Intra- and inter-individual variations in energy expenditure of 14–15-year-old schoolgirls as determined by indirect calorimetry

BY HANNELORE RIEPER, H. KARST, R. NOACK* AND D. JOHNSEN

Department of Energy Metabolism, German Institute for Human Nutrition, Bergholz-Rehbruecke, 0–1505, Arthur-Scheunert-Allee 114–116, Germany

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Eleven 14–15-year-old schoolgirls were investigated four times within 1 year to determine variations in energy expenditure between individuals (inter-individual variability) and within subjects (intra-individual variability). Indirect calorimetry was used to determine metabolic rates in the fasting and resting state (RMR), and during physical activities which were grouped into standardized and non-standardized activities. Analyses of variance supplied information about intra- and inter-individual variabilities of rates of energy expenditure. The mean resting metabolic rate in adolescent girls was 4·41 (SD 0·40) kJ/min. The overall coefficient of variation (9·1%) was approximately twice as high as the mean coefficient within subjects (4·3%). The reproducibility of the RMR of the girls was high (significant *F* value of variance analysis), even over a prolonged investigation of 1 year. This seemed to depend primarily on the constant body weight during the period of investigation. A workload of 30 W on a bicycle ergometer and walking at an individually chosen speed did not reveal significant differences between inter- and intra-individual variabilities of energy metabolic rates. However, in most non-standardized activities, e.g. relaxation at home, washing dishes and vacuum cleaning, inter-individual variability was significantly higher than intra-individual variability. There are true differences in energy expenditure rates between subjects which may be demonstrated by duplicated measurements. Conclusions on future experimental design were drawn, where differences between groups rather than between individuals are to be studied.

Energy metabolic rates: Indirect calorimetry: Schoolgirls

Whereas metabolic rates have been extensively studied in adults (for example, Garby *et al.* 1984; De Boer *et al.* 1987), investigations on the energy metabolism of adolescents and children are rare (Gajlish & Kondrat'eva, 1988; Ho *et al.* 1988). In setting up such studies in younger age-groups it must be taken into account that the variability of metabolic rates could be considerable, depending on individual differences in biological development as, for example, reflected in body mass and body composition.

Knowledge of the size of biological and methodological variations facilitates the estimation of sample size and the number of replications in planning experiments in growing subjects. Unfortunately, there is little information available on the reproducibility of energy expenditure measurements in children and adolescents.

The aim of the present study was to supply such results both at group and individual level. Thus, we investigated the energy expenditure of young girls in the resting and fasting state (RMR), and during several activities, by indirect calorimetry. Activity patterns (24 h) were recalled as well and used to estimate variabilities between and within subjects.

Habitual activities were involved both as standardized and individual activities. As an approach to determine variations in energy expenditure between individuals (inter-

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individual variability) as well as between repeated measurements in the same subject (intra-individual variability) eleven young girls were studied four times within 1 year (with time-intervals of between 6 and 8 weeks). These investigations are intended as a foundation for further studies on the relationship between energy metabolism and biological variables in adolescents.

MATERIALS AND METHODS

Subjects

Eleven 14–15-year-old schoolgirls agreed to participate in the energy metabolism investigations on a voluntary basis and supported by their parents. At the start of the study the purposes and objectives were explained in detail to the subjects. Specific instructions were given to them not to alter their habits, routine activities or the pattern of food intake.

Measurement of energy expenditure

Indirect calorimetry was used to determine RMR and the energy cost of special activities, which were grouped as follows: sitting, walking at an individually chosen speed, walking 5 km/h, doing schoolwork at home, vacuum cleaning and washing dishes, and standardized work on a bicycle ergometer. Measurements of oxygen uptake (V_{O_2}) and carbon dioxide production (V_{CO_2}) were obtained during rest and selected activities from each subject. The RMR of each subject was determined at 3-month intervals. Similarly selected activities were measured three or four times over 1 year.

