earlier Prediction Equation

There was a significant intercept difference (−10.2 kg) between SM calculated using the Lukaski equation and CT-measured SM (**Figure 2**). As shown in **Table 1**, SM calculated by the Lukaski equation (22.1 ± 5.3 kg) is significantly smaller than SM by CT (31.0 ± 4.5 kg, $P < 0.001$) for the subject group. A question growing out of these results is why the Lukaski equation underestimated SM by 28.7% for the subject group.

As mentioned above, a reliable 3MH-SM prediction equation must be based on an accurate measurement of total body SM. The Lukaski equation applied the Burkinshaw-Cohn neutron activation model as the reference (5). The main assumption of the Burkinshaw-Cohn model is the constancy of potassium to nitrogen ratios in SM (0.11 g/g) and non-SM-lean mass (0.052 g/g) (17). A model was derived to predict SM if total body potassium (TBK, in g) and total body nitrogen (TBN, in g) are assessed by ^{40}K whole-body counting and prompt-γ neutron activation analysis, respectively,

$$SM = 0.503 \times TBK - 0.0263 \times TBN. \quad [5]$$

Although much of the knowledge regarding SM in living humans has been derived using the Burkinshaw-Cohn model, SM estimates using this model are much lower than those observed in human autopsy studies including the Belgian Cadaver Study (15). Indeed one female subject in an earlier study using the Burkinshaw-Cohn model had a negative value (−2.35 kg) for calculated SM (17). Another study by our group strongly supported the long-standing suspicion that the Burkinshaw-Cohn model underestimates total body SM by an average of 20.1% (2). The main error source may be that the potassium concentration of SM is not a constant as suggested by Burkinshaw et al., but varies within a wide range between subjects. This could in-part explain why the Lukaski equation underestimates SM by average 28.7% for the subject group. Additionally, the Burkinshaw-Cohn model's assumed constant potassium to nitrogen ratios in SM and non-SM-lean mass were based on a literature review and an error in

Table 2. Comparison between Lukaski and present 3MH-SM prediction equations in young male non-athletes and wrestlers (mean ± SE)

Group	n	BW	FFM	3MH	SM (kg)		SM/FFM	
		kg	kg	μmol/d	Lukaski	Present	Lukaski	Present
Non-athletes	16	72.3 ± 2.7	61.5 ± 2.3	225 ± 8	23.1	31.8	0.376	0.516
Non-athletes	14	73.6 ± 2.4	62.3 ± 1.8	226 ± 10	23.2	31.8	0.373	0.510
Wrestlers	16	70.6 ± 9.1	63.3 ± 8.3	290 ± 5	30.8	37.5	0.486	0.593

Information based on references 5, 9, and 18.

BW, body weight; FFM, fat-free body mass; 3MH, 24-h urinary 3-methylhistidine excretion; SM, total body skeletal muscle mass calculated by the Lukaski equation or the present study's equation; SM/FFM, the ratio of SM to FFM in which SM was calculated by the Lukaski equation or the present study's equation.

the assumed population mean values might lead to a "bias" in predicted SM. The Burkinshaw-Cohn model, which has now largely been supplanted by CT, may thus lead to large SM errors both in individuals and in populations as a whole.

The 3MH-SM estimates by Lukaski, Mendez, and their colleagues are summarized in **Table 2** (5, 9, 18). The SM/FFM ratio in the non-athletes based on the Lukaski equation is only 0.38, which is much lower than the mean value (0.49) found in carcass analyses (19). According to the present 3MH-SM prediction equation, the SM/FFM ratio (0.51) is close to the value found by carcass analyses. This observation provides further support for the validity of the 3MH-SM prediction equation developed in the present study.

The 24-h urinary 3MH excretion (290 ± 5 μmol/d) in wrestlers was much higher than that in the non-athletes (226 ± 10 μmol/d; $P < 0.01$) (**Table 2**) (18). We recalculated SM from 3MH using equation 2 and found that the SM/FFM ratio in the wrestlers is higher than that in the non-athletes (0.59 *vs.* 0.51). This indicates that wrestling has a big influence on the SM/FFM ratio.

Conclusion

In the present study we explored the relationship between 24-h urinary 3MH excretion and total body SM. Our results suggest that under carefully controlled dietary conditions a good correlation exists between 3MH and SM measured by CT in adult men; and that urinary 3MH collected in adult men ingesting a meat-free diet can be used to estimate SM as SM = 0.0887 × 3MH + 11.8. An important question arising from these results is what role 3MH might play in SM assessment beyond that of easily measured 24-h urinary creatinine excretion (3). Lastly, future investigations are needed which include more subjects and consider the influence of multiple factors such as age, gender, race, and exercise level on 3MH-SM relations.

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CHAPTER 11

GENERAL DISCUSSION

Know the hows is important, but know the whys is more important.

BRIEF OVERVIEW OF THIS THESIS

The general introduction **[Chapter 1]** indicated that the study of human body composition is composed of three research areas. The nine research articles collected in this thesis fall into two of the three areas: body composition rules and body composition methodology. The author's effort can be briefly summarized in both the theoretical and the experimental aspects of the two areas.

Body Composition Rules

For ***the theoretical aspect***, this thesis proposed the five-level model of body composition **[Chapter 2]**. This model systematically organizes the rapidly accumulating information and knowledge that relate to the rules area of body composition study (Wang et al., 1992). The five-level model indicates that the ~40 major body components in the human body can be organized into five separate but interconnected body composition levels: I. atomic, II. molecular, III. cellular, IV. tissue-system, and V. whole-body. The body mass or weight (BW) can be expressed as the sum of major component masses on the five levels,

I. BW = O + C + H + N + Ca + P + S + K + Na + Cl + Mg;

II. BW = lipids + water + protein + bone mineral + soft tissue mineral + glycogen;

III. BW = cells mass + extracellular fluid + extracellular solids;

IV. BW = adipose tissue + skeletal muscle + skeleton + viscera + blood + others;

V. BW = head + neck + trunk + lower extremities + upper extremities.

Although each level and its multiple components are distinct, biochemical and physiological connections exist such that the five levels are consistent and function as a whole. This five level model provides an opportunity to define the concept of a steady-state of body composition in which relatively constant relationships are maintained between components at the same or different levels. The five-level model also provides a matrix for creating explicit body composition equations, and invites the development of multi-compartment methods.

Providing a comprehensive framework for body composition study, the proposed five-level model has now been widely accepted. The WHO Expert Committee stated that "Full appreciation of the utility of anthropometry requires an understanding of the organizational levels of human body composition. Recently, there have been major advances in conceptual models relating anthropometry to body composition, which provide insight into the physiological represented by anthropometry" (WHO Expert Committee, 1995).

