

Original Article

Prediction of extracellular water and total body water by multifrequency bio-electrical impedance in a Southeast Asian population

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Three different adult Indonesian population groups living on Sumatra (Palembang), Java (Depok) and Sulawesi (Makale) participated in a study on body composition. Body weight, body height and multifrequency bio-electrical impedance (1, 5, 50 and 100 kHz) were measured and in addition total body water (TBW) and extracellular water (ECW) were determined by dilution techniques, using deuterium oxide and sodium bromide, respectively, as tracers. In total 318 subjects, 159 males and 159 females, participated in the study. Predicting ECW and TBW from bio-electrical impedance, using existing prediction formulas from the literature, did not result in valid estimates of these parameters. Therefore new prediction equations for ECW and TBW were developed in this group and the prediction equations were validated in several subgroups. Extracellular water (kg) could be predicted with the formula $0.262 \times \text{height}^2/Z_1 + 2.7$ (SEE = 0.9 kg) and TBW with the formula $0.516 \times \text{height}^2/Z_{100} + 3.5$ (SEE = 1.6 kg), where Z_1 and Z_{100} are impedances at frequency 1 and 100 kHz, and SEE is the standard error of estimate. The prediction formulas showed good validity in the three geographical subgroups, in males and females separately and in two randomly selected subgroups. The absolute prediction error (kg) of the newly developed prediction equations is lower compared with values normally reported in the literature, but when expressed as coefficient of variation the errors are comparable with values from the literature. Addition of weight age and sex (for TBW only) improves the prediction equation. The prediction equations were compared with prediction equations developed in Dutch subjects. In the Indonesian subjects the slopes of the regression equations were not different from the Dutch equations but the intercepts were lower. This means that for the same body water compartment and for the same height Indonesians have lower impedance values. This could be explained by a slightly higher ECW/TBW ratio and a more slender body build among Indonesians. A higher environmental temperature and skin temperature of the subjects in this study could have added to the effect. The formulas should preferably be validated before applying them to other Asian populations.

Key words: body composition, bio-electrical impedance, extracellular water, total body water, Indonesia.

Introduction

Information on body composition is required in many situations, both in clinical settings as well as in epidemiological studies.^{1,2} There are a number of methods available to assess body composition.¹⁻⁴ Some of these techniques are very advanced and can only be used in well-equipped laboratories. Other techniques are less advanced, less laborious and therefore suitable in field studies and in epidemiological studies. Bioelectrical impedance is among the last group. Historically most methods are developed and validated in Caucasian populations, mostly in the USA and in Europe, and there is relatively little information available on the validity of assumptions in other ethnic groups.

Various studies have tested the validity of prediction formulas for body composition, for example body fat percentage (BF%), in different ethnic groups, and the results are not very consistent.⁵⁻¹⁰ Moreover, most of these studies are performed in ethnic groups living in the USA, Europe or Australia and the results could be biased by an adapted life style of the subjects. Only a limited number of validation studies have been performed in Asia. It is clear that the use of

invalid prediction formulas may lead to biased results, and conclusions drawn from such studies may be very misleading.^{6,10} Thus, there is a need to validate prediction formulas and if formulas are proven to be not valid, to develop new prediction formulas for the population under study.

As shown in earlier papers the relationship between body fat and body mass index (BMI) is different in Indonesians compared with Caucasians,^{6,12,13} and body fat percentage predicted from bioelectrical impedance using formulas developed in Caucasians is underestimated.¹⁰ As part of a large epidemiological project on obesity in Indonesia that covered a number of big cities, body composition was measured in subgroups using various methods including deuterium oxide

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dilution, bromide dilution, anthropometry and bioelectrical impedance.¹¹

The aim of the present study was to develop prediction formulas for extracellular water (ECW) and total body water (TBW) from impedance measurements at low and high frequency and to validate the formulas in several ethnic subgroups of the measured total population.

Subjects and methods

The data of 318 subjects are used for this study. The subjects were all apparently healthy, had normal body weights and varied in age from 17 to 74 years.

