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Calculating locomotor activity and energy utilisation factors from indirect calorimetric measurements ☆

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Abstract

Indirect calorimetric measurements have been used for years to determine energy expenditure (EE), respiratory quotient (RQ) and substrate oxidation rates of the subjects studied. This technique has now been extended to solve the problem of estimating locomotor activity (LA) and utilisation factors (UF). The method assumes (a) the organism rests at least once in a defined period, (b) this rest is long enough to reach basal CO_2 and O_2 concentrations in the calorimetric chamber, and (c) data acquisition occurs often enough to capture those minimum values.

Connecting and smoothing these minima separates LA from EE and leads to the time course of resting metabolic rate (RMR) + postprandial thermogenesis (ppTh). If the organism reaches a postabsorptive state without experiencing deep hunger, the ppTh is zero and so this value can be interpreted as RMR. Some tests gave the following relative errors ($r = s_d/\bar{x}$) showing the reliability of this technique: $\rho_{EE} = 3.2\%$, $\rho_{RQ} = 1.7\%$, $\rho_{LA} = 4.6\%$, $\rho_{RNU} = 4.6\%$.

Under some circumstances it is very useful to know to what extent a given amount of energy can be utilised. We developed a scheme to estimate energy and substrate UF and tested it with a diet comparable to one for prematurely born babies. Rats were placed in the indirect calorimetry unit for 4 days and were given that diet in the order of 450, 600, 750 and 0 kJ $kg^{-0.75}$. Energy and substrate balances ($BA = E_{intake} - E_{expenditure}$) were calculated for each individual and each energy intake (EI). Individual linear regression analyses $BA = f(EI)$ were calculated, giving estimation factors (r^2) of $99.71 \pm 0.13\%$ for energy utilisation. The

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correlation factors of that regression were 80.5 ± 4.4% with a relative reliability of ≈5.3%. They were then interpreted as UF.

Keywords: Calorimetry; Energy expenditure; Indirect calorimetry; Locomotion; Postprandial thermogenesis; Thermogenesis

1. Introduction

Indirect calorimetric measurements have been used for years to determine energy expenditure (EE) and respiratoric quotient (RQ) [1–4]. A review of the theoretical basis of indirect calorimetry is given in Ref. [5]. Usually, not only the oxygen consumption (M_{O_2}) and carbon-dioxide production (M_{CO_2}) are measured but also the amount of excreted nitrogen (N_{ex}). Assuming a standard composition of the oxidised substrates, the substrate oxidation rates can be calculated. Although one cannot say anything about the metabolic pathways of the substrates it is possible to calculate energy and substrate balances.

The energy and food utilisation can be determined by the classical long-term balance technique. It would be of interest to develop a method for estimating energy and substrate utilisation in short-term experiments by use of indirect calorimetry and to attribute food utilisation to the utilisation of the different substrates.

If EE is measured it would be of interest to divide EE into its components, basal metabolic rate (BMR), postprandial thermogenesis (ppTh) and locomotor activity (LA). To do so one has to determine LA. We have developed a method to estimate it from EE values.

Both methods are presented here.

2. Estimating utilisation factors

In this paper, a rapid method for the estimation of energy- and substrate-utilisation is presented. This method was developed on the occasion of a study of the utilisation of four protein diets. These diets had similar energy and substrate contents; they differed only in their amino acid spectrum. Classical balance techniques would need many animals and would last a long time in order to verify the small differences in this experiment.

2.1. Experimental

Groups of 8 to 11 male Wistar rats (≈200 g, fasted over 16 h) were held in calorimetric cages over a period of 4 × 23 h. They were fed with special diets in an ascending order of 450, 600, 750 and 900 kJ $kg^{-0.75}$, followed by a fasting day. The composition of the diets is given in Table 1. They differed mainly in the source of