For ***the experimental aspect***, the author and his colleagues explored a suggested body composition rule at the tissue-system level, i.e., the ratio of skeletal muscle mass to adipose tissue-free body mass (SM/ATFM) [**Chapter 3**]. Although limited data from elderly cadavers suggested a relatively constant SM/ATFM ratios in men (mean ± SD, 0.540 ± 0.046) and women (0.489 ± 0.049), there was little data in living humans that supports this hypothesis (Clarys et al., 1984; Clarys and Martin, 1985). This was because of the lack of *in vivo* methods for measuring the tissue-system level components. By using the 22 scan computerized axial tomography technique (CT) (Sjöström 1991; Chowdhury et al., 1994), the author and his colleagues were able to measure SM and ATFM and to explore the magnitude and constancy of the proportion of ATFM as SM in living human subjects. The SM/ATFM ratio (0.567 ± 0.035) in healthy young men was not significantly different from the value (0.540 ± 0.046) found in elderly male cadavers (Wang et al., 1997*b*). Multiple regression analysis indicated that the plot of SM versus ATFM had a non-zero intercept, and that the SM/ATFM ratio was significantly related to ATFM and body mass index (BMI), SM/ATFM = 0.531 + 0.0042 × BMI − 4.01/ATFM; r = 0.68, $P < 0.001$. This equation reveals that if ATFM is higher for some reason, for example in obesity, the SM/ATFM ratio would be higher. Conversely, when ATFM is lower for some reason, as for example with starvation, the SM/ATFM ratio would be lower. This suggests that SM can be altered easier than other lean tissues and organs.

Besides ATFM and BMI, other factors such as gender, race, physical activity, and importantly, disease states may also influence the relationship between SM and ATFM. Further investigation is now being done in our laboratory to explore the magnitude and constancy of the proportion of ATFM as SM in both men and women by using another accurate but safe method, multiscan magnetic resonance imaging (MRI).

Body Composition Methodology

For ***the theoretical aspect***, the author and his colleagues organized the currently applied body composition methodologies [**Chapters 4, 5**]. Although more and more body composition methods were developed over the years, there has remained a concept gap in the systematic organization of the methods. In this thesis, a classification for existing body composition methods is proposed in steps, beginning with the division of methods into *in vitro* and *in vivo* categories, advancing to organization by measurable quantity (Q, i.e., property and/or component), and ending with grouping of methods by mathematical function (f) which may be

descriptive or mechanistic (Wang et al., 1995, 1997*a*). A general formula was suggested to describe *in vivo* measurements of an unknown component C,

$$C = f(Q)$$

Further analysis on measurable quantity (Q) indicated that *in vivo* methods were organized into property-based, component-based, and combined methods. Based on analysis of mathematical functions (*f*), all of the methods were organized into descriptive (or statistically-derived) and mechanistic (or model-based) methods. All *in vivo* body composition methods can thus be classified into six categories: *descriptive* property-based, component-based, and combined methods; and *mechanistic* property-based, component-based, and combined methods.

This systematic organization of methods could facilitate communication regarding body composition methodology findings. Some methodology rules were summarized in these chapters. All *in vivo* methods are based on the measurement of body properties, so that property-based methods are the basis of component-based and combined methods.

For ***the experimental aspect***, the author and his colleagues explored the development of new methods and the evaluation of existing methods.

Method development. The simple estimation of total body oxygen was illustrated as the development of a mechanistic (model-based) method **[Chapter 6]**. Oxygen is the most abundant element in the human body, accounting for 61% of the 70 kg body weight of the Reference Man (Snyder et al., 1975). Oxygen is also a large proportions of several major molecular level components such as water (88.9%), protein (22.7%), glycogen (49.4%), and fat (10.9%) (Heymsfield et al., 1991). However, there were few investigations of the association of total body oxygen mass (TBO) with other body components. Although there are different possibilities for estimating TBO *in vivo*, the currently used methods are based on neutron activation analysis (Kehayias and Zhang, 1993; Mitra et al., 1995), which is expensive and invasive radiation is required. Based on theoretical considerations, a simple model for calculating TBO (in kg) was derived from the known relatively constant relationships among oxygen, body weight (BW, in kg), water (TBW, in kg), protein, and glycogen, TBO = 0.113 × BW + 0.845 × TBW (Wang et al., 1996*a*). The TBO calculated from the model was confirmed by TBO using *in vivo* neutron activation analysis as the reference method both in

healthy subjects and AIDS patients. Although AIDS is a wasting disease, it had no influence on the validity of the proposed model. This new method, which requires only the measurement of body weight and total body water, is simple and safe. This example shows that model derivation, combined with experimental verification, is a useful way to develop new mechanistic methods, including multi-compartment methods.

Method evaluation. With the rapid development of body composition research, additional methods were developed and some body components (e.g., skeletal muscle and fat) can be estimated by multiple methods at present. However, the difference between various methods were sometimes large for the same population. Comprehensive comparisons among currently applied methods is thus necessary. This thesis provides several examples to evaluate existing body composition methods.

Fat is a major body component on the molecular level. A large number of methods are now available for estimating total body fat mass. However, total body fat estimates by different methods sometimes show large differences (Wang J et al., 1993*a*). There thus remained a need for comprehensive inter-method comparisons of the currently used methods. In **Chapter 7**, a six-compartment molecular level model was applied, fat = body weight – (total body water + protein + bone mineral + soft tissue mineral + glycogen) (Heymsfield et al., 1991). A neutron activation analysis-based model was then derived and applied as the criterion, fat = body weight – (total body water + 6.525 × TBN + 2.709 × TBCa + 2.76 × TBK + TBNa + 1.43 × TBCl) (Wang ZM et al., 1993). Sixteen existing methods for fat estimation were compared with the reference model. These include 13 model-based and 3 statistically derived methods. Our results revealed that some multi-compartment model methods (Baumgartner et al., 1991; Heymsfield et al., 1996; Selinger, 1977; Siri, 1961) accurately measure total body fat mass with minimal technical error and maximal coefficient of reliability. These methods are applicable as reference standard in future studies. Dual energy X-ray absorptiometry (DXA) and two-compartment model methods (Benke et al., 1942; Brozek et al., 1963; Pace and Rathbun, 1945) show less agreement with the reference model, opening questions as to their specific roles in clinical evaluations and in research studies. This chapter indicates that statistically-derived methods (e.g. bioimpedance and anthropometry) and the total body potassium method show relatively poor associations with the reference model and may be of

value only for group estimates in field studies (Durnin and Womersley, 1974; Forbes et al., 1961; Segal et al., 1985; Steinkamp et al., 1965).

