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Intra-individual variability and measurement noise in estimates of energy expenditure by whole body indirect calorimetry

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1. Four men were each studied continuously over 12 d in a whole-body calorimeter. Dietary intake and daily activities were kept constant throughout the study.

2. Day-to-day coefficients of variation in energy expenditure within subjects were found to be 1·97% over 24 h, 5·93% during basal metabolic rate measurement, 2·40% overnight and 3·22% in exercise.

3. The contribution of measurement system noise to the observed variability was analysed and shown to be generally small. The source of this noise was considered.

4. The results reinforce and extend other comparable reports and show that within-subject variability forms a small part of reported observations of between-subject variability.

The recent introduction of new recommendations for predicting energy and protein requirements (Food and Agriculture Organization/World Health Organization/United Nation University, 1985) and equations for predicting basal metabolic rate (BMR) (Schofield *et al.* 1985) have generated an interest in the extent of inter-individual variability in energy expenditure (Dallosso *et al.* 1985). Contained within this is a component of intra-individual variability which has lacked comprehensive study. This variability is also of importance to designers of controlled trials involving measurements of energy expenditure. Whether each study subject acts longitudinally as his own control or is paired with a matched control subject, the sample size required to detect a particular level of metabolic response is a function of the intra-individual variability.

In the results of a study of four subjects, each confined within a calorimeter chamber for 12 d, we had a unique set of values which enabled us to analyse the intra-individual variability in measurements of heat production (HP) over 24 h, in BMR, in sleep and exercise. The results of comprehensive calibrations before the study enabled us to account for the component of variability arising from measurement-system noise and so to separate out the variability which was truly attributable to the subject.

METHODS

Subjects and experimental protocols

The primary aim of this study was to validate the doubly-labelled water ($^2H_2\,^{18}O$) method of estimating energy expenditure by comparing carbon dioxide production calculated from the differential isotope elimination rates with that measured in the calorimeter (Coward *et al.* 1985). Four male subjects were selected for this demonstration. Their physical characteristics are given in Table 1. All were non-smokers and a medical examination showed them to be fit and healthy. Ethical permission for the study was given by the Ethical Committee of the Dunn Nutrition Unit and each subject gave informed consent to his participation.

As the validation demanded a long period of study, each subject spent twelve consecutive days in the calorimeter. To make this acceptable to the subjects the protocol was adapted to meet each subject's requirements for periods spent standing, intensity and frequency of exercise, and duration of sleep. The acceptability of the protocol was then tested in a 36 h

Table 1. *Physical characteristics of subjects*

Subject no.	Age (years)	Height (m)	Wt (kg)	Percentage body fat from skinfolds
1	21	1·73	55·2	10·0
2	54	1·71	59·1	16·3
3	20	1·78	62·9	10·7
4	40	1·73	68·9	11·4

trial run in an 11 m^3 calorimeter before the main study. This also enabled the 24 h energy expenditure to be measured and the dietary intake adjusted in an attempt to avoid energy imbalance. Each subject was given three meals daily, equal in energy content and composition. In addition to his three main meals, subject no. 3 was given an evening snack containing 4% of his daily energy intake. Details of those elements of the protocol which varied from subject-to-subject are given in Table 2. Great care was taken to ensure that day-to-day variations in adherence to the protocol were avoided.

BMR was measured during the hour after waking. For subjects nos. 1, 3 and 4 this commenced at 08.00 hours and for subject no. 2 at 06.00 hours. In all cases this was 12 h after the last food intake. Obligatory periods of 0·5 h were spent in washing, undressing or dressing etc. after the BMR measurement and before going to bed.

The environmental temperature was maintained at 26° throughout the study. This is within the thermoneutral range for subjects at rest. Subjects wore their own choice of clothing overnight and jogging suits during the day.

Measurement of HP

Measurements were made in the 30 m^3 direct and indirect calorimeter at the Dunn Clinical Nutrition Centre (Murgatroyd, 1985). Although measurements of HP and heat loss were made simultaneously throughout the study, the results presented in the present paper are those of HP alone.