After a 10–12 h overnight fast, the subjects came to the laboratory where they rested 30 min before RMR was measured. Then the energy costs during rest and under a workload of 30 W on a cycle ergometer were measured. The energy costs of all field activities were measured in the post-prandial state. These included walking and cycling at their own typical stride and speed respectively, and walking at a standard speed of 5 km/h. Energy demands for housework activities, such as washing the dishes, vacuum cleaning and doing homework were measured at the subjects' home. Energy cost for comfortable sitting at home was also measured in the post-prandial state. Laboratory and home measurements were taken over periods of 7–14 min, and each exercise measurement over 5–7 min. The walking and riding were over distances of 500 and 1360 m respectively. In order to ensure steady-state conditions subjects walked and bicycled over 200 and 500 m respectively, before measurements were started. The backpack respirometer of Kofranyi Michaelis, of the Max Planck Institute for Work Physiology, Dortmund produced in Goettingen, combined with a face mask was used to measure the volume of expired air (V_E). Each respirometer was calibrated by the same dry-gas meter. Both at home and in the field the subjects wore the respirometers on their backs while performing activities, whereas during laboratory and home sitting measurements the device was placed beside the subject. Gas samples were collected in Douglas bags and the O_2 and CO_2 concentrations of expired air were analysed within 20–30 min after sampling using a Spirolyt (VEB Junkalor Dessau) containing a paramagnetic O_2 analyser and a infrared CO_2 analyser.

The O_2 and CO_2 analysers were adjusted before each measurement using room air, and calibration gas which was a certified gas mixture of O_2, CO_2 and nitrogen in the range of the expired air. The measured gas volume (V_E) was corrected to standard conditions (Body Temperature and ambient Pressure, Saturated (BTPS) as for V_E and Standard Temperature and Pressure, Dry (STPD) as for V_{O_2}). Energy expended was calculated from the V_{O_2}, V_{CO_2} and V_E values (Erbersdobler *et al.* 1976). Measured RMR values were compared with individually predicted basal metabolic rates (BMR) according to Kleiber (1967) and expressed as a proportion of the predicted values.

Recording of 24 h activity diaries

Continuous records of activity changes were collected from each subject during four separate 24 h periods, i.e. three school days with normal activity patterns and one school-free day. Activity diaries were performed by the subjects to obtain a minute-by-minute record of all daily activities. Before starting their diaries, the girls were given instructions by one of the investigators. The interviewer made certain that the girls understood the definitions of each type of activity, i.e. the time-period spent lying, standing or sitting, as well as how to differentiate between moderate and strenuous activities such as slow and fast walking and riding. The interviewer checked the subject's detailed activity record and classified the type of activity. The records were quantitatively evaluated, i.e. the duration of each classified activity expressed as a proportion of the total time-period spent awake, to estimate the daily individual activity level of the subject.

Estimating the individual energy cost at the standardized work-load of 30 W

Standardized exercise testing at the laboratory was performed using a bicycle ergometer. A fixed workload of 30 W was applied. V_{O_2} was measured by the same procedure as in the resting state. A metronome was used to achieve steady rates of 60 pedal rotations per min. A 5 min period of cycling was performed to achieve steady-state conditions before starting the 5–7 min test period.

Statistics

Mean is the arithmetic mean value, either of all observed values of one type, or of these data from all subjects at one time-point, or of all repeated measurements on one individual (M_t, M_b, and M_w respectively). The same applies to the standard deviations SD_t, SD_b, and SD_w of Table 1, the overall, between- and within-individual standard deviations respectively. Differences between the means (M_w) were tested for statistical significance using the *t* test for paired samples. Variability was investigated using one-way analyses of variance. The coefficient of variation within individuals (CV_w in Table 3) was derived from the one-way analyses of variation as the square root of the mean square MS_w, expressed as a proportion of the mean. Likewise, s_b^2 (see Table 2), the estimated between-component variance, comes from the analysis of variance being the difference of MS_b minus MS_w divided by the mean number of repeated measurements (*n* in Table 2).