Another example involved the estimation of total body skeletal muscle mass (SM). Skeletal muscle is one of the largest components at the tissue-system level, and the central engine for body movement. Although several methods were available for estimating SM, some methods have never been validated by an accurate method. By using the recently developed technique of 22 scan computerized axial tomography (CT) (Sjöström 1991; Chowdhury et al., 1994), the author and his colleagues were able to measure accurately SM. Four existing methods were evaluated including the Burkinshaw-Cohn neutron activation model (Burkinshaw et al., 1978; Cohn et al., 1980), dual energy X-ray absorptiometry (DXA, Heymsfield et al., 1990), 24-hour urinary creatinine excretion (Heymsfield et al., 1983), and 24-hour urinary 3-methylhistidine methods (Lukaski et al., 1981) **[Chapters 8-10]**. Our results revealed that the Burkinshaw-Cohn model underestimated SM by about 20%. This large error was caused by the assumption of the Burkinshaw-Cohn model that the potassium-to-nitrogen ratios of SM and non-SM lean tissue are constant (Wang et al., 1996*c*). The DXA method slightly overestimated SM by about 6% and thus needs further minor refinement. This indicates that DXA, when compared to CT and neutron activation, is a promising *in vivo* approach, having markedly less radiation exposure and lower cost (Wang et al., 1996*c*).

The urinary creatinine excretion method is one of the oldest *in vivo* body composition methods (Heymsfield et al., 1983). Although several equations have been suggested to predict SM from 24-hour urinary creatinine excretion, none was evaluated by an accurate method. By using the 22 scan CT technique (Sjöström 1991; Chowdhury et al., 1994), the author of the present thesis was able to explore the relation between 24-hour urinary creatinine excretion and SM. The results suggested that under carefully controlled dietary conditions a good correlation ($r = 0.92$) existed between SM (in kg) and creatinine excretion (in g/day), $SM = 18.9 \times \text{creatinine} + 4.1$ (Wang et al., 1996*b*). The 24-hour urinary creatinine in adult men ingesting a meat-free diet can thus be used to estimate SM in locations without advanced instruments such as CT or MRI.

3-Methylhistidine (3MH) is another urinary metabolite that is related to SM. Although a SM prediction equation from 3MH was developed by Lukaski et al. (1981), it has never been evaluated. By using the 22 scan CT technique (Sjöström 1991; Chowdhury et al., 1994), the

author of the present thesis explored the association of urinary 3MH excretion (in μmol/day) with total body SM (in kg), SM = 0.0887 × 3MH + 11.8 (Wang et al., 1997*c*). Although creatinine and 3MH arise primarily from muscle, their association with SM needs further examination with respect to factors that affect their pool sizes and turnover rates. Additional study is necessary in animals and human beings to discern the significance of contributions of non-SM sources of these metabolites to daily endogenous excretion. Two additional factors limit the general use of endogenous urinary creatinine and 3MH excretion to predict total body SM mass *in vivo*: there are a need to consume a meat-free diet and an accurate timed 24-hour urine collections.

THE INTERCONNECTIONS BETWEEN BODY COMPOSITION RULES AND METHODOLOGY

As described in the general introduction of this thesis, there are three areas of body composition study that are distinct from one to another. However, the three research areas are also interconnected. It is these interconnections that combine the three areas into the whole study of human body composition (**Figure 1**). Because the present thesis mainly involves body composition rules and methodology, the interconnection between these two areas is now discussed.

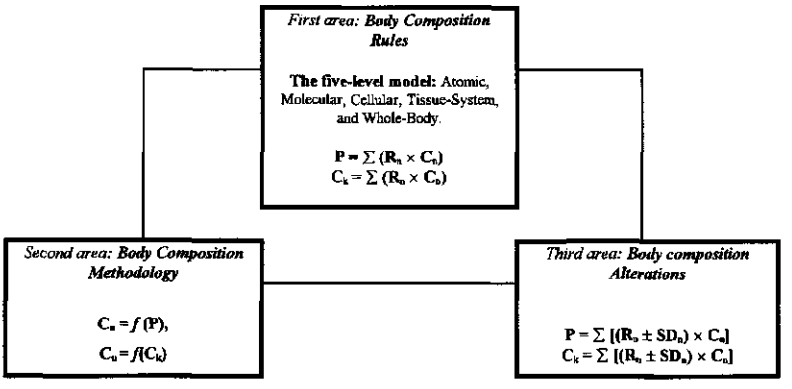

Figure 1. The study of human body composition: three research areas.

The rules-research area explores the models of human body composition, including property-component models and component-component models (**Table 1**). Property-component models and component-component models can be summarized by the following general formulas,

Property-component model, $\mathbf{P = \Sigma(R_n \times C_n)}$

Component-component model, $\mathbf{C_L = \Sigma(R_n \times C_n)}$

where P represents property; C is component at a given body composition level, C_L is component at low (or same) body composition level compared to C; R is coefficient linking C with P or C_L; and n is the component number. For known body composition models, the R value is constant or relatively constant within and between populations (**Table 1**).

These general formulas indicate that a property (P) can be expressed as a function of relevant components (C), and a low level component (C_L) can be expressed as a function of components at a high level (C). Some body composition methods are thus derived from the models. When n = 1, $\mathbf{P = R \times C}$, or $\mathbf{C_L = R \times C}$. The unknown component C can thus be calculated if P or C_L is measurable and R is known. When n = 2, $\mathbf{P = R_1 \times C_1 + R_2 \times C_2}$ or $\mathbf{C_L = R_1 \times C_1 + R_2 \times C_2}$. Two simultaneous equations are needed to resolve the two unknown components (C_1 and C_2) if R_1 and R_2 are known. This is the principle of multi-compartment model methods. Therefore, the study of body composition rules promotes the development of body composition methodology. More precisely, all mechanistic (or type II) body composition methods are based on well-established property-component or component-component models. On the other hand, development of body composition methodology provides necessary techniques for finding new body composition rules. Establishment of both property-component models and component-component models are dependent upon *in vivo* measurement of body components. In other words, new body composition rules cannot be found if the relevant components are not accurately measured. Therefore, *in vivo* methodology is the basis of the study of body composition rules. A good example is the exploration of the suggested rule of relatively constant ratio of total body skeletal muscle mass to adipose tissue-free body mass (SM/ATFM). Although this rule was found in elder cadavers, it was unknown if it is true for living young adults (Clarys et al., 1984, 1985). This is because of a lack of an accurate method for measuring SM *in vivo*. The development of multiscan CT technique in the past decade provided an opportunity to accurately measure the body components at tissue-system level, so

