Reproducibility of 24 h energy expenditure measurements using a human whole body indirect calorimeter

BY JANNA O. DE BOER, AREN J. H. VAN ES AND JOOP E. VOGT

Department of Animal Physiology, Agricultural University, Haarweg 10, 6709 PJ Wageningen, The Netherlands

AND JOOP M. A. VAN RAAIJ AND JOSEPH G. A. J. HAUTVAST

Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands

(Received 20 June 1986 – Accepted 17 September 1986)

1. Ten female subjects completed two similar experimental procedures (periods 1 and 2) to obtain values of reproducibility of energy intake and 24 h energy expenditure (24hEE) measurements in a whole body indirect calorimeter. The periods consisted of consumption of a provided weight-maintenance diet for 6–8 d, faeces and urine collection during the last 4 d and occupation of the calorimeter during the last 3 d. The daily routine inside the calorimeter simulated a sedentary day in normal life with some physical activity: 8 h sleep, 75 min bicycling and the remaining time spent on sedentary activities. The metabolizable energy (ME) content of the diet (14% energy as protein, 46% energy as carbohydrate, 40% energy as fat) was calculated using food tables. The actual ME intake as well as digestibility and metabolizability of the diet were obtained later by analyses of food, faeces and urine for energy. Three consecutive 24hEE measurements were performed during the stay in the calorimeter in each period. The time interval between the two periods varied from 2 to 24 months. Reproducibility was assessed at group and individual level.

2. Mean digestibility and metabolizability of the diet showed no significant difference between periods. The within-subject coefficient of variation of metabolizability between periods was 1·7%.

3. Mean 24hEE (MJ) over 3 d did not differ between period 1 (8·78 (SD 0·63)) and period 2 (8·73 (SD 0·66)). The within-subject coefficient of variation in mean 24hEE over three successive days between periods was 3·1% but decreased, after deletion of values for subjects who were less adapted to the calorimeter, to 1·9%.

4. The results are discussed with regard to length of trial and the number of subjects required to test a difference in energy metabolism using whole body indirect calorimeters.

To investigate the effects of thermogenic stimuli, for example, physical activity, cold or heat exposure, nutrient intake, smoking, drugs, etc., on 24 h energy expenditure, measurements are often performed on the same subject with and without the thermogenic stimulus (Dauncey, 1980; Blaza & Garrow, 1983; Dauncey & Bingham, 1983; Hofstetter *et al.* 1983; Dallosso & James, 1984; Van Es *et al.* 1984). It is important in such measurements to control the variables not under study. However there will remain sources of variation that cannot be controlled. The size of this variation is needed to estimate the size of the sample (number of subjects) for planning experiments on effects of thermogenic stimuli. For this estimation the investigator must decide on: (*a*) the size of the true effect (d) of the stimulus on 24 h energy expenditure, that is regarded as important; (*b*) the desired probability ($1-\beta$) of obtaining a significant result if the true difference is d; (*c*) the significance level α of the test, which may be either one- or two-tailed (Snedecor & Cochran, 1967).

In paired samples an assumption must be made of the standard deviation of the true difference in 24 h energy expenditure (24hEE). In this case knowledge of the within-subject variation or reproducibility of a single 24hEE measurement may be helpful.

At present there is little information available on the reproducibility of 24hEE measurements at group and individual levels. Dallosso *et al.* (1982) and Garby *et al.* (1984) found in male subjects with fixed physical activity within-subject coefficients of variation (CV_w)

of a single 24hEE measurement of 1·5 and 2·2% respectively, with an intervening period of 1 week.

From two 24hEE measurements in the control period in studies of Webb & Abrams (1983) and Webb & Annis (1983), values for CV_w of 3·3 and 6% respectively were calculated, the time-interval in the first study being about 14 d. Garrow & Webster (1985) stated that Blaza (1980) had found a mean difference between duplicate measurements of 2% in six obese women.

