Between-laboratory comparison of densitometry and bio-electrical impedance measurements

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Body composition was measured in nine healthy, normal-weight, weight-stable subjects in three different research centres. In each centre the usual procedures for the measurements were followed. It revealed that the measurement procedures in the three centres were comparable. Body composition was measured in each centre between 09.00 and 13.00 hours after a light breakfast by densitometry (underwater weighing) and bio-electrical impedance. A single, total-body-water determination by D_2O dilution was used as a reference value. Body fat determined by densitometry was significantly lower in one centre, which, however, could be completely explained by a lower body weight, probably due to water loss (the subjects refrained for a longer time from food and drinks before the measurements in that centre) and, thus, by violation of the assumptions of Siri's (1961) formula. Also, body impedance was slightly higher in that centre, indicating a lower amount of body water. Mean body fat from densitometry was also slightly lower in that centre compared with body fat determined by D_2O dilution. Individual differences between body fat from densitometry and from total body water were relatively large, up to 7% body fat. The relationship between fat-free mass from densitometry and bio-electrical impedance was not different between the centres. It is concluded that differences in the relationship between body composition and bio-electrical impedance, as reported in the literature, may be due to differences in standardization procedures and/or differences in reference population.

Body composition: Densitometry: Bio-electrical impedance

The assessment of body composition is important as an indicator of the nutritional status of a subject or a group (Moore *et al.* 1963; Forbes, 1987; Lukaski, 1987). Depending on the subjects and the circumstances, several aspects of body composition can be assessed for which, generally, several methods are available. Although in recent years new information has been made available by the use of modern techniques such as neutron-activation and computer tomography-scanning, most of the generally- and widely-used methods are still based on the results of the chemical analysis of a few human cadavers (Widdowson *et al.* 1951). From these chemical analyses three basic methods have been derived which have been accepted as reference methods. These are the densitometric method, the dilution method for total body water, and the ^{40}K method (Forbes, 1987). Each of these methods has its own assumptions, which are in fact only valid for healthy adults at a group level and which are known to be invalidated in many other groups of subjects (Slaughter *et al.* 1988;

van Raaij *et al.* 1989; Weststrate & Deurenberg, 1989; Deurenberg *et al.* 1989*a*, *c*). From the reference methods many 'indirect', in fact 'double indirect', methods were developed, such as skinfold thickness measurements (Durnin & Womersley, 1974) and impedance analysis (Lukaski *et al.* 1985).

Numerous studies have been published in which several methods are compared, cross-sectional (Blanchard *et al.* 1990; McNeill *et al.* 1991; Tagliabue *et al.* 1992) as well as longitudinal (Van der Kooy *et al.* 1992).

To our knowledge there have been no studies in which a comparison has been made between results from different laboratories using the same subjects and the same methods. This might be due to logistical problems. In The Netherlands we have three centres at which body composition can be measured by both a reference method (densitometry by underwater weighing) and by impedance analysis, and the travelling distances between these centres are relatively small. Therefore, a study was performed to investigate whether there are differences in results when the same subjects are assessed by the same method in different laboratories.

SUBJECTS AND METHODS

Nine subjects, seven females and two males, aged 22–55 years participated in the study. They had all had previous experience with the methods used as they had participated in several studies on body composition. Some physical characteristics of the subjects are given in Table 1. The three centres in which the body composition measurements were performed were the Department of Human Nutrition in Wageningen (centre A), the Department of Human Biology in Maastricht (centre B), and the TNO-Toxicology and Nutrition Institute in Zeist (centre C). The distance between the centres is 1–3 h drive by car.

All measurements were done in the morning between 09.00 and 13.00 hours, at least 2 h after a light breakfast. The measurements in four subjects were performed within 3 d, in three subjects within 6 d and in two subjects within 10 d. Body height was measured using a microtoise to the nearest 0·001 m. In all three centres body weight was measured in a swimsuit to at least the nearest 0·1 kg using a digital scale. Body impedance was measured at the left side of the body at a frequency of 50 KHz. In two centres a Xitron 4000 (Xitron Technologies, San Diego, CA, USA) impedance analyser was used, and in one centre (C) a RJL BIA 101 impedance analyser (RJL-systems, Detroit, MI, USA). The current injection electrodes were placed on the dorsal surfaces of hand and foot. The voltage drop was measured with electrodes placed between the distal prominences of the radius and the ulna and lateral malleoli at the ankle. Impedance was calculated as $(\text{resistance}^2 + \text{reactance}^2)^{0.5}$. The measurements were performed in duplicate. Body density was determined by underwater weighing, with simultaneous determination of the residual lung volume (accuracy 0·1 litres). In each centre the He dilution technique (Comroe *et al.* 1977) was used. The instruments were obtained from different companies but had comparable specifications. In two centres the underwater weight was measured with a digital scale to the nearest 1 g; in the other centre (C) the underwater weight was measured with an analogue scale. The accuracy of a reading in that centre was about 50 g. The mean value of two underwater weighing measurements (and two residual lung volumes) was used for the calculation of body density. From body density percentage body fat was calculated using Siri's (1961) formula.

