impedance in different (ethnic) groups and to relate the bias of predicted values to body water distribution and measures of body build.

Subjects and methods.
In total, 172 healthy subjects, 89 males and 83 females participated in the study. They were recruited in four countries (cities), Ethiopia (Addis Ababa), China (Beijing), Italy (Pavia) and the Netherlands (Wageningen). The subjects cannot be regarded as representative for their country, however they were not specifically selected. The aim of the study was explained to the subjects. The study protocol was approved by the local ethics committee of all the institutions. The Declaration of Helsinki of the World Medical Association (1989) and was approved by the Medical Ethical Committee of the Department of Human Nutrition, Wageningen, The Netherlands. In each centre the same study protocol was used.

All measurements were performed in the morning, in the fasting state after emptying the bladder. Body weight in underwear or swim suit was measured to the nearest 0.1 or 0.5kg (Ethiopia) and body height without shoes to the nearest 0.1 or 0.5cm (Ethiopia). Body mass index was calculated as weight/height squared (W/H2; kg/m2). After that, body impedance was measured on the left side of the body immediately after lying supine using a HUMANIM SCAN (Dietosystem, Milan, Italy) multi-frequency impedance analyzer. The self-adhesive electrodes (Lutman JM, 2325 VP, St. Paul MN, USA) with a surface area of about 5cm2 were attached as described by Lukaski et al18. From impedance, the impedance index was calculated and height squared impedance (H2/Z0, cm2/Ω). From impedance index at 1kHz and at 100kHz, extracellular water (ECW) and total body water (TBW) were predicted with the respectively formulas19:

ECW (kg) = 2.4253 * H2/Z0 + 7.04
TBW (kg) = 0.51303 * H2/Z0 + 6.3

In addition, ECW and TBW were calculated with prediction formulas that included not only impedance index but also weight and age, and, for TBW, also sex.

Total body water and extracellular water were determined by dilution techniques. A cocktail of an accurately weighed dose of about 15g deuterium oxide and 900mg bromide (1.34g as bromide) was taken orally by the subjects. After 2.5 to 3 hours dilution time a venous blood sample was taken, plasma was separated and stored at -80°C until analysis. Deuterium in plasma was determined after sublimation by infrared spectrometry18. TBW was calculated using a correction factor of 0.95 for non aqueous dilution1. Bromide in plasma was determined by HPLC after ultra filtration20. ECW was calculated using a correction factor of 0.9 for extracellular dilution and a correction factor of 0.95 for the DONNAEN effect21. All analyses were done in the same laboratory (Wageningen).

As a crude measure of body build, TBW/height and ECW/height were calculated. A slender subject will have lower values of these parameters compared to a more plump subject. Body weights measured in the four countries were compared with the values of these parameters of the population in which the prediction formulas were developed (reference population). The bias of predicted values of TBW and ECW was corrected for differences in body build and body water distribution compared to the reference population. The SPSS program22 was used for statistical analysis. Differences between measured and predicted values (bias) were tested for significance with the paired t-test. Correlations are Pearson’s moment correlation coefficients. Differences in variables between groups (countries) were tested by the NPAR (or ANCOVA) (analysis of co-variance). Multiple regression analyses were performed using country as a dummy variable. Regression equations were tested for differences in slope and/or intercept with the technique described by Kleinbaum and Kupper23. The validity of predicted values is described according to Bland and Altman24. Values are expressed as mean ± standard deviation (SD) except for regression coefficients for which the standard error of the mean (SE) is shown.

Table 1. Characteristics of the study groups.

<table>
<thead>
<tr>
<th>Country</th>
<th>Male</th>
<th>Female</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>24</td>
<td>26</td>
<td>33.5 ± 2.0</td>
<td>61.3 ± 9.0</td>
<td>163 ± 9.5</td>
<td>21.3 ± 1.7</td>
</tr>
<tr>
<td>China</td>
<td>30</td>
<td>35</td>
<td>32.5 ± 1.5</td>
<td>59.8 ± 4.0</td>
<td>163 ± 9.5</td>
<td>20.7 ± 1.3</td>
</tr>
<tr>
<td>Italy</td>
<td>32</td>
<td>34</td>
<td>30.5 ± 1.5</td>
<td>58.2 ± 4.0</td>
<td>163 ± 9.5</td>
<td>21.4 ± 1.4</td>
</tr>
<tr>
<td>Netherlands</td>
<td>30</td>
<td>35</td>
<td>29.0 ± 1.5</td>
<td>57.9 ± 4.0</td>
<td>163 ± 9.5</td>
<td>20.7 ± 1.3</td>
</tr>
</tbody>
</table>

Table 2. Regression coefficients for total body water and extracellular water against impedance index.

