Increased extracellular water compartment, relative to intracellular water compartment, after weight reduction

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OBESITY IS CHARACTERIZED by alterations in body composition. A part from an increase in fat mass (FM) and, to a lesser extent, increased fat-free mass (FFM), the extracellular water (ECW) compartment is enlarged compared with the intracellular water (ICW) compartment. Proposed explanations for the high ECW/ICW ratio are a high ECW/ICW of the adipose tissue (4, 19), an obesity-related edema, and hormonal responses related to adipose tissue (18). The observation that the ECW/ICW ratio does not normalize after weight reduction by surgery, and even increases after malabsorptive surgery (14, 21), led to the conclusion that obesity may be accompanied by a primary defect in fluid regulation and that morbid obesity leads to irreversible changes in the hemodynamic or fluid regulation. However, after surgical treatment, the ECW may have been expanded relative to ICW by other factors, e.g., malnutrition. Alternatively, decreased FFM may be a result of cell shrinking (8), which, in turn, results in a decrease of ICW.

To our knowledge, short- and long-term effects of weight reduction by diet and exercise on the water compartments have never been studied. Such a study would provide a valuable addition to the studies involving surgical treatment by increasing our understanding of fluid regulation in the obese. Therefore, we investigated the hydration of FFM and the ECW and ICW compartments in obese premenopausal women before and after a 12-wk weight-reduction program and again after a 9-mo weight-maintenance program.

MATERIALS AND METHODS

Participants and general study design. Thirty overweight women (body mass index range: 30–46 kg/m2; age range: 30–45 yr) volunteered for this study, which was approved by the Ethical Committee of the Urho Kaleva Kekkonen Institute for Health Promotion Research, Tampere, Finland. Written informed consent was obtained from all volunteers. All subjects were participants in a 3-mo weight-reduction program, including 2 mo of very-low-energy diet (Nutrilett, Nycomed Pharma, Oslo, Norway), followed by a 9-mo weight-maintenance program. The postweight reduction measurements were carried out 4–7 days after the low-energy diet was stopped and more than 3 wk after the very-low-energy diet was stopped. All subjects had been weight stable (± 3 kg) for at least 3 mo before the study. The subjects were not taking medication or oral contraceptives. Three subjects used an estrogen-releasing coil. The phase of the menstrual cycle was recorded. Measurement time points were not synchronized with respect to the women’s menstrual cycle. None of the subjects was physically active, smoking, pregnant, or lactating.

Weight and underwater weighing (UWW). Body weight was measured in underwear, after an overnight fast, on a high-precision scale (Sartorius F150S-D2, Goettingen, Germany). Body volume was measured by UWW after full exhalation (see Ref. 6). The residual lung volume was measured two to four times before the UWW with helium-dilution method. Body density was calculated by combining the results from UWW and body mass (BM).

Dual-energy X-ray absorptiometry (DEXA). Total bone mineral content (TBMC) was determined by a DEXA scanner (XR-26, Norland, Fort Atkinson, WI), as described earlier (5). Bone content was calculated by Norland total body composition scan software (version 2.5.2). According to repeated measurements at the Urho Kaleva Kekkonen Institute of 18 subjects, the in vivo coefficient of variation of the TBMC was 1.5%. The scanner was calibrated daily, and its performance was controlled with a quality-assurance program (17).

Total body water (TBW). TBW was determined by deuterium (\( \text{H}_2\text{O} \)) dilution, following the method described by Westerterp et al. (20). Subjects received an orally administered dose of \( \text{H}_2\text{O} \) of ~0.1 g/l estimated TBW. The appropriate amount of \( \text{H}_2\text{O} \) (99.8%, Akademie der Wissenschaften, Leipzig, Germany) was weighed out and diluted with tap water to 0.075 liter for intake. \( \text{H}_2\text{O} \) enrichment in the body fluid was measured in urine. Before dose administration,
background urine samples were taken. The dose was given at 2200. Urine samples were taken after 10 h of dose administration from the second voiding (first voiding at ~0700). Isotope abundances in urine were determined in duplicate with an isotope ratio mass spectrometer (Aqua Sira, Isogas, Cheshire, UK). TBW was calculated as the $^{2}$H$_2$O dilution space divided by 1.04, correcting for exchange of the $^{2}$H$_2$O label with nonaqueous hydrogen of body solids (16).

