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Multi-component body composition models: recent advances and future directions

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Objective: This overview examines concepts related to a category of body composition methods generally referred to as multi-component models, that is, those models that include three or more components. We summarize the rationale for, applications, and types of multi-component models along with sources of error. Our review presents the strengths and limitations of available models and identifies important future research directions.
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Introduction

An important aspect of the contemporary study of nutritional diseases is establishing the phenotypic characteristics of human subjects. These phenotypes are then linked with underlying genetic mechanisms. The process of simultaneously studying human phenotypes and genotypes has given rise to the increasingly important area of characterizing human body composition.

Early workers had relatively simple means of measuring the various body compartments, particularly total body fat. The so-called 'two-compartment model' served this process very well and was based on the concept that human body mass consists of two major components, fat and fat-free mass (Siri, 1961; Brozek *et al*, 1963). In order to divide body mass into these two components a number of assumptions were usually required. For example, the water content, the potassium content, and the density of fat-free mass were assumed stable and constant in all adult human subjects (Behnke *et al*, 1942; Siri, 1961). This assumption allowed development of various two-compartment models as water, potassium, and the density of fat-free mass were all measurable *in vivo* (Siri, 1961; Brozek *et al*, 1963). The two-compartment model using these various assumed constants served the field of clinical nutrition for over four decades.

Recently however, interest in developing more refined and accurate phenotypes has led to intense scrutiny of the two-compartment model. In particular, it is now clear that the various assumptions involved in the two-compartment model are not appropriate when examining subjects across wide age ranges and particularly between groups that differ in gender and ethnicity (Lohman, 1986; Mazariegos *et al*, 1994). This has led to the search for improved methods of phenotyping human subjects that are based on assumptions that are not violated by age, gender, and ethnic effects. From these endeavors has emerged the concept of multi-component models, the subject of the present report.

This overview describes the various families of multi-component models, that is, those methods of fractionating body mass that involve more than two body composition components. Additional details regarding these methods are presented in several earlier publications (Heymsfield *et al*, 1990,1991a; Wang *et al*, 1995,1999). In the present report we consolidate the ideas of these earlier studies and provide the reader with an overview of the various available multi-component methods.

The major body composition components at the atomic, molecular, cellular, and tissue-system levels of body composition are presented in Figure 1 (Wang *et al*, 1992). Each level can be formulated mathematically as a level equation. The equations for these four levels are summarized in Table 1.

Thus, each level of body composition can be characterized by a simple algebraic equation. These level models are the 4 fundamental equations that serve as the basis of formulating multi-component models.

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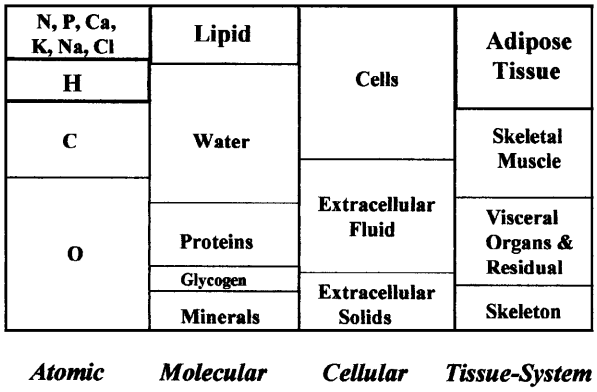

Figure 1 The first four body composition levels and their respective components. Adapted from Wang *et al* (1992), with permission.

Table 1 Body composition level equations

Level	*Equation*
Atomic	A1. BW = O + C + H + N + Ca + P + K + S + Na + Cl + Mg
Molecular	M1. BW = F + A + Pro + Ms + Mo + G
	M2. BW = F + A + Pro + M
	M3. BW = F + A + solids
	M4. BW = F + Mo + lean soft tissue
	M5. BW = F + FFM
Cellular	C1. BW = CM + ECF + ECS
	C2. BW = F + BCM + ECF + ECS
Tissue system	TS1. BW = AT + SM + bone + other tissues

Abbreviations: A, water; AT, adipose tissue; BCM, body cell mass; BW, body weight; 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. Modified from Heymsfield *et al* (1996) with permission.

Models

Atomic level

The atomic level is characterized by 11 main elements that comprise over 99% of body mass: oxygen, carbon, hydrogen, nitrogen, calcium, phosphorus, potassium, sulfur, sodium, chlorine, and magnesium (Table 1, A1) (Wang *et al*, 1992). All of these elements are now measurable *in vivo* by a variety of techniques, notably neutron activation analysis combined with whole body counting (Pierson *et al*, 1990; Heymsfield *et al*, 1991b; Kehayias *et al*, 1991). It is thus possible to completely reconstruct the fundamental molecular level model in human subjects.