RESULTS

Subjects' characteristics

The physical characteristics of the subjects are presented in Table 1. Members of the group completing the study ranged in age from 14 to 15 years, in weight between 49·6 and 64·4 kg and in height from 1·60 to 1·73 m. Neither height nor body weight were significantly different among the four time-periods within the period of investigation.

Reproducibility of RMR

Reproducibility is defined as the degree of agreement between repeated measurements, using one method on different subjects or on the same subject at different times. The reproducibility of RMR was investigated by repeated measurements of the same subject (intra-individual variability) as well as the inter-individual variability. The differences in energy expenditure were accepted as being real differences between subjects if the inter-individual variance (MS_b) was significantly higher than intra-individual variance (MS_w).

The individual values for the four repeated measurements of RMR as well as the standard deviations both within and between subjects are presented in Table 1. The mean values (kJ/min) for repeated RMR measurements in October, December, February and

Table 1. *Inter- and intra-individual variability of resting metabolic rate (kJ/min) measured at time-intervals for adolescent girls*

Subject no.	Mean height (m)	Mean body wt (kg)	Resting metabolic rate (kJ/min) Oct.	Dec.	Feb.	Apr.		M_w	SD_w	CV_w(%)
1	1·62	49·6	4·02	3·93	4·03	3·43		3·85	0·29	7·5
2	1·61	53·5	4·04	3·93	3·72	4·14		3·96	0·18	4·5
3	1·61	54·0	4·69	4·38	—	4·51		4·53	0·16	3·5
4	1·66	54·6	4·91	4·83	5·03	5·35		5·03	0·23	4·6
5	1·73	55·1	3·95	3·93	4·25	3·98		4·03	0·15	3·7
6	1·71	57·6	4·80	4·58	4·43	4·67		4·62	0·16	3·4
7	1·63	59·1	5·03	4·71	4·72	4·69		4·79	0·16	3·4
8	1·69	60·5	4·55	4·54	5·02	4·69		4·70	0·22	4·7
9	1·60	61·8	4·05	4·12	4·34	4·49		4·25	0·20	4·7
10	1·61	64·0	4·23	4·47	4·24	4·64		4·40	0·20	4·5
11	1·64	64·4	4·20	4·48	4·39	4·31		4·35	0·12	2·8
M_b	1·65	57·6	4·41	4·36	4·42	4·45	M_t	4·41		
SD_b	0·44	4·8	0·40	0·33	0·41	0·49	SD_t	0·40		
CV_b(%)	2·7	8·3	9·1	7·5	9·3	11·0	CV_t(%)	9·1		

M_t, M_b, M_w, overall, between-, and within-individual mean respectively; SD_t, SD_b, SD_w, overall, between-, and within-individual standard deviation respectively; CV_t, CV_b, CV_w, coefficient of variation for SD_t, SD_b and SD_w respectively.

Table 2. *Results of one-way analyses of variance with resting metabolic rate (RMR), body weight (BW), physical activity, and energy cost of workload (30 W) after four separated repeated measurements on adolescent girls**

	RMR (kJ/min)	BW (kg)	Activity† (%)	Work-load 30 W (kJ/min)
n	3·91	3·91	3·81	3·55
M_t	4·41	57·6	29·8	15·4
SD_t	0·40	4·8	10·0	1·2
N	43	43	42	19
MS_b	0·543	89·5	163·2	0·49
MS_w	0·037	1·65	79·8	1·7
F	14·5	54	2·04	0·29
s_b^2	0·129	22·5	21·9	0

n, mean number of repeated measurements $\left(n = \frac{N-(\Sigma k^2/N)}{n_i - 1}\right)$; N, total number of data; k, number of values within one measurement; n_i, number of observations for an individual subject; M_t, overall mean; SD_t, overall observed standard deviation; MS_b, mean squares between subjects; MS_w, mean squares within subjects; F, factor of variance; s_b^2, estimate of between component of variance $= (MS_b - MS_w)/n$.

* For details of subjects, see Table 1.

† Physical activity as percentage of the time-period spent awake.