ECW and ICW compartments. The ECW compartment was determined by bromide dilution. A known amount of sodium bromide (60 mg Br/l estimated TBW; Genfarma, Maarssen, The Netherlands) was mixed with the $^{2}$H$_2$O solution and thus administered simultaneously with the $^{2}$H$_2$O dose. Venous blood samples were obtained before intake and 10 h after ingestion of bromide (12). Bromide concentration in serum ultrafiltrate was determined with HPLC (15). Corrected bromide space was calculated according to Miller et al. (15).

ICW was determined by subtracting ECW from TBW.

Body composition models. Body fat was calculated by the four-component model (4C) using BM (in kg), body density (D$_{p}$, in g/cm$^3$) from UWW, TBW (in liters) by $^{2}$H$_2$O dilution, and TBMC (in kg) by DEXA. Multicomponent models are more accurate than the classic two-component (UWW) models, since these models account for interindividual variation in the water and mineral content of the fat-free body (11). FM (in kg) was according to the formula given by Fuller et al. (7)

\[
FM = 2.747 \text{BM} / D_{p} - 0.71 \text{TBW} + 1.46 \text{TBMC} - 2.05 \text{BM}
\]

The three-component model (3Cb) was also used to determine the hydration of the FFM. The 3Cb model reflects the hydration of the FFM, in which the TBW and FFM were determined independently. The hydration fraction is calculated by combining BM, TBW (by $^{2}$H$_2$O dilution), TBMC (by DEXA), and body volume (BV; by UWW)

\[
\text{Hydration fraction} = \frac{\text{TBW (by dilution)}}{\text{FFM (by 3Cb)}}
\]

Another way of calculating the hydration of FFM is applied as described by Fuller et al. (7), but there the numerator and the denominator are not completely independent. The hydration is calculated by combining BM, TBW (by $^{2}$H$_2$O dilution), TBMC (by DEXA), and body volume (BV; by UWW)

\[
\text{Hydration fraction} = \frac{\text{TBW/0.715 TBW + 3.070 BM}}{2.765 \text{BV} - 1.469 \text{TBMC}}
\]

Statistical analyses. Pearson correlation coefficients were used to test associations between variables. A repeated-measures one-way ANOVA was used to detect differences in any variables between baseline, month 3, and month 12. When significant differences were found, a Fisher post hoc test was used to determine the exact location of this difference. All results are expressed as means ± SD.

RESULTS

Body weight and body composition. Subject characteristics at the start of the experiments are shown in Table 1. The body mass index was on average 34.6 kg/m$^2$ (range: 30.1–45.6 kg/m$^2$), and the percentage body fat averaged to 45% (range: 36–52%).

All women lost weight during the weight-reduction period of 3 mo (Table 1). The average weight loss amounted to 12.8 kg, with a minimum weight loss of 7.1 kg and a maximum of 20.8 kg. During the 9-mo weight-maintenance period, the average weight change was not significant, with individual variation ranging from weight loss of 10.5 kg to a weight gain of 8.2 kg. During the whole period (weight loss plus weight maintenance), weight loss amounted to an average of 13.3 kg (range: 1.3–28.6 kg).

Mean FM was reduced from 42.0 kg at the start to 31.1 kg after 3 mo. The average difference of 10.9 kg shows that FM reduction amounted to 85.2% of the weight loss. During the weight-maintenance period, the FM loss was not significant (average 0.1 kg). During the whole study period, FM loss was on average 11.0 kg.

The change in BM during weight reduction was not related to the BM at the start. However, over the course of the whole study period (12 mo), the reduction in weight was significantly related to the BM at the start ($\Delta = -0.25 \text{BM} + 10.37$, where $\Delta$ is change; $r = 0.37, P < 0.05$).