Table 1
Characterisation of the tested diets

	Diet H	Diet P1	Diet P2	Diet C	Proportion per	
					Mass	Energy
Gross energy [a] in kJ g^{-1}	18.96	18.65	17.98	18.47		
Net energy [b] in kJ g^{-1}	15.90	16.07	16.11	15.73		
Protein					36.7 ± 0.3%	39.8 ± 0.3%
Starch					42.1 ± 0.4%	45.7 ± 0.5%
Fat					3.7 ± 0.1%	4.0 ± 0.1%
Lactose					8.6 ± 0.1%	9.4 ± 0.2%
Cellulose					2.0 ± 0.0%	0.00
Minerals					4.0 ± 0.0%	0.00
Vitamins					1.0 ± 0.0%	1.1 ± 0.0
Residual water					1.8 ± 0.0%	0.00

[a] Calculated from bomb calorimetric measurement (burned in pure oxygen). [b] Calculated from gross energy and bomb calorimetry of faeces and urea.

nitrogen which was human milk in diet H; synthetic amino acids and peptides in diets P1 and P2; and casein in diet C.

EE, RQ and substrate oxidation rates were calculated using standard equations. The balances of EE and CHO were calculated as

$$E_{\text{balance}} = E_{\text{uptake}} - \text{EE} \qquad \text{(stored energy)} \tag{1}$$

$$\text{CHO}_{\text{balance}} = \text{CHO}_{\text{uptake}} - \text{CHO}_{\text{oxidised}} \qquad \text{(stored CHO)} \tag{2}$$

Results of the measurements can be seen in Table 2. The measured values of EE and of real energy intake (EI) were used to perform individual linear regression analyses of the form

$$\text{Balance} = \alpha \times \text{Uptake} + \beta \tag{3}$$

where α is gradient and β the y intercept. The E_{balance} of diet C is shown in Fig. 1 as an example. The results of these regression analysis are displayed in Table 3.

2.2. Discussion

As an example, energy balances in the casein group are displayed versus energy intake for each individual and the total group in Fig. 1. The result for each individual on the four levels of energy intake fall very well on straight lines. The regression coefficients of determination are 99–100%. Because there were characteristic individual differences in the course of the regression line, a summarising regression resulted in worse regression parameters: the coefficient of determination was then about 98%. One can also see this characteristic in Fig. 1, comparing the individual values with the average line, for example, rat no. 21.

Table 2
EE, CHO and balances

Diet	Energy intake in kJ $kg^{-0.75}$ per day					Energy intake in kJ $kg^{-0.75}$ per day				
	450	600	750	900	0	450	600	750	900	0
	EE in kJ $kg^{-0.75}$ per day					$EE_{balance}$ in kJ $kg^{-0.75}$ per day				
Diet H	710 ± 36	737 ± 32	746 ± 37	787 ± 48	615 ± 40	−260 ± 36	−137 ± 32	4 ± 37	113 ± 48	−615 ± 40
Diet P1	714 ± 29	749 ± 32	758 ± 37	813 ± 25	623 ± 35	−264 ± 29	−149 ± 32	−8 ± 37	87 ± 25	−623 ± 35
Diet P2	709 ± 12	736 ± 16	762 ± 21	817 ± 35	627 ± 25	−259 ± 12	−136 ± 16	−12 ± 21	83 ± 35	−627 ± 25
Diet C	703 ± 54	723 ± 46	776 ± 34	823 ± 20	622 ± 31	−253 ± 54	−123 ± 46	−26 ± 34	77 ± 20	−610 ± 31
	CHO in g $kg^{-0.75}$ per day					$CHO_{balance}$ in g $kg^{-0.75}$ per day				
Diet H	7.7 ± 1.1	9.1 ± 1.3	10.6 ± 1.4	11.4 ± 0.7	4.3 ± 1.0	2.0 ± 1.1	4.0 ± 1.3	5.7 ± 1.4	8.1 ± 0.7	−4.0 ± 1.6
Diet P1	8.0 ± 0.8	9.5 ± 1.3	11.0 ± 1.2	11.9 ± 1.5	3.9 ± 0.8	1.8 ± 0.8	3.6 ± 1.3	5.3 ± 1.2	7.6 ± 1.5	−3.6 ± 1.4
Diet P2	8.7 ± 1.0	9.6 ± 1.1	11.5 ± 1.4	12.9 ± 0.6	4.3 ± 1.2	1.1 ± 1.0	3.4 ± 1.1	4.8 ± 1.4	6.7 ± 0.6	−3.9 ± 1.7
Diet C	8.1 ± 0.7	9.3 ± 0.8	11.3 ± 0.8	12.9 ± 0.8	4.7 ± 1.2	1.7 ± 0.7	3.8 ± 0.8	5.0 ± 0.8	6.6 ± 0.8	−4.3 ± 1.8