The data were collected at three different sites in Indonesia: 106 subjects were measured in Palembang, Sumatra; 109 subjects were measured in both Depok and Jakarta, Java; and 103 subjects were measured in Makale, southern Sulawesi. The subjects from Makale had Chinese ancestry, whereas the other two groups were of Malay ancestry. The Medical Ethical Committee of the Universitas Indonesia approved the study protocol and all subjects gave informed consent.

Subjects were invited to participate in the measurements that took place in the University of Indonesia (Depok) or in a health centre (Palembang, Makale) in a not air-conditioned room in which the temperature varied between 28 and 30°C. Subjects were all at least 2 hours postprandial, but most of them were in the fasting state from the preceding evening.

Body weight was measured in normal clothes without shoes to the nearest 0.1 kg using a digital scale. In order to correct for clothing, 1 kg was subtracted from the reading. Body height was measured to the nearest 0.5 cm without shoes using a wall mounted stadiometer. From body weight and body height body mass index (BMI, kg/m²) was calculated.

Tetra polar total body bioelectrical impedance was measured (HumanIm; Dietosystem, Milan, Italy) at four frequencies, 1, 5, 50 and 100 kHz, at the left side of the body, the subjects lying supine on a bed with legs and arms slightly spread. The two current injection electrodes were placed on the dorsal surfaces of the foot and hand and the two sensor electrodes were placed midway between the distal prominences of the radius and the ulna and between the medial and lateral malleoli of the ankle. Electrodes were self-adhesive ECG aluminium foil spot electrodes with a surface area of about 4 cm². Measurements were made within 2 min of the subject lying supine.

After the anthropometric and impedance measurements the subjects drank a cocktail of an accurately weighed dose of deuterium oxide (10 g) and sodium bromide (900 mg bromide). A venous blood sample was collected 2.5–3 h after ingestion of the cocktail and plasma was separated and stored at –20°C. Plasma was analysed for deuterium oxide after sublimation of the samples with infrared analysis.¹⁴ TBW was calculated from the given dose and the deuterium concentration in plasma assuming a 5% nonaqueous deuterium dilution.² Bromide was analysed after a 1:200 dilution of the plasma using ICPMS against a bromide standard provided by Merck Chemicals (Almere, Netherlands). Extracellular water was calculated from the given bromide dose and the plasma concentration, after correction for a 10% non-extracellular bromide dilution, a correction of 5% for the Donnan effect

and a correction of 5% for the concentration of water in plasma.²

Body water distribution was calculated as the ratio of ECW and TBW.

Statistical analysis was performed using SPSS for Windows.¹⁵ Differences between variables were tested with ANOVA. Stepwise multiple regression analyses were performed using either ECW or TBW as the dependent variable, and impedance index at different frequencies, weight, age and sex (coded as '0' for females and '1' for males) as independent variables.¹⁶ The prediction formulas were validated in two randomly assigned subgroups of the total population, in males and females separately and in the three different study sites (Palembang, Depok, Makale). Residuals (measured minus predicted values) were tested against zero (Student's *t*-test) and also tested using the Bland and Altman technique.¹⁷ Correlations are Pearson's product moment correlation coefficients. Results are given as mean ± SD, unless otherwise indicated. Significance is indicated if *P* < 0.05.

Results

Table 1 gives the characteristics of the subjects, for males and females separately. The normal differences between males and females in body composition parameters are observed. In addition, there were small differences in age, body height and extracellular water between the three study sites (results not shown), which were partly due to differences in sex distribution and partly to differences in age between the groups.