While measurement of elemental content *in vivo* is important in fields such as radiation physics, there is little value to quantifying the amounts of these elements in the field of clinical nutrition. Thus while it is possible to fully characterize the elemental model in human subjects, the amounts of these components present in a human subject provides only minimally useful clinical information. More importantly, elements are incorporated into molecular components and these established relationships form the important basis of multi-component molecular level models.

Molecular level

The molecular level is the most studied level in the field of body composition research. Numerous biological processes can be related to molecular level components and hence the widespread research interest in quantifying the major components. The classical two-component model consisting of fat and fat-free mass (Table 1, M5) is a molecular level model. Concern for the validity of the two component model in subjects who vary in age and ethnicity led to the development of three families of molecular multi-component models:

Neutron activation analysis-whole body counting 4-component model. The molecular level is one level up from the atomic level of body composition. Accordingly, close links exist between major atomic level elements and molecular level components. Nitrogen, calcium, carbon, and oxygen, are the main elemental components of protein, bone mineral, fat, and water, respectively (Wang *et al*, 1995). In order to estimate the amount of each molecular component present, the investigator must mathematically link the elements to their respective molecular components by means of simultaneous equations. These equations include terms that are assumed 'constants' consisting of stable chemical relationships (eg, carbon/fat = 0.77) (Figure 2). Since all of the major elements can be measured *in vivo* using neutron activation-whole body counting methods, it is possible to solve these equations in order to quantify each molecular level component. This ability to quantify the

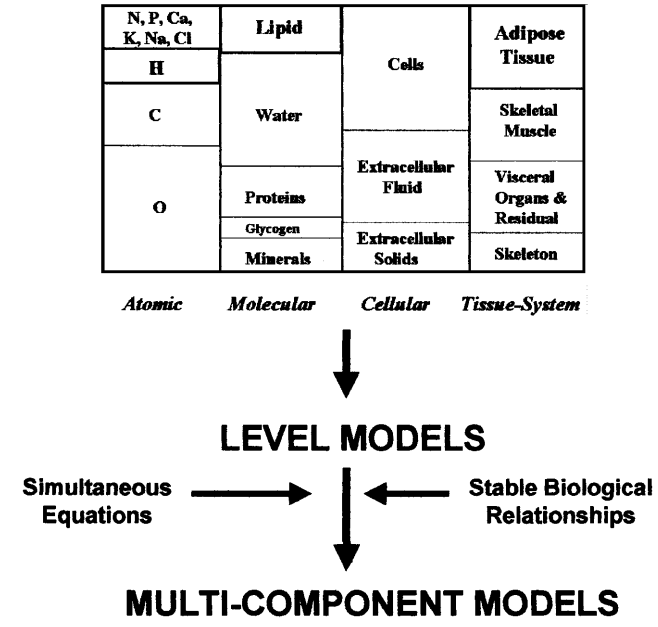

Figure 2 The general approach to preparing multi-component models.

major elements and their close linkage to molecular level species has given rise to the family of molecular level models based on neutron activation analysis combined with whole body counting. The earliest models consisted of four components (Table 1, M2) (Cohn *et al*, 1984) and newer models are recognized with up to six components (Table 1, M1) (Heymsfield *et al*, 1991b).

As each center has different neutron activation and whole body counting methods, there exist many different approaches to preparing neutron activation whole body counting multi-component models. In effect, the models used are typically unique to each center. A good example is the multi-component model base on neutron activation analysis and whole body counting applied at Brookhaven National Laboratory in New York (Pierson *et al*, 1990; Heymsfield *et al*, 1991b; Kehayias *et al*, 1991). The first component in this model, total body water, is quantified using either tritiated water or deuterium dilution. Total body nitrogen is next measured with the technique referred to as prompt gamma neutron activation analysis (Beddoe *et al*, 1984; Dutton, 1991; Kyere *et al*, 1982; Mernagh *et al*, 1977; Pierson *et al*, 1990; Varstsky *et al*, 1984). Total body nitrogen is then used to calculate total body protein mass. Total body calcium, sodium, and chlorine are next measured using delayed gamma neutron activation analysis (Dutton, 1991; Pierson *et al*, 1990). Total body calcium is then used to calculate bone mineral mass. As part of the delayed gamma neutron activation analysis method, whole body counting is carried out before and after activation. The whole body counter is also used to quantify ^{40}K and thus total body potassium. Total body sodium, chlorine, and potassium together are then used to calculate soft tissue mineral mass. Finally, total body carbon is measured using inelastic neutron scattering (Dutton, 1991; Kehayias *et al*, 1987; Pierson *et al*, 1990). Total body carbon, total body calcium, and total body nitrogen are used in solving simultaneous equations for total body fat (Heymsfield *et al*, 1991b). The Brookhaven approach, which takes about one day for all of the evaluations, thus allows calculation of total body fat, water, protein, and mineral components. The six component model includes two mineral components (ie, soft tissue and bone) along with an estimate of fasting glycogen mass (Heymsfield *et al*, 1991b).