The living space of the calorimeter was 4·25 m long and 2·95 m wide. It was carpeted and equipped with comfortable domestic furniture which included a divan bed, lounge chair, upright chair, desk, bookshelf and cycle ergometer. A colour television was viewed through a window. The calorimeter was illuminated by lights above windows in the ceiling. An airlock hatch enabled food, washing water, etc., to be given to the subject without disturbance to the gaseous equilibria. A telephone and an intercom provided vocal communication with experimenters and friends. Windows in the calorimeter made visual communication between experimenter and subject possible provided the experimenter was within the air-conditioned shell area surrounding the calorimeter. As the prolonged presence of the experimenter in the shell could potentially disturb the heat loss measurements such interactions were limited to those essential to the protocol.

HP was calculated from oxygen consumption and CO_2 production rates using a formula scaled from that of Weir (1949) to give results in watts,

$$\text{HP} = 275 \times \text{O}_2 \text{ consumption} + 77 \times \text{CO}_2 \text{ production},$$

where gas consumption and production rates are expressed as litres/min. A correction of $-0·105 \times$ mean daily urinary nitrogen loss (g) was made to account for protein oxidation. This term had a mean magnitude of $-0·96$% of the corrected HP.

O_2 and CO_2 production rates were calculated using the analysis of Brown *et al.* (1984) for suction-ventilated systems. O_2 concentrations were measured using a Servomex OA184

Table 2. *Details of inter-individual variations in protocol*

Subject no.	Period of sleep (h)	Cycling (no. 0·5 h periods)	Work rate (W)	Total standing time (h/d)	Metabolizable energy* (MJ/d)	Percentage energy from:		
						Protein	Fat	Carbohydrate
1	8	4	75	2·92	12·32	13·1	44·9	42·0
2	6·5	4	40	2·33	10·42	13·2	20·1	66·7
3	8	6	50	1·83	12·28	13·1	29·5	57·4
4	8	4	75	6·0	12·29	13·3	54·8	31·9

* Metabolizable energy was determined from analyses of duplicate meals and faeces by bomb calorimetry.

paramagnetic analyser and CO_2 by a GP Instrumentation IRGA40 infra-red analyser. The moisture content of the air was measured with an optical condensing dewpoint meter type DP5 (MBW Electronics). The calorimeter ventilation system maintained a constant air flow of 200 litres/min from the calorimeter. This air was passively replaced by conditioned fresh air from the shell area around the calorimeter. The flow was monitored on a Rotameter type 2100 (KDG Flowmeters) calibrated to better than 1 %.

Under a computer-controlled protocol the gas analysers sampled calorimeter air every 10 min, with reference to fresh air every hour and N_2 and a 1 % CO_2 in air calibration gases every 6 h. The calibration gas measurements revealed any analyser drift which was retrospectively corrected out of the final computations on the assumption that it occurred linearly between calibration observations. The sample interval of 10 min was determined by the available file size on the Hewlett Packard 1000 system computer. With a larger file-space more frequent sampling could have been employed with the advantage of better resolution of energy expenditure in short periods such as exercise and BMR.

The response time of measurements was such that a complete response to a step change in HP was observed in the second analysis after this event.

Calibrations

The calibration of the O_2 consumption and CO_2 production measurements was made by infusing a mixture of CO_2 and N_2 (20:80, v/v) to simulate the presence of a subject in the calorimeter. The infusion rate was measured with an oil-filled gas meter type DM3A (Alexander Wright & Co.) calibrated to ±0·25 %.

The expected O_2 consumption and CO_2 production values resulting from the infusion were calculated as

$$O_2 \text{ consumption (litres/min)} = \frac{F_t \cdot f_{oi} \cdot f_{nt}}{1 - f_{oi} - f_{ci}},$$

$$CO_2 \text{ production (litres/min)} = \frac{F_t[f_{ct} - f_{ct} \cdot f_{oi} - f_{ci}]}{1 - f_{oi} - f_{ci}},$$

Where F_t is the infusion test gas flow rate (litres/min STP dry), f_{oi}, f_{ci} are proportions of O_2 and CO_2 respectively in fresh air; f_{nt}, f_{ct} are proportions of N_2 and CO_2 respectively in the test gas. These formulas apply only to suction ventilated systems.

The accuracy of O_2 consumption measurements was found to be within the range +1·2 to −1 % in repeated calibrations while CO_2 production measurements were within ±0·5 % of the predicted value.

RESULTS

The standard deviation of 10 min measurements made over extended periods under steady-state calibration conditions was 6·61 W. It has been verified that the measurement noise standard deviation (SD_m) arising from the instrumentation is essentially Gaussian in distribution and so, as a component of longer periods of analysis, it can be predicted as:

$$\text{SD}_m = \frac{6 \cdot 61}{\sqrt{N}} \text{W},$$

where N is the number of measurements in the analysis period. The values of SD_m for the periods analysed in the present study were calculated and are given in Table 3.