Table 2 gives the regression coefficients and their standard error (SE) for the stepwise multiple regression of ECW at 1 and 5 kHz and TBW at 50 and 100 kHz. For ECW the prediction at 1 kHz is slightly better than the prediction at 5 kHz but the difference is only in the second decimal place of the standard error of estimate (SEE) and can not be read from the table. The validation of the prediction equation for ECW at 1 and 5 kHz in separate groups (Table 3) does not show remarkable differences. At both frequencies the residuals are significantly (*P* < 0.001) correlated with ECW and correlation coefficients are not different. The correlation of the residual for ECW with body water distribution (ECW/TBW) is slightly lower at 1 kHz (*r* = 0.51, *P* < 0.001) compared to 5 kHz (*r* = 0.54, *P* < 0.001), meaning that the

Table 1. Characteristics of the subjects (mean ± SD)*

	Females <i>n</i> = 159	Males <i>n</i> = 159
Age (years)	36.4 ± 10.4	39.2 ± 9.8
Height (cm)	152.0 ± 4.9	163.6 ± 5.8
Weight (kg)	51.9 ± 8.4	63.1 ± 9.7
Body mass index (kg/m ²)	22.4 ± 3.4	23.5 ± 3.2
Extracellular water (kg)	11.2 ± 1.3	14.5 ± 1.8
Total body water (kg)	24.4 ± 2.6	33.8 ± 4.2
Impedance (ohm)		
1 kHz	721 ± 74	598 ± 62
5 kHz	686 ± 75	570 ± 62
50 kHz	610 ± 71	497 ± 57
100 kHz	573 ± 67	466 ± 54

*All values different (*P* < 0.05) between males and females.

Table 2. Stepwise multiple regression analysis of extracellular water and total body water with impedance index at different frequencies, weight, age and sex*

Frequency (kHz)	Impedance index (cm ² /Ω)	Weight (kg)	Age (years)	Sex†	Intercept (kg)	SEE (kg)	R ²
Extracellular water							
1	0.262 (0.006)	–	–	–	2.7 (0.2)	0.9	0.85
	0.191 (0.009)	0.07 (0.01)	–	–	1.5 (0.2)	0.8	0.89
	0.186 (0.009)	0.08 (0.01)	0.02 (0.01)	–	1.9 (0.3)	0.7	0.90
5	0.251 (0.006)	–	–	–	2.6 (0.2)	0.9	0.86
	0.187 (0.008)	0.06 (0.01)	–	–	1.6 (0.2)	0.8	0.89
	0.183 (0.008)	0.07 (0.01)	0.03 (0.01)	–	2.1 (0.2)	0.7	0.90
Total body water							
50	0.555 (0.009)	–	–	–	3.3 (0.4)	1.6	0.92
	0.465 (0.013)	0.11 (0.01)	–	–	1.2 (0.5)	1.5	0.94
	0.358 (0.018)	0.14 (0.01)	–	2.1 (0.3)	3.5 (0.5)	1.3	0.95
	0.351 (0.017)	0.15 (0.01)	0.04 (0.01)	2.2 (0.5)	4.3 (0.5)	1.3	0.95
100	0.516 (0.008)	–	–	–	3.5 (0.4)	1.6	0.93
	0.429 (0.012)	0.11 (0.01)	–	–	1.3 (0.5)	1.4	0.94
	0.335 (0.016)	0.14 (0.01)	–	2.1 (0.2)	3.5 (0.5)	1.3	0.95
	0.327 (0.015)	0.15 (0.01)	0.03 (0.01)	2.1 (0.2)	4.3 (0.5)	1.2	0.96

*Values are mean (standard error); †females = 0, males = 1; SEE, standard error of estimate; R², explained variance.

prediction at 1 kHz is less dependent on water distribution of the subject.

The prediction for TBW at 100 kHz is slightly better than the prediction at 50 kHz. Also here the differences are minor (Table 2) and only in the second decimal place, which is in fact hardly relevant. The validation in the various groups shows, like for ECW, hardly differences between 50 and 100 kHz (Table 3) but notably is the difference in validity between Makale at the one hand and Palembang and Depok at the other hand. The correlation of the residuals at 50 and 100 kHz with TBW is significant ($P < 0.001$) and comparable, but the correlation of the residuals with body water distribution (ECW/TBW) is slightly lower at 100 kHz ($r = -0.33$, $P < 0.001$) compared to 50 kHz ($r = -0.35$, $P < 0.001$).