The important neutron activation–whole body counting multi-component models allow chemical analysis *in vivo* much as was carried out by chemical analysis of human cadavers in the early years of body composition research. An important feature of the neutron activation–whole body counting multi-component models is that most of the assumptions applied are very stable *in vivo* and thus concerns for violations of the underlying assumptions are minimal. For example, the 4- and 6-component models are not recognized to have terms that are potentially inaccurate across a wide age range or in different ethnic groups. Neutron activation whole body counting models and methods thus often serve as the reference for evaluating the validity of other body composition techniques.

Underwater weighing of family of 4-component molecular level models. Over a century ago the concept was advanced that the measured density of fish could be used to establish oil content (Bull, 1896). Behnke and others towards the middle of the century perfected the measurement for human use that included underwater weighing or 'hydrodensitometry' and residual lung volume as a means of quantifying body density (Behnke *et al*, 1942). Behnke advanced the two-component model hydrodensitometry that included molecular components fat and fat-free mass (Behnke *et al*, 1942). The model assumes stable or relatively stable densities of fat and fat-free mass (Siri, 1961). Underwater weighing was the initial method applied for measuring body density and volume and several additional approaches are now possible including air displacement plethysmography (Dempster *et al*, 1995; McCrory *et al*, 1995). The two-component underwater weighing model served as the reference body composition method at the molecular level for several decades. The underwater weighing and related methods are simple, safe, and relatively inexpensive thus leading to their widespread proliferation at research centers.

Although often considered the gold standard for body composition analysis at the molecular level, the two-component model has obvious limitations. While the density of fat or triglyceride, is very stable at approximately 0.9 kg/l, the fat-free compartment is a heterogeneous structure including at least three major components, protein, water, and minerals (Wang *et al*, 1999). The assumption of a constant density of fat-free mass of 1.1 kg/l includes the assumption that the proportion of the three major components also remains stable. This is because the densities of water ($\sim$ 1 kg/l), protein ($\sim$ 1.4 kg/l) and bone minerals ($\sim$ 3 kg/l) also varies. Not long after the introduction of the two-component model, the concept was advanced for improving the model using three components (Table 1, M3) (Siri, 1961). Total body water could be measured using either tritium or deuterium dilution at the time, and this permitted separate analysis of the water content of fat-free mass. Appropriate simultaneous equations can be written that employ both total body water and body density as a means of separating body mass into three components, fat, total body water, and 'solids' or residual mass (Wang *et al*, 1995). This three-component model solved an important problem in that small individual differences in hydration obviously lead to errors in the two-component model.

The main major remaining source of variability in the three-compartment model was the solids compartment consisting of minerals, protein, and a small amount of glycogen. Towards the mid 1980s the first practical means of quantifying bone minerals was made available to investigators through the introduction of dual photon-absorptiometry (Peppler *et al*, 1981) and later dual energy X-ray absorptiometry (Mazess *et al*, 1990). This advance led to the introduction and subsequent widespread application of the 4-compartment underwater weighing model consisting of fat, water, and mineral components combined

with a fourth residual component. The residual mass in this model is primarily protein with a small amount of glycogen in the fasting state. This 4-component model thus requires measurement of body density, total body water, and bone mineral mass. A stable relationship is assumed between bone mineral mass and the total mineral mass component.

As the means of developing the 4-component underwater weighing model are available at many research centers, this approach now often serves as a reference method against which other techniques are compared. This is particularly important, as the neutron activation whole body counting multi-component model is assessable to only a few investigators throughout the world.