Total body water was determined by D_2O dilution. The measurements were done on the same morning as the underwater weighing and impedance measurements in centre A. After a light breakfast and after voiding (background urine sample for D), a dose of 0·08 g D_2O (99·8% D_2O)/kg fat-free mass (FFM) was taken orally. After a 3 and 4 h dilution time a urine sample was collected. The D concentration in background and 4 h urine was

Table 1. *Age and physical characteristics of the subjects**
(Mean values and standard deviations for nine subjects, seven females, 2 males)

	Mean	SD
Age (years)	28·2	12·1
Body wt (kg)	67·1	8·1
Height (m)	1·73	0·08
Body mass index† (kg/m^2)	22·3	1·8

* Values as measured in centre A (see p. 310).
† Weight/height^2.

determined with an Aqua-Sira mass spectrometer (VG Isogas Ltd, Middlewich, Cheshire). From the difference in D concentration in urine before and after the dose, the amount of total body water (TBW) was calculated (Forbes, 1987). A correction for 40 g/kg non-aqueous dilution was used (Schoeller *et al.* 1980). FFM was calculated from TBW using a hydration factor of 0·73 (Forbes, 1987). Fat mass (FM) was calculated as the difference between body weight and FFM. Percentage body fat was calculated as FM/body weight. The percentage body fat derived from D_2O dilution was used as a relative standard.

Statistical calculations were performed with the SPSS-PC package (SPSS, 1988). The relation between FFM and impedance was analysed in each centre by linear regression. Differences in variables between centres were tested by ANOVA (repeated measurements) techniques. Differences in variables within each centre were tested by the paired Student's *t* test. A level of significance of $P < 0{\cdot}05$ was accepted. Correlations are Pearson's product-moment correlations. The Bland & Altman (1986) procedure was used to assess the relative validity of measurements. Body fat determined by D_2O dilution was used as the standard. Values are expressed as means and standard deviations.

RESULTS

Physical characteristics of the subjects are shown in Table 1, and body weight, body density and impedance as measured in each centre are given in Table 2. Centre B showed significant differences in body weight, body density, fat mass and fat-free mass compared with centres A and C. The differences are more pronounced between centres A and B. These differences remained when only the data for the seven females were used in the analysis. When the percentage body fat determined by underwater weighing was compared with body fat from D_2O dilution centre B showed a significantly lower percentage body fat (Table 3). Table 4 gives the Pearson's product-moment correlation coefficient for the percentage body fat between each centre together with the correlation with the body fat from deuterium oxide dilution. Fig. 1 shows the difference in percentage body fat determined by underwater weighing and D_2O dilution *v.* the percentage body fat by D_2O dilution. Although the mean differences as given in Table 3 were relatively small, individual differences were up to 7% body fat. There were no striking differences between males and females. Table 4 gives the correlation coefficient between percentage body fat by D_2O dilution and by densitometry in each centre.

The prediction equations for FFM from height^2/impedance did not differ significantly in intercept and slope between the centres (results not shown). Table 5 gives the difference in FFM as determined by underwater weighing and the FFM as predicted from impedance, when using the prediction equations based on the data measured in each centre.

Table 2. *Body composition variables as determined at each of the three centres for the same subjects*†

(Mean values and standard deviations for nine subjects)

Centre...	A		B		C	
	Mean	SD	Mean	SD	Mean	SD
Body wt (kg)	67·1	8·1	66·6*	7·9	67·3	8·2
Body density (kg/l)	1·0383	0·012	1·0436*	0·014	1·0418	0·010
Body impedance (ohms)	565	93	572	99	567	102
Percentage body fat‡	26·2	7·1	—	—	—	—
Percentage body fat§	26·8	5·6	24·4*	6·2	25·2	4·7
Fat-free mass (kg)§	49·2	8·3	50·5*	8·3	50·4	7·7

Mean values were significantly different from those for the other two centres (ANOVA): * $P < 0·05$.
† For details of subjects and procedures, see Table 1 and pp. 310–311.
‡ From deuterium oxide dilution.
§ By densitometry.