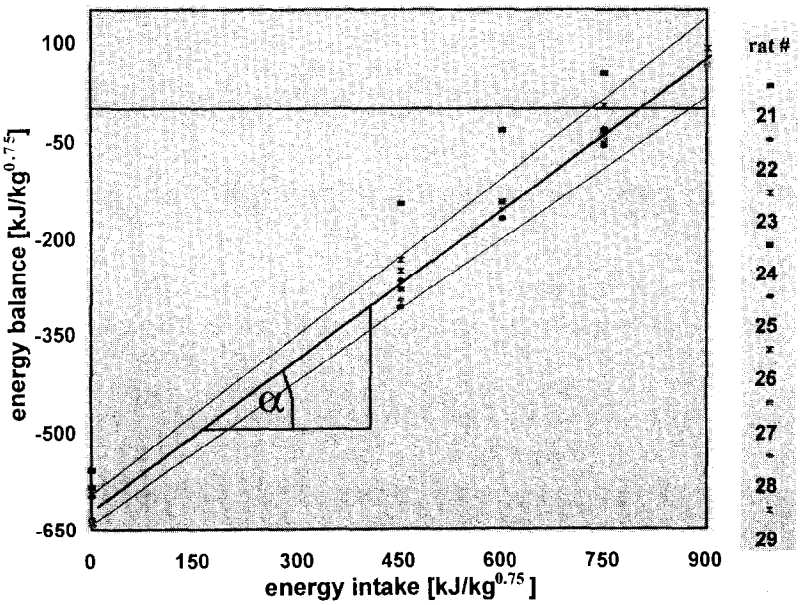

Fig. 1. The energy balance of Diet C.

Our interpretation of the results is that the y intercept of these lines is identical with EE (per day per metabolic body weight) on days without food intake ('lost energy' for the rats stores). The slope is interpreted as 'increase in the balance at rising uptake', in other words as the utilisation of that rising energy uptake or the utilisation factor. The X intercept can be interpreted as the daily maintenance requirement, the energy intake that equals the energy requirements.

Although there were only small differences in the utilization of energy from the different diets one can see the accuracy of the method by the dispersion of the averaged regression slopes of energy utilisation. They range between $77.7 \pm 3.9\%$ (diet C) and $81.3 \pm 3.3\%$ (diet H). The dispersions contain individual variabilities and variabilities in the measurement as well as differences in the growth phase of the rats! (The individual measurements lasted 4 days, that is about 1/35th of the growth phase of the rats life span.) The comparable values of protein utilisation range between $55.3 \pm 5.8\%$ (diet P2) $61.8 \pm 8.3\%$ (diet H).

To summarise, indirect calorimetry is suitable for the estimation of energy utilisation factors in the diets of rats. The subjects have to be fed over 3 whole days with approximately 80%, 100% and 120% of the maintenance EE. The energy utilisation factors within the prescribed feeding levels do not depend on the energy intake. This method is not applicable for the calculation of utilisation factors of fat and carbohydrates because of the partial mutual compensation of these substrates as a source of energy.

