

Comparison of commonly used procedures, including the doubly-labelled water technique, in the estimation of total energy expenditure of women with special reference to the significance of body fatness*

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According to the report of the World Health Organization (1985), total energy expenditure (TEE) in human subjects can be calculated as $BMR \times \text{physical activity level (PAL)}$. However, other reports have pointed out limitations in the suggested procedure related to the % body fat of the subjects. The purpose of the present study was to evaluate the World Health Organization (1985) procedure in thirty-four healthy women with BMI 18–39 kg/m². BMR and TEE were measured using indirect calorimetry (BMR_{meas}) and the doubly-labelled water method (TEE_{ref}) respectively. When assessed using the doubly-labelled water and skinfold-thickness methods, the women had 34 (SD 8) and 33 (SD 6) % body fat respectively. On the basis of guidelines provided by the World Health Organization (1985), 1.64 was selected to represent the average PAL of the women. Furthermore, PAL was also assessed by means of an accelerometer (PAL_{acc}), heart-rate recordings (PAL_{HR}) and a questionnaire (PAL_{q}). These estimates were: PAL_{acc} 1.71 (SD 0.17), PAL_{HR} 1.76 (SD 0.24), PAL_{q} 1.86 (SD 0.27). These values were lower than $TEE_{\text{ref}}/BMR_{\text{ref}}$, which was 1.98 (SD 0.21). BMR assessed using equations recommended by the World Health Organization (1985) ($BMR_{\text{predicted}}$) overestimated BMR by 594 (SD 431) kJ/24 h. However, when TEE was calculated as $BMR_{\text{predicted}} \times PAL_{\text{acc}}$, $BMR_{\text{predicted}} \times PAL_{\text{HR}}$ and $BMR_{\text{predicted}} \times PAL_{\text{q}}$ respectively, average results were in agreement with TEE_{ref} . Furthermore, TEE values based on $BMR_{\text{predicted}}$ and PAL_{acc} , PAL_{HR} , PAL_{q} as well as on $PAL = 1.64$, minus TEE_{ref} , were significantly correlated with body fatness. When the same PAL value (1.64) was used for all subjects, this correlation was particularly strong. Thus, the World Health Organization (1985) procedure may give TEE results that are biased with respect to the body fatness of subjects.

Basal metabolic rate: Body fatness: Doubly-labelled water: Physical activity level: Total energy expenditure

In 1985, the WHO recommended that estimates of human energy requirements should be based on assessments of energy expenditure rather than on energy intake as was common previously (World Health Organization, 1985). It was then suggested that total energy expenditure (TEE) should be calculated as $BMR \times \text{physical activity level (PAL)}$. Equations based on age, sex, weight and height for predicting BMR were provided, as well as guidelines on how to calculate PAL values (World Health Organization,

1985). These guidelines were based on the assumption that the PAL of individuals can be calculated using specific 'activity factors' for different kinds of bodily movements. When multiplied by the BMR of a subject, such a factor gives the energy expenditure of the subject when performing a particular activity. The World Health Organization (1985) recommended that information regarding such activity factors should be combined with information concerning the activity pattern of subjects in order to estimate

Abbreviations: acc, accelerometer; BF, body fat; BF_{DLW} , body fat calculated from estimates of total body water; BF_{STF} , body fat calculated from skinfold-thickness measurements; BMR_{meas} , BMR measured by indirect calorimetry; $BMR_{\text{predicted}}$, BMR predicted using World Health Organization (1985) equations; BW, body weight; DLW, doubly-labelled water; HR, heart rate; MET, metabolic equivalent; PAL, physical activity level; PAL_{acc} , physical activity level obtained using an accelerometer; PAL_{HR} , physical activity level obtained using heart-rate recording; PAL_{ref} , physical activity level obtained using a combination of doubly-labelled water and indirect calorimetry; PAL_{q} , physical activity level obtained using a questionnaire; TEE, total energy expenditure; TEE_{acc} , total energy expenditure obtained using an accelerometer; TEE_{HR} , total energy expenditure obtained using heart-rate recording; TEE_{ref} , total energy expenditure obtained using doubly-labelled water; TEE_{q} , total energy expenditure obtained using a questionnaire.

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their PAL values. The guidelines also provided average PAL values to be used for populations engaged in light, moderate and heavy work. However, it was recommended that as far as possible, users should calculate PAL values that are appropriate for their own group of subjects. The WHO approach to calculating TEE is attractive and has been widely adopted.

