A whole-body model to distinguish excess fluid from the hydration of major body tissues1–3

Paul W Chamney, Peter Wabel, Ulrich M Moissl, Manfred J Müller, Anja Bosy-Westphal, Oliver Korth, and Nigel J Fuller

ABSTRACT

Background: Excess fluid (ExF) accumulates in the body in many conditions. Currently, there is no consensus regarding methods that adequately distinguish ExF from fat-free mass.

Objective: The aim was to develop a model to determine fixed hydration constants of primary body tissues enabling ExF to be calculated from whole-body measurements of weight, intracellular water (ICW), and extracellular water (ECW).

Design: Total body water (TBW) and ECW were determined in 104 healthy subjects by using deuterium and NaBr dilution techniques, respectively. Body fat was estimated by using a reference 4-component model, dual-energy X-ray absorptiometry, and air-displacement plethysmography. The model considered 3 compartments: normally hydrated lean tissue (NH_LT), normally hydrated adipose tissue (NH_AT), and ExF. Hydration fractions (HF) of NH_LT and NH_AT were obtained assuming zero ExF within the diverse healthy population studied.

Results: The HF of NH_LT mass was 0.703 ± 0.009 with an ECW component of 0.266 ± 0.007. The HF of NH_AT mass was 0.197 ± 0.042 with an ECW component of 0.127 ± 0.015. The ratio of ECW to ICW in NH_LT was 0.63 compared with 1.88 in NH_AT. ExF can be estimated with a precision of 0.5 kg.

Conclusions: To calculate ExF over a wide range of body compositions, it is important that the model takes into account the different ratios of ECW to ICW in NH_LT and NH_AT. This eliminates the need for adult age and sex inputs into the model presented. Quantification of ExF will be beneficial in the guidance of treatment strategies to control ExF in the clinical setting.

KEY WORDS Excess fluid, tissue hydration, normal hydration, body composition, adipose tissue, ECW:ICW ratio

INTRODUCTION

Diseases such as cardiac impairment and kidney failure often lead to an accumulation of excess fluid (ExF), increasing the body’s state of hydration. ExF may be regarded as an expansion of the extracellular or total body fluid compartments of the body but is not required by the body to maintain homeostasis. In patients with kidney failure, it is essential to remove the ExF to avoid long-term cardiovascular mortality (1–4). Despite the occurrence of ExF, little progress has been made in the body-composition field to develop a method for its identification.

Well-established techniques, such as hydrometry (5, 6), dual-energy X-ray absorptiometry (DXA) (7–9), and underwater weighing (10, 11), are available to obtain estimates of fat-free mass (FFM). The major drawback with these methods, however, is that ExF cannot be distinguished from FFM. Although ExF may be reflected by a rise in ECW (12) or TBW, quantification of ExF is only possible once a hydration reference has been established. The hydration reference represents normal values of ECW and TBW found in healthy control subjects, although the proportions of ECW and TBW vary according to body composition. Where body composition is assumed constant in a given subject group, a hydration reference may be established allowing ExF to be calculated (13). The ratio of ECW to TBW also offers the basis of a hydration reference, and this approach has been applied in patients with corrections for age (14). The hydration of FFM (H_{FFM}), although regarded constant at 0.73 (15), may be influenced by several health factors (16) thus limiting its use for quantification of ExF. Additionally, in more recent work, the hydration of lean soft tissue and its relation with the ratio of ECW to ICW was investigated (17).

One factor influencing the ratio of ECW to ICW (and hence its use as a reference) is the diversity of major body tissues such as the distinct adipose tissue and lean (nonadipose) tissue (18–20). To take into account the dissimilar hydration of relevant tissues and satisfy the need for a method to quantify ExF, we developed a new body-composition model.

SUBJECTS AND METHODS

Subjects and measurements

Data were used from 89 healthy adults recruited in Kiel, Germany. These data were supplemented with those from 15 healthy adults gathered in a previous study conducted in Cambridge United Kingdom (Morgan M, Madden A, Jennings G, Elia M, 1 From the Research and Development department, Fresenius Medical Care, Bad Homburg, Germany (PWC, PW, and UMM); the Institut für Humanernährung und Lebensmittelkunde, Christian Albrechts-Universität, Kiel, Germany (MJM, AB-W, and OK); and the MRC Childhood Nutrition Research Centre, Institute of Child Health, London, United Kingdom (NJF). 2 Data from the Institut für Humanernährung und Lebensmittelkunde were obtained through funding from Fresenius Medical Care. 3 Reprints not available. Address correspondence to PW Chamney, Research and Development, Fresenius Medical Care, Bad Homburg, Germany. Received February 22, 2006. Accepted for publication August 23, 2006.)
Fuller N, unpublished observations). Ethical approval was obtained from the ethical board of the Christians Albrechts Universität. The subjects were specifically chosen such that the full extent of the body composition range (in terms of percentage fat) could be investigated. The subject characteristics derived by combining the data (n = 104) from the 2 centers are reported in Table 1.

Each subject fasted overnight for 10 h. At the end of this period, 5-mL venous blood samples were taken for assay calibration and to establish baseline concentrations of deuterium and bromide. A mixture of deuterium oxide (0.4g/kg body weight) and NaBr (50 mg/kg body weight) was administered to each subject. According to past studies, a homogenous distribution of bromide is achieved within 4 h (5). During this equilibration period any ingestion of food or water was prohibited. At the end of the equilibration period, 5-mL venous blood samples were taken for assay calibration.