All these estimates of CV_w in 24hEE measurements were derived from measurements with direct calorimeters, except for the value 1·5% which originates from indirect calorimetry (Dallosso *et al.* 1982). The CV_w includes errors of measurements, besides sources of variance like biological variability and differences in behaviour. Since techniques of direct and indirect calorimetry differ, values derived from direct calorimetry may not be appropiate for use in indirect calorimetry. Furthermore, most of the CV_w values originate from duplicate measurements with a time interval of 2 weeks or less. In the studies on thermogenic stimuli, however, the time interval between measurements varied from a few days to 1 month and, in studies on the effect of long-term overfeeding and underfeeding (for instance slimming) on energy metabolism, time intervals of 4 weeks and more are not uncommon (Norgan & Durnin, 1980; Bessard *et al.* 1983; Webb & Abrams, 1983; Webb & Annis, 1983).

The aim of the present paper is to present values relating to the reproducibility, at group level and individual level, of energy intake and 24hEE measured by indirect calorimetry. The values concern ten female subjects who were measured twice, each time during three consecutive days, with the same energy intake and the same standardized physical activity pattern, and with a time-interval of between 2 and 24 months.

MATERIALS AND METHODS

Subjects

The subjects were ten, apparently healthy women. Each completed two experimental periods (period 1 and period 2) of which the design is given later. Characteristics of the subjects are given in Table 1 (p. 205). Three subjects were smokers; these were allowed to continue smoking during the experimental periods.

Subjects nos. 1–6 had already spent 1 or 4 d in the calorimeter before experimental period 1. It may, therefore, be assumed that these subjects were familiar with, and well adapted to, the calorimeter. Subjects nos. 7–10 were familiarized by clear and careful explanation of the procedures and the calorimeter 1–2 months before and the evening before the measurements started.

Experimental design

Each subject was submitted to two experimental periods, period 1 and period 2. The subjects followed the same protocol in both experimental periods except for subjects nos. 1–3 whose experimental period 1 lasted only 6 d. Each experimental period consisted of the consumption of an experimental diet (for 6 or 8 d), which was designed to meet the individual energy requirement of the subject. Faeces and urine were collected during the last 4 d and the subjects occupied the calorimeter during the last 3 d (days no. 6, 7 and 8) of each experimental period. Three 24hEE measurements were made during each calorimetry session.

Some of the values originate from two different studies, which had both been approved by the Ethical Committee of the department of Human Nutrition, Agricultural University, Wageningen.

Diets

Each subject completed a weighed food record during normal life. The results of this food record were used to design the experimental diet. The metabolizable energy (ME) content of the experimental diet was initially calculated from the Dutch Food Composition Table (1981).

The contributions of energy from protein, fat and carbohydrate to the metabolizable energy intake of the experimental diet were 14, 40 and 46% respectively, simulating the composition of an average Dutch diet.

Food items, food preparation, storage and sampling procedures have already been described by Van Es *et al.* (1984). The food was weighed out to the nearest 0·1 g. The subjects had to eat and drink everything provided.

The diet during experimental period 1 was identical to that in period 2, except for subject no. 1, who consumed 1·2 MJ/d less in experimental period 2.

Gross energy intake was calculated by multiplying the weight of the provided foods with the energy content as assessed by bomb calorimetry (see later). Subtraction of energy losses in faeces and urine from gross energy intake yielded the digestible energy intake and ME intake respectively. Digestible energy and metabolizable energy, expressed as a percentage of gross energy intake, are called digestibility and metabolizability of the diet.

Collection of faeces and urine

Faeces and urine were collected during the last 4 d of each experimental period. Faeces were collected, refrigerated and subsequently pooled, weighed, freeze-dried and sampled. Urine collection started and ended after voiding in the morning. Urine was collected in 1 litre plastic bottles containing mercury iodide as a preservative. It was then stored in the refrigerator, pooled, weighed and sampled.

Measurements of energy expenditure

Apparatus: Two whole-body indirect calorimeters were used. The calorimeters and gas-exchange measurements have already been described by Van Es *et al.* (1984).

Three consecutive 24 h gas-exchange measurements were made during each experimental period. Each measurement started at 07.30 hours.

The 24 h composite samples of the air entering and leaving the calorimeters were analysed volumetrically for oxygen and carbon dioxide concentration using a Sonden apparatus.

