Hydration of fat-free body mass: review and critique of a classic body-composition constant[1–3]

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ABSTRACT The assumed "constancy" of fat-free body mass hydration is a cornerstone in the body-composition research field. Hydration, the observed ratio of total body water to fat-free body mass, is stable at ≈0.73 in mammals and this constancy provides a means of estimating total body fat in vivo. This review examines both in vitro and in vivo data that support the hydration constancy hypothesis and provides a critique of applied methodology. Biological topics of interest are then examined and critical areas in need of future research are identified. These are important issues because water dilution is the only method currently available for estimating body fat in all mammals, which range in body mass by a factor of 10^4. *Am J Clin Nutr* 1999;69:833–41.

KEY WORDS Hydration, fat-free body mass, total body water, body composition, mammals, dual-energy X-ray absorptiometry

INTRODUCTION

One of the primary aims of body-composition research is to identify quantitative relations between components that are relatively constant under most circumstances. These stable relations form the basis of many widely used body-composition methods and the origin of their constancy is of fundamental scientific interest (1).

Water, the largest chemical component in mammals, plays a central role in nutrient transport, waste removal, maintenance of cell volume, and thermal regulation. The water content or hydration of fat-free body mass (FFM) is among the best known and most widely applied of the body-composition constants. More than 5 decades ago, Pace and Rathbun (2) first proposed that total body water (TBW) is a constant fraction of FFM ($\bar{x}$ ± SD: 0.724 ± 0.021) on the basis of experiments in guinea pigs. Subsequent chemical analysis of mature animals supported a hydration magnitude of ≈0.73 with a range of between 0.70 and 0.76 for several mammal species that range in body size from that of mice to cattle—a body mass difference of 10^4 (3). Additional strong support for the observed FFM hydration magnitude in mammals is provided by whole-body chemical analysis of 9 human cadavers with a mean TBW:FFM of 0.737 ± 0.036 and a range of between 0.684 and 0.808 (Table 1).

The in-depth study of FFM hydration not only provides insight into basic biological processes but also enhances our understanding and application of the TBW method of quantifying total body fat. The relative stability of FFM hydration between species led to the wide use of the in vivo method, that is,

$$\begin{aligned} \text{Fat} &= \text{body mass} - \text{FFM} \\ &= \text{body mass} - \text{TBW}/0.73 \end{aligned} \qquad (1)$$

TBW can be measured by ^3H_2O, ^2H_2O, or $H_2{}^{18}O$ dilution (10). At present, no other body-composition measurement method is capable of providing fat estimates in mammals that range in body size from a few grams, such as the shrew, to several thousand kilograms, such as the elephant.

The importance of the TBW-based method of body-composition measurement led Sheng and Huggins in 1979 (3) to critically review available literature on methodology. Since then, many additional FFM hydration studies have been published, although no synthetic review summarized their findings. This report provides an overview and critique of existing FFM hydration studies. Our aim is to appraise investigators of both strengths and shortcomings in the TBW method of estimating fat mass in mammals.

PREVIOUS HYDRATION STUDIES

The current investigation is based on the 5-level body-composition model, which holds that the ≈40 major components in humans and other mammals can be organized into atomic, molecular, cellular, tissue-organ, and whole-body levels (1). When examining previous publications on FFM hydration, we found that the studies can be divided into 2 main categories: in vitro and in vivo. Studies can then be organized according to the body-composition level evaluated.

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TABLE 1
Fat-free body mass (FFM) hydration (TBW:FFM) evaluated in 9 adult human cadavers[1]

Sex	Age	Body mass	TBW	FFM	TBW:FFM	Cause of death	Reference and year
	y	*kg*	*kg*	*kg*			
M	46	53.8	29.7	43.3	0.686	Skull fracture	Forbes et al (4), 1953
M	60	73.5	37.2	53.0	0.702	Heart attack	Forbes and Lewis (5), 1956
M	25	71.8	44.4	61.1	0.726	Uremia	Widdowson et al (6), 1951
M	63	58.6	35.0	48.0	0.729	Esophageal cancer	Knight et al (7), 1986
F	59	25.9	13.3	18.2	0.731	Extreme cachexia	Knight et al (7), 1986
F	42	45.1	25.3	34.5	0.733	Drowning	Widdowson et al (6), 1951
M	48	62.0	43.9	59.3	0.740	Infectious endocarditis	Forbes and Lewis (5), 1956
M	35	70.6	47.9	61.7	0.776	Mitral insufficiency	Mitchell et al (8), 1945
F	67	43.4	32.0	39.6	0.808	Advanced malignancy	Moore (9), 1946
$\bar{x}$ ± SD	49 ± 14	50.1 ± 15.8	34.3 ± 10.8	46.6 ± 14.5	0.737 ± 0.036	—	—

[1]TBW, total body water.

In vitro

In vitro analysis, based on direct chemical assays of entire animal cadavers or isolated tissues and organs, is a classical approach used to investigate hydration of FFM. In vitro studies can be examined at 2 body-composition levels, whole body and tissue-organ.