Cobas Fara (Roche Diagnostic, Welwyn Garden City, United Kingdom). The appropriate corrections were then applied (23). Before any further analysis, the whole-body volumes of TBW and ECW were converted to mass by the density of water, $D_{BW}$, by using a value of 0.99371 kg/L at 36 °C (24).

Total body fat was measured by using DXA scanners (Hologic Inc, Waltham, Ma) models QDR-4500A V8.26a in Kiel and QDR-1000/W in Cambridge. Calculated values of body fat were also obtained from densitometry via Siri’s equation (25) and by application of a 4-component (4-C) model (24) with the use of measurements of body volume obtained either by air-displacement plethysmography with a Bod-Pod (Life Measurement Instruments, Concord, CT) in Kiel or by underwater weighing in Cambridge. It was shown that these measurements of body volume are equivalent in healthy adults (26).

**New 3-compartment model**

Several authors have suggested that the body should be divided into adipose tissue (AT) and nonadipose body mass or adipose-free mass (AFM) (18–20, 27). The considerable disparity between the hydration of AT and AFM shown in these studies formed the basis of our new model. In the present study, it was assumed that in a state of health these 2 tissues could be considered “normally hydrated.” A schematic of the new 3-component (3-C) tissue-based model is shown in Figure 1. It is consistent with the molecule level of the 5-level model classification described elsewhere (28), but modified to reflect the presence of excess fluid that may accumulate due to pathological reasons. It is clear from Figure 1 that the mass of ExF is a subcomponent of AFM. Therefore, to avoid ambiguity, AFM is divided into “normally hydrated lean tissue” (NH_LT) and MExF. Similarly, the term “normally hydrated adipose tissue” (NH_AT) is used in place of adipose tissue to emphasize how adipose tissue should be regarded.

**Table 1** Characteristics of healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 57)</th>
<th>Men (n = 47)</th>
<th>Combined (n = 104)</th>
<th>Combined range (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38.6 ± 16.12</td>
<td>43.7 ± 15.9</td>
<td>40.9 ± 16.1</td>
<td>21–68</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.06</td>
<td>1.78 ± 0.06</td>
<td>1.73 ± 0.08</td>
<td>1.54–1.96</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.1 ± 10.7</td>
<td>82.6 ± 11.1</td>
<td>75.2 ± 12.8</td>
<td>49.2–108.90</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 ± 3.7</td>
<td>26.0 ± 3.6</td>
<td>25.2 ± 3.7</td>
<td>17.5–36.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>33.9 ± 7.14</td>
<td>22.6 ± 7.14</td>
<td>25.7 ± 8.9</td>
<td>6.6–50.9</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>45.2 ± 5.2</td>
<td>63.4 ± 6.6</td>
<td>53.4 ± 10.8</td>
<td>34.5–78.0</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>32.7 ± 4.0</td>
<td>44.8 ± 4.7</td>
<td>38.2 ± 7.5</td>
<td>24.7–53.9</td>
</tr>
<tr>
<td>ECW\textsubscript{WB} (kg)</td>
<td>14.0 ± 1.9</td>
<td>18.2 ± 2.3</td>
<td>15.9 ± 3.0</td>
<td>10.1–24.1</td>
</tr>
</tbody>
</table>

1 FFM, fat-free mass; TBW, total body water; ECW\textsubscript{WB}, whole-body extracellular water.
2 ± SD (all such values).
3 Measured with dual-energy X-ray absorptiometry.
4 Women and men significantly different, $P < 0.001$.
5 Measured with deuterium assay.
6 Measured on the basis of excretion of sodium bromide.

It is consistent with the molecule level of the 5-level model classification described elsewhere (28), but modified to reflect the presence of excess fluid that may accumulate due to pathological reasons. It is clear from Figure 1 that the mass of ExF is a subcomponent of AFM.
eg, ascites. Regardless of how ExF is manifested, for the purposes of calculation it is convenient to consider ExF as a compartment separate from normally hydrated tissue as represented in Figure 1.

In the current model definition, NH_AT consists of stored lipids, essential lipids, water, and solids (some protein and nonosseous mineral). NH_LT tissue includes water, protein, osseous and nonosseous minerals, and essential lipids with some intracellular lipids. The ratio of bone mineral to total protein is assumed to be constant. An analysis of 3-C and 4-C models (24) shows that the error incurred as a result of this assumption is relatively small. Morse and Soeldner (20) concluded that the hydration parameters of adipose tissue and nonadipose tissue in healthy subjects (which is equivalent to NH_LT in the proposed new model) remain largely unchanged in obese or nonobese subjects. Consequently, our model was developed on the basis that in health a given mass of tissue could be associated with fixed proportions of intracellular water (ICW) and extracellular water (ECW) regardless of body composition. The consequence of these fixed hydration parameters is that the ratio of ECW to ICW is constant in a specific tissue. Fixed hydration parameters have the advantage that ExF can be identified by the new model by using 3 whole-body measurements of weight, ECW_{WB}, and ICW_{WB}. The details of this calculation are given in Appendices A and B. During childhood, the water content of adipose tissue is considered to decrease significantly (29, 30). Therefore, the use of this model is limited, at present, to adults, although introduction of age-specific adipose tissue hydration parameters could extend applicability of the model to children.