Brouwer's (1965) equation was used to calculate 24hEE from O_2 consumption, CO_2 production and urinary nitrogen (N_u):

$$24\text{hEE (kJ)} = 16{\cdot}18 \times O_2 \text{ consumption (l)} + 5{\cdot}02 \times CO_2 \text{ production (l)} - 5{\cdot}99 \times N_u \text{ (g)}.$$

Before calculation of 24hEE, O_2 consumed and CO_2 produced by burning cigarettes was subtracted from O_2 consumption and CO_2 production. The correction term for protein oxidation in the equation ($-5{\cdot}99 \times N_u$) lowered 24hEE by an average of 0·7%. This term was used only in the calculation of the mean 24hEE over 3 d. In single 24hEE values this correction term was neglected, thus resulting in about 0·7% higher 24hEE values.

Procedure in the calorimeter

The two calorimeters were used simultaneously. Subjects could see each other through a large window in the connecting wall between the calorimeters, and could talk to each other by intercom. Subjects wore their own clothes and adjusted their clothing to their own comfort. The ambient temperature was set at 21–22° during the daytime and at 19–20° at night, but was adjusted when subjects felt uncomfortable. Relative humidity was kept between 60 and 80%. Subjects entered the chamber in the evening 8–9 h before the gas-exchange measurement started. They were awoken at 07.45 hours and followed a standard daily activity schedule. This schedule included five times 15 min bicycling on a home-trainer at a speed of 24 km/h, without a load at 08.45, 12.15, 13.30, 17.30 and 22.30 hours. Subjects prepared for bed at 22.45 and were in bed from 23.15 until 7.45. The rest of the day was filled with sedentary activities and some spontaneous activities, like preparing coffee, tea or meals, washing dishes, etc. The schedule was meant to simulate a light housekeeping or sedentary working-day.

Analyses of food, excreta and cigarettes

Energy content of food items, urine, faeces and cigarettes was determined using a static bomb calorimeter. N content of urine was determined by the Kjeldahl method (International Organization for Standardization, 1979) using mercuric oxide as a catalyst.

Analyses of results

Reproducibility is defined as the degree of agreement between repeated measurements, using one technique on the same subject or on the same group of subjects at different times. It depends on the residual variance which includes variance due to errors in measurements, interaction terms, biological variability and behaviour. This residual variance will be referred to as within-subject variance. Reproducibility at the group level was tested using Student's *t* test for paired samples. When the difference between mean values was not significant ($P > 0{\cdot}05$), the measurement was considered to be reproducible at the group level.

Reproducibility at the individual level is expressed by CV_w. A small CV_w means that the measurement is highly reproducible. The CV_w was estimated as follows: the mean of the differences in individual data between periods and the variance of these differences (SD^2_{dif}) were calculated. Within-subject variance ($SD_w{}^2$) may be estimated as $SD_w{}^2 = SD^2_{dif}/2$. The CV_w (calculated as $SD_{w/mean} \times 100\%$) was calculated for all the values presented here.

Three different CV_w between periods were calculated for 24hEE; (1) CV_{w3} was calculated from the individual differences in the mean of three subsequent 24hEE measurements (days 6+7+8) between the two periods (in this calculation 24hEE was not corrected for protein oxidation); (2) CV_{w2} was calculated from the individual differences in the mean of two subsequent 24hEE measurements (days 6+7) between the two periods; (3) CV_{w1} was calculated from the individual differences in the 24hEE for 1 d (day 6) between the two periods. Analysis of variance was performed to test for an effect of the sequence of days spent in the calorimeter, and of the period of measurement on 24hEE. The CV_w within-periods was calculated from these computations. Calculations were performed using the Statistical Package for the Social Sciences (Nie *et al.* 1975).

RESULTS

Body-weight and body composition

Table 1 shows the change in body-weight and body composition of the individual subjects between the experimental periods. Subject 10 lost 13 kg in the 11-month interval between the experimental periods; the values were excluded from analyses on reproducibility between periods. Mean body-weight and body composition did not differ between periods 1 and 2. The CV_w in body-weight was $2{\cdot}0\%$ and that in body composition was $8{\cdot}0\%$.