Whole-body level

Most in vitro FFM hydration studies were carried out at the whole-body level, for which the entire animal cadaver was thoroughly homogenized. Aliquot samples were then used for chemical analysis to determine the contents of various molecular compounds. One can reasonably assume that the chemical composition of aliquot samples are identical to that of the whole body. FFM hydration can thus be calculated as follows:

$$\begin{aligned}\text{TBW:FFM} &= \text{sample's water content} \\ &\quad /\text{sample's FFM} \\ &= \text{sample's water content} \\ &\quad /\text{sample's (mass} - \text{fat content)}\end{aligned} \qquad (2)$$

Water content of the homogenate sample can be determined by freeze-drying or drying at 90°C to stable weight. Fat can be extracted from the homogenate sample by using solvents such as petroleum ether.

Pace and Rathbun (2) were the first authors to review chemical analytic data from several mammals. They calculated a mean TBW:FFM of 0.724 for 50 guinea pigs, whereas the widely quoted mean of 0.732 comes from combining available data for guinea pigs with limited data at that time for rats, rabbits, cats, dogs, and monkeys. Since then, many mammals have been investigated and there is substantial literature on this subject. We reviewed in vitro studies in 15 mammals (Table 2). Unfortunately, some investigators analyzed the animal's eviscerated carcass and their results may not be taken as indicative of whole-body FFM hydration. We review this concern in a later section. Only 9 mammals in this table were therefore considered, including mice, rats, hamsters, rhesus monkeys, baboons, goats, sheep, gray seals, and humans. A very strong correlation (r = 0.9999, P < 0.001) between TBW (kg) and FFM (kg) was observed across mammals (Figure 1):

$$\text{TBW} = 0.724 \times \text{FFM} + 0.255 \qquad (3)$$

The regression line slope ($\bar{x}$ ± SE: 0.724 ± 0.003) is significantly different from one ($P = 7.89 \times 10^{-17}$) and the intercept

TABLE 2
Fat-free body mass (FFM) hydration (TBW:FFM) evaluated in vitro in 15 mammal species[1]

Species	Body mass	TBW	FFM	TBW:FFM	Comment	Reference and year
	kg	*kg*	*kg*			
Mouse (*n* = 27)	0.0356 ± 0.0223	0.0198 ± 0.0114	0.0277 ± 0.0161	0.715 ± 0.016	Whole body	Holleman and Dieterich (11), 1975
Hamster (*n* = 34)	0.1274 ± 0.0064	0.0744 ± 0.006	0.1016 ± 0.0076	0.733 ± 0.006	Whole body	Kodama (12), 1971
Rat (*n* = 32)	0.168 ± 0.003	0.112 ± 0.005	0.149	0.758 ± 0.005	Whole body	Tisavipat et al (13), 1974
Guinea pig (*n* = 50)				0.724 ± 0.003	Carcass	Pace and Rathbun (2), 1945
Rabbit (*n* = 3)	2.07 ± 0.27	1.54 ± 0.18	2.02 ± 0.27	0.762 ± 0.013	Carcass	Harrison et al (14), 1936
Cat (*n* = 3)				0.769	Carcass	Spray and Widdowson (15), 1950
Dog (*n* = 2)	6.04 ± 0.33	3.60 ± 0.58	4.84 ± 0.73	0.744 ± 0.007	Carcass	Harrison et al (14), 1936
Rhesus monkey (*n* = 5)	12.42 ± 3.83	7.01 ± 1.32	9.55 ± 1.92	0.736 ± 0.013	Whole body	Wang et al (unpublished data)
Baboon (*n* = 23)	17.86 ± 4.22	11.80 ± 2.60	15.75 ± 3.57	0.751 ± 0.055	Whole body	Lewis et al (16), 1986
Goat (*n* = 10)	20.44 ± 9.6	13.2 ± 5.2	17.5 ± 6.8	0.756 ± 0.013	Whole body	Panaretto (17), 1963
Sheep (*n* = 9)	39.7 ± 17.4	22.0 ± 3.5	29.4 ± 5.8	0.751 ± 0.027	Whole body	Panaretto (17), 1963
Pig (*n* = 8)				0.770 ± 0.003	Whole body	Doornenbal (18), 1975
Cattle (*n* = 7)				0.765 ± 0.002	Whole body	Moulton (19), 1920
Human (*n* = 9)	50.1 ± 15.8	34.3 ± 10.8	46.6 ± 14.5	0.737 ± 0.036	Whole body	*see* Table 1
Gray seal (*n* = 4)	213.5 ± 58.8	87.2 ± 23.7	120.5 ± 31.4	0.724 ± 0.009	Whole body	Reilly and Fedak (20), 1990

[1]$\bar{x}$ ± SD. TBW, total body water.