April were 4·41 (SD_b 0·40), 4·36 (SD_b 0·33), 4·42 (SD_b 0·41) and 4·45 (SD_b 0·49) respectively. They were not significantly different from each other. The overall coefficient of variation (CV_t) of 9·1 % was approximately twice the intra-individual coefficient of variation (CV_w).

One-way analyses of variance supplied information on inter- and intra-individual variability of repeatedly measured RMR. Table 2 shows the variances for four separate

RMR determinations. The variance among the subjects (inter-individual variance; MS_b) was much higher than the residual variance (intra-individual variance; MS_w) with a coefficient of variance (F) of 14·5. On the one hand these values suggest a high reproducibility of our repeated RMR determinations, and on the other hand they indicate true differences between some subjects. Therefore, four separate measurements of this characteristic appeared to supply a correct estimation of the resting metabolism of 15-year-old girls over a time-interval of about 0·5 year.

Reproducibility of energy expenditure measurements of physical activities

Table 3 shows the energy expenditure of standardized and non-standardized activities of the young girls measured four times at two-monthly intervals. Variability of energy expenditure, sitting in a relaxed position at home as well as doing schoolwork, was compared with the variability of RMR obtained in the laboratory. The intra-individual variabilities of these energy metabolism results were quite similar (CV_w(%) 5·2, 5·9 and 4·4 respectively).

The mean energy costs (kJ/min) of the bicycle ergometer workload of 30 W, determined four times, were 14·9 (SD_b 0·7), 16·1 (SD_b 1·4), 16·3 (SD_b 1·1) and 14·4 (SD_b 0·9). These results were not significantly different from one another. The ratio of variances (F value) was 0·29 (Table 2). Hence, four repeated investigations failed to reveal clear differences among individuals or replications concerning the energy cost of this standardized activity.

Reproducibility of activity results

As Table 2 demonstrates, standard deviation of the girls' activity was very high. The individual activity of the same subject changed considerably from day-to-day, and so did the activity from subject-to-subject. The F value of the variance analysis of four repeated activity records was 2·04. It is slightly below the limit of significance on the 95% level (table value 2·2) with four replicates, and so one would have to conclude that differences between the subjects were not evident.

Reproducibility of biological variables

Tables 2 also shows the mean body weights of the subjects. On average, the eleven girls maintained their body weight during the whole investigation period. Mean body weights were 58·0 (SD_b 5·3), 57·7 (SD_b 5·4), 57·7 (SD_b 4·5) and 57·4 (SD_b 4·4) with the four repeated investigations. There was no significant difference between body-weight measurements.

DISCUSSION

The intra-individual coefficient of variation CV_w of 4·3% (ranging from 2·8 to 7·4%) in repeated RMR measurements was much smaller than the inter-individual (mean CV_b 9·1%, ranging from 7·5 to 11%). This demonstrates that there are true biological differences in RMR from subject-to-subject. Our results are in agreement with those of Murgatroyd *et al.* (1987) who investigated the BMR of adult men in a whole-body calorimeter. The overall CV_t of the RMR measurement in their study was about 6% and ranged from 4·3 to 7·6%. The variance attributable to the subject was between 2·2 and 6·6%. Analyses of values from a study of Dallosso & James (1984) with adult male subjects showed that in BMR measurements the CV_w was 3·1%. This value was in close agreement with the results of Soares & Shetty (1986). De Boer *et al.* (1987) also found in 24 h energy expenditure measurements of female subjects with indirect calorimetry an intra-individual coefficient of variation (CV_w) of 3·7 (range 2·5–7·1)%. The CV_w in well-adapted and consecutively measured subjects was smaller (CV 2·8%) but not significantly different from the former value.