Table 3
Results of regression analysis

Linear regression analysis of EE
All rats in one analysis

Diet	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	$Y_{std.err.}$	N
Diet H	0.816	−618.6	0.981	758.0	37.4	40
Diet P1	0.803	−623.5	0.985	776.1	32.6	42
Diet P2	0.806	−624.3	0.983	774.1	22.5	42
Diet C	0.776	−605.7	0.981	781.0	38.8	42

	Average values of individual regressions				*Dispersion of these individual regressions*				
Diet	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	N
Diet H	0.813	−627.0	0.998	771.5	0.033	41.9	0.003	44.3	11
Diet P1	0.809	−624.4	0.997	773.6	0.064	31.1	0.004	40.1	11
Diet P2	0.808	−624.3	0.999	773.6	0.034	23.3	0.002	30.2	11
Diet C	0.777 [a]	−617.9	0.995	796.4	0.039	24.8	0.008	27.6	11

Linear regression analysis of protein oxidation
All rats in one analysis

Diet	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	$Y_{std.err.}$	N
Diet H	0.626	−4.27	0.944	6.83	1.10	40
Diet P1	0.578	−3.95	0.936	6.83	1.07	42
Diet P2	0.562	−4.27	0.925	7.61	1.13	42
Diet C	0.594	−4.48	0.951	7.54	0.96	42

	Average values of individual regressions				*Dispersion of these individual regressions*				
Diet	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	Slope	$Y_{intercept}$	R^2	$X_{intercept}$	N
Diet H	0.618	−4.28	0.995	6.98	0.083	1.04	0.005	1.62	11
Diet P1	0.570	−3.90	0.990	6.92	0.071	0.63	0.011	1.34	11
Diet P2	0.553 [b]	−4.36	0.990 [b]	7.88	0.058	1.02	0.008	1.53	11
Diet C	0.594	−4.46	0.985	7.43	0.060	1.22	0.010	1.46	11

[a] $p < 0.05$. [b] $p < 0.10$.

3. Determination of locomotor activity

The estimation of the portion of energy expenditure used for locomotor activity is very important for the interpretation of calorimetric measurements. This portion is necessary to separate the estimated EE into its components BMR, ppTh and LA. There are several ways to solve this problem. Some authors make the assumptions that LA is constant on consecutive days [6,7], others have tried to estimate or measure LA [8–11] by diverse means. In Ref. [12], an evaluation of the different techniques for the measurement of LA is given. All these methods are either expensive, complicated or not very accurate. Therefore, we developed a method to estimate LA by mathematical means from the time-course of EE [13].

3.1. Theoretical basis

An example of a typical time-course of EE and the procedure of the method can be seen in Fig. 2. The normal procedure of measurement using indirect calorimetry in our group runs over 23 h per day with single measurements every 6 min. Therefore the time-course includes 230 single measurements. In Fig. 2 one can recognise 3 remarkable points: a distinctive rhythm with different periods; higher amplitudes and higher variabilities at night (night activity of the rats); and a higher level of EE up to approximately 3 am, caused by the ppTh.

This rhythm was also found in earlier experiments, indicating resting periods of at least 90 min and lasting at least 20 min. The gas in the calorimetric cages is removed within 15 min and the frequency of measurement is 10 per h so that each rest of the rats can be detected as temporary minima in the time-course of EE. These rests are periods without physical activity higher than basal values. So a link between these temporary minima separates LA from EE (see Fig. 2, 'minima'). The following assumptions were made: the rats rest at least every 2 h; a rest lasts at least 20 min; the air-stream through the chamber is high enough to achieve gas changing times of ≈ 15 min; there were 10 measurements per hour, performed to cover each rest of the rats with at least one measurement; the objects reach postabsorptive conditions without postprandial thermogenesis, but no deep hunger with metabolic changes occurring during the experiment.

A calculation within the spreadsheet program Quattro Pro 3.0 (Borland Inc.) was made

$$\mathrm{EE\,min}_i = \min(\mathrm{EE}_{i-7} \ldots \mathrm{EE}_{i+7}) \tag{4}$$

for $i = 0 \ldots 229$ (limits fixed). The stepwise line was then smoothed by gliding averages (Fig. 2, resting line)

$$\mathrm{RRrest}_i = \mathrm{mean}(\mathrm{EE\,min}_{i-7} \ldots \mathrm{EE\,min}_{i+7}) \tag{5}$$

for $i = 0 \ldots 229$ (limits fixed). This resting line was assumed to be the separation of the portion of EE that was used for locomotor activity, leaving only BMR and ppTh.