In contrast with the current model, an estimate of ExF can be obtained by considering the expansion of either ECW_{WB} or TBW_{WB}. These methods generally assume a fixed ratio, such as ECW_{WB}/ICW_{WB}, reflecting normal hydration status for a given population and are given in Table 2.

### Analysis

Because intracellular water (ICW_{WB}) was not measured directly, the difference between TBW and ECW_{WB} was used for calculations requiring the ICW_{WB}. ExF was calculated from the new model with Equation B5 (derived in Appendix B). The tissue

![FIGURE 1. A: The new 3-compartment model comprising normally hydrated adipose tissue mass ($M_{NH_{L,T}}$), normally hydrated lean tissue mass ($M_{NH_{L,T}}$), and excess fluid mass ($M_{ExF}$). B: Relation between the compartments of the new model and standard measures of body composition in terms of lean mass, fat mass, and fat-free mass (FFM).](https://academic.oup.com/ajcn/article-abstract/85/1/80/4649204/1)

<table>
<thead>
<tr>
<th>Method</th>
<th>Definition of ratio</th>
<th>Rearrangement for excess water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed $H_{FFM}$</td>
<td>$K_{H_{ffm}} = \frac{TBW - M_{ExF} \times H_{ExF}}{FFM - M_{ExF}}$</td>
<td>$M_{ExF} = \frac{TBW - K_{H_{ffm}} \times FFM}{H_{ExF} - K_{H_{ffm}}}$</td>
</tr>
<tr>
<td>Fixed $K_{E/I}$</td>
<td>$K_{E/I} = \frac{ECW_{WB} - M_{ExF} \times H_{ExF}}{ICW_{WB}}$</td>
<td>$M_{ExF} = \frac{ECW_{WB} - K_{E/I} \times ICW_{WB}}{H_{ExF}}$</td>
</tr>
<tr>
<td>Fixed $K_{E/T}$</td>
<td>$K_{E/T} = \frac{ECW_{WB} - M_{ExF} \times H_{ExF}}{TBW - M_{ExF} \times H_{ExF}}$</td>
<td>$M_{ExF} = \frac{ECW_{WB} - K_{E/T} \times TBW}{(1 - K_{E/T}) \times H_{ExF}}$</td>
</tr>
</tbody>
</table>

1 The ratio for each method is defined in the second column, including excess water, which may then be rearranged to yield the mass of excess fluid shown in the third column. The constants $K_{H_{ffm}}, K_{E/I}$, and $K_{E/T}$ are derived from data of healthy subjects with $M_{ExF}$ assumed to be zero, i.e., $K_{H_{ffm}} = TBW/FFM, K_{E/I} = ECW_{WB}/ICW_{WB}$, and $K_{E/T} = ECW_{WB}/TBW$. $H_{FFM}$, hydration of fat-free mass; TBW, total body water; $M_{ExF}$, mass of excess fluid; $H_{ExF}$, hydration of excess fluid; FFM, fat-free mass.
hydration parameters were calculated according to Equations C5, C6, and C9-12, which are shown in Appendix C. A summary of all abbreviations is given in Appendix D. Parameter value CIs were obtained through the bootstrap process. One-half of the data records (n = 52) were selected randomly from the total data pool. Each data record selected was replaced in the data pool enabling possible reselection. After retrieving 52 data records (one-half the total number of records), a set of hydration parameters was calculated. The process of random selection and parameter set calculation was repeated an arbitrary 2000 times. The CIs for the hydration parameters were obtained from the SD of the resulting distribution of each parameter.

Box plots were used to identify significant statistical differences in $H_{FPM}$ and the ratios of ECW$_{wb}$ to ICW$_{wb}$ and of ECW$_{wb}$ to TBW in different body composition ranges. Measurement precision was measured for each input measurement as SD/$\sqrt{N}$ (where SD represents the SD of the intrasubject measurement reproducibility). This allowed the precision of ExF to be estimated by propagation of errors, based on a reference man weighing 73 kg and with 20% body fat (31).

RESULTS

After normalization of fat, TBW, ECW$_{wb}$, and ICW$_{wb}$, a clear reduction in body water compartments was observed with increasing body fat content as shown in Figure 2. By linear regression of normalized TBW and ECW$_{wb}$, as defined by Equations C4 and C8 against DXA fat mass, the following parameters were obtained: $a_{TBW} = -0.672$, $b_{TBW} = 0.703$, $a_{ECW} = -0.185$, and $b_{ECW} = 0.266$. Using these regression parameters, the tissue hydration parameters were calculated with Equations C5, C9, C11, and C12. The process was repeated by using fat mass calculated with the Siri equation and the 4-C model. However, these particular methods are not entirely independent of either body weight or TBW and so a small degree of mathematical coupling occurs, which slightly influences bias. The hydration parameters obtained with all 3 methods for fat determination are shown in Table 3 along with those derived by using DXA for comparison. Literature values obtained with the use of in vitro techniques are shown in Table 4. By substitution of the DXA hydration parameters given in Table 3 into Equation B5, the expression for the mass of ExF may be reduced to the following:

$$M_{ExF} = 1.136 \times ECW_{wb} - 0.430 \times ICW_{wb} - 0.114 \times M_{WB}$$

Similarly, the mass of NH_LT given by Equation B2 may be simplified to the following:

$$M_{NH_LT} = 2.725 \times ICW_{wb} + 0.191 \times M_{ExF} - 0.191 \times M_{WB}$$