Energy intake

Individual gross energy intake, digestibility and metabolizability in each experimental period are presented in Table 2. Mean differences in gross energy intake, digestibility,

Table 1. *Characteristics of ten female subjects at the time of experimental periods 1 and 2 including individual differences, between periods 1 and 2, in body-weight (BW; kg)* and body fat percentage (BF)† (ΔBW and ΔBF respectively)*

Subject no.	Age‡ (years)	Height (m)	BW 1	BW 2	ΔBW	BF 1	BF 2	ΔBF	Time interval (months)
1	22	1·77	63·0	62·6	0·4	22·5	23·4	−0·9	13
2	23	1·73	71·1	69·9	1·2	30·1	—	—	2
3	23	1·66	68·7	71·5	−2·8	32·7	36·4	−3·7	12
4	28	1·73	54·1	55·0	−0·9	20·8	19·0	1·8	7
5§	36	1·63	59·0	60·8	−1·8	24·0	—	—	5
6§	24	1·68	67·3	65·0	2·3	35·4	31·8	3·6	7
7	25	1·63	54·4	55·4	−1·0	25·5	26·7	−1·2	10
8	25	1·62	63·5	64·9	−1·4	28·5	32·5	−4·0	4
9§	29	1·76	60·4	58·4	2·0	24·2	21·1	3·1	24
10	31	1·66	113·2	100·6	12·6	40·9	36·3	4·6	11
Mean‖	27	1·69	62·4	62·6	−0·2	27·1	27·3	−0·2	10
SD	5	0·06	6·0	5·9	1·8	5·4	6·5	3·1	6

* Mean BW during 3 d calorimetry session.
† Derived from underwater weighing.
‡ Age at time of experimental period 1.
§ Smokers.
‖ Values for subject no. 10 were excluded from mean BW 1, BW2, ΔBW, BF 1, BF 2 and ΔBF, because of large BW change.

Table 2. *Gross energy intake (GE; MJ) and energy digestibility (D) and metabolizability (M) of ten female subjects during experimental periods 1 and 2, including differences between periods 1 and 2 (ΔGE, ΔD and ΔM respectively)*

Subject no.	GE 1	GE 2	ΔGE	D 1	D 2	ΔD	M 1	M 2	ΔM
1*	11·29	9·72	1·57	92·7	95·0	−2·3	88·6	91·2	−2·6
2	10·28	9·99	0·29	93·5	93·4	0·1	89·7	89·4	0·3
3	10·29	10·24	0·05	92·8	92·0	0·8	88·4	88·0	0·4
4	9·64	9·48	0·16	93·0	92·2	0·8	88·9	88·4	0·5
5	10·70	10·60	0·10	96·9	91·8	5·1	92·6	87·9	4·7
6	10·28	10·41	−0·13	93·8	94·3	−0·5	89·2	90·5	−1·3
7	10·49	10·71	−0·22	94·6	93·7	0·9	90·8	89·8	1·0
8	10·31	10·37	−0·06	93·9	93·3	0·6	89·8	89·0	0·8
9	10·50	10·94	−0·44	95·2	92·1	3·1	90·9	88·3	2·6
10	11·19	11·39	−0·20	91·7	94·5	−2·8	86·8	90·1	−3·3
Mean†	10·31‡	10·34‡	−0·03‡	94·0	93·1	1·0	89·9	89·2	0·7
SD	0·31	0·45	0·23	1·4	1·1	2·1	1·3	1·2	2·1

* GE lower in experimental period 2 for experimental reasons.
† Values for subject no. 10 not included because of large weight change.
‡ Values for subject no. 1 excluded.

Table 3. *Individual 24 h energy expenditure (24hEE) values (MJ) and individual differences (Δ) in 24hEE (MJ) between periods 1 and 2*

(Δ1, 24hEE day 6 period 1 − 24hEE day 6 period 2; Δ2, mean 24hEE days 6+7 period 1 − mean 24hEE days 6+7 period 2; Δ3, mean 24hEE days 6+7+8 period 1 − mean 6+7+8 period 2)

Subject no.	Period 1			Period 2					
	6*	7	8	6	7	8	Δ1	Δ2	Δ3
1	8·49	8·45	8·51	8·38	8·17	8·42	0·11	0·19	0·16
2	8·23	8·07	8·02	7·96	8·20	8·10	0·27	0·07	0·03
3	8·32	8·36	8·57	8·76	8·41	8·82	−0·44	−0·24	−0·24
4	8·31	7·85	7·99	8·23	7·98	8·18	0·08	−0·02	−0·08
5	9·14	8·79	9·13	8·56	9·05	8·80	0·58	0·17	0·22
6	9·56	9·37	9·28	9·59	9·78	9·98	−0·03	−0·22	−0·38
7	9·46	9·66	8·99	8·34	8·57	8·58	1·12	1·10	0·87
8	8·94	8·51	9·18	9·03	9·04	8·87	−0·09	−0·32	−0·10
9	9·66	10·11	—†	9·81	10·12	9·78	−0·15	−0·08	—†
10	14·04	13·93	13·16	12·66	12·42	12·57	1·38	1·44	1·23
						Mean‡	0·16	0·07	0·06
						SD_{dif}§	0·46	0·43	0·38
						SD_w‖	0·32	0·30	0·27