Following publication of the World Health Organization (1985) procedure, several reports have, however, provided evidence for its limitations. The system based on 'activity factors' for calculating energy expenditure has been further developed by Ainsworth *et al.* (1993, 2000), who also pointed out that such factors were intended to provide an activity classification system for use in epidemiological research, rather than for estimating the precise energy costs of physical activity for individuals. Furthermore, several authors (Black, 2000a; Staten *et al.* 2001) have pointed out that the recommended equations will overestimate the BMR of subjects with a high body fat (BF) content. In addition, Racette *et al.* (1995) demonstrated that the kind of 'activity factors' recommended by the World Health Organization (1985) need modification when applied to such subjects, and their observations are supported by the results of Staten *et al.* (2001). These findings are of concern, as the prevalence of overweight and obesity is currently increasing in many countries. It is thus relevant to examine the significance of % BF for the validity of the World Health Organization (1985) procedure in groups with a BF content typical of contemporary populations. The purpose of the present study was to provide an example in which this procedure is evaluated in such a population of women and where PAL was estimated using an accelerometer, a heart-rate (HR) recorder and a questionnaire, as well as where a constant PAL value, assumed to represent the average for the group of subjects in the study, was used.

Subjects and methods

Subjects

Thirty-four healthy, non-smoking women were recruited through the healthcare system, or by advertising in the local press, to a study on energy metabolism during pregnancy. All studies reported in the present paper were conducted before conception. The subjects were employed in the areas of office work, childcare and nursing. Only a few took part in physically demanding activities on a regular basis during their leisure time. The study was approved by the Ethics Committee at the University of Linköping.

Study outline

In the morning of the first day of the experiment, the BMR of the subject was measured by indirect calorimetry after an overnight fast and 45 min of rest (BMR_{meas}). The woman came to the hospital by car to keep physical activity to a minimum. Later the same morning, her skin-fold thicknesses were measured to assess BF. The subject then performed six standardised activities (sitting, walking at two speeds, jogging at two speeds and jumping), each

for 5 min, while wearing an accelerometer and an HR recorder. A metronome was used to set the speed of the activities (beats per min: walking 58 and 98, jogging 118 and 130, jumping 141). The six activities were assigned different metabolic equivalent (MET) factors (Ainsworth *et al.* 1993): sitting 1.5, slow walking 2.5, brisk walking 4.0, slow jogging 4.5, quick jogging 7.0, jumping 12.0). (The definition of a MET factor is the same as that given by the World Health Organization (1985) for an 'activity factor', i.e. the energy expenditure of a subject when performing this activity divided by the BMR of the subject.) Linear relationships between recorded HR, or number of counts per min as measured by the accelerometer, v. the MET factors for the six activities were established for each subject. Before leaving the hospital, the subject was given a dose of doubly-labelled water (DLW) and asked to collect urine samples during the following 14 d. The DLW method was used to measure TEE during this period of time (TEE_{ref}), and a PAL value covering this period was calculated as TEE_{ref}/BMR_{meas} (PAL_{ref}). The subject was requested to wear an accelerometer during the complete 14 d period and an HR recorder during the first 7 d of this period. These devices were to be worn all the time while in the waking state except when in water. The subject was asked to use a notebook for recording any period of time when either of the two recorders was taken off, and also to indicate the kind of activity performed during this time (i.e. sleeping, showering). At the end of the 14 d period, the subject returned to deliver urine samples, the recorders and the notebook. On this occasion, she was interviewed via a questionnaire about her physical activity during the preceding 14 d period.

BMR

CO_2 production and O_2 consumption were measured for 20 min using a ventilated hood system (Deltrac Metabolic Monitor; Datex Instrumentarium Corp, Helsinki, Finland). On the day of measurement, 15 and 18 women were in the pre- and post-ovulatory phases, respectively. BMR was calculated according to Weir (1949) to obtain BMR_{meas} . BMR was also predicted from age, weight and height using the equations from the World Health Organization (1985) to obtain $BMR_{predicted}$.

Doubly-labelled water method

Each subject was given an accurately weighed oral dose of isotopes (0.05 g 2H and 0.15 g ^{18}O per kg body weight (BW)) after collection of two or three background urine samples during a 2–7 d period before dosing. Another five urine samples were collected on days 1, 4, 8, 11 and 15 after the day of dosing. The date and time of day of each sample collected was always noted. Urine samples were stored in glass vials with internal Al-lined screw-cap sealing at +4°C until sample collection was completed. They were then stored at –20°C until analysed. Isotopic enrichments of dose and urine samples were analysed using an isotope-ratio MS fitted with a $CO_2-H_2-H_2O$ equilibrium device (Deltaplus XL; Thermoquest, Bremen, Germany). The procedure described by

Thielecke & Noack (1997) was followed, except that the equilibration times for H₂ and CO₂ were 180 and 840 min respectively. Isotope dilution spaces (N_D and N_O) were calculated from zero-time enrichments obtained from the exponential isotope disappearance curves that provided estimates for the rate constants, k_D and k_O , for ²H and ¹⁸O, respectively. The MS response was standardised using Vienna standard mean ocean water. Dose and urine samples from the same subject were always analysed on the same occasion within the same equilibrium device, when a linear MS response was also confirmed for both isotopes. Analytical precision in the measurement range, for results expressed in ppm (Speakman, 1997), was 0.44 for ²H and 0.15 for ¹⁸O. N_D/N_O for our present thirty-four subjects was 1.037 (SD 0.007). Total body water was the average of N_D/1.04 and N_O/1.01. CO₂ production was calculated using equation 5 as described by Coward (1988), with N_D and N_O as the distribution spaces matching their respective rate constants for isotopic disappearance and assuming 30% of water losses to be fractionated. To obtain TEE_{ref} from CO₂ production, the food quotient was assumed to be 0.85 (Black *et al.* 1986).

