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Day-to-Day Energy Expenditure Variability in Low Birth Weight Neonates

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ABSTRACT. We estimated the metabolic rate of 13 low birth weight infants over a 9-day period, using indirect calorimetry in conjunction with serial measurements of oxygen consumption, carbon dioxide production, and total urinary nitrogen excretion. The mean percent error for oxygen consumption and carbon dioxide production measurements (determined by alcohol combustion experiments) assignable to the open-circuit system was 0.4 and 3.8%, respectively. Error in the total urinary nitrogen excretion measurement was <1% by the Kjeldahl technique. In the clinical setting, however, the range of deviation of measured oxygen consumption, carbon dioxide production and total urinary nitrogen excretion was ±12, 12, and 15% of the mean value respectively for an individual patient under standardized controlled conditions. The variability of metabolic rate between infants may be as much as 76%. Factors that had a small effect on metabolic rate were difficult to detect because of the variability inherent in the short-term measurement of metabolic rate. It was virtually impossible to control the sources of variation in the resting metabolism of low birth weight neonates over extended experimental periods. Day-to-day variations in resting energy expenditure may explain, in part, the widely different growth rates of premature infants receiving similar caloric intakes. (*Pediatr Res* 21: 66–71, 1987)

Abbreviations

$\dot{M}R$, metabolic rate
$\dot{V}O_2$, oxygen consumption
$\dot{V}CO_2$, carbon dioxide production
TUN, total urinary nitrogen excretion
NPRQ, nonprotein respiratory quotient
EOG, electrooculogram

There has been an increased interest in the sources of variation in energy expenditure that occur among very low birth weight infants in the modern clinical setting (1–6). Recently, Schulze *et al.* (7–9) made an assessment of minute ventilation, heart rate, and gaseous metabolism in relation to the state of activity and feeding cycle of growing low birthweight infants. They showed that the infants were prone to significant (4.7 to 24.6%) swings in energy expenditure even under (apparently) strict basal conditions, and concluded that steady state conditions do not occur in growing low birth weight infants. Abdulrazzaq and Brooke (10) measured respiratory metabolism of premature infants and discussed body weight, activity, and energy intake in relation to 24-h measurements of energy expenditure. These authors found that $\dot{V}O_2$ measured over a 3-h period might lie anywhere between 13.8% above to 12.6% below the value obtained from the 24-h mean $\dot{V}O_2$. In contrast, Rutter *et al.* (11) and Gudinchet *et al.* (12) found only small variations (3–6%) in repeated measurements of resting $\dot{V}O_2$ within infants over a 24-h period. Similarly, Catzeflis *et al.* (13) found a stable pattern of energy expenditure in very low birth weight infants fed at constant intermittent intervals and extrapolated single 90-min to 3-h measurements of energy expenditure to 24 h.

Interest in the mechanisms responsible for these conflicting results led us to make longitudinal replicate measurements of $\dot{V}O_2$, $\dot{V}CO_2$, and total urinary nitrogen to determine with indirect calorimetry the average day-to-day variability of metabolic rate within and between low birth weight infants under controlled conditions.

PATIENTS AND METHODS

The 13 very low birth weight infants were without major medical problems, were tolerating feeds and gaining weight. Informed parental consent was obtained for each child. Data regarding weight, age, nutritional intake, and complications before measurements are provided in Table 1. Body weight was determined daily on a beam balance accurate to 10 g. Each baby received ready-to-feed formula at three hourly intervals by orogastric or nasogastric lavage. We weighed the containers before and after feeding to calculate caloric intake from the declared energy value of the formula. The dietary intake provided approximately 2½ times maintenance energy requirements (3) and remained constant (per kg body weight) throughout the 9-day study period. All patients received 1 ml Polyvisol (Mead Johnson, Evansville, IN) daily. Patient 11 was treated with caffeine citrate (5 mg kg^{-1} po) daily for apnea and patient 13 received 1 tsp of rice cereal (3.75 kcal g^{-1}) with feeds to prevent gastroesophageal reflux.

Having been admitted into the study, each infant was scheduled to complete six sequential measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ to determine the range of resting energy expenditure over a period of 9 consecutive days. The study protocol used to measure metabolic rate required the infant's body temperature to be stable in conditions approximating the neutral thermal environment with the infant lying supine for 2 h. Measurements started 1 h after a feed and at the same time each afternoon. Heart rate, respiration rate, and activity level were continuously monitored. Included in the metabolic rate measurements were periods of deep sleep, light sleep [determined by standard behavioral and physiologic criteria (14)], and quiet wakefulness with a minimum of gross body movements (but exclusive of crying).