Table 1. Examples of body composition models

General formula	n	Model	Reference
Property-component model	1	$\mathbf{P = R \times C}$	Forbes, 1987
		γ dpm (1.46 MeV) = $1.68 \times 10^6 \times 0.0118\% \times$ TBK = $198 \times$ TBK	
$\mathbf{P = \sum (R_n \times C_n)}$	2	$\mathbf{P = R_1 \times C_1 + R_2 \times C_2}$	Behnke et al., 1942.
		BV = fat/0.900 + FFM/1.100	
	≥ 3	$\mathbf{P = R_1 \times C_1 + R_2 \times C_2 + ... + R_n \times C_n}$	
		BW = O + C + H + N + Ca + P + S + K + Na + Cl + Mg	Heymsfield et al., 1991
		BW = lipid + TBW + Pro + Mo + Ms + Gly.	Heymsfield et al., 1991
		BW = cells mass + extracellular fluid + extracellular solids	Wang et al., 1992
		BW = adipose tissue + skeletal muscle + skeleton + others	Wang et al., 1992
		BV = fat/0.900 + TBW/0.994 + Pro/1.34 + Mo/2.982 + Ms/3.317	Heymsfield et al., 1991
Component-component model	1	$\mathbf{C_L = R \times C}$	
		TBN = $0.160 \times$ Pro	Snyder et al., 1975
$\mathbf{C_L = \sum (R_n \times C_n)}$		TBCa = $0.364 \times$ Mo	Burnell et al., 1982
		TBK = $0.00266 \times$ FFM	Forbes and Lewis, 1956
		TBW = $0.732 \times$ FFM	Pace and Rathbun, 1945
		TBK = $0.00307 \times$ BCM	Moore et al., 1963
		SM = $0.540 \times$ ATFM (men); SM = $0.489 \times$ ATFM (women)	Clarys et al., 1984
	2	$\mathbf{C_L = R_1 \times C_1 + R_2 \times C_2}$	
		TBP = $0.456 \times$ TBCa + $0.555 \times$ TBK	Heymsfield et al., 1991
	≥ 3	$\mathbf{C_L = R_1 \times C_1 + R_2 \times C_2 + ... + R_n \times C_n}$	
		TBC = $0.759 \times$ fat + $0.532 \times$ Pro + $0.444 \times$ Gly + $0.180 \times$ Mo	Kehayias et al., 1991
		TBO = $0.119 \times$ fat + $0.889 \times$ TBW + $0.227 \times$ Pro + $0.400 \times$ Mo	Wang et al., 1992

ATFM, adipose tissue-free body mass (kg); BCM, body cell mass (kg); BV, body volume (liter); BW, body weight (kg); C, body component at a given level; C_L, component at low or same body composition level compared to C; FFM, fat-free body mass (kg); Gly, glycogen (kg); Mo, bone mineral (kg); Ms, soft tissue mineral (kg); n, component number; P, measurable property; Pro, protein (kg); R, coefficient; SM, skeletal muscle (kg); TBC, total body carbon (kg); TBCa, total body calcium (kg); TBK, total body potassium (kg); TBN, total body nitrogen (kg); TBP, total body phosphorus (kg); TBO, total body oxygen (kg); and TBW, total body water (kg).

that the author and his colleagues were able to explore the proportion of ATFM as SM in living young subjects [**Chapter 3**].

The following example reveals the interconnection between the rules and methodology areas of body composition study. Although *in vivo* neutron activation analysis can measure total body oxygen (TBO), it is done with radiation-exposure and high cost (Kehayias and Zhang, 1993; Mitra et al., 1995). In order to develop a new simple method of estimating body oxygen, known models with relatively constant relationships between TBO, total body water (TBW), body weight (BW), fat, protein, and glycogen are concerned in this thesis. Based on these models, a simple equation was derived, $TBO = 0.113 \times BW + 0.845 \times TBW$. This is a mechanistic (or type II) model and can be used to estimate TBO (Wang et al., 1996*a*). This example reveals that the study of rules and methodology areas promote each other.

THE STUDY OF HUMAN BODY COMPOSITION: A SPECIAL BRANCH OF HUMAN BIOLOGY

The study of human body composition is a branch of human biology. A further question could be what kind of relationship exits between body composition research and the other biology branches? This is not an accidental question. Many scientists in other branches of human biology such as biochemists and physiologists often ask: "What is the study of human body composition? What is its purpose?" And body composition researchers may be unable to distinguish clearly the study of body composition from the other branches of human biology. In this last section, the relationship between body composition research and other branches of human biology is briefly discussed.

Human biology, defined as a science of the living human organism, consists of morphology, anatomy, biochemistry, histology, physiology, body composition research, and so on. These branches investigate the characteristics of the human body from two aspects, qualitative or quantitative. Most branches of human biology explore and investigate qualitative characteristics of various body components such as their configuration, structure, metabolism, function, and modulation. In other words, each of these branches studies the qualitative characteristics of human components at a given body composition level. For instance,

— Biochemistry studies at the molecular level and focuses on *in vivo* chemical changes of components such as fat, water, protein, mineral, and glycogen.

— Cytology, cytochemistry, and cytophysiology study at the cellular level; and these branches focus on the structure, metabolism, function, and reproduction of the various cells such as adipocytes, neurons, and skeletal muscle cells.

— Histology and anatomy study at the tissue-system level; and these two branches mainly deal with the structure and distribution of the various tissues and organs, such as muscle, skeleton, and adipose tissue.

— Physiology studies at the tissue-system level; and it focuses on the function of various organs and systems, and their regulation.

— Morphology studies at the whole-body level, and it focuses on the configuration characteristics of the human body as a whole.

Despite massive accumulation of information, these branches of human biology do not or only briefly mention the quantitative characteristics of the human body. Specifically,

— How many components exist in the human body? Are there any relatively stable quantitative relationships between these components in the healthy human body?

— How can the body components be safely and accurately assessed in the living human body?

— What is the effect of various *ab inter* and *ab extra* factors upon the quantitative relationships between body components?

The answer to these questions is the objective of the study of human body composition, a special branch of human biology, which focuses on the quantitative characteristics of human body, and is involved with all five of the levels.

In the theoretical aspects, body composition research thus fills a big knowledge gap in other branches of human biology. As shown in the Body Composition Study Chronological Table (see Appendix), more and more information and knowledge have been accumulated since 1850'. All of these achievements are involved with understanding the quantitative characteristics of the human body, and can be organized into three research areas, rules, methodology, and alteration of body composition.

In practical aspects, body composition research can be very useful for two reasons. First, alteration in body composition may mediate morbidity and mortality, and body composition study can play an important role in characterizing the nature and degree of some diseases such as obesity and undernutrition. For example, although marathon runners and patients with anorexia nervosa may have similar body weight and body mass index, they are clearly

distinguishable groups by markedly great skeletal muscle mass of the marathon runners. Second, by assessing body composition changes with treatment, the clinician is better able to monitor treatment safety and progress.

CONCLUSION

In this thesis, recently developed methods such as multiscan CT, multi-component methods, and *in vivo* neutron activation analysis are used as the references to evaluate existing methods for estimating total body skeletal muscle, fat, and oxygen masses. However, the main contribution of this thesis is to establish a comprehensive framework for the study of human body composition. Some conclusions are drawn as follows,

— As a branch of human biology, the study of human body composition focuses on the quantitative relationships between body components. The study of human body composition is composed of three distinct but interconnected research areas, body composition rules, body composition methodology, and body composition alterations.