Rearrangement of Equation A1 leads to the mass of NH_AT, as follows:

$$M_{NH_AT} = M_{WB} - M_{ExF} - M_{NH_LT}$$

Finally, by combining the parameters $H_{TBW}$ and $K_{AR}$ from Equation A8, the fat mass may be determined as follows:

$$M_{Fat} = 0.753 \times M_{NH_AT}$$
TABLE 3
Hydration parameters of normally hydrated lean tissue (NH_LT) and normally hydrated adipose tissue (NH_AT) measured in vivo with 3 different methods\(^1\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obtained by using DXA estimate of fat</th>
<th>Obtained by using the Siri model of fat</th>
<th>Obtained by using a 4-component model of fat (24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_{TW,NH,LT})</td>
<td>0.703 ± 0.009(^2)</td>
<td>0.692 ± 0.007</td>
<td>0.712 ± 0.004</td>
</tr>
<tr>
<td>(H_{TW,NH,AT})</td>
<td>0.197 ± 0.042</td>
<td>0.239 ± 0.030</td>
<td>0.184 ± 0.022</td>
</tr>
<tr>
<td>(H_{ECW,NH,LT})</td>
<td>0.266 ± 0.007</td>
<td>0.262 ± 0.006</td>
<td>0.267 ± 0.006</td>
</tr>
<tr>
<td>(H_{ECW,NH,AT})</td>
<td>0.127 ± 0.015</td>
<td>0.139 ± 0.012</td>
<td>0.126 ± 0.011</td>
</tr>
<tr>
<td>(H_{ICW,NH,LT})</td>
<td>0.437</td>
<td>0.430</td>
<td>0.445</td>
</tr>
<tr>
<td>(H_{ICW,NH,AT})</td>
<td>0.070</td>
<td>0.100</td>
<td>0.058</td>
</tr>
<tr>
<td>(ECWNH,LT)/ICWNH,LT)</td>
<td>0.63</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>(ECWNH,AT)/ICWNH,AT)</td>
<td>1.88</td>
<td>1.43</td>
<td>2.19</td>
</tr>
</tbody>
</table>

\(^1\) \(H_{TW,NH,LT}\), fractional mass of total water in normally hydrated lean tissue; \(H_{TW,NH,AT}\), fractional mass of total water in normally hydrated adipose tissue; \(H_{ECW,NH,LT}\), fractional mass of intracellular water in normally hydrated lean tissue; \(H_{ECW,NH,AT}\), fractional mass of intracellular water in normally hydrated adipose tissue; \(H_{ICW,NH,LT}\), fractional mass of intracellular water in normally hydrated lean tissue; \(H_{ICW,NH,AT}\), fractional mass of intracellular water in normally hydrated adipose tissue; \(ECWNH,LT\)/ICWNH,LT\), ratio of extracellular water in normally hydrated lean tissue to intracellular water in normally hydrated adipose tissue; \(ECWNH,AT\)/ICWNH,AT\), ratio of extracellular water in normally hydrated lean tissue to intracellular water in normally hydrated adipose tissue. 

\(^2\) \(\bar{x} \pm SD\) (all such values). 

\(^3\) Calculated as the difference between total tissue water and the extracellular component. 

\(^4\) Calculated as \(H_{ECW,LT}/H_{ICW,LT}\). 

\(^5\) Calculated as \(H_{ECW,AT}/H_{ICW,AT}\).

Estimated for ECWWB, ICWWB, and \(M_{WB}\) respectively. By propagation of these errors, the overall precision of the new method for calculation of \(M_{WB}\) (Equation B5) was thus found to be \(\sim 0.5\) kg.

The subjects were categorized into 1 of 3 body composition groups according to percentage fat: lean (<20%), normal (20–30%), and obese (>30%). Significant differences were found in the ECW/WB-to-ICW/WB and ECW/WB-to-TBW hydration ratios at the extremes of body composition, as shown in Figure 3. \(H_{FFM}\) did not differ significantly between the lean and obese subgroups but was accompanied by a relatively large variation.

The bias occurring with the use of the different methods for calculation of MExF in the categorical body composition subgroups is shown in Table 5. The difference in MExF between the lean and obese subjects was not significant in results calculated from a fixed \(H_{FFM}\); however, the method was found to be subject to considerable variability within each body-composition group, which was apparent from the SD. Significant underestimation of ExF in lean subjects and overestimation of ExF in obese subjects was observed for methods involving fixed ECW/WB-to-ICW/WB and ECW/WB-to-TBW ratios. In the new model, there was no significant bias between the lean and obese subgroups.

In order to obtain an impression of how the new model performed in pathologic cases, the Equation 2 to Equation 5 were applied to 3 groups of 11 malnourished patients from a previous study (32). The analysis exposed considerable quantities of ExF in these patients, as shown in Table 6. The results indicated an