If it is assumed that the variability in the energy expenditure of a subject undertaking the same activity on different occasions is also Gaussian in distribution, and uncorrelated with the measurement noise, then the contribution of the subject, SD_s, to the total observed standard deviation, SD_t, is given by

$$\text{SD}_s = \sqrt{(\text{SD}_t^2 - \text{SD}_m^2)}.$$

Table 3. *Contribution of measurement noise standard deviation,* SD_m*, to various analytical periods*

Period	Duration (h)	SD_m (W)
One sample	10 min	6·61
Exercise	20 min	4·67
	30 min	3·82
BMR	1	2·7
Sleep	6	1·10
	7·5	0·99
Whole day	24	0·55

BMR, basal metabolic rate. $\text{SD}_m = \frac{6\cdot61}{\sqrt{N}}$ where N is the number of 10 min measurements in each analysis period.

Table 4. *Day-to-day variability in energy expenditure measured over 24 h*

Subject no.	No. of days studied	Mean energy expenditure MJ/d	Mean energy expenditure W	SD_t (W)	CV_t (%)	SD_s (W)	CV_s (%)
1	11	11·69	135·34	2·91	2·15	2·86	2·11
2	11	9·48	109·73	1·91	1·74	1·84	1·68
3	12	12·10	140·05	2·63	1·88	2·57	1·84
4	10	13·95	161·41	3·37	2·09	3·32	2·06
Group values		11·80	136·63	2·76	1·97	2·70	1·93

SD_t, SD_s, total observed standard deviation and subject standard deviation respectively; CV_t, CV_s, coefficient of variation for SD_t and SD_s respectively.

Table 5. *Day-to-day variability in energy expenditure during measurement of basal metabolic rate*

Subject no.	No. of days studied	Mean energy expenditure kJ/min	Mean energy expenditure W	SD_t (W)	CV_t (%)	SD_s (W)	CV_s (%)
1	11	4·37	72·77	3·13	4·30	1·58	2·17
2	12	4·33	72·13	5·46	7·57	4·75	6·59
3	12	4·82	80·27	4·64	5·78	3·77	4·70
4	11	4·24	70·65	3·97	5·62	2·91	4·12
Group values		4·44	73·96	4·38	5·93	3·45	4·67

SD_t, SD_s, total observed standard deviation and subject standard deviation respectively; CV_t, CV_s, coefficient of variation for SD_t and SD_s respectively.

From this expression the component of SD_s in SD_t has been calculated and is shown for each subject over 24 h (Table 4), in BMR (Table 5), overnight (Table 6) and in exercise (Table 7). The values of SD_t and SD_s are also expressed as coefficients of variation (CV). The group mean energy expenditures and the root mean square (pooled) SD and CV values are also shown where appropriate. The overnight measurements exclude the first 0·5 h after 'lights out' to allow the subject to fall asleep but include any subsequent periods of disturbed sleep and occasions when subjects rose to pass urine, these being regarded as part of the variability in overnight energy expenditure. One night when subject no. 2 was out of bed

Table 6. *Day-to-day variability in overnight energy expenditure*

Subject no.	No. of nights studied	Duration (h)	Mean energy expenditure (W)	SD$_t$ (W)	CV$_t$ (%)	SD$_s$ (W)	CV$_s$ (%)
1	11	7·5	77·21	1·45	1·88	1·06	1·37
2	10	6	75·59	1·65	2·18	1·23	1·63
3	12	7·5	81·56	1·36	1·67	0·93	1·14
4	11	7·5	78·65	2·71	3·45	2·52	3·21
Group values			78·25	1·87	2·40	1·57	2·01

SD$_t$, SD$_s$, total observed standard deviation and subject standard deviation respectively; CV$_t$, CV$_s$, coefficient of variation for SD$_t$ and SD$_s$ respectively.