* Day of experiment.
† No values on 24hEE due to technical failure.
‡ Values for subject no. 10 not included because of large weight change between periods.
§ Standard deviation of the individual differences.
‖ Within-subject standard deviation.

Table 4. *Within-subject coefficients of variation (CV_w%) between periods when one, two or three subsequent 24 h energy expenditure measurements per period were performed*

(Within-subject standard deviation is presented in parentheses (kJ)).

	n 9*	*n* 6†
CV_{w1}	3·7 (323)	2·8 (238)
CV_{w2}	3·4 (301)	1·6 (133)
CV_{w3}	3·1 (270)	1·9 (164)

* Values for subject no. 10 excluded.
† Excluding values for subjects less-well adapted to the calorimeter.

metabolizability and ME intake between periods were not significantly different from zero. CV_w of digestibility and metabolizability were 1·7 and 1·7% respectively.

Energy expenditure

Values for 24hEE measurements are shown in Table 3. There was no indication that the 24hEE within experimental periods differed systematically between days. The within-subject coefficient of variation of 24hEE measurements in experimental period 1 was 2·6% and that in period 2 was 1·8% (values for subject no. 10 included).

Mean 24hEE (corrected for protein oxidation) over 3 d (MJ) in period 1 was 8·78 (SD 0·63) and in period 2 was 8·73 (SD 0·66). Analysis of variance showed that the variance due to experimental period did not reach significance. The CV_{w3} was 3·1% (SD_{w3} 270 kJ). The larger CV_{w1} in 24hEE in period 1 (2·6%), compared with that in period 2 (1·8%), suggests that the subjects in experimental period 1 may have been insufficiently adapted to the calorimeter. Values for subjects nos. 7–9 were therefore excluded from analysis (as already mentioned, these subjects were less familiar with the calorimeter). After exclusion of their values, the CV_{w3} diminished to 1·9% (SD_{w3} 164 kJ; Table 4). Table 4 presents values of the CV_{w2} and CV_{w1} of 24hEE, based on values for all subjects (except no. 10) and on the values from the well-adapted subjects.

DISCUSSION

Energy intake

Mean gross energy intake remained constant at group level as expected since the diets were the same in both periods. The variation in gross energy intake on an individual level (CV_w 1·6%) may be ascribed to variation in energy density of the foods between experiments and to errors in measurement. Taking into account this variation and the variation in metabolizability, ME intake may show a CV_w of

$$\sqrt{(1{\cdot}6^2+1{\cdot}7^2)} = 2{\cdot}3\% \text{ (95\% confidence interval 1·5–4·4\%)}.$$

It is concluded that this variation in energy intake is small and is likely to have very little impact on 24hEE.

Energy expenditure

Our results show that mean 24hEE over 3 d with a standardized daily activity pattern is highly reproducible for groups, provided no significant change in weight or in body composition has occurred. The fact that the CV_w decreased after adaptation to the calorimeter indicates that reproducibility at the individual level improves after adaptation of the subjects to the calorimeter. A 1 d adaptation some weeks before the start of the experiment may be sufficient.