<table>
<thead>
<tr>
<th>Country</th>
<th>Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>-0.5 ± 0.3</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>China</td>
<td>-0.4 ± 0.3</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Italy</td>
<td>-0.9 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Netherlands</td>
<td>-0.8 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Table 3. Differences between measured and predicted total body water and extracellular water.

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>-0.5 ± 1.8</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>China</td>
<td>-0.6 ± 1.5</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Italy</td>
<td>-0.8 ± 2.0</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>Netherlands</td>
<td>-0.9 ± 1.5</td>
<td>1.3 ± 0.4</td>
</tr>
</tbody>
</table>

In Table 2 the coefficients of the regression equations between TBW and H2/Z0 and ECW and H2/Z0 are given for the four groups. The four regression equations for TBW and the four regression equations for ECW did not differ in slope and intercept. Figure 1 and Figure 2 show the relationship between TBW and H2/Z0 and ECW and H2/Z0, respectively for all subjects combined.

In Table 3 the differences measured and predicted TBW and ECW (bias) from impedance index alone, using prediction equations from the literature, are given. Although the bias for ECW was significantly different from zero in all groups it was relatively small and did not differ between the countries. The bias of ECW and TBW correlated with body water distribution (ECW/TBW) and with body build (TBW.getHeight and ECW.height). The prediction formulas can be used to overestimate total body water and extracellular water.
impedance in different (ethnic) groups and to relate the bias of predicted values to body water distribution and measures of body build.

Subjects and methods.
In total, 172 healthy subjects, 89 males and 83 females participated in the study. They were recruited in four countries (cities), Ethiopia (Addis Ababa), China (Beijing), Italy (Pavia) and the Netherlands (Wageningen). The subjects cannot be regarded as representative for their country, however they were not specially selected. The aim of the study was explained to the subjects. The study protocol was approved with the guidelines of the Declaration of Helsinki of the World Medical Association (1989) and was approved by the Medical Ethical Committee of the Department of Human Nutrition, Wageningen, The Netherlands. In each centre the same study protocol was used.

All measurements were performed in the morning, in the fasting state after emptying the bladder. Body weight in underwear or swimsuit was measured to the nearest 0.1 or 0.5kg (Ethiopia) and body height without shoes to the nearest 0.1 or 0.5cm (Ethiopia). Body mass index was calculated as weight/height squared (W/H² kg/m²). After that, body impedance was measured at 10kHz (H2Qa. cm²/Q). From impedance index at 1kHz and at 100kHz, extracellular water (ECW) and total body water (TBW) were predicted with the respectively formulae 2.

ECW (kg) = 0.24253*H2Qa/1kHz  
TBW (kg) = 0.51303*H2Qa + 6.3

In addition, ECW and TBW were calculated with prediction formulas that included not only impedance index but also weight and age, and, for TBW, also sex.

Total body water and extracellular water were determined by dilution techniques. A cocktail of an accurately weighed dose of about 15 mgtritiated deoxyribose 900mg g and 90mg g was taken orally by the subjects. After 2.5 to 3 hours dilution time a venous blood sample was taken, plasma was separated and stored at -80°C until analysis. Deuterium in plasma was determined after sublimation by infrared spectroscopy. 3. TBW was calculated using a correction factor of 0.95 for non aqueous dilution 4. Bromide in plasma was determined by HPLC after ultra filtration 5. ECW was calculated using a correction factor of 0.9 for extracellular water distribution and a correction factor of 0.95 for the DONNAN effect 6. All analyses were done in the same laboratory (Wageningen).

As a crude measure of body build, TBW/height and ECW/height were calculated. A smaller subject will have lower values of these parameters compared to a more plump subject. Body water ratios measured in body build in the country groups were compared with the values of these parameters of the population in which the prediction formulas were developed (reference population). The bias of predicted values of TBW and ECW was corrected for differences in body build and body water distribution compared to the reference population.

The SPSS program 7 was used for statistical analysis. Differences between measured and predicted values (bias) were tested for significance with the paired t-test. Correlations were Pearson’s product moment correlations. Differences in variables between groups (countries) were tested by the NANOVA or ANCOVA (analysis of co-variance). Multiple regression analyses were performed using country as a dummy variable. Regression equations were tested for differences in slope and intercept with the technique described by Kleinbaum and Kupper 8. The validity of predicted values is described according to Bland and Altman 9. Values are expressed as mean ± standard deviation (SD) except for regression coefficients for which the standard error of the mean (SE) is shown.