Body weight and body fat

BW without clothes was recorded before breakfast using a balance (KCC150; Mettler-Toledo, Germany). BF was estimated using the values for total body water obtained by means of the DLW method (BF_{DLW}). Fat-free mass was calculated as total body water divided by 0.732. BF_{DLW} was calculated as BW – fat-free mass. Body density was calculated from measurements of skinfold thickness at the triceps, biceps, subscapular and suprailliac sites, as described by Durnin & Womersley (1974), and was converted to BF (BF_{SFT} (%)) using the equation of Siri (1961). Skinfold thickness measurements were taken using a Harpenden skinfold-thickness caliper (Practical Metrology; Lancing, West Sussex, UK) as described by Harrison *et al.* (1988).

Assessment of physical activity level and total energy expenditure using the accelerometer

The subjects wore the uniaxial accelerometer (Activity monitor 7164; Computer Science and Applications Inc., Shalimar, FL, USA) on a belt around the waist. Movements were registered by the accelerometer and the recorded information was transferred to a computer and converted to number of counts per min. Satisfactory readings were obtained for 11, 12, 13 and 14 d for one, five, five and twenty-three subjects respectively. The readings obtained during these days, covering 98 (SD 1) % of all time in the waking state, were used to assess PAL_{acc}. For each subject, the MET factor corresponding to any particular number of counts per min was obtained based on the relationship between MET factors and number of counts established before the experiment. The amount of time spent sleeping was obtained from the notebook. Sleep was assigned a MET factor of 0.9. Time not recorded was assumed to be time spent on activities with a MET factor of 1.4. The coefficient of correlation for the linear

relationship between MET factors and number of counts per min, as obtained during calibration, was 0.96 (SD 0.04) (n 34). TEE_{acc} was PAL_{acc} × BMR_{predicted}.

Assessments of physical activity level and total energy expenditure using heart-rate recording

An HR recorder (Polar Vantage NV; Polar Sverige AB, Stockholm, Sweden), consisting of a chest belt and a receiver worn around the wrist, was used. Recorded HR was transferred to a computer. Satisfactory readings were obtained for 4, 5, 6, and 7 d for one, two, nine and twenty-two subjects respectively. These readings, covering 90 (SD 6) % of all time in the waking state, were used to assess PAL_{HR}. PAL_{HR} was calculated as described earlier for PAL_{acc}, except that the relationship between MET factors and recorded HR established initially was used. The coefficient of correlation for the linear relationship between MET factors and HR was 0.95 (SD 0.04). TEE_{HR} was PAL_{HR} × BMR_{predicted}.

Assessment of physical activity level and total energy expenditure by means of a questionnaire

Each subject estimated how much time she had spent in the following six activity categories: sleeping, very light activity (e.g. office work), light activity (e.g. washing-up), moderate activity (e.g. vacuum cleaning or walking at normal pace), vigorous activity (e.g. cycling or cleaning windows) and very vigorous activity (e.g. aerobics or running) during the preceding 14 d period. A person in charge of the experiment recorded the information given by the subject on a questionnaire form. PAL_q was calculated by assigning appropriate MET factors (Ainsworth *et al.* 1993) to the different activity categories (sleeping 0.9, very light activity 1.4, light activity 2.4, moderate activity 3.0, vigorous activity 4.5, very vigorous activity 8.0). TEE_q was PAL_q × BMR_{predicted}.

Assessment of physical activity level and total energy expenditure (total energy expenditure_{1.64}) for the subjects as a group

A value of 1.64, i.e. that suggested for women whose occupational work is classified as moderate by the World Health Organization (1985), was selected to represent PAL for this group of subjects. TEE_{1.64} was calculated as 1.64 × BMR_{predicted}.

Statistical analysis

Values are given as means and standard deviations. Significant differences between mean values were identified by *t* test for paired observations or by repeated ANOVA with subsequent *post hoc* analysis using Tukey's multiple comparison test (Hassard, 1991). Agreement between results obtained using different methods and the appropriate reference results was examined according to Bland & Altman (1986). Linear regression, as well as correlation and multiple regression analyses, were performed as described by Hassard (1991). Significance was accepted

at the $P < 0.05$ level. All statistical analyses were done using Statistica software, version 6.0 (statsoft; Scandinavia AB, Uppsala, Sweden).

Results

Subjects

As shown in Table 1, the women in the study varied considerably with respect to BW, BMI and BF (%). The variation in BF_{SFT} (%) was, however, smaller than in BF_{DLW} (%). Two of the women had a BMI > 30 and twelve had a BMI > 25.