Table 3. *Energy expenditure (kJ/min) of standardized and non-standardized activities measured in four separated repeated measurements on adolescent girls**

Activities	*n*	Oct.	Dec.	Feb.	Apr.	M_t	CV_w(%)	*F*
Standardized								
RMR	43	4·41	4·36	4·42	4·44	4·41	4·4	14·5
Walking (5 km/h)	29	17·2	18·1	—	17·5	17·6	7·2	4·8
30 W workload	19	14·9	16·1	16·3	14·4	15·4	8·5	0·3
Non-standardized								
Walking (individually chosen speed)	32	18·5	19·3	—	18·7	18·8	11·1	1·3
Relaxation at home	17	5·3	5·2	5·4	5·0	5·2	5·2	8·4
Writing at home	17	5·7	6·1	6·1	5·6	5·8	5·9	7·0
Washing the dishes	14	7·2	9·1	8·7	8·6	8·6	7·4	7·7
Vacuum cleaning	16	13·2	15·4	15·1	12·7	13·9	13·5	8·6

n, number of single values; M_t, overall mean; CV_w, within-individual coefficient of variation; *F*, factor of variance; RMR, resting metabolic rate.
* For details of subjects, see Table 1.

Table 4. *Inter- and intra-individual variability of energy expenditure of walking at 5 km/h for adolescent girls**

Subject no.	Energy expenditure (kJ/min) Oct.	Dec.	Apr.		M_w	SD_w	CV_w(%)
1	12·7	15·5	14·8		14·3	1·5	10·2
2	17·9	—	21·0		19·5	2·2	11·3
3	16·9	17·2	16·5		16·9	0·4	2·1
4	—	16·8	15·8		16·3	0·7	4·4
5	15·7	—	16·0		15·9	0·2	1·3
6	18·7	17·1	16·8		17·5	1·0	5·8
7	17·3	18·0	16·4		17·2	0·8	4·6
8	19·9	18·9	16·5		18·4	1·8	9·5
9	18·2	19·3	19·8		19·1	0·8	4·3
10	17·2	—	18·0		17·6	0·6	3·2
11	17·9	21·8	20·5		20·1	2·0	9·9
M_b	17·2	18·1	17·5	M_t	17·6		
SD_b	2·0	1·9	2·1	SD_t	2·0		
CV_b(%)	11·6	10·5	12·0	CV_t(%)	11·4		

M_t, M_b, M_w, overall, between-, and within-individual mean respectively; SD_t, SD_b, SD_w, overall, between-, and within-individual standard deviation respectively; CV_t, CV_b, CV_w, coefficient of variation for SD_t, SD_b and SD_w respectively.
* For details of subjects, see Table 1.

Other authors found smaller intra-individual coefficients. Dallosso *et al.* (1982) reported results of 24 h energy expenditure measurements of standardized physical activity in seven male subjects receiving a fixed diet over 1 week preceding the measurements. The average within-subject variation in 24 h energy expenditure was 1·5 (range 1·1–2·2)%. Garby *et al.* (1984) estimated within-subject variability of energy expenditure at rest to be 2·2 and 2·4% in male subjects. In the latter investigations some of the sources of variation effective in our experiments, such as seasonal, nutritional or growth influences, were absent or limited. The values of CV_w also depend on methodological conditions, e.g. whether field or laboratory

Table 5. *Inter- and intra-individual variability of energy expenditure of walking at individually chosen speed for adolescent girls**

Subject no.	Energy expenditure (kJ/min) Oct.	Dec.	Apr.		M_w	SD_w	CV_w(%)
1	13·7	19·3	19·2		17·4	3·2	18·4
2	17·0	17·2	18·5		17·6	0·8	4·6
3	17·6	17·5	17·6		17·6	0·1	0·3
4	19·8	18·7	17·9		18·8	1·0	5·1
5	19·2	19·1	16·7		18·3	1·4	7·7
6	19·7	19·3	21·4		20·1	1·1	5·6
7	21·4	18·5	12·9		17·6	4·3	24·5
8	20·4	19·7	18·8		19·6	0·8	4·1
9	18·2	19·6	21·5		19·8	1·7	8·4
10	17·4	—	18·8		18·1	1·0	5·5
11	18·6	24·0	22·4		21·7	2·8	12·8
M_b	18·5	19·3	18·7	M_t	18·8		
SD_b	2·1	1·9	2·6	SD_t	2·2		
CV_b(%)	11·4	9·8	13·9	CV_t(%)	11·5		

M_t, M_b, M_w, overall, between-, and within-individual mean respectively; SD_t, SD_b, SD_w, overall, between-, and within-individual standard deviation respectively; CV_t, CV_b, CV_w, coefficient of variation for SD_t, SD_b and SD_w respectively.