Water compartments. At the start of the experiments (month 0), the ECW/CW amounted to 0.78 ± 0.10, and the hydration of the FFM was 0.74 ± 0.1.

Although body weight and body fat showed a clear reduction during the weight-reduction program and remained the same after the weight-maintenance period, the water compartments showed a different picture. TBW did not change significantly during weight reduction and weight maintenance. Neither did ECW. After the 3 mo of weight reduction. On the contrary, 12 mo after the start, ECW was significantly increased from 16.7 liters at the start and 17.0 liters at 3 mo to 17.7 liters (Table 1). The mean increase in ECW was 0.71 ± 1.1 liters during weight maintenance and 1 ± 1.7 liters during the whole study period.

### Table 1. Body composition and water compartmentation of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 3 mo</th>
<th>After 12 mo</th>
<th>P Value by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>93.3 ± 10.6</td>
<td>80.5 ± 10.4*</td>
<td>80.0 ± 10.5*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>34.6 ± 4.0</td>
<td>29.8 ± 3.9*</td>
<td>29.7 ± 3.8*</td>
<td>0.0001</td>
</tr>
<tr>
<td>TBW, liters</td>
<td>38.3 ± 2.9</td>
<td>35.3 ± 3.5</td>
<td>38.2 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>ECW, liters</td>
<td>16.7 ± 1.9</td>
<td>17.0 ± 1.2</td>
<td>17.7 ± 1.6†</td>
<td>0.005</td>
</tr>
<tr>
<td>ICW, liters</td>
<td>21.6 ± 1.9</td>
<td>21.3 ± 3.0</td>
<td>20.5 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>ECW/ICW</td>
<td>0.78 ± 0.10</td>
<td>0.81 ± 0.11</td>
<td>0.87 ± 0.12*</td>
<td>0.01</td>
</tr>
<tr>
<td>Hydration FFM</td>
<td>0.74 ± 0.01</td>
<td>0.77 ± 0.02*</td>
<td>0.77 ± 0.03*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hydration FFM</td>
<td>0.74 ± 0.03</td>
<td>0.81 ± 0.06*</td>
<td>0.83 ± 0.07*</td>
<td>0.0001</td>
</tr>
<tr>
<td>FM 3Cb, kg</td>
<td>41.6 ± 8.0</td>
<td>33.1 ± 8.6*</td>
<td>33.5 ± 9.3*</td>
<td>0.0001</td>
</tr>
<tr>
<td>FM 4C, kg</td>
<td>42.0 ± 8.0</td>
<td>31.1 ± 8.1*</td>
<td>31.0 ± 8.6*</td>
<td>0.0001</td>
</tr>
<tr>
<td>FM 4C, %</td>
<td>44.7 ± 4.1</td>
<td>38.0 ± 5.7*</td>
<td>38.1 ± 6.2*</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SD. TBW, total body water; ECW, extracellular water; ICW, intracellular water; FFM, fat-free mass; FM, fat mass; 3Cb, 3-component model; 4C, 4-component model. *Significant difference compared with baseline (month 0); †significant difference compared with month 3. NS, not significant.
ICW tended to decrease during the course of the study, but the reduction was not significant. The ECW/ICW ratio did not change significantly during weight reduction but was significantly increased at the end of the whole study (mean increase: 0.1 ± 0.16). The increase in ECW/ICW was significantly positively related to the change in FM from month 0 to month 3 (ΔECW/ICW = 0.02 ΔFM + 0.25, r = 0.36, P < 0.05). Finally, the hydration of the FFM increased significantly during weight reduction, from a mean of 74 ± 1 to 77 ± 2% [calculated according to Fuller et al. (7)], or 0.81 [calculated according to the ratio TBW/FFM (3Cb)]. After 9 mo of weight maintenance, the hydration of FFM was still significantly higher, compared with the start of the study.