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Table 1. *Clinical and nutritional characteristics of 13 low birthwt infants*

			At start of study						Daily intake (mean ± SD)		
Patient	Birth wt (g)	Gestational Age (wk)	Age/sex (days)	Wt (g)	% Ideal body wt (%)*	Daily wt gain (g kg^{-1})	Complications before measurements†	Formula‡	Volume (ml kg^{-1})	Calories (kcal kg^{-1})	Protein (g kg^{-1})
1	1150	28	29/M	1320	77	30	HMD, IVH	SC	163 ± 0	129 ± 3	3.6 ± 0
2	1000	32	26/F	1280	47	23		P	168 ± 0	109 ± 3	4.0 ± 0
3	1320	29	9/F	1180	85	15	TTNB	PM	175 ± 7	115 ± 4	2.8 ± 0.1
4	1280	31	8/M	1150	67	18		PM	163 ± 3	108 ± 6	2.6 ± 0
5	1050	30	32/M	1280	52	20	Pneumonia	SC	138 ± 4	109 ± 3	3.1 ± 0.1
6	1470	32	17/F	1480	66	9	HMD, pneumonia, PDA	PM	174 ± 4	115 ± 5	2.8 ± 0.1
7	1110	34	21/F	1475	51	8		PM	171 ± 1	113 ± 2	2.7 ± 0
8	780	28	48/F	1375	55	15	TTNB, IVH, apnea	PM	174 ± 2	113 ± 5	2.8 ± 0
9	1130	30	21/F	1245	65	26		SC	176 ± 9	138 ± 5	3.9 ± 0.2
10	590	34	40/M	1060	33	18	Asphyxia, hernia	SC	149 ± 0	118 ± 3	3.3 ± 0
11§	790	29	36/F	1260	57	15	HMD, pneumonia, PIE, reflux, apnea	SC	146 ± 1	117 ± 2	3.2 ± 0
12	1390	32	20/M	1490	60	23		SC	146 ± 2	115 ± 4	3.2 ± 0
13	1540	31	15/M	1570	82	23	HMD, reflux, apnea	SC	153 ± 2	132 ± 5‖	3.4 ± 0
Mean	1123	31	25	1320	61	19			161	118	3.2
SD	283	2	12	151	15	6			13	9	0.5
SEM	79	0.6	3	42	4	2			4	2.6	0.1

* Wt expressed as a percent of a normal infant of the same age, using the 50th percentile of the Lubchenco standard (37).

† Abbreviations used: HMD, hyaline membrane disease; IVH, intraventricular hemorrhage, TTNB, transient tachypnea of the newborn; PDA, patent ductus arteriosus; PIE, pulmonary interstitial emphysema.

‡ SC, Special Care; PM, PM 60/40 (Ross Laboratories); P, Portagen (Mead Johnson). Manufacturer's Stated Values: Special Care, 81 kcal dl^{-1}; Portagen, 67 kcal dl^{-1}; PM 60/40, 67 kcal dl^{-1}.

§ Patient 11 received caffeine citrate (5 mg kg^{-1} po) daily throughout the study.

‖ Patient 13 also received 1 tsp rice cereal (3.8 kcal g^{-1}) with each feed.

Oxygen consumption and carbon dioxide production were determined with an open-circuit technique described in detail in the companion paper (15). Respiratory gas exchange was analyzed every 100 s from a continuous recording of the output from the paramagnetic O_2 and IR CO_2 gas analyzers, and $\dot{V}O_2$ and $\dot{V}CO_2$ subsequently calculated as mean values for each observation period. During the study, three 24-h urine samples per infant were collected and analyzed (in duplicate) for total nitrogen by the micro-Kjeldahl method (16). Gaseous metabolism and urine collection periods were contiguous. $\dot{M}R$ and the NPRQ were calculated according to standard assumptions of indirect calorimetry using Jéquier's formula (17).