There are now a relatively large number of 4-component molecular level models published in the scientific literature. However, all of these models provide very similar estimates for total body fat and the differences between them are relatively small (Baumgartner *et al*, 1991; Heymsfield *et al*, 1990; Lohman, 1986; Selinger, 1977). All of these models assume stable and relatively constant densities of the major components. Thus, while these four component and even more, up to six component models, continue to be refined, they all share relatively common features and provide similar body composition estimates. The main improvements occurring in the four and related component models include facilitation of body density measurements such as those provided by air displacement plethysmography (Dempster *et al*, 1995; McCrory *et al*, 1995). The final unresolved lingering assumption, not a major one, is that a stable relationship exists between soft tissue and total bone mineral mass (Heymsfield *et al*, 1996). A method is therefore eventually needed to separate these two mineral components and this topic awaits additional investigation.

Dual energy X-ray absorptiometry (DXA) 3-component model. The DXA multi-component level molecular model includes three components, fat, lean soft tissue, and bone mineral (Table 1, M4) (Heymsfield *et al*, 1996). Thus, DXA alone is capable of providing estimates for three separate components. The lean soft tissue component consists of water, protein, glycogen, and soft tissue minerals.

The DXA three component model is an important advance as DXA methodology is widely available, evaluations are relatively inexpensive to carry out, and the procedure is safe for subjects of all ages. While in theory changes in lean soft tissue hydration can influence DXA fat estimates, in two recent studies we were able to show that hydration effects produce only minimal errors in percent fat estimation. The DXA method is also based on a number of additional assumptions and these are reviewed in detail by Pietrobelli *et al* (1996, 1998).

Cellular level

The cellular level consists of three main components, cells, extracellular fluids, and extracellular solids (Table 1, C1). While these are the three classic components at the cellular level, a more functional model is the one originally proposed by Moore and colleagues consisting of fat, body cell mass, extracellular fluids, and extracellular solids (Table 1, C2) (Moore *et al*, 1963). According to Moore's model, the cell mass component is further divided into the body cell mass or 'protoplasmic tissues' and the fat content within cells. The extracellular solids component consists primarily of bone minerals and to a lesser extent other solid components such as collagen, reticular and elastic fibers.

Estimation at the four major components at the cellular level are as follows: body cell mass can be estimated from total body potassium (Pierson *et al*, 1990); extracellular fluid can be measured with a specific marker such as by bromide dilution (Ma *et al*, 1996) or by a combination of total body water and total body potassium (Pierson *et al*, 1990); and extracellular solids can be measured by assuming a constant relationship between bone mineral content and extracellular solids with bone mineral content measured by DXA (Heymsfield *et al*, 1996). Fat is then calculated as the difference between the three estimated components and body weight. As with all multi-component models, several options are available for estimating each of the components but the approach suggested is practical for centers that have whole body counting and DXA capabilities.

The cell level model is important in physiological studies because cells are the basic functioning biological units. Evaluation of each of the major terms in the cell model allows physiological insights into a wide array of biological processes.

Tissue system level

During the 19th century anatomists were able to easily quantify the tissue system level of body composition by cadaver analysis. Early in the 20th century many studies of organ and tissue weights were carried out allowing us to establish with reasonable certainty the various weights and distributions of the tissues and organs making up the primary aspects of body mass (Allen *et al*, 1959; Keys *et al*, 1953; Brozek *et al*, 1963). The complexity of cadaver analysis and the diseases that were almost invariably present in cadavers led investigators away from this analysis of tissues and organs to the easier and clear molecular and cellular level models that were of biological interest. The introduction of imaging methods, computerized axial tomography (CT) and magnetic resonance imaging (MRI) changed the perception of the tissue system level as a 19th century method and brought it forward into modern times.

Cross-sectional images can be prepared that allow viewing of the major tissues and organs of the body which can then be quantified as respective areas (Despres *et al*, 1996; Foster *et al*, 1984; Ross *et al*, 1992; Sjostrom *et al*, 1986). With appropriately spaced slices the areas can be converted to volumes and mass can then be calculated by assuming a stable density of each tissue and organ. The tissue system multi-component model thus potentially includes adipose tissue and its subcomponents, major organs including the brain, heart, liver, kidneys, spleen, lung, skeletal muscle, and smaller tissues and organs such as the thyroid and adrenal gland (Table 1, TS1) (Gallagher *et al*, 1998). The

radiation exposure provided by whole body CT precluded its use in children and women in their childbearing years. It was only after the introduction of MRI in the early 1980s that multi-component tissue system level models proliferated in the study of human body composition and physiology (Chowdhury *et al*, 1994; Foster *et al*, 1984; Ross *et al*, 1992; Seidell *et al*, 1990). With MRI it is possible to scan multiple slices from head-to-toe and thus reconstruct with reasonable accuracy all of the major tissues and organs in the body (Ross, 2000). The procedure requires several hours for producing all of the images for a whole body including organs and an additional several hours for analysis of the images.