Table 3. *Difference in percentage body fat derived from deuterium oxide dilution and by densitometry at each of the three centres for the same subjects*†‡

(Mean values and standard deviations for nine subjects)

Centre	Mean	SD	Range
A	−0·6	1·7	−3·2–+2·1
B	+1·8*	1·7	−1·4–+3·7
C	+1·0	3·7	−5·4–+7·5

Mean values were significantly different from those for the other two centres (paired t test): * $P < 0·05$.
† For details of subjects and procedures, see Table 1 and pp. 310–311.
‡ Body fat from total body water minus body fat from densitometry.

Table 4. *Pearson's product-moment correlation coefficients between body fat (fat %) derived by densitometry and body fat obtained by deuterium oxide dilution at each of the three centres (A, B, C) for the same subjects**†

	Fat %			
Fat %	D_2O	A	B	C
D_2O	—	0·99	0·98	0·88
A		—	0·97	0·87
B			—	0·90

* All values were significant ($P < 0·01$).
† For details of subjects and procedures, see Table 1 and pp. 310–311.

DISCUSSION

Many studies describe the comparison between different methods of body composition measurements (Blanchard *et al.* 1990; McNeill *et al.* 1991; Tagliabue *et al.* 1992; Van der Kooy *et al.* 1992). To our knowledge, however, no study has yet been focused on possible differences when the same subjects are measured in different laboratories. This may be

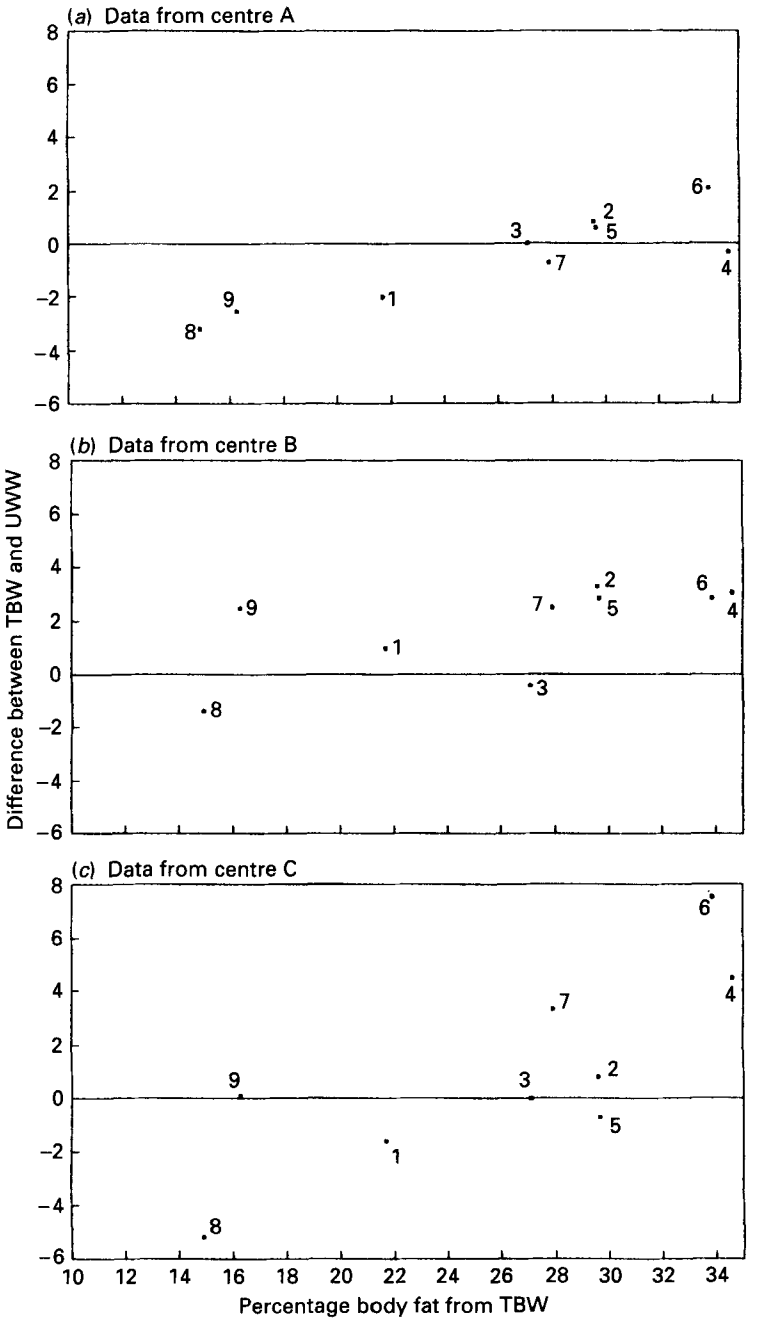

Fig. 1. Individual differences between percentage body fat estimated from deuterium oxide dilution and densitometry at each of the three centres for the same subjects. Difference = body fat from total body water minus body fat from densitometry. For details of subjects and procedures, see Table 1 and pp. 310–311. Subjects nos. 8 and 9 were male, nos. 1–7 were female.