In Table 4 predicted values of TBW and ECW are given using prediction formulas from the literature. As indicated, the predictions of some of the formulas differ significantly from the measured values, showing the necessity for the population specific prediction formulas.

Table 3. Residuals of extracellular water and total body water prediction at different frequencies in different sub-groups (mean ± SD)

	ECW _{1 kHz}	ECW _{5 kHz}	TBW _{50 kHz}	TBW _{100 kHz}
Sex				
Females	0.0 ± 0.6	0.0 ± 0.6	0.0 ± 1.0	0.0 ± 1.0
Males	0.0 ± 0.9	0.0 ± 0.8	0.0 ± 1.5	0.0 ± 1.4
Random (n = 153)	0.0 ± 0.8	0.0 ± 0.7	0.0 ± 1.3	0.0 ± 1.2
Group (n = 165)	0.0 ± 0.7	0.0 ± 0.7	0.0 ± 1.3	0.0 ± 1.3
Study sites				
Palembang	0.0 ± 0.8	0.0 ± 0.8	-0.2 ± 1.3	-0.2 ± 1.3
Depok	0.0 ± 0.8	0.0 ± 0.8	-0.3 ± 1.2	-0.3 ± 1.2
Makale	0.0 ± 0.6	0.0 ± 0.6	0.5* ± 1.1	0.5* ± 1.1

* $P < 0.05$ compared to Palembang and Depok.

Discussion

In earlier studies in small groups of Indonesian subjects it was found that existing prediction formulas for body fat, based on bioelectrical impedance, underestimated body fat compared to values determined by deuterium oxide dilution.^{6,10} Calculated body fat from deuterium oxide dilution assumes a constant and stable hydration of the fat free mass of 0.73¹⁸ and violation of this assumption will automatically result in a biased value of body fat. Hydration in healthy subjects is fairly constant and it can be calculated that if the actual hydration levels in the present population were as low as 0.71, body fat percentage would have been overestimated by only 1.7%.¹⁹ This is much less than the difference of 3–4% observed in an earlier study,¹⁰ meaning that differences in hydration of the fat free mass, in fact violation of assumptions, can not be the only reason for the found differences. In addition, dehydration is coincided with a loss of mainly ECW,² and the ECW/TBW ratio is not lower compared with values found in earlier studies (e.g. in Netherlands) using the same methodologies.^{20,21} Thus, there is no reason to assume that body fat per cent by deuterium oxide is an overestimation of the actual value.

Extracellular water could be predicted by bioelectrical impedance analysis at 1 and 5 kHz with equal precision and

Table 4. Measured and predicted extracellular water and total body water using different formulas from the literature (mean ± SD)

	Extracellular water	Total body water
Measured by dilution	12.9 ± 2.3	29.1 ± 5.9
Predicted by formula (reference)		
Kushner <i>et al.</i> (23)	–	28.5* ± 5.9
Lukaski <i>et al.</i> (24)	–	31.3* ± 6.4
Segal <i>et al.</i> (22)	11.9* ± 3.4	34.0* ± 6.2
Deurenberg <i>et al.</i> (21)	13.5* ± 2.0	32.1* ± 5.7

* $P < 0.001$ compared with the measured value.