Body fatness

A significant linear relationship between BF_{DLW} (%) and BF_{SFT} (%) was found ($n = 34, r = 0.86, P < 0.0001$). BF_{DLW} (%) was significantly correlated with BMI ($BF_{DLW} = 1.4973 \times BMI - 1.931, r = 0.824, P < 0.0001$).

BMR

Table 2 shows BMR_{meas} and BMR_{predicted}. The Bland & Altman (1986) graph for BMR_{meas} v. BMR_{predicted} is shown in Fig 1. On average, BMR_{meas} was significantly lower than BMR_{predicted} by almost 600 kJ/24 h. BF_{DLW} (%) was significantly correlated with BMR_{predicted} - BMR_{meas} (kJ/24 h), the regression equation being: $BMR_{predicted} - BMR_{meas} = 27.031 BF_{DLW} - 326.3 (r = 0.47, P = 0.0048)$. The relationship between BF_{SFT} (%) and BMR_{predicted} - BMR_{meas} (kJ/24 h) was, however, not significant ($r = 0.28, P = 0.106$).

Physical activity level

Table 2 also shows PAL_{acc}, PAL_{HR}, PAL_q and PAL_{ref}. The Bland & Altman (1986) graphs for PAL_{acc}, PAL_{HR} and PAL_q as compared with PAL_{ref} are shown in Fig. 2. On average, PAL_{acc} and PAL_{HR} were 1.71 and 1.76 respectively, and both were significantly lower than the average PAL_{ref}, which was 1.98. The corresponding value for PAL_q was 1.86, which was also lower than the average

Table 1. Age, weight, height, BMI, fat-free mass and body fatness of Swedish women*
(Mean values, standard deviations and ranges for thirty-four subjects)

	Mean	SD	Range
Age (years)	30	4	21–41
Weight (kg)	67	10	50–95
Height (m)	1.67	0.07	1.54–1.78
BMI (kg/m ²)	24	4	18–39
Fat-free mass (kg)	43	5	35–52
BF (%)			
BF _{DLW}	34	8	19–51
BF _{SFT}	33	6	20–43

BF, body fat; BF_{DLW}, body fat calculated from estimates of total body water; BF_{SFT}, body fat calculated from skinfold-thickness measurements.
* For details of procedures, see pp. 962–963.

Table 2. BMR, physical activity level (PAL) and total energy expenditure (TEE) obtained using different methods in Swedish women|| (Mean values and standard deviations for thirty-four subjects)

	Mean	SD
BMR (kJ/24 h)		
BMR _{predicted}	6010*	490
BMR _{meas}	5420	560
PAL		
PAL _{acc}	1.71†††	0.17
PAL _{HR}	1.76†	0.24
PAL _q	1.86	0.27
PAL _{ref}	1.98	0.21
TEE (kJ/24 h)		
TEE _{acc}	10 300§	1430
TEE _{HR}	10 580	1630
TEE _q	11 210	2000
TEE _{ref}	10 670	1370

BMR_{predicted}, BMR predicted from equations of the World Health Organization (1985); BMR_{meas}, BMR measured by indirect calorimetry; PAL, physical activity level; PAL_{acc}, physical activity level obtained using an accelerometer; PAL_{HR}, physical activity level obtained using heart-rate recording; PAL_q, physical activity level obtained using a questionnaire; PAL_{ref}, physical activity level obtained using a combination of doubly-labelled water and indirect calorimetry; TEE, total energy expenditure; TEE_{acc}, total energy expenditure obtained using an accelerometer; TEE_{HR}, total energy expenditure obtained using heart-rate recording; TEE_q, total energy expenditure obtained using a questionnaire; TEE_{ref}, total energy expenditure obtained using doubly-labelled water.

Mean value was significantly different from BMR_{meas}: * $P = 0.000015$.
Mean values were significantly different from PAL_{ref}: † $P = 0.00030$, †† $P = 0.00014$.
Mean value was significantly different from PAL_q: ‡ $P = 0.015$.
Mean value was significantly different from TEE_q: § $P = 0.013$.
||For details of subjects and procedures, see Table 1 and pp. 962–963.

PAL_{ref} but the difference was not significant. PAL_{acc} - PAL_{ref}, PAL_{HR} - PAL_{ref} and PAL_q - PAL_{ref} were -0.27, -0.22 and -0.12, respectively. The limits of agreement (Fig. 2) were large in all three cases (PAL_{HR}, PAL_{acc} 0.50; PAL_q 0.64). The value 1.64 - PAL_{ref} was found to be -0.34 (SD 0.21), i.e. two SD equalling only 0.42. Only weak linear relationships were found between PAL_{ref} and BF_{DLW} (%) ($r = 0.309, P = 0.075$), and between PAL_{ref} and BF_{SFT} (%) ($r = 0.341, P = 0.048$). However, (TEE_{ref} - BMR_{meas})/BW (kJ/kg) was significantly correlated with BF_{DLW} (%) ($r = 0.688, P = 0.000007$) as well as with BF_{SFT} (%) ($r = 0.652, P = 0.000029$). None of the linear relationships between BF_{DLW} (%) and PAL_{acc}, PAL_{HR} or PAL_q, or between BF_{SFT} (%) and PAL_{acc}, PAL_{HR} or PAL_q, were significant.