The hydration of FFM (at baseline, month 3, or month 12) was significantly (P < 0.02) related to the percentage body fat, when the 3Cb method was used for assessment of body fat. These methods, however, are not fully independent, because the hydration of FFM also affects the validity of assumptions underlying the use of the 3Cb method. Moreover, there was no relation when the 4C model (also not completely independent from the hydration) or the body fat from DEXA was used.

DISCUSSION

Because, in the obese, ECW/ICW ratio is elevated compared with controls (18), it would at first seem logical that after weight reduction the ECW/ICW ratio would drop. Contrary to these expectations, we found an increase in the ECW/ICW ratio up to a year after the start of the 3-mo weight-reduction program followed by 9 mo of weight maintenance. Thus the ratio between these compartments did not “normalize.”

The hydration of the FFM increased after weight reduction, but, surprisingly, did not return to baseline levels after 9 mo of weight maintenance.

As in other studies carried out with obese subjects, we found high levels of ECW/ICW (average value at the start of the study: 0.78 ± 0.18) compared with normal controls (Table 2). In the six studies indicated in Table 2, mean ECW/ICW ratios of obese subjects ranged from 0.76 to 0.89 and in controls from 0.60 to 0.68. The lowest values are those of children, but differences between obese children and controls are similar to those in adults. An interesting finding is that the ECW/ICW variation in the adult studies was much larger in the obese than in the lean controls (see the SD values in Table 2). This suggests that the obese subjects are a much more heterogeneous group than the lean subjects with respect to water compartmentation.

This study is the first in which ECW and ICW were determined independently in obese subjects who underwent a weight-reduction program, although it is not ideal that ICW is calculated by the difference between TBW and ECW. It would be better to determine ICW independently. However, such a method is not readily available. What makes this study interesting is that a successful weight-maintenance period followed after the weight-reduction program. This minimized the direct effects of weight reduction (e.g., undernutrition). Notwithstanding these facts, we found an increase in ECW/ICW during the weight-maintenance period (Table 1). Previous studies looking at ECW/ICW ratio after weight reduction were special cases of morbidly obese subjects, who underwent surgical treatment (14, 21). Only in the study of Mazariegos et al. (14), the ECW and ICW were determined independently. Both studies showed an increase in the ECW/ICW after surgical treatment. Mazariegos et al. found a change after a gastric restriction operation from 0.81 ± 0.14 to 0.83 ± 0.14 and after a malabsorption operation from 0.82 ± 0.20 to 1.09 ± 0.25. Zimmerman et al. (21) report a change from 0.89 ± 0.18 (preoperative) to 1.07 ± 0.43 at 6 mo after gastroplasty.

Relative expansion of the ECW can possibly be explained by malnutrition, the ECW/ICW ratio of the adipose tissue, a defect in hemo- and fluid regulation, cell shrinking, and/or an insulin-mediated sympathetic stimulation.

ECW/ICW ratio can be an indicator of nutritional status. The few studies carried out on body composition in total starvation show that the changes in body content include a large decrease in FM, a decrease in body cell mass (or ICW), and no changes in absolute ECW, but, because of the reduced body weight, a relative increase in ECW (1, 9). In our study, we found comparable average results. However, from our data and from the other studies mentioned, it is clear that there is a very large variation in the ECW/ICW ratio in the obese subjects. After weight reduction, the variation is even larger. The general usefulness of the ECW/ICW ratio as an indicator of nutritional status for the individual must thus be questioned. Moreover, since ECW/ICW significantly increased even during the weight-maintenance period, malnutrition is unlikely to be only explanation of the ECW/ICW changes we report.