The multi-component tissue system level model provides important insights into biological processes. The present limitation of the model is that access to MRI and CT scanners is limited and cost in most centers in prohibitive. Additionally, the time required for analysis of the images for a whole body scan can be substantial. Nevertheless, computerized reconstruction algorithms may eventually lead to rapid image analysis and thus facilitate the use of this approach in body composition research.

Rationale

An important question is why measure so many components with multi-component models? The following summarizes the rationale and applications for multi-component models (Heymsfield *et al*, 2000):

- The research problem is best solved and greatest insights are gained using concurrent measurement of several interrelated components such as with growth hormone administration (ie, Table 1, C2; fat, body cell mass, extracellular fluid, and extracellular solids/bone minerals).
- Provide body composition estimates with greater accuracy than with two compartment methods (Heymsfield *et al*, 1996). Importantly, this applies in pathological states when the potential for model error is large (eg, assumed fat-free mass hydration = 0.732). Multi-component methods may also serve as a reference standard when evaluating other less accurate methods.
- Provide a new approach that replaces an older method involving radiation or some other potential hazard.

Table 2 Representative measurement errors for multi-component molecular-level methods[a]

Quantity	*Measurement method*	*Errors (CV.%)**	*Reference*
Components			
Total body nitrogen	PGNA	3.6	Dutton (1991)
		2.7	Pierson *et al* (1990)
		4.1	Beddoe *et al* (1984)
		3.0	Mernagh *et al* (1977)
Total body hydrogen	PGNA	0.4	Dutton (1991)
Total body potassium	WBC	1.5	Pierson *et al* (1990)
Total body carbon	INS	4.2	Dutton (1991)
		3.0	Pierson *et al* (1990)
Total body calcium	DGNA	2.6	Dutton (1991)
		0.8	Pierson *et al* (1990)
Total body phosphorus	DGNA	5.1	Dutton (1991)
		3.0	Pierson *et al* (1990)
Total body sodium	DGNA	2.5	Pierson *et al* (1990)
Total body chlorine	DGNA	1.5	Dutton (1991)
		2.5	Pierson *et al* (1990)
Total body water	$^{3}H_2O$ dilution	1.5	Wang *et al* (1973)
	$^{2}H_2O$ dilution	1.5	Pierson *et al* (1990)
		4.0	Bartoli *et al* (1993)
	$H_2{}^{18}O$ dilution	2.0	Scholler & Jones (1987)
Total body fat methods	TBC method	NA	Kehayias *et al* (1991)
			Heymsfield *et al* (1991b)
	3-C UWW	NA	
	4-C IVNA	NA	Cohn *et al* (1984)
	4-C UWW	NA	
	6-C IVNA	NA	Heymsfield *et al* (1991b)
Bone mineral	DXA	1.28	Heymsfield *et al* (1990)
Properties			
Body weight	Scale	< 1%	Heymsfield & Waki (1990)
Body volume/density	Hydrodensitometry	0.5% BF	Withers, personal comm.
Stature	Stadiometer	0.2	Heymsfield *et al* (1991a)

Abbreviations: BF, body fat; C, component; CV, coefficient of variation; DXA, dual energy X-ray absorptiometry; DGNA, delayed-γ neutron activation analysis; INS, inelastic neutron scattering; IVNA; *in vivo* neutron activation analysis; PGNA, prompt-γ neutron activation analysis; TBC, total body carbon; UWW, underwater weighing; WBC, ^{40}K-whole body counting.

*CVs represent between-measurement variation in phantoms or humans on the same or different days.

[a]Modified from Heymsfield *et al* (1996) with permission.

Errors

A general rule is that with more measurements there exists greater potential for measurement error. On the other hand, as more measurements are added the potential for assumption or model error usually tends to decline. Multi-component models trade off between these two sources of error. Some representative measurement errors are summarized in Table 2. When applying multi-component models as a means of improving accuracy and reproducibility 'error' becomes a central issue. The 'error' area of multi-component model development needs additional exploration.

Conclusions

Multi-component body composition methods, while of interest for over four decades, rapidly matured over the past decade. The remarkable progress can be attributed to the ever-increasing demand for improved accuracy when evaluating subjects differing widely in phenotypic characteristics and to the important advances in measurement technology. While on the one hand the model development area has reached a mature level, on the other there remain many unresolved questions and potential investigative topics that await future investigation.

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