Table 7. *Day-to-day variability in energy expenditure during exercise*

Subject no.	Mean energy expenditure (W)	SD$_t$ (W)	CV$_t$ (%)	SD$_s$ (W)	CV$_s$ (%)
1	425·20	12·68	2·98	12·54	2·95
2	198·89	4·67	2·35	4·26	2·14
3	304·80	13·92	4·57	13·83	4·54
4	414·44	10·22	2·47	10·84	2·42
Group values		10·96	3·22	10·81	3·15

SD$_t$, SD$_s$, total observed standard deviation and subject standard deviation respectively; CV$_t$, CV$_s$, coefficient of variation for SD$_t$ and SD$_s$ respectively.

for 1·5 h and one when subject no. 4 was out of bed for 0·5 h have been excluded. The 0·5 h exercise periods are analysed over the last 20 min to allow the subject and the calorimeter time to respond fully to the commencement of exercise.

Table 8 shows the variability in the cost of exercise within the day. Each expenditure value is the mean of the values for that time on each day of the study.

DISCUSSION

Measurement noise

Measurement noise at the level experienced in the present study only plays a large part in the total variability in measurements of energy expenditure analysed over 1 h or less. However, as these include BMR measurements and individual exercise sessions, it is necessary to reduce the measurement noise as far as possible.

Measurement noise arises predominantly from apparent gas concentration changes in the form of random noise on the analyser output signals. When rapid gas concentration changes occur the evaluation of gas production or consumption rates is dominated by an expression of the form

$$\frac{\text{d concentration}}{\text{d time}} \times \text{calorimeter volume},$$

and it is this expression which amplifies analyser noise to the extent that it limits the precision of energy expenditure measurement. As the calorimeter volume is a multiplier the problem of noise is greater in large calorimeters and size is traded for performance in calorimeter design.

Brown *et al.* (1984) have analysed the sensitivity of energy-expenditure estimates to

Table 8. *Mean energy expenditure in exercise for each period in the day*

Subject no.	Period.....	1	2	3	4	5	6
1	Energy expenditure (W)	416·21^{a}	423·31^{b}	421·26^{c}	441·82^{a,b,c}		
	Time of day (hours)	12.30	15.30	18.30	21.30		
	Time since meal (h)	3·0	2·0	5·0	2·0		
2	Energy expenditure (W)	195·15^{a}	193·42	202·88	207·08^{a}		
	Time of day (hours)	10.00	12.00	16.30	20.00		
	Time since meal (h)	1·5	3·5	3·5	2.0		
3	Energy expenditure (W)	304·18	297·01^{a,b}	307·04^{a,d}	306·07	315·28^{b,c}	294·88^{c,d}
	Time of day (hours)	10.30	12.30	14.30	16.30	18.30	23.00
	Time since meal (h)	1·0	3·0	1·0	3·0	1·0	3·5
4	Energy expenditure (W)	406·59^{a}	416·57	415·82	418·50^{a}		
	Time of day (hours)	12.00	14.30	18.30	21.30		
	Time since meal (h)	3·0	1·0	5·0	2·0		

$^{a-d}$ For any subject, period values with similar superscript letters were significantly different ($P < 0·05$).

changes in gas concentrations and have shown that they are over sixty times more sensitive to changes in O_2 concentration than CO_2 concentration. In practice this is fortuitous as the best O_2 analysers generally exhibit a noise level at least an order of magnitude better than the best CO_2 analysers. Brown *et al.* (1984) discuss the problem of computing the value of the rate of change of gas concentration and recommend ways to minimize the impact of analyser random noise on this computation.

24 h Energy expenditure

The pooled CV in 24 h energy expenditure within the group found in the present study was 1·97% and showed little variability from subject to subject, the range being 1·74–2·15%. The noise component of this measurement was small, and its removal reduced the group CV to 1·93%. In spite of attempts to match energy intake and expenditure, subjects nos. 1 and 3 were in positive energy balance by 5% and subject no. 2 by 10%. Subject no. 4 was in negative energy balance by 12%. The resulting changes in body composition would have affected metabolic rate and this effect could be expected to account for part of the variability. There is, however, no significant relation between observed variability and energy balance.

De Boer (1985) has made measurements of energy expenditure over three successive days in a group of ten female subjects, with a repeated study on a later occasion. A CV of 2·6% was found for the first study and 1·8% for the repeat experiment. The protocol was comparable to that of the present study in all respects except temperature, which was 21–22° in the daytime and 19–20° overnight, and the smaller size of the calorimeters which had a volume of 12 m^3.

In repeated 24 h measurements separated by 1 week, Dallosso *et al.* (1982) found a CV of 1·5%. These measurements were made in 11 m^3 calorimeters on a somewhat more rigid activity protocol than that adopted in the present study or that of De Boer (1985). Garby *et al.* (1984), using direct calorimetry measurements, found a CV of 2·2% in energy-expenditure measurements separated by 1 week on a protocol whose rigidity was comparable to that of Dallosso *et al.* (1982). Garby *et al.* (1984) indicate a contribution of 0·5% to this value from measurement noise.