Table 2. ECW/ICW comparison between obese and lean subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Obese</th>
<th>Controls</th>
<th>n</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>0.78 ± 0.18</td>
<td>0.68 ± 0.04</td>
<td>30</td>
<td>Bromide, H2O2</td>
<td>This study, controls: Ref. 13</td>
</tr>
<tr>
<td>Women</td>
<td>0.77 ± 0.08</td>
<td>0.6 ± 0.08</td>
<td>25</td>
<td>Bromide, H2O2</td>
<td>3</td>
</tr>
<tr>
<td>Men, women</td>
<td>0.89 ± 0.18</td>
<td>0.6 ± 0.18</td>
<td>17:6</td>
<td>Bromide, H2O2</td>
<td>21</td>
</tr>
<tr>
<td>Women</td>
<td>0.82 ± 0.17</td>
<td>0.63 ± 0.06</td>
<td>25</td>
<td>35SO4, H2O2</td>
<td>14</td>
</tr>
<tr>
<td>Women</td>
<td>0.81 ± 0.18</td>
<td>0.63 ± 0.07</td>
<td>26</td>
<td>35SO4, H2O2</td>
<td>18</td>
</tr>
<tr>
<td>Children</td>
<td>0.76 ± 0.09</td>
<td>0.61 ± 0.19</td>
<td>10:11</td>
<td>Bromide, H2O2</td>
<td>2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects per group. Note: Mazariegos et al. (14) and Waki et al. (18) probably had the same control group.
The second explanation of the increased levels of ECW/ICW in obese subjects is the ECW/ICW ratio of the adipose tissue. Because adipose tissue consists of ~14% of water and has an ECW/ICW of ~3.7 (19), whereas the FFM consists of ~72% of water with an ECW/ICW of ~0.82, this could explain the difference between the obese subjects and controls. The more adipose tissue, the larger the relative contribution of ECW. Indeed, we found a relationship between the FM and the hydration and a change in FM and the ECW/ICW ratio during the weight-reduction program. However, after weight reduction, the ECW/ICW did not change significantly, and after weight maintenance the ECW/ICW even increased. Thus the ECW/ICW of the adipose tissue alone does not explain ECW/ICW found after weight reduction.

Excluding malnutrition and the contribution of the ECW/ICW of adipose tissue as primary causes of the post-weight-reduction ECW/ICW change, we can conclude with others (13, 14, 21) that obesity may be accompanied by a primary (irreversible) alteration in hemodynamics and fluid regulation. However, the reason or a mechanism of this defect is not known. One mechanism could be cell shrinking of tissues of the FFM, which results in a decrease of ICW (8), relative to ECW.

Landsberg and Krieger (10) show that an insulin-mediated sympathetic stimulation in the obese may cause increased Na+ levels (10). Indeed, Mazariégos et al. (14) found that the elevated exchangeable Na+ in obese subjects remained elevated after weight loss, which may reflect a nonreversible effect on ECW/ICW in obesity, possibly of a hormonal nature. Salt intake itself could also play a role. It is to be expected that the absolute amount of salt intake is higher during weight maintenance than during weight reduction. Data on dietary salt intake are not available.

In line with the changes in ECW/ICW are the results of the hydration of FFM. If it was the contribution of water in the adipose tissue that caused the relative expansion of water in the FFM, the weight reduction would have caused a decreased hydration of FFM. Nevertheless, we did find a relationship between hydration of FFM and the percent body fat, and the results from weight reduction show an opposite effect, namely, an increase in hydration of the FFM.

It is generally assumed that the nutritional status can be determined by body composition measurement. However, many methods rely on assumptions about the hydration or water compartmentalization in the body (e.g., 2H2O dilution for calculation of percent body fat or single-frequency bioimpedance for calculation of water compartments). Our results, and those of others [e.g., Mazariégos et al. (14)], clearly indicate that the hydration of FFM and the ECW/ICW can vary substantially between individuals. The composition of weight loss is not uniform, so that assumptions underlying body composition methods that rely on water compartmentalization do not hold.

In summary, our study shows an increase in ECW/ICW in obese women a year after the start of the 3-mo weight-reduction program that was followed by a 9-mo period of weight maintenance. The ratio between these compartments did not return to baseline values or nonobese (lower) ratios. The hydration of the FFM increased after weight reduction but did not return to baseline levels after 9 mo of weight maintenance. Possible explanations are discussed, but the main cause of these findings is still an enigma and requires further study.

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