— The ~40 human body components are distributed at five levels of increasing complexity: atomic, molecular, cellular, tissue-system, and whole-body. Body composition rules, i.e., constant or relatively constant quantitative relationships, exist including property-component and component-component models.

— All *in vivo* methods can be systematically organized. There are six categories of *in vivo* body composition methods, descriptive and mechanistic property-based methods, descriptive and mechanistic component-based methods, and descriptive and mechanistic combined methods.

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APPENDIX

BODY COMPOSITION STUDY CHRONOLOGICAL TABLE

There is no limit to knowledge.

—— Confucius

1850' Justus von Liebig (1803 - 1873) found that the human body contained many substances present in food and that body fluids contained more sodium and less potassium than tissues.

1857 A. von Bezold discovered that animal growth was accompanied by a decrease in water and an increase in ash content.

1859 Moleschott first reported values for the amounts of protein, fat, extractives, salts and water per 1,000 parts of the human body.
J. B. Lawes and J. H. Gilbert documented that animal body water varied inversely with fat content.

1863 E. Bischoff analyzed adult human cadavers for water content.

1871 L. A. J. Quetelet first observed that among adults weight seemed to increase in proportion to the square of stature and established Quetelet's Index that was renamed as Body Mass Index by A. Keys et al. (1972).

1876 H. Fehling analyzed fetuses and newborns for water content.

1887 L. Pfeiffer noted that the variation in water content of animal bodies can be reduced if the data were expressed on a fat-free basis.

1896 J. Katz reported detailed chemical analysis of muscle.

1900 W. Camerer and Söldner estimated the chemical composition of fetuses including water, fat, nitrogen, and major minerals.

1901 E. Voit (1901) and M. Rubner (1902) announced the concept of "active protoplasmic mass" related with certain physiological functions.

1905 O. Folin hypothesized that urinary creatinine was a qualitative indicator of body composition.

1906 A. Magnus-Levy announced for the first time the concept of "fat-free body mass".

1907 E. P. Cathcart found that nitrogen was lost from the body during fasting.

1909 P. A. Shaffer and W. Coleman used urinary creatinine excretion as an index of muscle mass.

1914 E. Benjamin reported that infants retained nitrogen as they grew.

1915 N. M. Keith, L. G. Rowntree, and J. T. Geraghty first determined plasma and blood volume by dilution of dies (Vital Red and Congo Red).

1916 D. Du Bois and E. F. Du Bois proposed the height-weight equation to estimate whole body surface area.

1921 J. Mateiga derived an anthropometric model to estimate total body muscle mass.

1923 C. R. Moulton advanced the concept of "chemical maturity of body composition".
W. McK Marriott advanced the concept of "anhydremia" and initiated the modern study of body fluids.

1934 G. von Hevesy and E. Hofer used deuterium to estimate total body water volume.

1938 N. B. Talbot estimated that 1 g of creatinine excreted during a 24 hour period was derived from approximately 18 kg of muscle mass.
V. Iob and W. W. Swanson reported chemical composition in fetus and newborn.

1940 H. C. Stuart, P. Hill, and C. Shaw first used two-dimensional standard radiography to estimate adipose tissue and skeletal muscle shadows.

1942 A. R. Behnke, Jr., B. G. Feen, and W. C. Welham estimated the relative proportion of lean and fat in the human body based on Archimedes' principle.

1945 N. Pace and E. N. Rathbun found the relatively constant ratio of total body water to fat-free body mass and suggested a method for estimating body fat from total body water.
H. H. Mitchell, T. S. Hamilton, F. R. Steggerda, and H. W. Bean first reported whole body composition analysis on the molecular level (water, fat, protein, ash, Ca, and P) for an adult human cadaver.

1946 F. D. Moore introduced the concept of total body exchangeable sodium and potassium.

1951 R. A. McCance and E. M. Widdowson determined extracellular fluid volume by using dilution techniques with thiocyanate.
E. M. Widdowson, R. A. McCance, and C. M. Spray first reported whole body composition analysis on the atomic level (Ca, P, K, Na, Mg, Fe, Cu, and Zn) for adult human cadavers.

1953 A. Keys and J. Brozek provided a detailed analysis of the densitometric technique.
F. Fidanza, A. Keys, and J. T. Anderson reported remarkable uniformity in the density (0.90 g/cm^3) of human fat across individuals.

1955 N. Lifson, G. B. Gordon, and R. McClintock measured total body water and total body carbon dioxide production by using $D_2{}^{18}O$ dilution method.

1958 I. S. Edelman, J. Leibman, M. P. O'Meara, and L. W. Birkenfeld first observed that in normal extracellular fluid the total cation forms a linear relationship with total osmotic solute.
R. Kulwich, L. Feinstein, and E. C. Anderson (1958) and E. C. Anderson and W. Langham (1959) reported the existence of a correlation between natural ^{40}K concentration and fat-free body mass.

1960 J. M. Foy and H. Schneider determined total body water by using tritium dilution method.

1961 W. E. Siri developed three-compartment model to estimate total body fat mass.
G. B. Forbes, J. Hursh, and J. Gallup (1961) estimated fat and lean contents by using whole body ^{40}K counting.

1962 A. Thomasset first introduced bioimpedance analysis (BIA) method.

1963 First Body Composition Symposium of the Society for Study of Human Biology was held by New York Academy of Sciences.
J. F. Brozek, F. Grande, J. T. Anderson, and A. Keys developed two-compartment model to estimate total body fat mass.
F. D. Moore et al. suggested the concept of body cell mass (BCM).
J. R. Cameron and J. A. Sorenson developed single photon absorptiometry (SPA) method.

1964 J. Anderson et al. (1964) and S. H. Cohn and C. S. Dombrowski (1971) developed *in vivo* neutron activation method for body composition analysis.

1966 J. Rundo and L. J. Bunce established the first *in vivo* prompt-γ neutron activation facility for measuring body hydrogen mass.

1970 R. B. Mazess, J. R. Cameron, and J. A. Sorenson developed dual photon absorptiometry (DPA) method.

1973 W. Harker first introduced total body electrical conductivity (TOBEC) method.

1975 W. S. Snyder et al. introduced the Reference Man concept.

1979 S. B. Heymsfield, R. P. Olafson, M. H. Kutner, and D. W. Nixon first used computed axial tomography (CT) for body composition analysis.

1981 H. C. Lukaski, J. Mendez, E. R. Buskirk, and S. H. Cohn developed urinary 3-methylhistidine method to estimate total body skeletal muscle mass.

1983 CT is used in whole body composition analysis (G. A. Borkan et al., 1983; K. Tokunaga et al., 1983; and L. Sjöström et al., 1986).