Table 1 provides characteristics of the subjects of the four countries. The sex distribution was comparable between the countries. Most pronounced differences between the groups were the lower age of the Italians, the low values of body water compartments in the Ethiopianis and the high body water, body height and body mass index of the Dutch subjects.

Table 1. Characteristics of the study groups.

<table>
<thead>
<tr>
<th>Country</th>
<th>Male (kg)</th>
<th>Female (kg)</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>ECW (kg)</th>
<th>TBW (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>80</td>
<td>74</td>
<td>34.2 ± 6.3</td>
<td>56.4 ± 16.1</td>
<td>163 ± 135</td>
<td>21.3 ± 3.4</td>
<td>34.7 ± 7.0</td>
<td>53.7 ± 10.7</td>
</tr>
<tr>
<td>China</td>
<td>43</td>
<td>52</td>
<td>10.1 ± 8.3</td>
<td>64.5 ± 15.1</td>
<td>170 ± 99</td>
<td>22.5 ± 4.8</td>
<td>39.6 ± 9.7</td>
<td>59.7 ± 14.3</td>
</tr>
<tr>
<td>Italy</td>
<td>44</td>
<td>48</td>
<td>18.3 ± 2.4</td>
<td>54.5 ± 15.1</td>
<td>170 ± 85</td>
<td>22.8 ± 3.4</td>
<td>37.4 ± 8.2</td>
<td>62.8 ± 14.3</td>
</tr>
<tr>
<td>Netherlands</td>
<td>45</td>
<td>48</td>
<td>20.1 ± 2.3</td>
<td>67.4 ± 16.4</td>
<td>170 ± 85</td>
<td>22.1 ± 4.3</td>
<td>39.4 ± 9.7</td>
<td>61.5 ± 14.3</td>
</tr>
</tbody>
</table>

Table 2. Regression coefficients for total body water and extracellular water against impedance index.

<table>
<thead>
<tr>
<th>Country</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>0.25</td>
<td>0.00</td>
<td>0.0</td>
<td>0.21</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>China</td>
<td>0.25</td>
<td>0.00</td>
<td>0.0</td>
<td>0.22</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>Italy</td>
<td>0.25</td>
<td>0.00</td>
<td>0.0</td>
<td>0.21</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0.25</td>
<td>0.00</td>
<td>0.0</td>
<td>0.21</td>
<td>0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

In Table 1 the coefficients of the regression equations between TBW and ECW and H2Qa are given for the four groups. The four regression equations for TBW and the four regression equations for ECW did not differ in slope and intercept. Figure 1 and Figure 2 show the relationship between TBW and H2Qa and ECW and H2Qa, respectively for all subjects combined.

In Table 3, the measures of body build in the country groups were compared with the values of these parameters of the population in which the prediction formulas were developed (reference population). The bias of ECW was significantly different from zero in all groups it was relatively small and did not differ significantly from the country groups. The bias of ECW and TBW correlated with body water distribution (ECW/TBW) and with body build (TBW/height and ECW/height).

Table 3. Differences between measured and predicted total body water and extracellular water.

<table>
<thead>
<tr>
<th>Country</th>
<th>TBW (kg)</th>
<th>ECW (kg)</th>
<th>TBW/TBW corrected</th>
<th>ECW/ECW corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia</td>
<td>-0.5</td>
<td>0.1</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>China</td>
<td>-0.5</td>
<td>0.1</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Italy</td>
<td>-0.5</td>
<td>0.1</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 4. Correlation of the bias of predicted total body water and extracellular water with body water distribution and measures of body build.

When prediction formula not only containing the impedance index but also weight, age and sex were applied to the subjects, the bias of ECW was -0.8 ± 1.0, -0.6 ± 1.0, -0.5 ± 1.0 and -0.8 ± 1.0 kg. The bias of TBW was -0.8 ± 1.0, -0.6 ± 1.0, -0.5 ± 1.0 and -0.8 ± 1.0 kg for Ethiopian, Chinese, Italian and Dutch subjects respectively. The bias of ECW showed comparable correlations with body water distribution as in Table 4, but was only in the Chinese group correlated with ECW/height. The bias of TBW was not correlated with body water distribution, nor with TBW/height in either group.

Table 5. Body water distribution and body build in the four groups in comparison with the population in which the prediction formulas were developed (reference population).