Total energy expenditure

Table 2 also shows TEE_{acc}, TEE_{HR}, TEE_q and TEE_{ref} of the subjects in the present study. The Bland & Altman (1986) graphs for TEE_{acc}, TEE_{HR} and TEE_q v. TEE_{ref} are shown in Fig. 3. On average these four estimates of TEE were similar and neither TEE_{acc}, TEE_{HR} nor TEE_q were significantly different from TEE_{ref}. The mean differences (kJ/24 h) between TEE_{acc}, TEE_{HR} and TEE_q and TEE_{ref} were -370 for TEE_{acc}, -83 for TEE_{HR} and 544 for TEE_q. The limits of agreement (Fig. 3) were large in all three cases (TEE_{HR} 2706, TEE_{acc} 2930, TEE_q 3862 kJ/24 h). TEE_{1.64} was 9860 (SD 800) kJ/24 h and TEE_{1.64} - TEE_{ref} was -804 (SD 1217) kJ/24 h, i.e. two SD equalling only 2434 kJ/24 h.

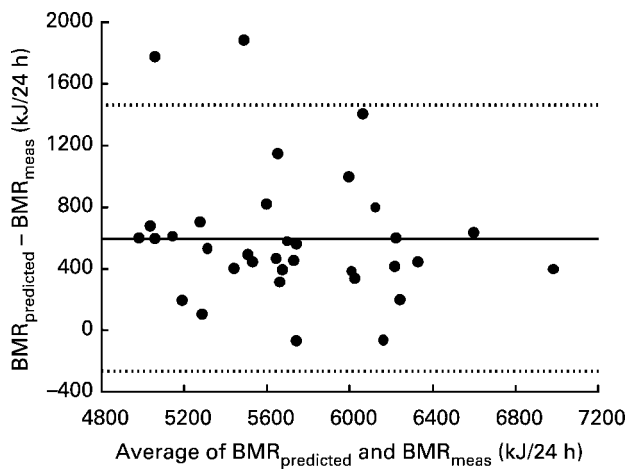


Fig. 1. Comparison of BMR obtained using prediction equations ($BMR_{predicted}$; World Health Organization, 1985) and BMR measured by means of indirect calorimetry (BMR_{meas}), according to Bland & Altman (1986). Mean difference ($BMR_{predicted} - BMR_{meas}$) = 594 (2SD 862) kJ/24 h. —, Mean value;, + or - 2SD. For details of subjects and procedures, see Table 1 and pp. 962–963.

Body fatness and total energy expenditure

Table 3 shows the results obtained when we investigated whether BF of the subjects contributed to a bias in TEE_{acc} , TEE_{HR} , TEE_q and $TEE_{1.64}$, i.e. the results obtained when $TEE_{acc} - TEE_{ref}$, $TEE_{HR} - TEE_{ref}$, $TEE_q - TEE_{ref}$ and $TEE_{1.64} - TEE_{ref}$ were correlated with BF_{DLW} (%) and BF_{SFT} (%) respectively. As indicated in Table 3, the correlation coefficients for seven of the eight linear regression equations were significant. None of the four dependent variables shown in Table 3 was significantly correlated with BW. The multiple regression equation with $TEE_{1.64} - TEE_{ref}$ (kJ/24 h) as the dependent variable and BF_{DLW} (%) and BW (kg) as independent variables was: $y = 141.3 \times BF_{DLW} - 48.8 \times BW - 2367$ (adjusted coefficient of determination 38.4 %, $P=0.0002$). When maintaining $TEE_{1.64} - TEE_{ref}$ (kJ/24 h) as the dependent variable while using BF_{SFT} (%) and BW (kg) as

Table 3. Correlation between variables related to total energy expenditure and body fatness in Swedish women* (Results based on thirty-four subjects)

Dependent variable (kJ/24 h)	Independent variable (%)	Correlation coefficient	Statistical significance (P)
$TEE_{acc} - TEE_{ref}$	BF_{DLW}	0.470	0.0051
	BF_{SFT}	0.348	0.0439
$TEE_{HR} - TEE_{ref}$	BF_{DLW}	0.287	NS
	BF_{SFT}	0.411	0.0158
$TEE_q - TEE_{ref}$	BF_{DLW}	0.431	0.0110
	BF_{SFT}	0.387	0.0238
$TEE_{1.64} - TEE_{ref}$	BF_{DLW}	0.582	0.0003
	BF_{SFT}	0.476	0.0044

TEE_{acc} , total energy expenditure obtained using an accelerometer; TEE_{ref} , total energy expenditure obtained using doubly-labelled water; TEE_{HR} , total energy expenditure obtained using heart-rate recording; TEE_q , total energy expenditure obtained using a questionnaire; $TEE_{1.64}$, total energy expenditure obtained by using physical activity level 1.64; BF_{DLW} , body fat calculated from estimates of total body water; BF_{SFT} , body fat calculated from skinfold-thickness measurements.