1984 Brussels cadaver study (12 men and 13 women) on tissue-system level was published by J. P. Clarys, A. D. Martin, and D. T. Drinkwater.
M. A. Foster, J. M. S. Hutchison, J. R. Mallard, and M. Fuller were among the first to demonstrate that magnetic resonance imaging (MRI) could accurately measure body composition.

1986 The First International Symposium on *In Vivo* Body Composition Studies was held in New York.

1987 J. J. Kehayias, K. J. Ellis, S. H. Cohn, and J. H. Weinlein established the first inelastic scattering facility for estimating total body carbon and oxygen.

1990 S. B. Heymsfield SB et al. estimated appendicular skeletal muscle by dual energy X-ray absorptiometry (DXA).
J. J. Kehayias et al. developed a method for assessing total body fat mass from total body carbon mass by *in vivo* neutron activation analysis.
K. J. Ellis introduced the Reference Women concept.

1992 ZM. Wang, R. N. Pierson, Jr., and S. B. Heymsfield proposed the five-level model of human body composition.

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SUMMARY

The study of human body composition is a branch of human biology which focuses on the *in vivo* quantification of body components, the quantitative relationships between components, and the quantitative changes in these components related to various influencing factors. Accordingly, the study of human body composition is composed of three interconnecting research areas. The present thesis reflects the author's effort during the recent years at both theoretical and experimental aspects within the areas of body composition rules and methodology.

Theoretical Investigations

It is said that science without organization and taxonomy is bland. The major contributions of this thesis was to suggest a comprehensive five-level body composition model and a systematic classification of body composition methodology.

The Five-Level Body Composition Model In the research area of body composition rules, a five-level body composition model was proposed which grows from the need to organize the rapidly accumulated knowledge and information that relate to the study of human body composition [**Chapter 2**]. The ~ 40 body components were systematically organized into five body composition levels of increasing complexity: I. atomic; II. molecular; III. cellular; IV. tissue-system; and V. whole-body. Although each level and its multiple components are distinct, interconnections exist such that the model is consistent and functions as a whole. This model provides the opportunity to clearly define the concept of a body composition steady-state in which quantitative associations exist over a specified time interval between components at the same or different levels. This model is intended to be a foundation on which future studies can refine or expand selected definitions or equations. The five-level model serves in this organizational capacity and also stimulates a broader view of body composition research as a whole.

The Systematic Organization of Body Composition Methodology In the research area of body composition methodology, a systematic classification of body composition methods was suggested [**Chapters 4, 5**]. Methods used to quantify body components were first divided into two groups, *in vitro* and *in vivo*. A basic formula, $\mathbf{C} = f(\mathbf{Q})$, can be used to define two parts that are characteristic of all *in vivo* body composition methods. The first part is the measurable

quantity (Q), and there are two main categories, property and component. The second part of the basic formula is a mathematical function (*f*), and two types, referred to as statistically-derived (i.e., descriptive) and model-based (i.e., mechanistic) are found. All body composition methods can be organized according to this system. This organization should allow investigators to systematically organize and communicate their research findings within body composition methodology. The approach should also allow students to base their learning of body composition methodology on concepts rather than on simple listing devoid of an underlying theme.

Experimental Investigations

Based on the comprehensive overview of the theoretical consideration, several experimental studies were collected in this thesis within the research areas of body composition rules and methodology.

Proportion of Adipose Tissue-Free Body Mass as Skeletal Muscle In the research area of body composition rules, a suggested model, the proportion of adipose tissue-free body mass (ATFM) as skeletal muscle (SM), was studied in healthy men **[Chapter 3]**. Until recently autopsy was the only method available to accurately measure the quantitative relationships between SM and other tissue-system level components. Based on multiscan computerized axial tomography (CT) technique, this thesis explored the magnitude and constancy of the SM/ATFM ratio in 24 healthy men. It was found that the proportion of ATFM as SM was 0.567 ± 0.035 (mean $\pm$ SD) which was not significantly different from the value found in 6 elderly male cadavers (0.540 ± 0.046). Multiple regression analysis indicated that the SM/ATFM ratio was significantly related to both ATFM ($P < 0.028$) and body mass index ($P < 0.04$). However, the SM/ATFM ratio was not significantly related to age and did not differ between young and elderly men.

Measurement of Total Body Oxygen Mass In the research area of body composition methodology, a simple and safe method was developed for estimating total body oxygen mass (TBO) **[Chapter 6]**. The main purpose of this study was to illustrate the development of model-based body composition methods. Although oxygen is the most abundant element of the human body, there are very few reports concerning TBO measurement. All available methods require *in vivo* neutron activation analysis facilities which are very expensive and expose subjects to radiation. Based on the known elemental stoichiometries and proportions of

relevant body components at the molecular level, a TBO (in kg) prediction equation was derived from body weight (BW, in kg) and total body water mass (TBW, in kg): TBO = 0.113 × BW + 0.845 × TBW. The difference between BW and the sum of the 8 elements (C, H, N, Ca, P, K, Na, and Cl) measured by *in vivo* neutron activation analysis and whole-body ^{40}K counting was used to estimate TBO and thereby to evaluate the proposed TBO model. The results confirmed the adequacy of the proposed model for both healthy subjects and AIDS patients which provides a simple, low cost, and safe method of assessing TBO *in vivo*.

Inter-Method Comparison of Fat Estimation Methods Fat is a major body component at the molecular level of body composition. Although numerous methods are available, total body fat mass estimated by various methods differ from each other, and there remained at a comprehensive inter-method comparisons of the currently used methods. For the purpose, a six-compartment body composition model was proposed based on *in vivo* neutron activation analysis, and was used as the criterion [**Chapter 7**]. Thirteen model-based methods and three statistically-derived methods were then compared to the criterion model. The results indicated that some multi-compartment model methods (e.g., Siri, Selinger, Baumgartner, and Heymsfield) are very close to the six-compartment model and can thus provide accurate total body fat measurement. DXA and some two-compartment model methods (e.g., Pace, Brozek, and Behnke) are suitable for healthy subjects. More studies are needed to find if DXA method can be applied in different disease status. Two-compartment TBK model, anthropometric, and bioimpedance methods are not accurate estimation of total body fat mass, although they are often used in the field studies.

Evaluation of Burkinshaw-Cohn Model and DXA Methods of Estimating Total Body Skeletal Muscle Mass Although skeletal muscle is a major body component of the tissue-system level, whole body measurement methods remain limited and inadequately investigated. One aim of this thesis was to evaluate the Burkinshaw-Cohn *in vivo* neutron activation analysis-whole body ^{40}K counting model and dual-energy X-ray absorptiometry (DXA) method of estimating total body SM by comparison to adipose tissue-free SM measured using multiscan computerized axial tomography (CT) [**Chapter 8**]. In the Burkinshaw-Cohn model the potassium-to-nitrogen ratios of SM and non-SM lean tissue are assumed constant; in the DXA method the ratio of appendicular SM to total SM is assumed constant at 0.75. Compared with measurement of SM by CT, the Burkinshaw-Cohn model substantially

underestimated and the DXA method slightly overestimated SM. Because of substantially less radiation exposure, low cost, and availability at many research and clinical centers, this study suggested that the DXA method may be a practical alternative to CT or MRI as a means of quantifying SM *in vivo*.