DATA ANALYSIS

The values for $\dot{V}O_2$, $\dot{V}CO_2$, and TUN used to predict $\dot{M}R$ incorporate a correction for the overall methodological and instrumentation error calculated as follows. $\dot{V}O_2$, $\dot{V}CO_2$, and TUN measurements were made over the range encountered in the clinical studies using absolute alcohol combustion (15) and stock urea solutions (16) respectively, as laboratory standards. The relation between the measured values and the true values made under ideal conditions was estimated by linear regression. The fitted line and its 95% confidence band was plotted using a high resolution graphics procedure (SAS GRAPH, Statistical Analysis System software package, version OS 82.3, SAS Institute, Inc., Cary, NC) (Fig. 1). The equation for the fitted line was in the general form $Y = \beta_0 + \beta_1 X$. Where: Y = measured value (dependent variable); X = true value (independent variable); β_1 = slope of the fitted line; β_0 = intercept of line on vertical axis.

For each infant, the mean values and 95% confidence intervals of $\dot{V}O_2$, $\dot{V}CO_2$, and TUN measurements were calculated from the following equation (18):

$$\bar{Y} \pm t^{\alpha/2}_{df} \sqrt{\frac{MSE}{n}}$$

where: $\bar{Y}$ = mean value of $\dot{V}O_2$, $\dot{V}CO_2$, TUN from (n) measurements per infant; n = number of measurements per infant; MSE = mean square error (this is a measure of the average within subject variation); df = degrees of freedom used to determine t; t = t statistic with the degree of "confidence" that is associated with the resulting interval. We chose $\alpha = 0.05$ (two-tailed).

Figure 1 shows how the best estimate of the true mean value ($\bar{X}$) of $\dot{V}O_2$, $\dot{V}CO_2$, and TUN was determined from the measured value ($\bar{Y}$). The 90% confidence interval of $\dot{V}O_2$, $\dot{V}CO_2$, and TUN for each infant was determined by the projection of the 95% confidence interval of $\bar{Y}$ through the 95% confidence band of the regression line to the X axis (Fig. 1) (18). The 95% confidence intervals of NPRQ and $\dot{M}R$ were obtained from large sample approximations (19).

RESULTS

As shown in Table 1, the 13 patients who met the selection criteria represent a heterogenous population of low birth weight infants whose ages ranged from 8 to 48 days (eight were female and five male). All but two patients had surpassed their birth weight; but both of these (patients 3 and 4) had established weight gain. There was a previous history of undernutrition (mean % ideal body weight = 61%, range 33–85%) in all patients coincident with their extreme prematurity or serious illness in the first weeks of life. All infants gained weight during the study although the rate of daily weight gain was very variable from one

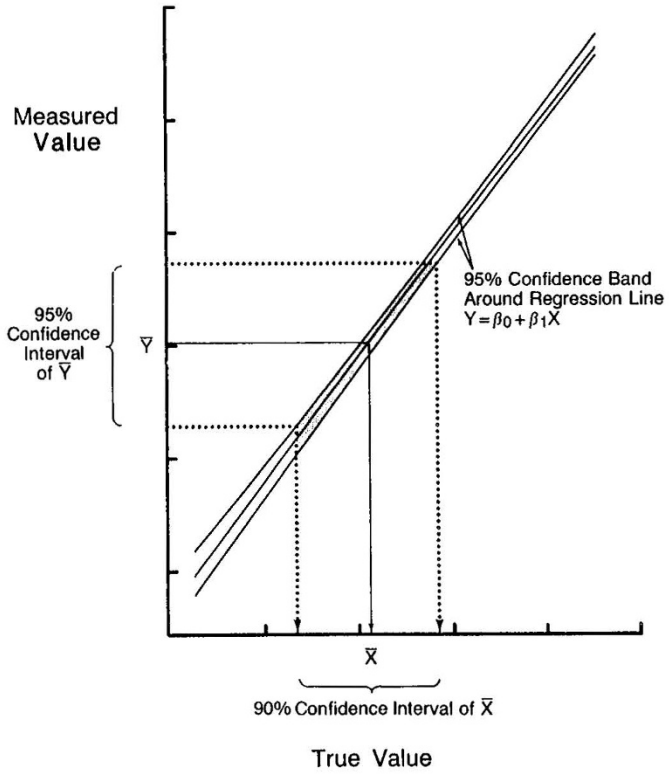

Fig. 1. Use of the regression graph with confidence bands to compute the best estimate of the true mean value ($\bar{X}$) and its 90% confidence interval for $\dot{V}O_2$, $\dot{V}CO_2$, and TUN. The regression line ($Y = \beta_0 + \beta_1 X$) and its 95% confidence bands was estimated from measured values related to true values using laboratory standards (15, 16). $\bar{Y}$ represents the mean value of (n) measurements per infant. The *dotted lines* show the 95% confidence interval of $\bar{Y}$ and their intersection with the 95% confidence bands of the regression line. Projection to the X axis determines the 90% confidence interval around the best estimate of the true mean value ($\bar{X}$) (18).