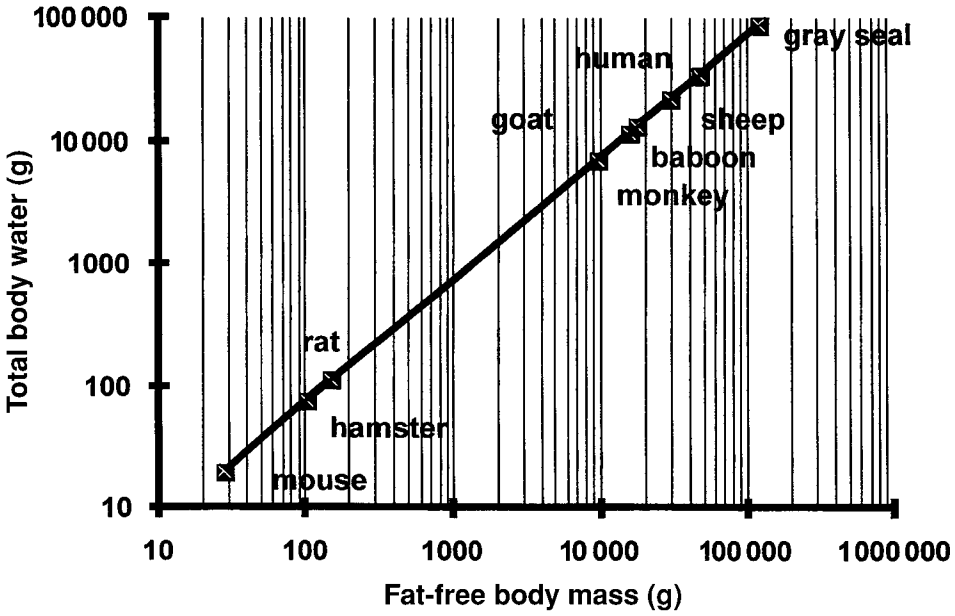

FIGURE 1. Total body water versus fat-free body mass, both expressed in logarithms, for humans and 8 mammal species. Body masses are those of mature animals presented in Table 2.

(0.255 ± 0.131) is not significantly different from zero (P = 0.088). The mean TBW:FFM for the 9 mammals is 0.739 ± 0.015 with a CV of 2.0%, indicating hydration stability between species. Note in Figure 2 that FFM hydration constancy applies to various mammals from 0.04-kg mice to 214-kg gray seals. The correlation between TBW:FFM and body mass for the 9 mammals is not significant (r = 0.18, P > 0.50).

Similar chemical analysis data are available for humans (Table 1). The chemical composition of adult human cadavers was reported by several investigators and a strong correlation (r = 0.987, P < 0.001) between TBW (in kg) and FFM (in kg) was observed (Figure 3):

$$\text{TBW} = 0.737 \times \text{FFM} \qquad (4)$$

The mean TBW:FFM for the 9 human cadavers is 0.737 ± 0.036 with a CV of 4.9%.

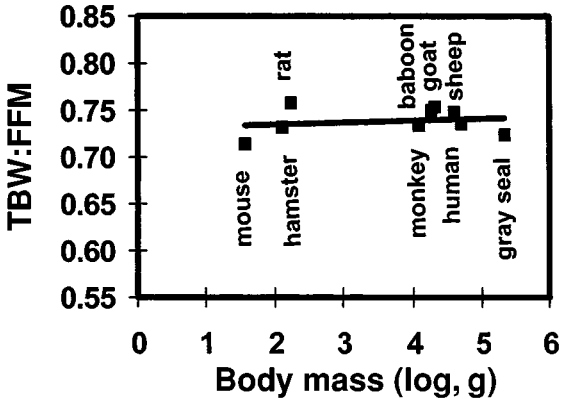

FIGURE 2. Fat-free body mass hydration (TBW:FFM) versus body mass, expressed as a logarithm, in 9 mature mammals. Body masses are those of mature animals presented in Table 2.

Tissue-organ level

Hydration studies were also carried out on isolated tissues and organs. The animal cadaver was first anatomically separated into various components, including, for example, skeletal muscle, adipose tissue, skin, skeleton, brain, liver, heart, lung, and kidneys. Each isolated tissue and organ was weighed and thoroughly homogenized. The contents of molecular components such as water and fat were next determined by chemical analysis. Whole-body FFM hydration was then calculated by summing the water and FFMs of all isolated tissues and organs.

Chemical analysis at the tissue-organ level provides valuable insights into the magnitude and variability in observed FFM hydration. Whole-body FFM hydration at this level is equal to the sum of individual tissue water contents (W_i) divided by the sum of individual tissue FFMs (FFM_i):

$$\text{TBW:FFM} = \Sigma W_i / \Sigma \text{FFM}_i \qquad (5)$$

where i represents individual tissues and organs. The FFM_i term can be expressed as $f\text{FFM}_i \times \text{FFM}$, where $f\text{FFM}_i$ is the fraction of whole-body FFM as individual tissue and organ. Similarly, W_i can be expressed as FFM hydration of individual tissues and organs (H_i), $W_i = H_i \times \text{FFM}_i$. On the basis of the definition $\Sigma(f\text{FFM}_i) = 1$, whole-body FFM hydration can be expressed as

$$\begin{aligned}\text{TBW:FFM} &= \Sigma(H_i \times f\text{FFM}_i) \\ &\quad \times \text{FFM}/\Sigma(f\text{FFM}_i) \times \text{FFM} \\ &= \Sigma(H_i \times f\text{FFM}_i) \qquad (6)\end{aligned}$$

Equation 6 indicates that whole-body FFM hydration is determined by 2 factors, hydration of individual tissues and organs (H_i) and fractions of FFM as individual tissues and organs ($f\text{FFM}_i$).