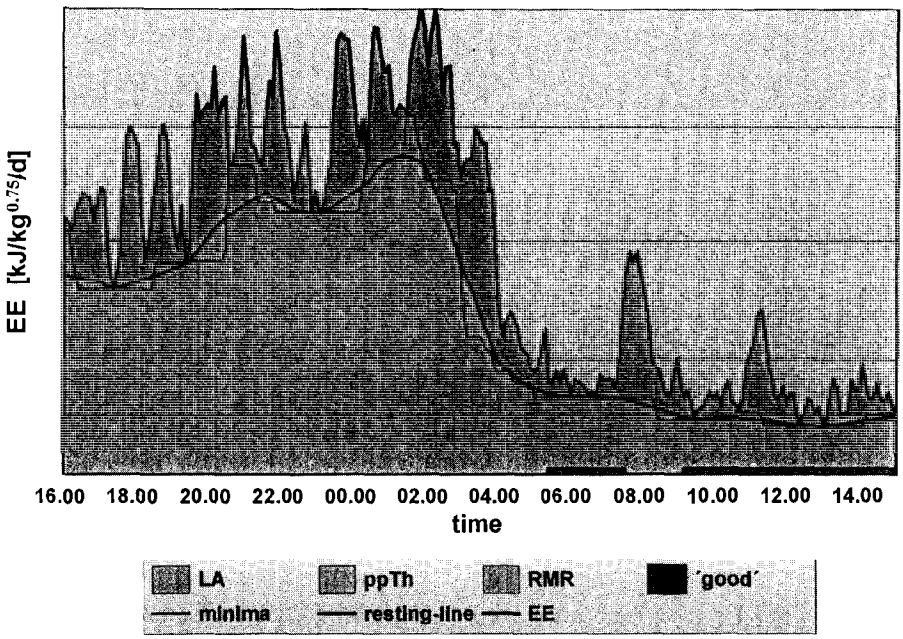

Fig. 2. A typical EE time-course and the normal measurement procedure.

If the rats had restricted feeding, they reach postabsorptive conditions within the 23 h period of measurement. Under these conditions the resting line represents only BMR. The detection of such conditions was very complex; we established a routine calculating an empirical value 'test' in the spreadsheet. This value tested for (a) the level of the resting line which has to be 'near its daily minimum', and (b) the gradient of the resting line which has to be unvarying. The test was calculated as

$$\text{Test}_i = \text{std.dev.}(\text{RRrest}_{i-7} \ldots \text{RRrest}_{i+7})(\text{RRrest}_i \times \text{MBW})^6 \quad (6)$$

for $i = 0 \ldots 229$, and compared with a defined limit. All cycles with test below the limit and resting line within the minimum and 15% of its span (Fig. 2, "good') were used to calculate an average value of resting line. Projecting this level onto the whole period was assumed to be BMR.

3.2. Results

This method has been used by our group for more than two years. Results of an experiment with a high CHO diet (82% of energy from CHO, 12% from fat, 6% from protein) are shown in Table 4 and in Fig. 2 as an example. This was an experiment with 7 small 7-week-old rats of Wistar strain, from the German Institute of Human Nutrition. The rats were fed ad libitum with a diet containing 0.1% of the neurotransmitter tryptophane. Their energy intake was quite similar and below their maintenance requirement. The RQ resulting from the CHO diet was 0.914.