infant to another (range 8–30 g kg^{-1}). These findings were in accord with those from a larger study of catch-up growth in low birth weight infants recovering from their acute illness (20). The daily values for the volume, caloric, and protein intake of milk formula for each infant are also presented in Table 1. The average coefficient of variation of the daily energy intake throughout the study was 3% (range 1.7–5.6%) and was accounted for by the mass of formula lost by vomitus, burps, or spillage. We assumed that the small variation in caloric intake during the study was of no consequence. All patients completed the study and there were no adverse clinical events.

The infants were usually asleep during measurements so that sleep accounted for >90% of the total measurement time. The coefficient of variation (mean ± SEM) during each 2-h measurement of gaseous metabolism within the same infant was 10 ± 1.2% and 12 ± 1.9% for $\dot{V}O_2$ and $\dot{V}CO_2$, respectively. This spontaneous variability of respiratory gas exchange within individual measurements may be attributed to the expected relationship between $\dot{V}O_2$, $\dot{V}CO_2$, and state of activity (quiet sleep, active sleep, and quiet wakefulness) over the measurement period and was within the range of previous findings (21–24). Table 2 summarizes the results from the longitudinal measurements of $\dot{V}O_2$, $\dot{V}CO_2$, and TUN excretion for each infant. Seventy-eight measurements, or six measurements per infant, of $\dot{V}O_2$ were made; $\dot{V}CO_2$ measurements from three infants were not obtained because of technical difficulties, so there was a total of 62 $\dot{V}CO_2$ measurements. The values for $\dot{V}O_2$ and $\dot{V}CO_2$ in Table 2 are the mean of six 2-h observation periods (range 40–173 min), whereas values for TUN are the mean of three 24-h urine collection periods (range 18–24 h). The daily total urinary nitrogen loss for individual infants ranged from 53 to 112 mg kg^{-1} (mean ± SEM, 70 ± 5 mg kg^{-1}). This was slightly lower than the mean value for TUN reported in the literature (25) but yet within the normal range particularly when the type of formula used and the infant's prior intake of protein was taken into account (26, 27). Expressed in terms of protein utilization, the total urinary nitrogen excreted was between 9 and 23% (mean ± SEM, 14 ± 1%) of the daily protein intake of ~3.2 g kg^{-1} (protein = nitrogen × 6.25). The volume of urine collected during the measurement period was 80 ± 4 ml kg^{-1} (mean ± SEM).

Also presented in Table 2 are the 90% confidence intervals around the best estimate of the true mean values for $\dot{V}O_2$, $\dot{V}CO_2$, and TUN excretion. By minimizing the variation due to methodological and instrumentational errors, together with standardizing the test conditions as precisely as possible, the within-infant variability presented in Table 2 can be considered to be those due mainly to biological day-to-day variations in expenditure. The MR varied considerably from patient to patient, ranging from 1.86 to 3.27 kcal kg^{-1} h^{-1}, reflecting considerable physiological differences between infants. Of interest are the data from patient 11 with apnea spells who received caffeine citrate daily (5 mg kg^{-1} po), a methylxanthine and β-adrenergic stimulant known to effect the metabolic rate in the direction we observed.

DISCUSSION

Longitudinal measurements of energy expenditure made on each infant at regular intervals, in the same laboratory, and with constant methodology permits correction for the inevitable inter-individual differences of MR related to weight, gestational age, postnatal age, growth rate, and nutritional intake (28). Our results of energy expenditure variability from serial determinations of MR on the same infant tend to agree with the previous observations (7–9) that measurements of gaseous metabolism in resting low birth weight infants, even under carefully controlled conditions, was considerable. Our analysis showed variations of some 10% within a measurement of $\dot{V}O_2$ lasting 2 h, whereas $\dot{V}O_2$ measured on the same infant repeated on another day under as nearly as possible the same conditions may have been 12% below or 11% above the best estimate of the true mean value. Allowing for a minimum measurement error of ± 4% (29), we may conclude that the absolute biological day-to-day variation of $\dot{V}O_2$ within an infant under these conditions was over a range of ±8% for a measurement lasting 2 h. Alternatively, we could also reasonably conclude that the open-circuit indirect calorimetry technique is less reproducible in the clinical setting.