There are few reported in vitro FFM hydration studies at the tissue-organ level. Mitchell et al (8) studied a male cadaver 35 y of age, and later in 1953 and 1956, Forbes et al (4) and Forbes

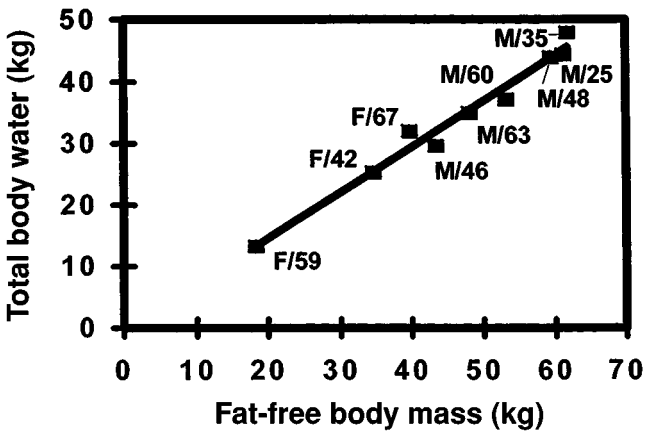

FIGURE 3. Total body water versus fat-free body mass for 9 adult human cadavers. Data are from Table 1.

and Lewis (5) reported anatomic and chemical analysis data for 2 male cadavers aged 46 and 60 y, respectively. Whole-body FFM hydration values calculated by these authors for the 3 cadavers were 0.778, 0.696, and 0.695 with a mean of 0.723 ± 0.038, a value close to the well-recognized value of ≈0.73.

In addition to whole-body FFM hydration, tissue-organ level studies provide information regarding hydration of individual tissues and organs. As an example of this approach, we calculated H_i and $f\text{FFM}_i$ values for 16 tissues and organs using reference man data (Table 3) (21). The sum of ($H_i \times f\text{FFM}_i$) for the 16 tissues and organs is 0.714 and the sum of $f\text{FFM}_i$ values is 0.975. According to equation *6*, whole-body FFM hydration can be calculated as $\Sigma(H_i \times f\text{FFM}_i)/\Sigma(f\text{FFM}_i) = 0.714/0.975 = 0.732$, which is equal to the well-recognized value (2). Note in Table 3 that no individual tissue or organ has an FFM hydration equal to 0.73. The observed whole-body FFM hydration value of 0.73 is the integrated result of low hydration components (eg, skeleton and skin) and high hydration components such as skeletal muscle and visceral organs.

TABLE 3
Reference man's fat-free mass (FFM) hydration calculated from various tissues and organs[1]

Tissue or organ	FFM	$f\text{FFM}_i$	H_i	$H_i \times f\text{FFM}_i$
	kg			
Skeleton	8.10	0.143	0.407	0.058
Connective tissue	1.58	0.028	0.633	0.018
Tendons and fascia	1.39	0.025	0.633	0.016
Skin	2.34	0.041	0.684	0.028
Adipose tissue	3.00	0.053	0.767	0.041
Liver	1.68	0.030	0.774	0.023
Pancreas	0.09	0.002	0.778	0.001
Spleen	0.18	0.003	0.778	0.003
Lung	0.99	0.018	0.788	0.014
Heart	0.30	0.005	0.800	0.004
Skeletal muscle	27.38	0.483	0.804	0.388
Blood	5.46	0.096	0.806	0.078
Kidney	0.29	0.005	0.828	0.004
Gastrointestinal tract	1.13	0.020	0.841	0.017
Brain	1.25	0.022	0.880	0.019
Urinary bladder with contents	0.12	0.002	0.844	0.002
Sum	55.28	0.975	—	0.714

[1] $f\text{FFM}_i$, fraction of whole-body FFM (56.7 kg) as individual tissue's FFM; H_i, FFM hydration of individual tissue or organ. Whole-body FFM hydration can be calculated by using equation *6*: TBW:FFM = $\Sigma (H_i \times f\text{FFM}_i)/\Sigma (f\text{FFM}_i) = 0.714/0.975 = 0.732$. Data from reference 21.

Is there a difference in hydration between whole-body measures and carcass analysis?

An animal's carcass is usually considered the difference between the whole animal and removed visceral organs and tissues. The question posed here is important because most reports provide plentiful data on FFM hydration for the carcass rather than the entire body (3). Even the 50 guinea pigs used in Pace and Rathbun's (2) classic study were eviscerated. The animal's body mass can be reduced by up to 25% when the viscera are removed. Criticisms of carcass studies thus arise because it is questionable whether FFM hydration data for a carcass can be applied to the entire body. The FFM hydration of the entire body and carcass were compared in several studies of different animal species. Panaretto (17) reported entire-body FFM hydration values of 0.756 ± 0.005 for sheep and 0.756 ± 0.004 for goats, which were significantly higher than those of the carcass FFM, 0.736 ± 0.003 for sheep and 0.726 ± 0.004 for goats. Reilly and Fedak (20) also reported an FFM hydration of 0.722 ± 0.009 for entire gray seals, which is significantly higher than that observed for carcass FFM, 0.700 ± 0.010.