Evaluation of 24-Hour Urinary Creatinine Excretion Method of Estimating Total Body Skeletal Muscle Mass The 24-h urinary creatinine excretion is one of the oldest *in vivo* methods for estimating SM. The value of the creatinine-SM method is that urinary creatinine is simple to quantify in locations without advanced instruments such as CT or MRI. In this thesis the relationship between 24-h urinary creatinine excretion and SM was explored **[Chapter 9]**. The results suggested that under carefully controlled dietary conditions a good correlation ($r = 0.92$) exists between SM measured by multiscan CT and daily urinary creatinine excretion in adult men; and that urinary creatinine (in g/day) collected in adult men ingesting a meat-free diet can be used to estimate SM (in kg) as $SM = 21.8 \times$ creatinine or $SM = 18.9 \times$ creatinine + 4.1. Finally, this study identified the need to evaluate large and more diverse subject populations and to examine basic questions related to creatine and creatinine metabolism.

Association of 24-Hour Urinary 3-Methylhistidine Excretion with Total Body Skeletal Muscle Mass Besides creatinine, 3-methylhistidine (3MH) is another urinary metabolite that related to SM. The association of 24-h urinary 3MH excretion and SM was explored in the thesis **[Chapter 10]**. The results suggested that under carefully controlled dietary conditions a good correlation exists between 3MH and SM measured by multiscan CT in adult men; and that 24-h urinary 3MH (μmole/day) collected in adult men ingesting a meat-free diet can be used to estimate SM (in kg) as $SM = 0.0887 \times 3MH + 11.8$. Further investigations are needed which include more subjects and concern the influences of multiple factors (such as age, gender, race, and exercise level) on the 3MH-SM relations.

SAMENVATTING

De studie van de lichaamssamenstelling van de mens is een onderdeel van de humane biologie die zich vooral richt op de kwantitatieve bepaling van lichaamscomponenten '*in vivo*', de kwalitative relaties tussen de componenten en de kwantitatieve veranderingen in deze componenten in relatie met verschillende factoren die hierop van invloed zijn. Dientengevolge kunnen bij de bestudering van de lichaamssamenstelling drie onderling gerelateerde onderzoeksgebieden onderscheiden worden. Dit proefschrift beschrijft het theoretisch en praktisch werk van de auteur gedurende de laatste jaren op het gebied van wetmatigheden en methodologie bij lichaamssamenstellingsonderzoek.

Theoretische Aspecten

Er wordt weleens gesteld dat wetenschap zonder systematiek en taxonomie blind is. Een belangrijke bijdrage in dit proefschrift is de introductie van een allesomvattend model van lichaamssamenstelling op 5 niveaus ('five level body composition model') en een systematische rangschikking van methoden van lichaamssamenstelling.

Het 'Five Level Body Composition Model' Als gevolg van de behoefte aan een classificeringssyteem door een steeds groeiende kennis en informatie op het gebied van lichaamssamenstelling is een model op 5 abstractieniveaus ontwikkeld **[Hoofdstuk 2]**. De ongeveer 40 lichaamscomponenten zijn systematisch gerangschikt in 5 niveaus van toenemende complexiteit: I. atomair; II. moleculair; III. cellulair; IV. weefselniveau; en V. totaal lichaamsniveau. Ofschoon elk niveau en zijn componenten uniek zijn, bestaan er onderlinge samenhangen en is het model in zijn geheel consistent en functioneel. Het model heeft de mogelijkheid een 'stationaire toestand' van lichaamssamenstelling te beschrijven waarin duidelijk kwantitatieve verbanden bestaan binnen een bepaald tijdsinterval tussen componenten op hetzelfde dan wel op een ander abstractieniveau. Het model is bedoeld als basis waarop toekomstige studies kunnen terugvallen voor definities of vergelijkingen. Het model voldoet voor dit doel en kan ook een verbreding van de horizon op het gebied van lichaamssamenstellingsonderzoek stimuleren.

De systematische rangschikking van lichaamssamenstellingmethodologie Op het gebied van lichaamssamenstellingsmethodologie is een systematische rangschikking van methoden voorgesteld **[Hoofdstuk 4, 5]**. Methoden die gebruikt worden voor kwantitatieve bepaling van

lichaamscomponenten zijn eerst verdeeld in twee groepen, '*in vitro*' en '*in vivo*' methoden. Een algemene formule, $\mathbf{C} = f(\mathbf{Q})$, kan gebruikt worden om twee charateristieken voor alle '*in vivo*' methoden te definiëren. Het eerste is de meetbare hoeveelheid (Q) en er zijn twee hoofdcategoriëen te onderscheiden; lichaamseigenschap en lichaamscomponent. Het tweede deel van de formule is de mathematische functie (*f*) en er kunnen weer twee types worden onderscheiden, een statistische (descriptieve) functie en een functie die gebaseeerd is op een model (een mechanistische functie). Alle lichaamssamenstellingsmethodieken kunnen binnen dit systeem gerangschikt worden. Deze classificering geeft onderzoekers de mogelijkheid hun gegevens systematisch weer te geven. Voor studenten heeft de classificering het voordeel dat methoden van lichaamssamenstelling bestudeerd kunnen worden op basis van een concept in plaats van een willekeurige opsomming van methodieken.

Experimenteel onderzoek Gebaseerd op bovenstaande theoretische beschouwingen zijn verschillende experimentele studies uitgevoerd.

Spiermassa als proportie van vetweefsel-vrij gewicht Op het gebied van lichaamssamenstellingswetmatigheden is het model 'spiermassa (SM) als proportie van vetweefsel-vrij gewicht' (ATFM) onderzocht in een groep gezonde mannen [**Hoofdstuk 3**]. Tot voor kort was autopsie de enige mogelijkheid om nauwkeurig de kwantitatieve relatie tussen SM en andere weefselcomponenten te bestuderen. Gebruik makend van axiale tomografie (CT) technieken werd een constante SM/ATFM ratio in gezonde mannen gevonden van 0.567 ± 0.035 (gemiddelde ± SD). Deze waarde verschilde niet van de waardes die gevonden zijn na autopsie in 6 overleden oudere mannen (0.540 ± 0.046). Multipele regressie wees uit dat de SM/ATFM ratio significant gerelateerd was aan zowel ATFM ($P < 0.028$) als de body mass index ($P < 0.04$). De SM/ATFM ratio correleerde echter niet met leeftijd en verschilde niet tussen oudere en jongere mannen.