* For details of subjects and procedures, see Table 1 and pp. 962–963.

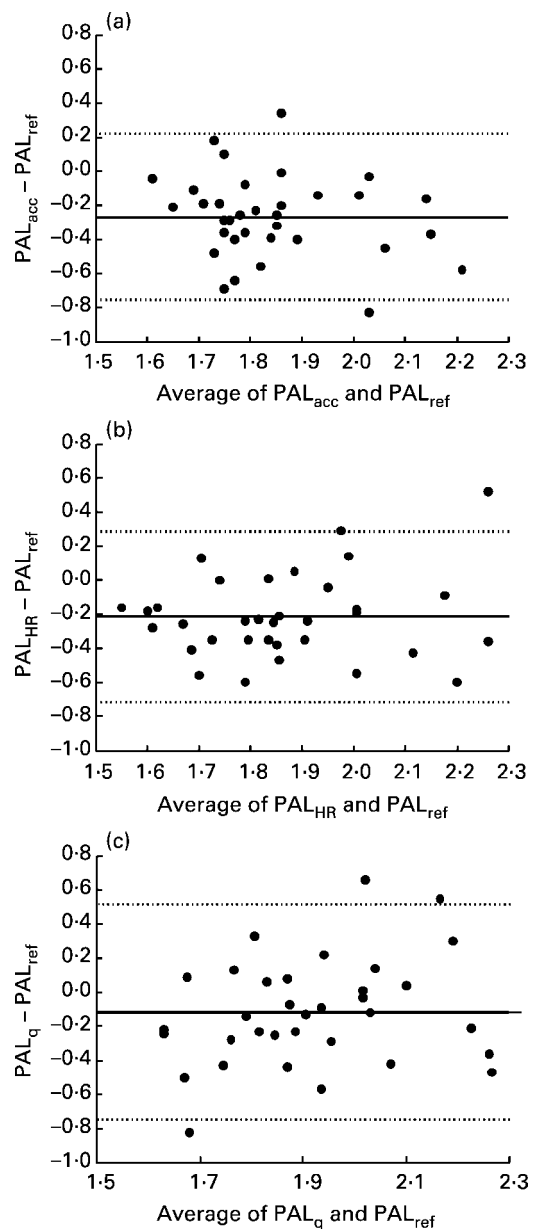


Fig. 2. (a), Comparison of physical activity level obtained using the accelerometer (PAL_{acc}) and physical activity level measured using a combination of the doubly-labelled water method and indirect calorimetry (PAL_{ref}), according to Bland & Altman (1986). Mean difference ($PAL_{acc} - PAL_{ref}$) = -0.27 (2SD 0.50). (b), Comparison of physical activity level obtained with the heart-rate recorder (PAL_{HR}) and PAL_{ref} , according to Bland & Altman (1986). Mean difference ($PAL_{HR} - PAL_{ref}$) = -0.22 (2SD 0.50). (c), Comparison of physical activity level obtained using the questionnaire (PAL_q) and PAL_{ref} , according to Bland & Altman (1986). Mean difference ($PAL_q - PAL_{ref}$) = -0.12 (2SD 0.64). —, Mean value;, + or - 2SD. For details of subjects and procedures, see Table 1 and pp. 962–963.

independent variables, the multiple regression equation was: $y = 142.3 \times BF_{SFT} - 28.7 \times BW - 3516$ (adjusted coefficient of determination 21 %, $P=0.0099$). Using $TEE_{acc} - TEE_{ref}$, $TEE_{HR} - TEE_{ref}$ or $TEE_q - TEE_{ref}$ as the dependent variable (kJ/24 h), while BF (%) and BW (kg) were the independent variables, the adjusted coefficients of determination of the corresponding multiple