These observations can be explained by the tissue-organ level FFM hydration model summarized by equation *6*. The entire body can be divided into viscera and carcass. Assume that H_V and H_C are FFM hydration of viscera and carcass, and f_V and f_C are the fractions of FFM as viscera and carcass, respectively. Because $f_V + f_C = 1$, equation *6* can be converted and simplified to be

$$\begin{aligned}\text{TBW:FFM} &= f_V \times H_V + f_C \times H_C \\ &= f_V \times (H_V - H_C) + H_C\end{aligned}$$

and

$$\text{TBW:FFM} - H_C = f_V \times (H_V - H_C) \qquad (7)$$

Because visceral organs have high hydration values (Table 3), entire-body FFM hydration is higher than that of the carcass alone. The difference between entire-body and carcass hydration (ie, TBW:FFM $- H_C$) is mainly determined by the fraction of viscera removed. The larger the fraction of removed viscera, the larger the hydration difference between the entire body and the carcass. Equation *7* thus indicates that carcass FFM hydration data may not be applied to the entire body if the fraction of viscera removed is large.

Limitations of in vitro analysis

There is general agreement that in vitro chemical analysis is accurate and should be considered the criterion when studying FFM hydration. However, this technique is also prone to biological and measurement errors. First, human cadavers that were analyzed postmortem often suffered from severe illnesses before death (Table 1). It is difficult to judge the effects of terminal illness on FFM hydration, and the extent to which TBW:FFM measured in cadavers represents hydration in healthy adults remains uncertain.

Second, underestimates of FFM hydration may be caused by insensible water loss between the time of death and the chemical analysis of homogenate samples. Conversely, overestimates of FFM hydration can result from loss of volatile solids during drying of homogenate samples.

A third concern is that investigators differ in their choice of lipid extraction solvent. The type of solvent used has a large effect on the amount of material extracted. Fat or triacylglycerols are bound in tissues by weak van der Waal's forces or hydrophobic bonds and are usually extracted with nonpolar solvents such as ethyl ether or petroleum ether. The residual lipids, including phospholipids and sphingolipids, may form hydrogen bonds and electrostatic associations with proteins that require polar solvents such as methanol and acetone for disruption and tissue extraction (22). Many in vitro studies of FFM hydration used nonpolar solvents, hence, the extracted lipid consisted primarily of triacylglycerol or fat. Some other studies, however, were based on total lipid extraction protocols that used mixtures of nonpolar and polar solvents such as chloroform:methanol (2:1, by vol) or 45% chloroform, 10% methanol, and 45% heptane (7). Dobush et al (23) pointed out that although chloroform:methanol removes total lipid, under some conditions it also extracts a substantial amount of nonlipid compounds. For example, Dobush et al measured the percentage fat of homogenate samples of snow geese. The measured mean (±SE) percentage fat was 29.1 ± 0.27% with petroleum ether and 30.1 ± 0.22% with diethyl ether, respectively. Compared with nonpolar solvents, polar solvents such as chloroform:methanol extracted relatively large amounts of material (34.6 ± 0.6%, $P < 0.05$ compared with the other 2 methods). When combinations of nonpolar and polar solvents are used, therefore, the observed hydration of FFM will be higher than that when a nonpolar solvent is used (23, 24). Comparisons among studies must be interpreted cautiously.

Last, appropriate chemical analyses of entire animals or isolated tissues is difficult and requires substantial resources for completion. Accordingly, FFM hydration information from in vitro studies is limited, especially from humans and large animals.

In vivo

Whole-body level

Compared with in vitro studies, in vivo analysis avoids difficult homogenization and chemical analyses and can be carried out on a large scale in well-characterized and clinically stable living humans and animals. In vivo studies are thus widely used in FFM hydration investigations, especially when biological factors that may influence hydration such as age and adiposity are examined.

Inspection of published hydration studies often reveals contradictory findings with respect to hydration magnitude and stability. For example, in vivo data suggest that the aging process may or may not influence FFM hydration. Some authors report that FFM hydration does not change significantly in old adults (25–27). However, studies of very old adults (≤84 y old) show a significantly higher ($P < 0.01$) TBW:FFM than that observed in young adults (28). In contrast, an opposite effect was reported by Virgili et al (29), who found that hydration steadily decreases with age in men from the seventh decade (0.702 ± 0.077) to the 10th decade (0.659 ± 0.082). These discrepant results may be caused by population differences, the sample size analyzed, or the measurement methods applied.

The principle of studying in vivo hydration is simple: TBW and FFM are measured separately and the ratio of TBW to FFM is then calculated. The accuracy of observed hydration is closely related to the quality of the TBW and FFM measurements.

Total body water measurement

Although several methods are available for estimating TBW, the accuracy of these methods differ. For example, TBW is sometimes estimated by anthropometric and bioimpedance analysis methods in field studies (30). The validity of observed FFM hydration values by these methods is obviously questionable because of their high measurement error. Antipyrine was used in the past as a dilution tracer but because of conflicting results, its use was discontinued in favor of labeled water isotopes (31).