Table 6. Mean bias of total body water and extracellular water corrected for body water distribution and body build.
Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups
Paul Deurenberg, Anna Tagliabue, Jingzhong Wang, Zewdie Wolde-Gebriel

Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups

Paul Deurenberg, Anna Tagliabue, Jingzhong Wang, Zewdie Wolde-Gebriel

Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups

Paul Deurenberg, Anna Tagliabue, Jingzhong Wang, Zewdie Wolde-Gebriel

Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups

Paul Deurenberg, Anna Tagliabue, Jingzhong Wang, Zewdie Wolde-Gebriel

Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups

Paul Deurenberg, Anna Tagliabue, Jingzhong Wang, Zewdie Wolde-Gebriel
Multi-frequency bioelectrical impedance for the prediction of body water compartments: validation in different ethnic groups

Paul Deurenberg, Anna Tagliafure, Jingzhong Wang, Zewdie Wolde-Gebriel


Discussion
In this study, prediction formulas for TBW and ECW developed in a Caucasian population were validated in several independently measured groups, partly with a different ethnic background. These groups cannot be regarded as representative for the entire population of that country, but they were not specially selected. The study design and the methodology used in this study was equal for all groups and the chemical analysis to obtain reference values for ECW and TBW were performed in the same laboratory. The impedance instruments used were from the same manufacturer. It is known from the literature that different impedance instruments can give different readings, even when the same subject is measured. In three out of four groups (Ethiopia, China, The Netherlands) impedance was measured by the same investigator. Thus it is likely that differences found between groups are not due to differences in methodology and/or standardisation.

The inclusion of other independent variables than impedance index in a prediction formula generally lowers the prediction error. However, it also makes a prediction equation more population specific. Therefore the main statistical analyses were performed using prediction equations containing only the impedance index as independent variable. The results show (Table 3) that the mean bias, in all four groups, was relatively small and comparable, for TBW as well as for ECW. This was to be expected as the regression equations for TBW and ECW did not differ between the groups (Table 2). The bias for TBW and ECW was not correlated with the mean value of predicted and measured ECW and TBW respectively, except for the Italian subjects for TBW. This is an important condition for a valid prediction.18, 20 However, generally the bias of ECW was positively correlated with body water distribution and the bias of TBW was negatively correlated with body water distribution. This was found by us in earlier studies.19 For ECW it can be explained that, at low frequency, the current is partly conducted along the cell membrane, resulting in biased (lower) impedance values and hence causing an over prediction of ECW. For TBW it is likely that body impedance is influenced by the different specific resistivities of intra and extracellular fluid.19 The phenomenon is discussed in detail elsewhere.21 The bias of predicted TBW and ECW was also correlated with measures of body fat and age (data not shown in the statistical point of view, slender subjects have higher impedance values compared to subjects with a plumper body build. As most of the water is located in the trunk which only shows a minor contribution to total body impedance,22, 23 plump subjects will have relatively high impedance values compared to their amount of subcutaneous water. Hence, prediction formulas developed in plump subjects will overestimate body water in more slender subjects. This is confirmed by the positive correlation between bias and TBW/height or ECW/height.

After correction for differences in body water distribution (ECW/TBW) and/or for differences in body build (TBW/height or ECW/height) between the groups under study and the group in which the prediction formulas were developed, the bias decreased and was not statistically significant any more. This clearly confirms the dependency of body impedance on body build and shows that ideally population specific prediction formulas have to be used. The fact that, also in the Dutch group, the validity of the prediction was affected by body water distribution and body build, shows that even in a very comparable group these effects have an impact. The comparison in the four different groups confirms an earlier statistical analysis of the Italian and Dutch groups26 where the differences in the relationship between body water compartments and impedance were studied in relation to a number of anthropometric variables.

When prediction formulas for TBW and ECW were used that also contained body weight, age and (for TBW) sex, the bias in predicted ECW generally decreased, but the bias for TBW slightly increased. With these prediction formulas, however, there was no effect of body build any more. It seems likely that including other parameters in the regression equation, especially weight, the effect of body build is taken into account.

Summary
Prediction formulas for TBW and ECW, developed in a Dutch Caucasian population, showed generally valid values in Ethiopian, Chinese and Italian subjects as well as in another group of Dutch subjects. The small bias was generally dependent on body water distribution and measures of body build, and decreased after correction for differences in body water distribution and body build.

Acknowledgments
We would like to thank the participants for volunteering in the study, Mr Frans JM Schouten for the chemical analyses of the body water samples and Mrs Hellaas Cena, Mr Aftab Tesema and Mrs Xiaogai Wang for their help in the conduct of the study. The study was funded by part by Dietenosystems (Milan, Italy).

References