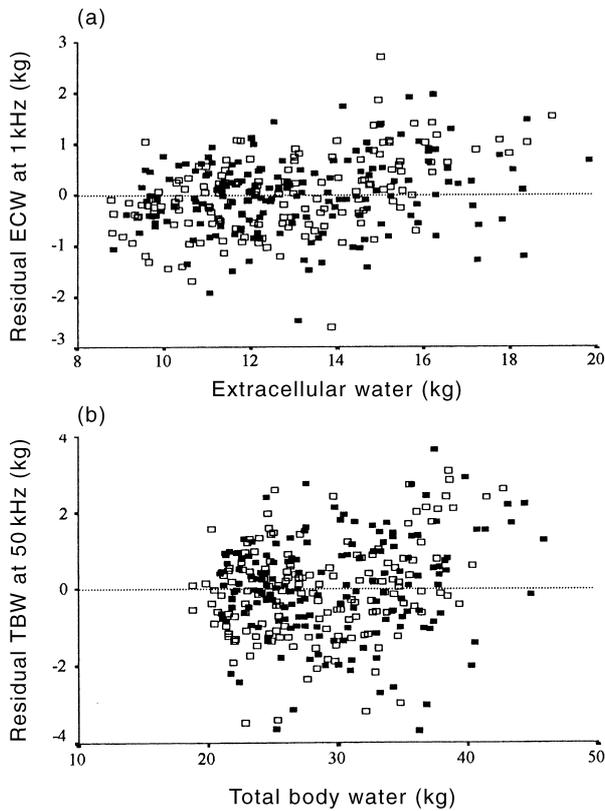


Figure 1. Relationship of residuals of extracellular water (ECW) and total body water (TBW) at 1 and 100 kHz with body water distribution. Open and closed squares symbolise the two validation groups. (a) $R = 0.51$, $P < 0.01$; (b) $R = -0.33$, $P < 0.01$.

from the present study no conclusion can be drawn as to what frequency is the most suitable. Theoretically the frequency should be as low as possible to avoid any penetration of the current through the cell membrane, as this would enable conductance by intracellular fluids. The prediction from impedance index alone already has a low SEE and is lower compared to other studies,^{21,22} but expressed as coefficient of variation (CV%) the difference is comparable to values reported in the literature. Bland and Altman analysis shows that the residuals are only slightly related to the level of ECW (Fig. 1a,b), but the validity of the predicted value is strongly dependent on body water distribution (Fig. 2).¹⁷ This limits the use of the equations under extreme conditions where water distribution is disturbed. This has to be kept in mind in tropical countries, in particular, where dehydration may occur more easily compared with places with more moderate climates.

Total body water could be predicted well at higher frequencies, slightly better at 100 kHz compared with 50 kHz (Table 2). For TBW the prediction error is lower compared with prediction errors reported in other studies, but expressed as CV percentage it is comparable to values reported in the literature.^{21–24} The prediction is hardly dependent on the level of TBW (Fig. 1b) but is negatively correlated with body water distribution (ECW/TBW). Thus at relatively higher levels of ECW/TBW, TBW is overestimated.

The underestimation of ECW and the overestimation of TBW at higher levels of the ECW/TBW ratio is found in other studies.^{20,21} For ECW it can be explained by the fact