TABLE 4
Hydration parameters of normally hydrated lean tissue (NH_LT) and normally hydrated adipose tissue (NH_AT) from the literature\(^1\)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>(H_{TW,NH,LT})</td>
<td>0.8 ± 0.032(^2)</td>
<td>0.689 ± 0.023 (obese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.697 ± 0.024 (nonobese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(H_{TW,NH,AT})</td>
<td>0.14 ± 0.014</td>
<td>0.151 ± 0.052 (obese)</td>
<td>0.25§</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.154 ± 0.048 (nonobese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(H_{ICW,NH,LT})</td>
<td>0.24 ± 0.017</td>
<td>0.233 ± 0.023 (obese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.248 ± 0.02 (nonobese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(H_{ICW,NH,AT})</td>
<td>0.11 ± 0.011</td>
<td>0.132 ± 0.054 (obese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.118 ± 0.041 (nonobese)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(H_{ECW,NH,LT})</td>
<td>0.56</td>
<td>0.45</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(H_{ECW,NH,AT})</td>
<td>0.03</td>
<td>0.03</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(ECWNH,LT)/ICWNH,LT)</td>
<td>0.43</td>
<td>0.53</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(ECWNH,AT)/ICWNH,AT)</td>
<td>3.67</td>
<td>4.00</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) \(H_{TW}\), fractional mass of total water; \(H_{ECW}\), fractional mass of extracellular water; \(H_{ICW}\), fractional mass of intracellular water. Some authors used the term “non-adipose tissue,” which is equivalent to NH_LT in studies of healthy subjects in which no excess fluid is assumed to be present.

\(^2\) \(\bar{x} \pm SD\) (all such values).

\(^3\) Mean value of adipose tissue hydration.

\(^4\) Calculated as the difference between total tissue water and the extracellular component.
is that the hydration parameters in a group of healthy control subjects may be established by using any alternative dilution reference of choice such as total body potassium (TBK). Nevertheless, the hydration parameters obtained in our study for NH_LT and NH_AT were in the range reported in other studies (19, 20, 30, 33). NH_AT was found to have a much lower water content but a higher ratio of ECW to ICW than did NH_LT, which is consistent with the findings of others (19, 20). By definition, \( H_{TW,NH_LT} \) cannot exceed \( H_{FFM} \) because adipose water is included in FFM but not in NH_LT. Because the range of \( H_{FFM} \) in healthy control subjects is 0.69–0.77 (16), the value of 0.8 for \( H_{TW,NH_LT} \) obtained by Wang et al (19) may be slightly overestimated.

Although Morse and Soeldner (20) observed no difference in the hydration of NH_AT between obese and nonobese subjects, as seen in Table 4, a more recent study by Martin et al (33) indicated the contrary. This suggests that more detailed investigation of the hydration properties of adipose tissue may be necessary in differing degrees of obesity, not only in terms of total water content but also in terms of intra- and extracellular phases.

The model developed in the present study assumed fixed hydration parameters for adipose tissue, and Figure 2 indicates that this serves as a good approximation to the measured data. Furthermore, an improvement in the reproducibility of the measurement methods is necessary before a more complicated model of adipose tissue can be justified.

It is evident from Figure 2 that whereas the ECW/WW in clearly lower than the ICW/WW when NH_LT dominates body weight, the converse is observed as NH_AT becomes the principle body weight component. This would appear to be the basis of the relative expansion of ECW/WW in obese subjects observed in other studies (27). As the ratio of ECW to ICW in NH_LT is at least twice that of NH_LT, then the whole-body proportions of NH_LT and NH_AT would explain the significant differences in the ratios of ECW/WW to ICW/WW and ECW/WW to TBW between the lean and obese subjects, as shown by categorical divisions of body fat (Figure 3).

Although the fat content of female subjects tends to be higher than that of male subjects, as seen in Table 1, these differences are reflected in the relative proportions of NH_LT and NH_AT.

**TABLE 5**

Comparison of different methods to calculate excess fluid (\( M_{ExF} \)) in lean, normal, and obese body-composition subgroups.

<table>
<thead>
<tr>
<th>( M_{ExF} ) method</th>
<th>Lean: &lt;20% fat (n = 20)</th>
<th>Normal: 20–30% fat (n = 40)</th>
<th>Obese: &gt;30% fat (n = 44)</th>
<th>( \Delta ) Bias (lean - obese)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECW/WW: ICW/WW, ( M_{WW} )(^3)</td>
<td>(-0.28 \pm 1.76^4)</td>
<td>(-0.08 \pm 2.34)</td>
<td>(0.04 \pm 2.35)</td>
<td>(-0.31)</td>
<td>0.637</td>
</tr>
<tr>
<td>( H_{FFM} )</td>
<td>(-0.33 \pm 8.27)</td>
<td>(-1.78 \pm 6.80)</td>
<td>(0.84 \pm 6.15)</td>
<td>(-1.17)</td>
<td>0.574</td>
</tr>
<tr>
<td>ECW/WW: ICW/WW, ( M_{WW} )(^5)</td>
<td>(-2.46 \pm 1.97)</td>
<td>(-0.79 \pm 2.84)</td>
<td>(0.83 \pm 2.70)</td>
<td>(-3.29)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ECW/WW: TBW(^5)</td>
<td>(-2.75 \pm 3.19)</td>
<td>(-0.56 \pm 2.76)</td>
<td>(0.98 \pm 2.63)</td>
<td>(-3.73)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\(^1\) ECW/WW, mass of whole-body extracellular water; MCW/WW, mass of whole-body intracellular water; \( M_{WW} \), body weight; \( H_{FFM} \), hydration of fat-free mass. Because healthy subjects were measured in this study, the \( M_{ExF} \) should be close to zero with no significant bias in the body-composition (in terms of relative fat) subgroups. All models exhibit a degree of scatter (SD), which reflects a combination of differences in body hydration between the subjects due to fluid gain and excretion as well as random measurement errors. In some models, particularly \( M_{ExF} (H_{FFM}) \), random measurement errors propagate to large variations in \( M_{ExF} \).

\(^2\) Comparison of lean with obese subjects.

\(^3\) Calculated with the parameters given in Table 3.