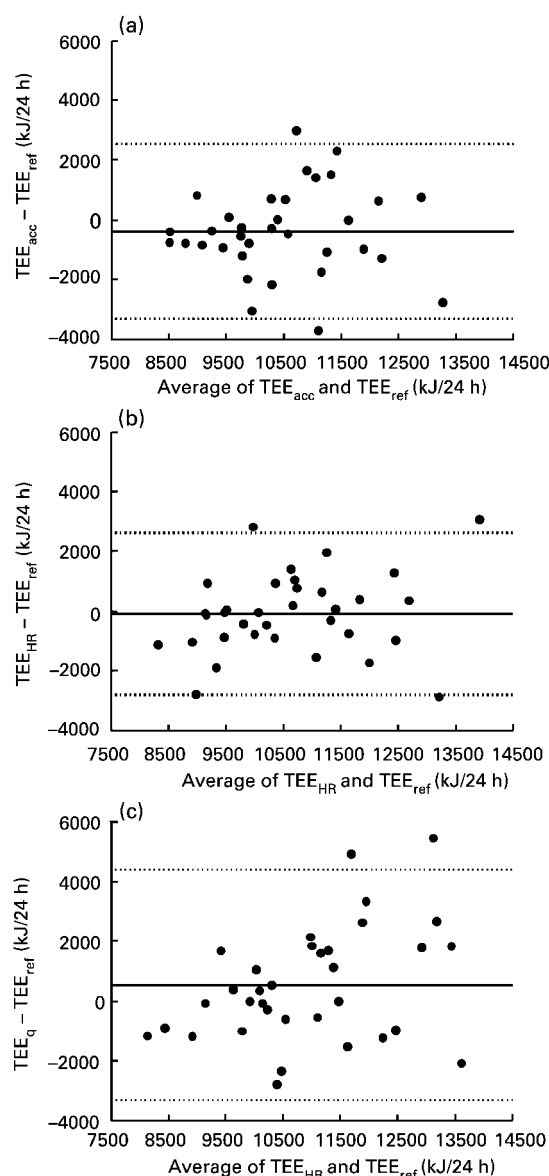


Fig. 3. (a), Comparison of total energy expenditure obtained using the accelerometer (TEE_{acc}) and total energy expenditure obtained using the doubly-labelled water method (TEE_{ref}), according to Bland & Altman (1986). Mean difference ($TEE_{acc} - TEE_{ref}$) = -370 ($2SD$ 2930) kJ/24 h. (b), Comparison of total energy expenditure obtained using the heart-rate recorder (TEE_{HR}) and TEE_{ref} , according to Bland & Altman (1986). Mean difference ($TEE_{HR} - TEE_{ref}$) = -83 ($2SD$ 2706) kJ/24 h. (c), Comparison of total energy expenditure obtained using the questionnaire (TEE_q) and TEE_{ref} , according to Bland & Altman (1986). Mean difference ($TEE_q - TEE_{ref}$) = 544 ($2SD$ 3862) kJ/24 h. —, Mean value; , + or $-2SD$. For details of subjects and procedures, see Table 1 and pp. 962–963.

regression equations were $\leq 13\%$ irrespective of whether BF_{DLW} (%) or BF_{SFT} (%) was used as the estimate of body fatness.

Discussion

Recent estimates (National Board of Health and Welfare, 2002) show that about 8 and 22 % of Swedish adult

women have BMI >30 and >25 respectively. The situation in many other Western countries is similar (International Obesity Task Force, 2003). Thus, with respect to the variation in body fatness, our subjects are typical for women in Western populations. Their average TEE_{ref} was also comparable with previous results for TEE obtained by means of the DLW method in Swedish women (Forsum *et al.* 1992) and in women in other countries (Goldberg *et al.* 1993). The corresponding value for 136 women with the same age range as those in our present study, but with a BMI <25 , was 5–10% lower (Food and Nutrition Board and Institute of Medicine, 2002).

In the present study, $BMR_{predicted}$ overestimated BMR_{meas} by, on average, about 10%. An even greater discrepancy between BMR measured using indirect calorimetry and BMR predicted by means of the equation from the World Health Organization (1985) was found by Staten *et al.* (2001) in a group of female subjects with an average BMI 28 kg/m^2 . Since BMR_{meas}/kg fat-free mass in our present subjects was comparable with results from other studies (Forsum *et al.* 1992; Bronstein *et al.* 1996; Butte *et al.* 2003), this overestimation can be explained by the comparatively high level of body fatness in our present subjects.

The finding that our estimate of PAL_{ref} was higher than our estimates of PAL_{acc} , PAL_{HR} and PAL_q motivates the following comments. The subjects did not wear the recording devices the whole time that they were awake, and for time not monitored we assumed that their activities had a MET factor of 1.4. We consider it probable that time without recorders tended to be time spent doing light rather than more vigorous activities. Likewise, if the subjects failed to report any activities when the questionnaire was administered, it is highly unlikely that activities with high MET factors were forgotten. The obvious explanation for our low PAL values is rather that the MET factors used are too low for our relatively heavy and fat subjects. Although such factors were originally assumed to be constant across a wide range of BW (World Health Organization, 1985), Haggarty *et al.* (1997) reported that the energy cost of different activities expressed as a multiple of BMR increases with increasing BW. In addition, recent findings by Howell *et al.* (1999) as well as by Staten *et al.* (2001) support the statement by Racette *et al.* (1995) that standard calculations of the energy costs of physical activity need modification when applied to subjects with a high BF content, as available MET factors underestimate the energy expenditure of such subjects.

With respect to the precision of our estimates of PAL_{acc} , PAL_{HR} and PAL_q as well as of TEE_{acc} , TEE_{HR} and TEE_q , the following comparisons are relevant. With regard to HR recording, our present results compare favourably with those reported by Fogelholm *et al.* (1998) and by Livingstone *et al.* (1990). This is despite the fact that our present subjects recorded their HR during only one of two experimental weeks, that several of them were unable to wear the HR recorder as much as we wanted since the device was perceived as quite uncomfortable, and that we did not establish individual relationships between HR and O_2 uptake as is commonly done when this method is applied.