Table 4
Results of the estimation of LA ($N = 6$)

		average	std.dev.	rel.std.
Body weight	BW	115.1 ± 2.3	in g	2.0%
Metabolic body weight	MBW	0.198 ± 0.003	in $kg^{0.75}$	1.5%
Energy intake	EI	741 ± 2	in kJ $kg^{-0.75}$	0.3%
Energy expenditure	EE	886.3 ± 40.6	in kJ $kg^{-0.75}$	4.6%
Respiratory quotient	RQ	0.914 ± 0.008		0.9%
Locomotor activity	LA	119.6 ± 9.3	in kJ $kg^{-0.75}$	7.8%
Postprandial thermogenesis	ppTh	148.2 ± 12.2	in kJ $kg^{-0.75}$	8.2%
Resting metabolic rate	RMR	628.4 ± 33	in kJ $kg^{-0.75}$	5.3%
Portions of energy	RMR/EE	70.1 ± 1.1	in %	1.6%
	ppTh/EI	20.0 ± 1.7	in %	8.5%
	LA/EE	13.4 ± 1.0	in %	7.3%
	LA/EI	16.1 ± 1.2	in %	7.5%

The method works quite well, as can be seen for the levels of LA, ppTh and RMR of EE and EI. RMR is expected to be a constant proportion of EE and the variation of RMR/EE is very small. In comparison to LA, ppTh is expected to be a constant proportion of EI. The variation of ppTh/EI is higher as can be seen in Table 4. This is caused by the fact, that ppTh is calculated as EE-LA-RMR and all variations of EE, LA and RMR influence ppTh. The variation of LA/EE or LA/EI is very similar. So there seems to be no correlation between EE and LA, although LA is calculated from the variation of EE. Some tests gave relative errors of LA and RNU of 4.6% and 4.6%, respectively, whereas the relative errors of EE and RQ were 3.2% and 1.7%. These values show the variations of LA and RNU only, and any possible systematic error cannot be estimated.

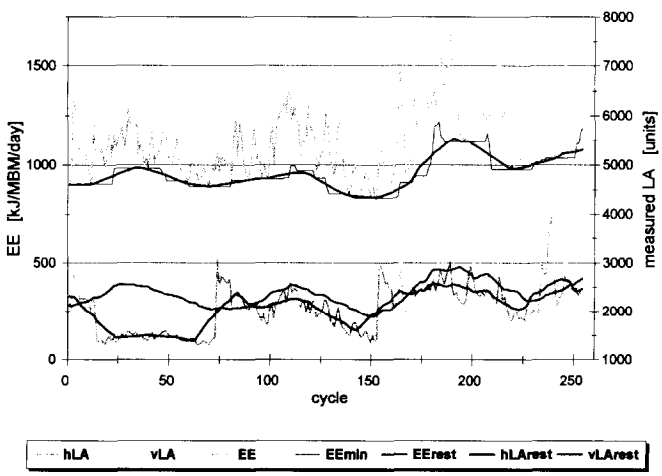

Fig. 3.

Fig. 3 gives a comparison of this method with the results of an experiment we did a few years ago. At this time we had a technique for the measurement of LA on rats [13], dynamically calculating the weight and position of a rat on the bottom of a cage by triangular weighting. To be able to compare measured horizontal (hLA) and vertical (vLA) locomotor activity with the calculated LA we had to smooth vLA and hLA. vLA appears very similar to EE or the calculated LA; a linear regression analysis (excluding the first and last 10 cycles because of the exceptions at the limits) of the calculated LA in dependence of vLA and hLA resulted in the following coefficients of determination: $R^2_{LA(hLA)} = 26.6\%$; $R^2_{LA(vLA)} = 79.4\%$; and $R^2_{La(hLA,vLA)} = 84.0\%$. Within that experiment the rats were fed ad libitum. Because the EI was not fixed in the experiment the greater variability of EE (RNU + ppTh + LA) was not a surprise, which is why a coefficient of determination higher than 85% cannot be expected.

To summarise one can state that locomotor activity can be calculated from frequent measurements of energy expenditure on rats; the frequency of measurement and external conditions have to be chosen such that the phases of resting and activity can be detected; and a useful separation of locomotor activity from energy expenditure can be generated by a smoothed link of temporary minima of EE.

4. Summary

Two methods for the mathematical estimation of interesting parameters from indirect calorimetric measurements are described. One method is useful for rapid evaluations of utilisation factors of energy and protein. The second method enables estimation of the portion of locomotor activity on energy expenditure only from frequently measured energy expenditure on rats. This method utilises the distinctive rhythm of the activity of rats.

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