\(^4\) SD (all such values).

\(^5\) Calculated from the expressions given in Table 2 and based on fixed whole-body-ratios of \( K_{E,1} = 0.759 \), \( K_{Fi,1} = 0.727 \), and \( K_{E,T} = 0.429 \) derived from the mean values of the study data.
Therefore, there was no need to differentiate by sex in our model, because body composition (in terms of fat content) is taken into account from the input measurements of ECW\textsubscript{WB}, ICW\textsubscript{WB}, and body weight. It is also unlikely that age contributes fundamentally to the ratio of ECW\textsubscript{WB} to ICW\textsubscript{WB}, but is simply due to the proportions of NH\textsubscript{LT} and NH\textsubscript{AT}, as suggested by Wang et al. It can be argued that age-associated increases in fatness may lead to a higher proportion of NH\textsubscript{AT}.

In our study, the mean value of H\textsubscript{FFM} was found to be 0.723, which is consistent with the findings of others (24, 34, 35). In circumstances where ExF accumulates, a rise in H\textsubscript{FFM} can be expected (16), but the lack of sensitivity of H\textsubscript{FFM} to variations in ExF has not been emphasized in past studies. This can be illustrated considering, for example, a subject with 55 kg FFM and 40 L TBW, which leads to a H\textsubscript{FFM} of 0.727. If this subject now gains 5 kg ExF, H\textsubscript{FFM} rises to 0.750, a change of just 3%. It is clear that because ExF appears in both the FFM and the TBW, the effect of ExF largely cancels out in H\textsubscript{FFM}. This renders H\textsubscript{FFM} a poor choice for providing a reliable hydration reference against which any ExF can be detected.

A significant difference in the ECW\textsubscript{WB}-to-ICW\textsubscript{WB} and ECW\textsubscript{WB}-to-TBW hydration ratios was observed between the lean and obese subgroups, as seen in Figure 3. A similar rise in ratio of ECW\textsubscript{WB} to ICW\textsubscript{WB} has been observed in other studies with increasing BMI (27) and with increasing age (36). Therefore, any method for estimation of ExF that assumes fixed ECW\textsubscript{WB}-to-ICW\textsubscript{WB} or ECW\textsubscript{WB}-to-TBW values for the entire population will result in bias errors at extremes of relative fat, as shown in Table 5. In the method described by Lopot et al (14), such effects of body composition have been taken into account by introduction of corrections for age and sex in the ratio of ECW\textsubscript{WB} to TBW.

Considerable quantities of ExF were found to be present on application of the new model in a previous study of malnourished patients (32), as seen in Table 6. These results are reasonable given the high ratios of ECW to ICW resulting in these subjects coupled with the observation that a significant decrease in the ratio of plasma volume to ECW occurred in the severely malnourished subgroup (32). Methods to obtain the mass of LT or FFM via DXA, for example, do not differentiate ExF from these tissues (37). If a large proportion of the LT or FFM is occupied by ExF, the estimated protein content of these tissues will be reduced. This could lead to ambiguous conclusions regarding nutritional status. In the new model, by contrast, the mass of NH\textsubscript{LT} applies regardless of the degree of ExF presented; that is, removal or accumulation of large volumes of fluid do not change the mass of NH\textsubscript{LT}. It is proposed, therefore, that estimation of NH\textsubscript{LT} mass may offer a more reliable alternative to measures such as LBM and FFM in disease.

**APPENDIX A**

**General model definitions**

Whole-body mass (M\textsubscript{WB}) is given by the sum of the 3 compartments, namely normally hydrated adipose tissue mass (M\textsubscript{NH,AT}), normally hydrated lean tissue mass (M\textsubscript{NH,LT}), and excess fluid (ExF) mass (M\textsubscript{ExF}), as follows:

\[
M_{WB} = M_{NH,AT} + M_{NH,LT} + M_{ExF} \quad (A1)
\]

The total water (TW), intracellular water (ICW), and extracellular water (ECW) components of M\textsubscript{NH,LT} and M\textsubscript{NH,AT} are defined by Equations A2–A4 and A5–A7, respectively.

\[
H_{TW, NH, LT} = M_{TW, NH, LT}/M_{NH, LT} \quad (A2)
\]

\[
H_{ECW, LT} = M_{ECW, NH, LT}/M_{NH, LT} \quad (A3)
\]

\[
H_{ICW, NH, LT} = M_{ICW, NH, LT}/M_{NH, LT} \quad (A4)
\]

\[
H_{TW, NH, AT} = M_{TW, NH, AT}/M_{NH, AT} \quad (A5)
\]

\[
H_{ECW, NH, AT} = M_{ECW, NH, AT}/M_{NH, AT} \quad (A6)
\]

\[
H_{ICW, NH, AT} = M_{ICW, NH, AT}/M_{NH, AT} \quad (A7)
\]

The fat mass (M\textsubscript{Fat}) is related to the mass of normally hydrated adipose tissue (M\textsubscript{NH,AT}) with equation A8:

\[
M_{Fat} = M_{NH, AT} - M_{NH, AT} \times H_{TW, NH, AT} - M_{NH, AT} \times K_{AR}
= M_{NH, AT} \times (1 - H_{TW, NH, AT} - K_{AR}) \quad (A8)
\]

where K\textsubscript{AR} is the ratio of residual adipose components (solids, mainly protein and mineral) to M\textsubscript{NH,AT} with a value of typically 0.05 (30, 33).
APPENDIX B