Table 5. *Measured fat-free mass (FFM; kg; by underwater weighing) and predicted FFM (kg; by impedance) using centre-specific prediction formulas for each of the three centres and the same subjects*†

(Mean values and standard deviations for nine subjects)

	Measured FFM at centre		Difference in predicted FFM with formula from centre‡					
			A		B		C	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A:	49·2	8·3	—		−1·5*	1·9	−1·1	2·3
B:	50·5	8·3	1·5	2·4	—		0·4	2·5
C:	50·4	7·7	1·1	2·8	−0·4	2·6	—	

Mean value was significantly different from those for the other two centres: * $P < 0{\cdot}05$.
† For details of subjects and procedures, see Table 1 and pp. 310–311.
‡ Measured minus predicted.

attributable to logistical problems. In The Netherlands we have at relatively close proximity three centres where body composition measurements are carried out regularly, using several techniques. Therefore, the present study describes the between-laboratory comparison of body composition measurements in the same subjects.

At each centre the measurements were carried out in their usual way, without any discussion beforehand on standardization. In each institute all instruments were calibrated as usual. It appeared that there were no differences in the placement of the electrodes during the impedance measurements. Table 2 shows that there were slight and sometimes significant differences in the mean values of measured body composition between the three centres; centre B tended to have lower values for percentage body fat, fat mass and body weight and higher values for FFM. These lower values could be explained completely, however, by the fact that the measurements in centre B took place about 2–3 h later in the morning compared with the measurements in centres A and C. The subjects refrained from eating and drinking during that time. Thus, loss of body water could be the reason for the lower body weight. The water loss will also have caused an increase in the density of the FFM (Forbes, 1987). Using Siri's (1961) equation under these conditions will result in a lower estimate for percentage body fat. Also, the slightly higher mean impedance value indicates a lower body water content during the measurements at centre B. Individually the differences between the centres were sometimes large.

The data in Tables 3 and 4 and in Fig. 1 show that the variation between the centres in percentage body fat derived by densitometry compared with D_2O dilution was large, especially for centre C. This shows that an accurate measurement of the underwater weight is absolutely essential. An accuracy in the underwater weighing of 50–100 g, as occurred in centre C, seems not to be appropriate. This is indicated also by the approximately fivefold larger variability in the duplicate measurements of the body density at centre C compared with centres A and B (results not shown). The significant difference in body fat derived from total body water and density at centre B may, as discussed previously, again be the result of invalidating the assumptions for Siri's (1961) formula. The relatively low difference between body fat derived by densitometry and body fat obtained by D_2O dilution at centre A may be due to the fact that both techniques were performed on the same morning.

When 'centre-specific' prediction formulas were calculated from impedance and the FFM (as determined by underwater weighing; $FFM = a \times height^2/impedance + b$), these

formulas did not differ in slope and intercept (results not shown). Applying these 'centre-specific' prediction formulas to the data measured at the other centres revealed slight differences in predicted mean FFM up to 1·5 kg (Table 5). Larger differences between measured FFM and predicted FFM using prediction formulas from the literature are reported by several authors (Deurenberg *et al.* 1991; Svendson *et al.* 1991). The present study shows that prediction formulas developed at different institutes for the same subjects reveal the same results. In the present study there were no apparent differences in placements of electrodes, which has a large impact on the measured impedance value (Baumgartner *et al.* 1989; Scheltinga *et al.* 1991), and the differences in reference value (FFM by underwater weighing) were only small but significant. Although these differences could be explained completely by the longer duration of the fasting state for subjects measured at centre B, it indicates that a measuring procedure not carefully standardized can easily lead to errors which can become important, specially in longitudinal studies. The impedance instruments used at the three centres were tested against each other and showed no differences in readings. In other studies differences between different instruments have been reported (Deurenberg *et al.* 1989*b*; Heitmann, 1990).

The present study shows that when standard procedures are used for the same subjects the relationship between total body impedance and FFM derived by densitometry is not different between research centres. Thus, differences between measured and calculated body composition derived from impedance formulas from the literature may be caused by differences in the reference population or by differences in the standardization procedure. Prediction formulas from the literature have to be applied with care. Ideally a prediction formula must be tested against a reference method for the subjects or population group under study before it can be applied.

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