TBW can be accurately measured by using tritium (^3H_2O) and deuterium (^2H_2O) dilution and in some laboratories by ^{18}O-labeled water ($H_2{}^{18}O$) (10). Each isotope measures a specific dilution volume. For tritium and deuterium the dilution volume is larger than actual TBW volume because the labeled hydrogen atoms exchange with hydrogen atoms associated with carboxyl, hydroxyl, and amino groups (32, 33). Similarly, ^{18}O exchanges with labile oxygen atoms in carboxyl and phosphate groups (34, 35). The overexchange rate is ≈4–5% for tritium and deuterium and 0–1% for $H_2{}^{18}O$. The usual approach today is to assume a 4% and 1% TBW overestimate for tritium and deuterium and ^{18}O-labeled isotopes, respectively (10). This is a critical assumption because, for example, selecting 4% or 5% for tritium TBW overestimation correspondingly affects hydration by ≈1% (eg, 0.73 compared with 0.72). With correction for overexchange and careful attention to detail, TBW can be measured with a precision and accuracy of 1–2% (10). Even a measurement error of this magnitude may influence the observed TBW:FFM, particularly when small populations are studied.

Another problem is that a considerable difference in TBW estimations is observed when in vivo and in vitro studies are compared (3). After estimating TBW in vivo with tritium, animals in 5 separate studies (9, 17, 31, 36, 37) were killed and TBW was also estimated by chemical analysis. Although in vivo and in vitro methods obtained approximately the same TBW values for rabbits, sheep, and goats (9, 17, 31), in vivo methods measured a TBW value significantly larger (4–15% of body mass) than that produced by in vitro methods for rats, pigs, dogs, and cattle (36, 37). Only 0.5–2.0% of the overestimation by in vivo methods can be explained by the exchange of hydrogen between tritiated water and tissue organic compounds (3). Although technical errors may influence the observed TBW estimate, the remainder of the difference between in vivo and in vitro studies is still not explained fully.

Fat-free body mass measurement

Accurate in vivo measurement of FFM may be even more difficult than that of TBW. Although FFM can be estimated by total body potassium, anthropometry, and bioimpedance methods (29, 30), their value in investigating FFM hydration is obviously limited because of their low accuracy. Streat et al (38), for example, estimated FFM by skinfold thickness anthropometry. The estimated TBW:FFM (0.690 ± 0.075) is much lower than that measured by the neutron activation method (0.739 ± 0.028, $P < 0.001$). Moreover, the range of FFM hydration estimated by anthropometry (0.52–0.90) is clearly outside of accepted biological limits.

Currently available methods for measuring FFM include 2-, 3-, and 4-compartment densitometry models based on summary equations *8–10*. These approaches are derived from correspond-

ing 2-, 3-, and 4-compartment models for measuring total-body fat mass (39, 40):

$$\text{FFM} = 5.50 \times \text{body mass} - 4.95 \times \text{body volume} \quad (8)$$

$$\text{FFM} = 2.351 \times \text{body mass} + 0.78 \times \text{TBW} - 2.118 \times \text{body volume} \quad (9)$$

$$\text{FFM} = 3.037 \times \text{body mass} + 0.714 \times \text{TBW} - 1.129 \times \text{BM} - 2.75 \times \text{body volume} \quad (10)$$

where TBW (in kg) is measured by dilution methods; BM is bone mineral (in kg) measured by dual-energy X-ray absorptiometry (DXA); and body volume (in L) is measured by hydrodensitometry. FFM can also be measured by DXA as the difference between body mass and total body fat (41).

It has been suggested that multiple-component methods can be used as the reference to assess FFM; however, these methods may not be ideal for the analysis of FFM hydration. As shown in equations *9* and *10*, 3- and 4-compartment model approaches require TBW measurement so that water measurement error may be propagated to FFM estimation. Therefore, ideally, FFM should be estimated independently from water measurement in hydration studies. The 2-compartment densitometry model (equation *8*) and DXA do not require water measurement. However, the 2-compartment densitometry model is based on an FFM density of 1.10 g/cm^3, which was derived from an assumed FFM water fraction of 0.73. DXA also assumes a uniform hydration of 0.73 and electrolyte constancy of FFM (42). If the measurement of FFM is based on DXA or the 2-compartment densitometry model, with an assumed FFM hydration of 0.73, estimated TBW:FFM will be in error for subjects that deviate from assumed hydration. Hewitt et al (28) compared the values of FFM hydration estimated by 2-, 3-, and 4-compartment models. The TBW:FFM values from 3- and 4-compartment models were significantly less than TBW:FFM values from the 2-compartment model in prepubescent subjects and elderly adult females ($P < 0.001$). In young adults, however, TBW:FFM values from the 3- and 4-compartment models (0.714 ± 0.012 and 0.710 ± 0.010) were greater than that from the 2-compartment model (0.690 ± 0.026, both $P < 0.01$).

Pietrobelli et al (43) recently evaluated errors arising in DXA body-composition estimates as a result of soft tissue hydration changes. The magnitude of this error is small (ie, a percentage fat error of 1%) unless the relative amount of added water or electrolyte solution is large.