Calculation of excess fluid

The sum of the intracellular components of $M_{\text{NH,LT}}$ and $M_{\text{NH,AT}}$ yields ICW_{WB}, as given by Equation B1:

$$ICW_{WB} = M_{\text{ICW,LT}} + H_{\text{ICW,LT}} × M_{\text{NL,AT}}$$

Introducing the intracellular water mass fraction of normally hydrated lean tissue ($H_{\text{ICW,LT}}$), substituting $M_{\text{NL,AT}}$ by Equation A1 and rearranging for the lean tissue mass $M_{\text{NL,LT}}$ leads to the following equation:

$$M_{\text{NL,LT}} = [ICW_{WB} + H_{\text{ICW,AT}}] × (M_{\text{Fat}} - M_{\text{WB}}) / (H_{\text{ICW,LT}} - H_{\text{ICW,AT}})$$

By introducing relevant constants, the mass of excess water ($M_{\text{ExW}}$ not to be confused with $M_{\text{ExF}}$) may be calculated as the difference between ECW_{WB} and the sum of the extracellular components of NH_AT and NH_LT. By substituting of $H_{\text{ECW,LT}}$ and $H_{\text{ECW,AT}}$ in the respective tissues then gives the following equation:

$$M_{\text{ExF}} = ECW_{WB} - H_{\text{ECW,LT}} × M_{\text{NL,LT}}$$

Because ExF contains dissolved proteins and minerals in addition to ExW, a factor $H_{\text{ExF}}$ may be applied which denotes the ratio of the mass of excess water, $M_{\text{ExW}}$ to the mass of excess fluid, $M_{\text{ExF}}$. A mean value for $H_{\text{ExF}}$ of 0.98 was assumed in the current model as suggested by Wang et al (16). Introducing $H_{\text{ExF}}$ to convert $M_{\text{ExW}}$ to $M_{\text{ExF}}$ and rewriting Equation B3 by re-expressing $M_{\text{NL,LT}}$ from Equation B1 leads to the following equation:

$$M_{\text{ExF}} = [ECW_{WB} - H_{\text{ECW,LT}} × M_{\text{NL,LT}} - H_{\text{ECW,AT}} × M_{\text{NL,AT}}] × (M_{\text{WB}} - M_{\text{NL,LT}} - M_{\text{Fat}})/H_{\text{ExF}}$$

Substituting the expression for $M_{\text{NL,LT}}$ (Equation B2) into Equation B4 and rearranging for $M_{\text{ExF}}$ yields the following equation:

$$M_{\text{ExF}} = [ECW_{WB} - H_{\text{ECW,AT}} × M_{\text{WB}} + k × (ICW_{WB} - H_{\text{ICW,AT}} × M_{\text{WB}})] / H_{\text{ExF}}$$

where $k$ is defined by the following:

$$k = (H_{\text{ECW,LT}} - H_{\text{ECW,AT}}) / (H_{\text{ICW,LT}} - H_{\text{ICW,AT}})$$

APPENDIX C

Calculation of principal body-composition parameters

The purpose of the following derivation was to extract the principal body-composition parameters defined by Equations A2–A7. To proceed, it was assumed that the ExF in healthy control subjects has a mean value of zero, i.e., $M_{\text{ExF}} = 0$. By introducing Equation A1, the mass of total body water, TBW, may be expressed as follows:

$$TBW = H_{\text{TW,LT}} × M_{\text{NL,AT}} + H_{\text{TW,LT}} × M_{\text{NL,LT}}$$

By rewriting Equation C1 in terms of measurable input quantities via Equations A1 and A8, with $M_{\text{ExF}} = 0$, then the following equation is obtained:

$$TBW = (H_{\text{TW,LT}} - H_{\text{TW,AT}})/(1 - K_{AR}) × M_{\text{Fat}} + H_{\text{TW,LT}} × M_{\text{WB}}$$

The normalized TBW and fat, $N_{\text{TBW}}$ and $N_{\text{Fat}}$, respectively, are obtained by dividing Equation C2 by $M_{\text{WB}}$, which leads to the following equation:

$$N_{\text{TBW}} = (H_{\text{TW,LT}} - H_{\text{TW,AT}})/(1 - K_{AR}) × N_{\text{Fat}} + H_{\text{TW,LT}}$$

By normalizing the measured $M_{\text{Fat}}$ and TBW data to body weight, the following linear relation between $N_{\text{Fat}}$ and $N_{\text{TBW}}$ was readily derived from regression analysis:

$$N_{\text{TBW}} = a_{\text{TBW}} × N_{\text{Fat}} + b_{\text{TBW}}$$

where $a_{\text{TBW}}$ and $b_{\text{TBW}}$ are the regression coefficients. Because Equations C4 and C5 are equivalent expressions, the tissue parameters $H_{\text{TW,LT}}$ and $H_{\text{TW,AT}}$ could thus be solved by comparing coefficients, as follows:

$$a_{\text{TBW}} = (H_{\text{TW,LT}} - H_{\text{TW,AT}})/(1 - H_{\text{TW,LT}} - K_{AR})$$

$$b_{\text{TBW}} = H_{\text{TW,LT}}$$

$H_{\text{TW,LT}}$ and $H_{\text{TW,AT}}$ are obtained directly from Equations C5 and C6.