The observed within-period CV_w values in 24hEE (2·6 and 1·8%) are well within the range of values found by other investigators (Blaza, 1980; Dallosso *et al.* 1982; Webb & Abrams, 1983; Webb & Annis, 1983; Garby *et al.* 1984). These CV_w values are, however, not entirely comparable with those of the other investigators, because they originate from consecutive measurements. They may therefore be more strongly influenced by autocorrelation in 24hEE than the CV_w values of the others. The most comparable CV_w values are those presented in Table 4. It may be assumed that these 24hEE measurements within subjects were independent, as the interval between measurements was rather long. The value for CV_{w1} of 3·7% (*n* 9, 95% confidence interval 2·5–7·1%) was obtained using similar methods to those of the other investigators, except that the interval between measurements was much longer. This CV_{w1} is higher than the 1·5, 2·0 and 2·2% mentioned by others (Dallosso *et al.* 1982; Blaza, 1980; Garby *et al.* 1984 respectively). The CV_w of the well-adapted subjects (2·8%, 95% confidence interval 1·7–6·9%) is not significantly different from two of the earlier-mentioned estimates: 2·0 and 2·2%. It should be noted that in our experiment sources of variation were probably present that were absent or limited in the experiments of other investigators. First, physical activity, body-weight, body composition and living circumstances may have changed, thus influencing energy metabolism. Body-weight showed a small CV_w; body fat percentage, however, showed a relatively large CV_w. This CV_w reflects changes in body composition. Assuming that the observed variation in body-weight (SD_w 1·3 kg) is entirely reflected in change in fat mass (mean fat mass 17 kg), a CV_w of body fat percentage of 7·7% might be expected. The other part of variation is due to errors in body fat measurement (CV 2–3%). Second, spontaneous activities and behaviour of the subjects in the calorimeter may have differed between days and periods, although a standard activity pattern was prescribed. Third, another source of variation may

Table 5. *Number of female subjects required to test a change in 24 h energy expenditure (24hEE) within subjects of 3 (260 kJ), 5 (430 kJ) and 10% (860 kJ), with significance levels of $\alpha = 0{\cdot}05$ or $\alpha = 0{\cdot}01$ and a probability $(1-\beta)$ of 80 and 90%, when one, two or three consecutive 24hEE measurements were performed per subject.*

(The within-subject coefficients of variation used are 2·8% (238 kJ) for 1 d, 1·6% (133 kJ) for 2 d, and 1·9% (164 kJ) for 3 d measurements (plots from Owen (1962) were used))

Expected change in 24hEE (%) ...	3		5		10	
β ...	0·2	0·1	0·2	0·1	0·2	0·1
No. of 24hEE measurements						
$\alpha = 0{\cdot}05$ (one-tailed)						
1	8	9	4	5	3	3
2	4	5	3	3	3	3
3	5	6	3	4	3	3
$\alpha = 0{\cdot}01$ (one-tailed)						
1	12	14	6	7	4	4
2	6	7	4	4	3	3
3	7	9	5	5	3	4

have been the time of 24hEE measurement with regard to the menstrual cycle. Bisdee & James (1983) reported that sleeping metabolic rate increased, from the preovulatory to premenstrual stage of the cycle, by 6%. Webb (1985) mentions that the effect of the stage of the ovulatory cycle is strong in women in their 20's (7–15% increase after ovulation) but that this effect is reduced or absent in women in their 40's.

The calorimetry sessions in our experiment were planned between menstruation periods, but since in some subjects the length of the menstrual cycle was unpredictable, this planning failed in some instances. Garby *et al.* (1984) and Dallosso *et al.* (1982) did not have this source of variation, because they used men. Webb & Abrams (1983) and Webb & Annis (1983), however, also used female subjects; this may be one of the reasons for the higher CV_w in 24hEE in their experiments.

Table 4 shows that the CV_w in 24hEE between periods decreased as the number of subsequent measurements is increased. The CV_{w2}, however, was smaller than the CV_{w3} in the six well-adapted subjects. This seems likely to be an artefact, but the CV_w values given in Table 4 were used to calculate the sample size required to test a change in 24hEE induced by, for example, a thermogenic stimulus. The chosen levels of significance (α) were 0·05 and 0·01 (one-tailed) and the chosen probability (1-β) 80 and 90%. The expected change in 24hEE was selected as 3, 5 or 10%. The sample sizes required when one, two or three consecutive measurements are performed, are given in Table 5. It is clear from this table that no beneficial effect is to be expected from increasing the number of measurements per subject, when the effect to be tested amounts to 10% or more of the 24hEE. However, in calorimetric studies intended to detect small differences in 24hEE, i.e. 5% or less, with a high level of significance and probability, performing several consecutive measurements per subject may be advantageous. The values in Table 5 show no benefits to be gained by performing three rather than two measurements per subject.