It has been suggested that FFM can be calculated from body mass, total body carbon (TBC), nitrogen (TBN), and calcium (TBCa, all in kg), which are measured by the neutron-activation method (44), as follows:

$$\text{FFM} = \text{body mass} - 1.30 \times \text{TBC} + 4.45 \times \text{TBN} + 0.065 \times \text{TBCa} \quad (11)$$

This approach avoids errors caused by TBW measurement in 3- and 4- compartment models and errors caused by model assumptions in 2-compartment models and the DXA method. However, this approach is not completely TBW independent because the measurement of total body nitrogen by neutron activation is dependent for calibration on the TBW value. In addition, neutron-activation analysis, because of the radiation exposure involved, cannot be used in the study of children and premenopausal women.

An ideal approach, although one that is not always practical, is to apply TBW-independent FFM measurement methods in hydration studies. An example of the method is dilution of fat-soluble inert gases such as cyclopropane or ^{85}Kr (45). However, the application of fat-soluble inert gas methods is limited because of expense and restricted access to instruments.

In summary, both in vitro and in vivo studies make major contributions to the investigation of FFM hydration. In vitro studies reveal that FFM hydration of ≈0.73 is a universal body-composition rule that applies widely in mammals. In vivo studies additionally identify various biological factors that influence FFM hydration. An important consideration for both in vitro and in vivo hydration research is selection of appropriate subjects in adequate numbers and application of carefully planned body-composition analysis methods. These are critical considerations when evaluating within- or between-group hydration differences because under normal conditions TBW:FFM varies by only a few percentage points.

AREAS IN NEED OF ADDITIONAL RESEARCH

There are many important unanswered questions related to the constancy of FFM hydration. We have selected several of the common questions for review to highlight the need for more research in this area.

Does body adiposity influence hydration?

It is often concluded that FFM hydration of adult animals is independent of adiposity and there is a sizable database in support of this view in humans and other mammals (5, 6, 8). In contrast, small and unimpressive increases in FFM hydration with greater body adiposity were reported for guinea pigs and rats (2). Lewis et al (16) tested 13 female baboons by chemical analysis and found a high correlation ($r = 0.98$, $P < 0.01$) between percentage body fat and FFM hydration. However, their conclusion is questionable because the FFM hydration values (0.85 and 0.92) observed in 2 animals are obviously beyond the upper limit of normal biological variation.

The influence of adiposity on FFM hydration may be explained with the aid of the tissue-organ level hydration model (equation *6*). Body mass can be divided into adipose tissue (AT) and adipose tissue–free body mass (ATFM) on the tissue-organ level. Reference man, with whole-body TBW:FFM of 0.741, has an adipose tissue hydration (H_{AT}) of 0.7667, which is higher than that of adipose tissue–free body mass (H_{ATFM}) at 0.7393 (21). The difference between H_{AT} and H_{ATFM} (0.0274) indicates that the more adipose tissue an individual has, the higher the FFM hydration. If one assumes that f_{AT} and f_{ATFM} are the fractions of FFM as fat-free AT and fat-free ATFM, respectively, and that $f_{AT} + f_{ATFM} = 1$, the tissue-organ level hydration model (equation *6*) can be rewritten as

$$\begin{aligned}\text{TBW:FFM} &= f_{AT} \times H_{AT} + f_{ATFM} \times H_{ATFM} \\ &= f_{AT} \times (H_{AT} - H_{ATFM}) + H_{ATFM} \\ &= 0.0274 \times f_{AT} + H_{ATFM}\end{aligned} \quad (12)$$

This equation indicates that when H_{ATFM} is maintained stable, the fraction of FFM as the nonfat portion of adipose tissue (f_{AT}) is directly proportional to whole-body TBW:FFM.

For example, reference man contains 56.7 kg FFM, 15 kg adipose tissue, and 3 kg nonfat adipose tissue, $f_{AT} = 3/56.7 = 0.053$. Even though f_{AT} doubles from 0.053 to 0.106, according to equa-

tion *11*, FFM hydration only increases from 0.741 to 0.742. Therefore, although body adiposity theoretically influences FFM hydration, the change in TBW:FFM may be too small to identify using available in vivo methods. However, the model presented in equation *11* assumes a constant ATFM hydration at all levels of adiposity. Organ proportions may change with increasing body mass and, additionally, edema is often observed in very obese subjects. Hence, our estimates of adiposity effects on TBW:FFM based on equation *11* should only serve as a guide for planning future hydration studies.

Is there an association between age and hydration?

In vivo studies indicate that FFM hydration may be influenced by biological factors such as age. Moulton (46), in his classic investigation, summarized chemical analysis results of 9 mammals, including mice, rats, guinea pigs, rabbits, cats, dogs, pigs, cattle, and humans. At birth, all mammals show a high FFM hydration and low concentrations of protein and mineral. FFM hydration then rapidly declines and protein and mineral content increase from early life until chemical maturity is reached.

Although TBW:FFM decreases rapidly during growth and then stabilizes in young adults, it is not clear whether senescence influences FFM hydration and previous studies are contradictory on this important issue. For example, in vitro cadaver studies (Table 1) do not show a significant correlation (r = 0.15, P > 0.50) between FFM hydration and age. The number of cadaver analyses, however, is small (n = 9) and there are no subjects >67 y of age. Moreover, the subjects presented in Table 1 all died from illnesses or conditions that potentially alter FFM hydration.