The consistency of these findings, with a range in CV from 1·5 to 2·6%, suggests that intra-individual variability on a 24 h basis is indeed small. Garby (1985) has cautiously suggested that on a fixed, low-activity protocol, the physical activity index (24 h energy expenditure:BMR; PAI) is not greatly influenced by body-weight. Thus 24 h energy expenditure can, with some justification, be normalized in the same way as BMR, to lean body mass. The inter-individual variability found by Dallosso *et al.* (1985), in analysis of values from ten calorimetry studies of subjects on fixed activity protocols, was 6% when the energy expenditure was expressed on a lean body mass basis. This suggests that inter-individual variability in energy expenditure over 24 h may not be entirely explained in terms of intra-individual variability. However, the variability in estimation of lean body mass and the inter-individual variability in the composition of lean body mass are uncertain and inseparable quantities which may together account for some of the residual inter-individual variability (Bakker & Struikenkamp, 1977).

BMR

The overall CV of the BMR measurement in this study ranged from 4·3 to 7·57%. The component of variability attributable to the subject was 2·17–6·59%. These values are higher than expected. The overall group value of 5·93% compares with 3·4% found by Garby & Lammert (1984) and 3·17% reported by Soares & Shetty (1986) in subjects measured at weekly intervals. The noise-free variabilities are 4·67, 2·4 and 2·9% for the present study, Garby *et al.* (1984) and Soares & Shetty (1986) respectively. The measurements of Garby *et al.* (1984) and Soares & Shetty (1986) were made by analysing directly expired air from the subjects who were thus able to be closely supervised. In our measurements we were not able to supervise the subjects so closely for fear of incurring the interaction between the observer and heat-loss measurements described previously (p. 348). The repetition on twelve successive days of this 1 h period of measurement, in which the subjects were asked to remain awake while lying on their backs, was undoubtedly a stress. Subject no. 4 informed us on one occasion that he lapsed into sleep and it is possible that other subjects did so on occasions too. Analysis of values from a study by Dallosso & James (1984), made in our 11 m^3 indirect calorimeters where supervision of subjects is much more straightforward, shows that in BMR measurements the CV was 3·14% including measurement noise. This value is in close agreement with the observations of Garby *et al.* (1984) and Soares & Shetty (1986) and confirms that the variability found in the present study was unusually large.

A CV of about 3% in BMR measurement repeated in the same individual again shows that intra-individual variation is smaller than inter-individual variation in BMR which Dallosso *et al.* (1985) found to be 6% in measurements expressed per unit lean body mass. Separating the intra-individual from inter-individual variability suggests that there is a variability in basal metabolism, even after correction for lean body mass, which may amount to 5%, although once again the variability in the estimation of the quantity and composition of lean body mass may account for this.

Overnight metabolic rate

The pooled CV for overnight measurements was 2·40%, reducing to 2·01% when the noise component was excluded. Although there is no literature in whose context these values can be considered, they are of similar magnitude to the values reported for 24 h measurements.

Exercise

The CV of the mean daily energy expenditure during exercise was 3·22% for the group. As the measurement noise level is constant, and independent of the energy expenditure, it forms a very small component of the variability of exercise measurements, and the group CV was only reduced to 3·15% when it was removed. The variability in the cost of exercise contains variability due to the limited precision with which the ergometer workload could be set, and the reliability with which subjects cycled in time to a metronome beat. The influence of these components cannot be separately quantified.

Within each day there was further variability between exercise periods. The exercise within 2 h of the evening meal, which was served hot, was always associated with the highest energy expenditure. Paired *t* tests on the full set of values showed expenditure in this period of exercise to be significantly greater than in some of the other periods for all subjects. Multivariate analysis has suggested that the temperature of the meal was a much greater influence on expenditure in this period than the combined effects of time of day and time-interval since the meal. Confirmation of this is being sought in further studies.

Conclusions

The results of the present study show that, within individuals, day-to-day variability in 24 h energy expenditure and in all the component activities tested is generally small. The variability in BMR and 24 h expenditures is so much smaller than in comparable analyses of variability between individuals, even when these are normalized for lean body mass, that a residual variability of about 5% between individuals is revealed.

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