Bepaling van totaal lichaamszuurstof Op het gebied van lichaamssamenstellings-methodologie is een eenvoudige en veilige methode ontwikkeld voor de schatting van de totale hoeveelheid lichaamszuurstof [TBO, **Hoofdstuk 6**]. Het hooddoel van deze studie was de praktische waarde van een, op een model gebaseerde lichaamssamenstellingsmethode te demonstreren.

Ofschoon zuurstof het meest voorkomende chemisch element in het lichaam is, zijn er maar enkele studies naar gedaan. Alle beschikbare methoden maken gebruik van *in vivo neutronen*

activatie, een methode die erg kostbaar is en inherent is aan stralingsbelasting. Gebaseerd op bekende stöchiometrische verhoudingen van relevante lichaamssamenstellingscomponenten op moleculair niveau is een voorspellingsformule voor TBO (kg) ontwikkeld, uitgaande van gewicht (BW, kg) en totaal lichaamswater (TBW, kg): $TBO = 0.113 \times BW + 0.845 \times TBW$. Het verschil tussen lichaamsgewicht en de som van 8 elementen (C, H, N, Ca, P, K, Na, en Cl), gemeten met behulp van *in vivo neutronen activatie* is gebruikt om TBO te schatten en het model te evalueren. De resultaten van de studie bevestigen de betrouwbaarheid van het model in zowel gezonde vrijwilligers als ook in patiënten met AIDS. Het model is een eenvoudige, goedkope en veilige methode voor de schatting van TBO.

Methodenvergelijking voor het schatten van lichaamsvet Vet is een belangrijke component op het moleculair niveau van lichaamssamenstelling. Ofschoon er talrijke schattingsmethoden beschikbaar zijn, verschillen de resultaten al naar gelang de gebruikte methode en een uitputtende methodenvergelijking ontbreekt. Dertien methoden gebaseerd op een lichaamssamenstellingsmodel en drie methoden gebaseerd op een statistische relatie werden vergeleken met een 6-compartimenten model gebaseerd op *in vivo neutronen activatie* [**Hoofdstuk 7**]. Uit de resultaten van deze studie blijkt dat sommige meer-compartimenten modellen (bv Siri, Selinger, Baumgartner, Heymsfield) nagenoeg dezelfde resultaten geven als de referentie methode en dus een nauwkeurige schatting geven van lichaamsvet. DXA en sommige twee-compartimenten modellen (bv Pace, Brozek, Behnke) zijn geschikt voor gezonde personen. Meer studies zijn nodig om de geschiktheid van DXA als schattingsmethode voor vet bij verschillende ziektes te bestuderen. Het twee-compartimenten model gebaseerd op totaal lichaamskaliumbepaling, anthropometrie en de bio-impedantiemethode geven geen nauwkeurige schatting van de totale vetmassa ofschoon zij vaak gebruikt worden in veldstudies.

Evaluatie van het Burkinshaw-Cohn model en DXA methoden voor de schatting van de totale lichaamsspiermassa Ofschoon spiermassa een belangrijke component is van de lichaamssamenstelling op weefselniveau, zijn metingen ervan op totaal lichaamsniveau beperkt en onvoldoende onderzocht. Het doel van deze studie [**Hoofdstuk 8**] was het Burkinshaw-Cohn model (gebaseerd op *in vivo neutronen activatie* en bepaling van totaal lichaamskalium), en *dual energy X-ray absorptiometry* (DXA) methode voor de schatting van spiermassa te evalueren door het te vergelijken met vetweefselvrije spiermassa bepaald door middel van

multiscan computer tomografie (CT). In het Burkinshaw-Cohn model wordt de ratio kalium:stikstof van spiermassa en spiermassavrij vetvrij weefsel constant verondersteld; bij de DXA methode wordt de ratio appendiculaire spiermassa:total spiermassa constant verondersteld op 0.75. Vergeleken met de spiermassa gemeten met behulp van CT onderschat het het Burkinshaw-Cohn model de spiermassa substantieel en de DXA methode overschat de spiermassa. Vanwege de belangrijk lagere stralingsbelasting, lagere kosten en betere beschikbaarheid in veel klinische en research centra wordt uit deze studie geconcludeerd dat de DXA methode een praktisch alternatief is voor de bepaling van spiermassa in vergelijking met CT of MRI.

24 Uurs creatinine uitscheiding als schatter van de spiermassa

De bepaling van de hoeveelheid creatinine in 24 uurs urine is een van de oudste *in vivo* schattingsmethoden voor spiermassa. De waarde van de methode is vooral gelegen in het feit dat urinair creatinine gemakkelijk te bepalen is zonder geavanceerde apparatuur. In **Hoofdstuk 9** is de relatie tussen 24 uurs creatinine uitscheiding en spiermassa beschreven. De resultaten laten zien dat onder goed gecontroleerde omstandigheden (na consumptie van een vleesvrije voeding) de relatie tussen spiermassa gemeten met multiscan CT en 24 uurs urinair creatinine in een groep volwassen mannen hoog is ($r = 0.92$) en dat urinair creatinine (in gram/dag) kan worden gebruikt om de spiermassa te schatten: SM (kg) = 21.8 × creatinine, of: SM (kg) = 18.9 × creatinine + 4.1. Uit deze studie bleek verder dat het wenselijk is de relatie tussen creatinine uitscheiding en spiermassa in grotere en meer heterogene groepen te onderzoeken en dat er behoefte is aan onderzoek naar creatine en creatinine metabolisme.

Relatie tussen 24 uurs 3-methyl-histidine uitscheiding en spiermassa Behalve creatinine is ook urinair 3-methyl-histidine gerelateerd aan spiermassa en is in een studie beschreven in deze thesis nader onderzocht [**Hoofdstuk 10**]. De resultaten suggereren dat onder zorgvuldig gecontroleerde omstandigheden de correlatie tussen 24 uurs urinair 3-methyl-histidine (umol/dag) en spiermassa gemeten met behulp van CT goed is en dat de spiermassa berekend kan worden uit: SM (kg) = 0.0887 × 3MH + 11.8. Verder onderzoek met meerdere proefpersonen is nodig om de relatie in meer detail te onderzoeken en de invloed van factoren als leeftijd, geslacht, ethniciteit en mate van lichamelijke activiteit te bestuderen.

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ABOUT THE AUTHOR

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From 1992 until 1997, he carried out a series of studies described in this thesis with experimental work in New York and final phases work in the Department of Human Nutrition and Epidemiology, Wageningen Agricultural University, The Netherlands.

He is an active member of several professional societies including American Institute of Nutrition, American Society for Clinical Nutrition, and New York Academy of Sciences. He is also an active member of Chinese Biochemical Society, Chinese Physiological Society, and China Sports Science Society.

He married Hong-Bing Yao; and they have two daughters, Ying and Wei.

He who has once in his life experienced this joy
of scientific creation will never forget it.

—— Prince Kropotkin

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