* For details of subjects, see Table 1.

investigations. For such studies relating only to experimental error and day-to-day variations of RMR, changes in body weight, body composition and living conditions were excluded. Therefore, the activity programme, food intake and meal frequency during the week preceding the measurements are often standardized. However, in our investigation food energy intake preceding the measurement was not kept fixed but 'habitual'. A further explanation for higher values for variation may be the variable time-point of our investigations with regard to the menstrual cycle. Webb (1986) estimated that the effect of the various ovulatory cycle stages increased the metabolic rate by 8–16%.

Analyses of variance supplied information about the intra-: inter-individual variability ratio in measured energy expenditure rates (Table 3). Typical differences between subjects with higher reproducibility will be reflected by significantly higher variances between than within subjects ($MS_b > MS_w$; high F value). True inter-individual differences existed, for example, in the metabolic rates of non-standardized activities like relaxation at home, writing at home, washing the dishes and vacuum cleaning (F 8·4, 7·0, 7·7, 8·6 respectively).

A lower inter-individual variance was found in standardized activities such as walking at 5 km/h (Table 4) and cycling at 30 W. A standardized bicycle ergometer workload of 30 W did not produce characteristic inter-individual differences nor regular intra-individual variation (F 0·3). The walking activity was standardized by speed but there were differences between individuals which can be accounted for by body weight differences (significant correlation between the intra-individual means of body weight and 5 km/h walking energy expenditure with r 0·68, $P < 0·05$). So the inter-individual variance in standardized walking substantially exceeded the intra-individual variance (F 4·8).

In the case of walking at individually chosen speeds (Table 5) the impact of body weight was obviously overridden by the effect of variable pace. This led to a rather low inter-individual variance compared with the intra-individual (F 1·3) and differences between individuals were not evident.

From these findings we conclude that metabolic rates, like RMR, and energy costs of walking at a constant speed or of some home activities, which are all predominantly related to body weight, show true and reproducible individual differences. These cases are characterized by high F values from analyses of variance. For these activities, as for body weight itself, we consider two separate measurements to be appropriate to obtain reliable values for girls of the investigated age level.

In other instances, such as walking at an individually chosen speed or the daily physical activity, even four repeated determinations appear to be insufficient to reveal probable differences of individual energy metabolic rates with this number of subjects.

The statistical information obtained from the present investigation will be useful in selecting experimental designs for studies which are to detect differences not simply between individual subjects but between various groups of subjects. The aim will then be to predict the most efficient combination of the acceptable minimum number of individuals per group and the necessary number of repeated measurements.

If, for instance, the question is whether girls of different body composition or body build show a typical different pattern of energy metabolism or not, it could be difficult to recruit a number of about ten individuals of a desired but not so widespread type within the given population.

In the case of BMR, where a difference of 10%, that is about 0·4 kJ/min, could be expected between groups, the variance of a group means should not be higher than 0·05 kJ/min in order to detect this supposed difference. If the group size would be five and the number of repeated measurements two, then we will get a variance of the group mean from our data (see Table 2) of $(s_b^2+(MS_w/n))/k = 0{\cdot}03$. From this the confidence interval of the group mean has a width of $\pm 0{\cdot}215$ at the 95% level. Thus, a difference in the group mean of 0·4 kJ/min would prove significant, but a difference of merely 0·2 kJ/min would not. Increasing the number of repeated measurements to four would not considerably improve the reliability of group mean differences, because the group mean variance would increase to 0·028 and the related confidence interval width would become $\pm 0{\cdot}208$ instead of $\pm 0{\cdot}215$. Thus, a 10% difference between group means of BMR appears to be detectable using two repeated investigations on two groups of five young girls.

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