In conclusion, considering the CV_w in 24hEE, the reproducibility of 24hEE is high, even over a long period of time. This is true provided that (1) body-weight and body composition have not changed significantly during the interval, (2) energy intake and physical activity during the measurements are the same and (3) the subjects are already well adapted to the calorimeter. Part of the CV_w is due to errors in measurement, suggesting that energy metabolism is a fairly constant phenomenon under comparable circumstances. Because the reproducibility of the 24hEE measurements in female subjects is high, one measurement on relatively few subjects may suffice to test an expected change of 10% or more in 24hEE. To test smaller changes in 24hEE (5% or less) it may become profitable to increase the number of consecutive 24hEE measurements, rather than increasing the number of subjects.

The authors wish to thank Mrs C. A. Roovers, Mr W. A. van Kranenburg, Mr M. J. N. Los, Academy Diedenoort and students of the department of Human Nutrition for their careful assistance in preparing meals, care for the experimental subjects and analytical work. The authors also wish to thank the volunteers who participated in the study. This work was supported by The Netherlands Heart Foundation (grant no. 31.005).

REFERENCES

Bessard, T., Schutz, Y. & Jequier, E. (1983). *American Journal of Clinical Nutrition* **38**, 680–693.

Bisdee, J. T. & James, W. P. T. (1983). In *Program and Abstracts of Fourth International Congress on Obesity*, New York, 52A.

Blaza, S. E. (1980). Thermogenesis in lean and obese individuals. PhD Thesis, CNAA.

Blaza, S. & Garrow, J. S. (1983). *British Journal of Nutrition* **49**, 171–180.

Brouwer, E. (1965). In *Proceedings of 3rd Symposium on Energy Metabolism, European Association of Animal Production. Publication* no. 11, pp. 441–443 [K. L. Blaxter editor]. London: Academic Press.

Dallosso, H. M. & James, W. P. T. (1984). *British Journal of Nutrition* **52**, 49–64.

Dallosso, H. M., Murgatroyd, P. R. & James, W. P. T. (1982). *Human Nutrition: Clinical Nutrition* **36C**, 25–39.

Dauncey, M. J. (1980). *British Journal of Nutrition* **43**, 257–269.

Dauncey, M. J. & Bingham, S. A. (1983). *British Journal of Nutrition* **50**, 1–13.

Dutch Food Composition Table (1981). *Nederlandse Voedingsmiddelen Tabel*. 33rd ed. Den Haag: Voorlichtingsbureau voor de Voeding.

Garby, L., Lammert, O. & Nielsen, E. (1984). *Human Nutrition: Clinical Nutrition* **38C**, 391–394.

Garrow, J. S. & Webster, J. D. (1985). In *Human Energy Metabolism: Physical Activity and Energy Expenditure Measurements in Epidemiological Research Based Upon Direct and Indirect Calorimetry, Euro-Nut Report* no. 5, pp. 215–224 [A. J. H. Van Es, editor]. Wageningen: The Netherlands Nutrition Foundation.

Hofstetter, A., Schutz, Y., Wahren, J. & Jequier, E. (1983). In *Program and Abstracts of Fourth International Congress on Obesity*, New York, 28A.

International Organization for Standardization (1979). *International Standard* no. 5983.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H. (1975). *Statistical Package for the Social Sciences*, 2nd ed. New York: McGraw-Hill Book Co.

Norgan, N. G. & Durnin, J. V. G. A. (1980). *American Journal of Clinical Nutrition* **33**, 978–988.

Owen, D. B. (1962). *Handbook of Statistical Tables*. Reading, Mass.: Addison-Wesley.

Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, 6th ed. Ames, Iowa: Iowa State University Press.

Van Es, A. J. H., Vogt, J. E., Niessen, Ch., Veth, J., Rodenburg, L., Teeuwse, V., Dhuyvetter, J., Deurenberg, P., Hautvast, J. G. A. J. & Van der Beek, E. (1984). *British Journal of Nutrition* **52**, 429–442.

Webb, P. (1985). In *Human Energy Metabolism: Physical Activity and Energy Expenditure Measurements in Epidemiological Research Based Upon Direct and Indirect Calorimetry. Euro-Nut Report* no. 5, pp. 30–31 [A. J. H. Van Es, editor]. Wageningen: The Netherlands Nutrition Foundation.

Webb, P. & Abrams, T. (1983). *Human Nutrition: Clinical Nutrition* **37C**, 271–282.

Webb, P. & Annis, J. F. (1983). *Human Nutrition: Clinical Nutrition* **37C**, 117–131.

Printed in Great Britain