With regard to the precision of the accelerometer measurements, our present results were slightly better than those reported by Fogelholm *et al.* (1998), while the precision of our estimates of PAL_q was better than for the corresponding values reported, for example, by Conway *et al.* (2002) for physical activity records and recall questionnaires. Thus, our PAL_{acc} , PAL_{HR} and PAL_q values have a precision comparable with that reported by other investigators.

In the present study, BF was estimated using two different methods that were independent of each other. Estimates based on assessment of total body water represent an accurate method for assessing BF, while the skinfold-thickness method has the advantage of providing estimates of body fatness independent of BW. There was a strong correlation between results obtained using the two methods. However, a comparison according to Bland & Altman (1986) showed that BF_{SFT} (%) tended to be lower than BF_{DLW} (%) for subjects with high levels of body fatness. This is supported by the results in Table 1 showing that the fattest woman in the study contained 51 % BF_{DLW} but only 43 % BF_{SFT} .

The World Health Organization (1985) procedure may at first sight appear to give satisfactory estimates of TEE in our present subjects since, on average, TEE_{acc} , TEE_{HR} as well as TEE_q were all in reasonable agreement with TEE_{ref} . However, this was apparently an effect of errors in estimates of both BMR and PAL that cancelled each other out. In addition, the results presented in Table 3 show that there is a risk that TEE values calculated using the World Health Organization (1985) procedure will be biased with respect to BF of the subjects. This observation has, to the best of our knowledge, not been reported previously. It should be noted that all relationships in Table 3 were examined using two estimates of BF that are independent of one another as independent variables. One of these, BF_{DLW} (%), requires estimates of BW, a variable that is also a component of the dependent variables in Table 3, a situation involving a risk for obtaining spurious correlations. However, significant correlations with the dependent variables were also identified for BF_{SFT} (%), clearly showing that the bias identified in this Table is independent of BW estimations. The relationships in Table 3 demonstrate that the risk for obtaining biased estimates of TEE is present irrespective of the method used to assess PAL, but that it is especially pronounced when a constant PAL value is used. This observation is of interest, since a similar kind of bias will be present for any constant PAL value used to calculate TEE by means of the World Health Organization (1985) procedure. Furthermore, the results obtained in the present study can be used to demonstrate that for subjects where BW and BF (%) vary to the extent they do in our subjects, there is a tendency to underestimate the TEE of lean subjects, while the TEE of overweight subjects tends instead to be overestimated. Thus, there is a risk that the World Health Organization (1985) procedure may produce misleading results. For example, this kind of bias may have important implications in relation to the observation that obese subjects tend to underestimate their dietary energy intake. Hill & Davies (2001) have compiled studies based on the DLW method showing that this statement is valid and

well documented. However, the World Health Organization (1985) procedure is often used to calculate TEE in studies evaluating the validity of energy intake measurements in human subjects (Black *et al.* 1991; Goldberg *et al.* 1991; Johansson *et al.* 1998, 2001; Black, 2000b). As our present results demonstrate, such a procedure is likely to influence the validity of the results, especially if, as is often the case, all subjects are assumed to have the same PAL. The consequence may well be incorrect conclusions regarding the significance of BF for the validity of energy intake reports.

One obvious explanation for our finding that TEE, estimated by means of the World Health Organization (1985) procedure, will be increasingly too high as BF increases is that $BMR_{predicted}$ then becomes increasingly too high. However, when multiplied by PAL, obtained using currently available MET factors, this overestimation is counterbalanced, since these factors tend to be increasingly too low when body fatness increases. However, to explain our finding, additional considerations may also be relevant. Our present results showed weak correlations between PAL_{ref} and BF (%), and when expressing $TEE_{ref} - BMR_{meas}$ per kg BW, significant linear relationships with BF (%) were obtained. In their study on Pima Indians, Rising *et al.* (1994) observed similar but stronger correlations, findings that were interpreted as a decrease in physical activity with increasing body fatness. In our present study, we were unable to demonstrate any significant decrease in PAL_{HR} , PAL_q or PAL_{acc} with increasing BF (%). Nevertheless, it is possible that failure to correctly measure a decrease in physical activity associated with increasing body fatness has contributed to our observation that the World Health Organization (1985) procedure tends to provide estimates of TEE that are biased with respect to body fatness.

In conclusion, the present study shows that although the World Health Organization (1985) procedure produced apparently satisfactory estimates of average TEE for a group of women with a variation in body fatness typical for Western populations, it also produced erroneous results. Thus, BMR values calculated using the recommended equations were too high for these women, while PAL values estimated according to the World Health Organization (1985) procedure tended to be too low, because the recommended procedure was obviously inappropriate for our relatively heavy and fat subjects. Furthermore, when BMR was multiplied by PAL according to the World Health Organization (1985) procedure, the TEE results obtained were biased with respect to the body fatness of the subjects. This observation is potentially important in many situations, for example when the validity of dietary intake data is evaluated.

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