The ECW parameters were obtained in the same way by normalization of ECW_{WB} and $M_{\text{Fat}}$ to body weight. Taking the form of Equation C5 and making appropriate substitutions leads to the following equation:

$$N_{\text{ECW}} = (H_{\text{ECW,LT}} - H_{\text{ECW,AT}}) × [N_{\text{Fat}}/(1 - H_{\text{TW,LT}} - K_{AR})] + H_{\text{ECW,LT}}$$

The equivalent regression equation involving ECW may be written as follows:

$$N_{\text{ECW}} = a_{\text{ECW}} × N_{\text{Fat}} + b_{\text{ECW}}$$
By comparing coefficients of Equation C8 with those of Equation C7, then we obtain the following equations:

\[ a_{ECW} = (H_{ECW,NH,LT} - H_{ECW,NH,LT})/(1 - H_{TW,NH,LT} - K_{AR}) \]  
(\text{C9})

\[ b_{ECW} = H_{ECW,NH,LT} \]  
(\text{C10})

\[ H_{ECW,NH,LT} \text{ and } H_{ECW,NH,AT} \text{ are obtained from Equations C9 and C10. Finally, the parameters } H_{ECW,NH,LT} \text{ and } H_{ECW,NH,AT} \text{ can be readily found with the following equations:} \]

\[ H_{ICW,NH,LT} = H_{TW,NH,LT} - H_{ECW,NH,LT} \]  
(\text{C11})

\[ H_{ICW,NH,AT} = H_{TW,NH,AT} - H_{ECW,NH,AT} \]  
(\text{C12})

APPENDIX D

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{BW}</td>
<td>Density of body water</td>
</tr>
<tr>
<td>ECW</td>
<td>Extracellular water of an arbitrary tissue</td>
</tr>
<tr>
<td>ECW_{WB}</td>
<td>Mass of whole-body extracellular water</td>
</tr>
<tr>
<td>ExF</td>
<td>Excess fluid</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat-free mass</td>
</tr>
<tr>
<td>H_{ECW,NH,AT}</td>
<td>Fractional mass of extracellular water in normally hydrated adipose tissue</td>
</tr>
<tr>
<td>H_{ECW,NH,LT}</td>
<td>Fractional mass of extracellular water in normally hydrated lean tissue</td>
</tr>
<tr>
<td>H_{ExF}</td>
<td>Hydration of excess fluid</td>
</tr>
<tr>
<td>H_{ExM}</td>
<td>Hydration of fat-free mass</td>
</tr>
<tr>
<td>H_{ICW,NH,AT}</td>
<td>Fractional mass of intracellular water in normally hydrated adipose tissue</td>
</tr>
<tr>
<td>H_{ICW,NH,LT}</td>
<td>Fractional mass of intracellular water in normally hydrated lean tissue</td>
</tr>
<tr>
<td>H_{TW,NH,AT}</td>
<td>Fractional mass of total water in normally hydrated adipose tissue</td>
</tr>
<tr>
<td>H_{TW,NH,LT}</td>
<td>Fractional mass of total water in normally hydrated lean tissue</td>
</tr>
<tr>
<td>ICW</td>
<td>Intracellular water of an arbitrary tissue</td>
</tr>
<tr>
<td>ICW_{WB}</td>
<td>Mass of whole-body intracellular water</td>
</tr>
<tr>
<td>K_{AR}</td>
<td>Ratio of residual adipose components to M_{NH,AT}</td>
</tr>
<tr>
<td>K_{ECW,ICW}</td>
<td>Fixed ratio of ECW_{WB} to ICW_{WB}</td>
</tr>
<tr>
<td>K_{ECW,TBW}</td>
<td>Fixed ratio of ECW_{WB} to TBW</td>
</tr>
<tr>
<td>K_{FFM}</td>
<td>Fixed ratio of TBW to FFM, equivalent to a mean value of H_{FFM} in a control population</td>
</tr>
<tr>
<td>M_{ExF}</td>
<td>Mass of excess fluid</td>
</tr>
<tr>
<td>M_{ExW}</td>
<td>Mass of excess water</td>
</tr>
<tr>
<td>M_{fat}</td>
<td>Fat mass</td>
</tr>
<tr>
<td>M_{NH,AT}</td>
<td>Mass of normally hydrated adipose tissue</td>
</tr>
<tr>
<td>M_{NH,LT}</td>
<td>Mass of normally hydrated lean tissue</td>
</tr>
<tr>
<td>M_{WB}</td>
<td>Body weight</td>
</tr>
<tr>
<td>N_{b-fat}</td>
<td>Fat mass normalized to body weight</td>
</tr>
<tr>
<td>NH_{AT}</td>
<td>Normally hydrated adipose tissue</td>
</tr>
<tr>
<td>NH_{LT}</td>
<td>Normally hydrated lean tissue</td>
</tr>
<tr>
<td>TBW</td>
<td>Total body water</td>
</tr>
<tr>
<td>TW</td>
<td>Total water content of an arbitrary tissue</td>
</tr>
</tbody>
</table>

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PWC and PW developed the concepts for excess fluid calculation and prepared the manuscript. UMM contributed to technical aspects of the data analysis. MJM organized the study in Kiel and made a number of recommendations to simplify the manuscript. AB-W and OK undertook the studies in Kiel, performed all of the measurement assays and provided valuable scientific support. NJF contributed to refinement of the concepts, and input for alterations to the draft manuscript. PWC, PW, and UMM are employed at the Research and Development department at Fresenius Medical Care. None of the other authors has a conflict of interest to disclose.

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