FFM hydration change may not be identified by in vivo studies, particularly with small subject groups, because the expected change may be within the range of measurement error. Schoeller (25) suggested that there is little or no effect of aging on FFM hydration through the age of 70 y. Visser et al (47) studied the FFM hydration in a large cohort of individuals aged 20–94 y. No relation was observed between the FFM hydration and age. The correlation coefficients were −0.02 (P = 0.67) for women and −0.07 (P = 0.23) for men. Baumgartner et al (48) also did not observe an age-related change in FFM hydration in 98 subjects aged 65–94 y. Moreover, Goran et al (49) did not observe a significant difference in FFM hydration between young (0.716) and elderly (0.723) men. Mazariegos et al (50) compared FFM hydration between young and older women matched for body mass and height. The TBW:FFM value was similar in the young (0.735 ± 0.020) and older (0.725 ± 0.030) women.

However, a significantly higher TBW:FFM than in young adults (0.708 ± 0.012) was observed in elderly men ≤age 84 y (0.725 ± 0.014, P < 0.01) (28). Bergsma-Kadijk et al (51) observed that FFM hydration was lower in young females (0.723 ± 0.010) than in elderly women aged 65–78 y (0.737 ± 0.025, P < 0.001). The contradictory observations reported by previous investigators on the relation between FFM hydration and the aging process may have been caused by varying population characteristics, including differences in subject body mass, physical activity level, and health status.

Fomon et al (52) and Ellis (53) reported TBW and FFM for children from birth (≈0.81) to age 10 y (≈0.75) and in adults from age 20 to 85 y, respectively. FFM hydration decreases markedly during growth and the "constancy" of FFM hydration can therefore only be assumed in nonelderly adults. Although FFM hydration may also change with senescence, the change is probably small and may be difficult to quantify by in vivo studies. Clarification of these issues awaits longitudinal studies with appropriately selected methods and adequate numbers of subjects (54).

Is hydration in nonmammals also stable at <0.73?

Previous studies show that FFM hydration is remarkably constant across mammal species. An interesting question thus arises: do nonmammal vertebrates have the same FFM hydration of ≈0.73? Up to now, to our knowledge, there are no chemical analysis reports that describe FFM hydration in lower vertebrates. Thorson (55–59), however, provided systematic reports on a related body-composition index, the ratio of TBW to body mass, in poikilothermous vertebrates (Table 4). The fraction of body mass as water in different species depends on habitat. In general, fresh water animals tend to have a higher ratio of water to body mass whereas the reverse applies in marine and terrestrial animals. Another important factor is evolutionary hierarchy.

TABLE 4
Fractions of body mass as water (TBW:body mass) in various vertebrate species[1]

Species	TBW:body mass
Freshwater animals	
Osteichthyes	
Teleostei	0.714 ± 0.025[2]
Chondrostei	0.732 ± 0.014
Amphibia	
Ranidae	0.790 ± 0.013
Cryptobranchi	0.791 ± 0.013
Proteidae	0.811 ± 0.015
Reptilia	
Alligator	0.729 ± 0.010
Turtle	0.729 ± 0.008
Crocodilia	0.730 ± 0.012
Marine animals	
Agnatha	
Sea lamprey	0.756 ± 0.005
Chondrichthes	
7 Species, summary	0.748 ± 0.016
Osteichthyes	
Teleostei	0.708 ± 0.026
Reptilia	
Green turtle	0.648
Atlantic turtle	0.649 ± 0.013
Mammalia	
Gray seals	0.408
Terrestrial animals	
Amphibia	
Bufonidae[3]	0.741 ± 0.019
Reptilia	
Gopher snake	0.700 ± 0.006
Iguana	0.708 ± 0.009
Boa	0.710 ± 0.001
Mammalia	
Sheep	0.554
Mouse	0.556
Hamster	0.584
Human[4]	0.600

[1] Data from references 55–59. TBW, total body water.
[2] $\bar{x}$ ± SD.
[3] Relatively terrestrial species.
[4] Reference man data from reference 21.

In general, lower animal classes tend to have higher ratios of water to body mass than higher animals. Note that the fractions of body mass as water are similar in animal species of the same class that share similar habitats.

Because body mass is the sum of FFM and fat, TBW:FFM must be larger than the ratio of TBW to body mass for all animal species. Therefore, although Table 4 only provides data for the ratio of TBW to body mass, one can still appraise the FFM hydration of different species. Lower animals living in fresh water may have higher FFM hydration (>0.80) whereas higher animals living in sea water and on land have lower hydration (≈0.73). Therefore, even though chemical analysis is still lacking for individual species of lower animals, one can make a preliminary conclusion: FFM hydration of ≈0.73 may not be a characteristic of all vertebrates.

SUMMARY AND CONCLUSION

In the present report, we examined in vitro and in vivo studies and concluded that, even though methodologic limitations preclude a highly accurate analysis, adult mammals, including humans, share in common a relatively constant hydration of FFM. We also examined some common questions to highlight the need for more research on the hydration of FFM. Additional questions also prevail, such as do sex and race influence the constancy of FFM hydration? More importantly, why is FFM hydration equal to ≈0.73 in humans and other mammal species? Does the constancy of FFM hydration of ≈0.73 reflect physiologic regulatory mechanisms? These are all important topics for future investigation.

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