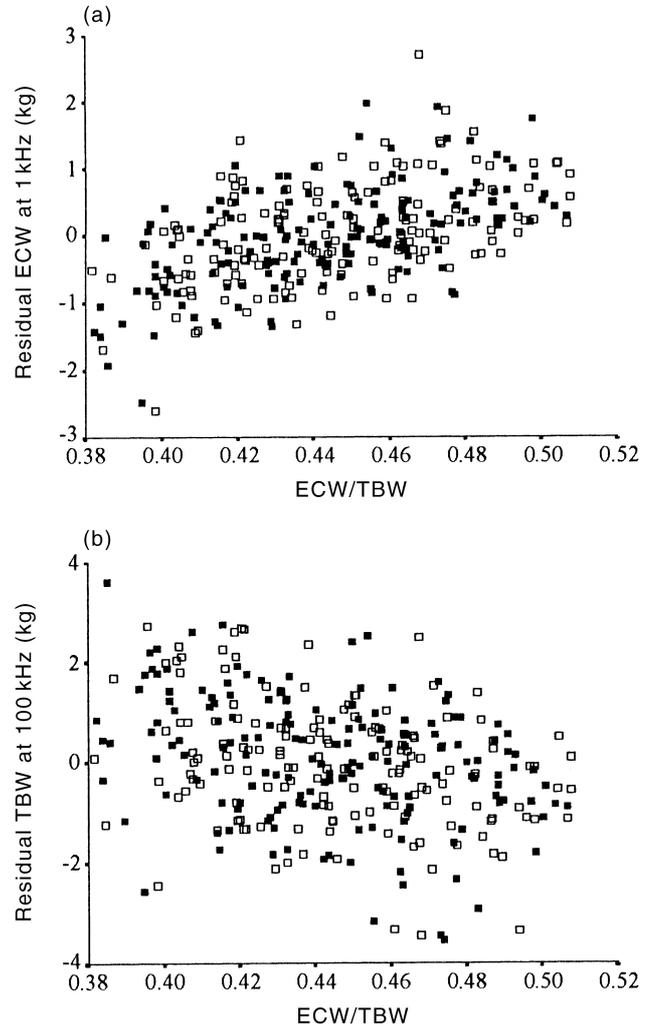


Figure 2. Relationship of residuals of extracellular water (ECW) and total body water (TBW) at (a) 1 and (b) 100 kHz with body water distribution. Open and closed squares symbolise the two validation groups. (a) $R = 0.51$, $P < 0.01$; (b) $R = -0.33$, $P < 0.01$.

that at lower frequencies there is conductance by the cell membrane.²⁵

When the ratio of ECW/TBW is relatively high the conductive effect of the cell membrane can be assumed to be relatively low and hence impedance at low frequency is relatively high. The impedance index will be lower, resulting in an underestimation of ECW. For total body water the overestimation at higher levels of ECW/TBW ratio can be explained by the lower specific resistivity of extracellular fluids compared with intracellular fluids.^{21,26} When there is relatively more extracellular fluid, the total resistivity will be lower and thus impedance index will be higher.

The prediction formulas of the present study were compared with prediction formulas developed in a Dutch population in which the same reference techniques and instruments were used. Compared with the 'Dutch' prediction formulas, the intercepts of the regression equations using only impedance index are significantly lower but the slopes are not different. This means that at the same level of body water compartment (ECW or TBW) and the same height the impedance is lower. There might be several explanations for this.

Firstly, the ECW/TBW ratio in this Asian population is higher compared to the ratio generally found in the Dutch

population, and at a relatively higher level of extracellular water the specific resistivity of the total body fluid is lower.²⁶ This should have a lowering effect only on the TBW prediction. It can be calculated that the prediction error for ECW, using the Dutch prediction formula and assuming an equal water distribution in the present population, would increase from 0.7 to 1.0 kg and the prediction error for TBW would decrease by 0.4 kg to 2.6 kg.

A second reason may be that the environmental temperature during the impedance measurements was much higher than is normally found in any laboratory condition in more moderate climates. The room temperature during the measurements was between 28 and 30°C. It can be assumed that skin temperature and skin humidity of the subjects was higher (although there was no obvious sweating), and both factors would lower impedance values. Thirdly, slenderness will have an impact on body impedance values. As most of the water is located in the trunk, which only shows a minor contribution to total body impedance, plumper subjects will have relatively high impedance values compared with their amount of water. Hence prediction formulas developed in plump subjects will overestimate body water in slender subjects. Body build in the Indonesian subjects (expressed as TBW/height, for example) was indeed more slender compared with the Dutch.²⁷

The differences in residuals of TBW between Makale and the other two sites can be partly explained by body build (values of TBW/height corrected for sex in Palembang, Depok and Makale are 0.18 kg/cm, 0.19 kg/cm and 0.18 kg/cm, respectively). In Dutch subjects these values are substantially higher at 0.22 kg/cm.²⁷

In summary, the results of this study show that the impedance technique can provide valid estimates of total body water and extracellular water as long as population specific prediction formulas are used. The observed bias in predicted body water using Caucasian prediction formulas may be partly due to differences in water distribution, partly due to differences in body build and partly due to the extreme environmental condition in which the impedance measurements were performed.

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