The results of the present investigation seem to contradict those obtained by Rutter *et al.* (11) who measured the resting rate of oxygen consumption of small babies over a period of 3 to 15 min. They found only small variations in serial VO_2 measurements repeated over 24 h and from day-to-day. The disparities observed were probably partially due to the differences between measurement periods. The longer the sampling interval, the more likely it was that multiple behavioral states, periods of wakefulness, or body movements were included in the sample. Such factors, if present, greatly increase the variability of the sample. In two previous studies using calorimetry systems designed for prolonged (24 h) continuous $\dot{V}O_2$ measurements, Abdulrazzq and Brooke (10) reported differences of about 13% between highest and lowest $\dot{V}O_2$ measurements during a 24-h study period. They drew attention to the possibility of considerable error in assuming that short term measurements (5–15 min) were representative of minimal resting metabolism or of energy expenditure over prolonged periods. In contrast, Gudinchet *et al.* (12) reported a stable pattern of $\dot{V}O_2$ over a 24-h period on 2

Table 2. *Indirect calorimetry data for 13 low birth wt infants; $\dot{V}O_2$, $\dot{V}CO_2$ measurd by open-circuit system (15) and TUN measured by micro-Kjeldahl method (16); NPRQ and $\dot{M}R$ derived by indirect calorimetry methods (17)*

	$\dot{V}O_2$ (ml kg^{-1} min^{-1} STPD)					$\dot{V}CO_2$ (ml kg^{-1} min^{-1} STPD)					TUN (mg kg^{-1} h^{-1})					NPRQ				$\dot{M}R$ (kcal kg^{-1} h^{-1})			
			90% confidence intervals§		Band-width‖			90% confidence intervals§		Band-width‖			90% confidence intervals§		Band-width‖		95% confidence limits		Band-width‖		95% confidence limits		Band-width‖
Patient	$\bar{Y}$(n)†	$\bar{X}$‡	Lower	Upper	(%)	$\bar{Y}$(n)†	$\bar{X}$‡	Lower	Upper	(%)	$\bar{Y}$(n)†	$\bar{X}$‡	Lower	Upper	(%)	Mean¶	Lower	Upper	(%)	Mean¶	Lower	Upper	(%)
1	8.46 (6)	8.70	7.71	9.44	9.9	8.48 (6)	8.19	7.29	8.96	10.2	2.23 (3)	2.25	1.81	2.64	18.4	0.94	.82	1.06	12.8	2.58	2.43	2.70	5.2
2	7.69 (6)	8.05	7.10	8.91	11.2						4.66 (3)	4.63	4.20	5.07	9.4								
3	7.75 (6)	8.18	7.06	9.25	13.4						4.33 (3)	4.32	3.89	4.79	10.4								
4	7.69 (6)	8.16	7.00	9.22	13.6	8.63 (6)	8.37	7.24	9.47	13.3	3.27 (3)	3.24	2.80	3.70	13.9	1.03	.89	1.16	13.1	2.47	2.29	2.65	7.3
5	7.91 (6)	8.23	7.14	9.14	12.2	8.59 (6)	8.25	7.29	9.22	11.7	2.20 (3)	2.19	1.78	2.60	18.7	1.00	.88	1.12	12.0	2.48	2.32	2.64	6.5
6	7.30 (6)	7.59	6.66	8.44	11.7	7.46 (6)	7.22	6.39	8.04	11.4	2.89 (3)	2.88	2.50	3.30	13.9	0.95	.84	1.06	11.6	2.25	2.11	2.39	6.3
7	6.82 (6)	7.12	6.23	7.91	11.8	6.38 (6)	6.15	5.25	7.00	14.2	2.79 (3)	2.78	2.40	3.18	14.0	0.86	.75	0.98	13.4	2.07	1.93	2.21	6.8
8	8.78 (6)	8.96	8.08	9.79	9.5	8.43 (6)	8.07	7.20	8.95	10.8	2.38 (3)	2.38	1.96	2.80	17.6	0.90	.81	1.00	10.6	2.63	2.48	2.77	5.6
9	8.41 (6)	8.70	7.63	9.67	11.7	8.46 (6)	8.17	7.48	9.15	10.2	2.42 (3)	2.39	1.97	2.90	19.5	0.94	.83	1.05	11.7	2.58	2.42	2.74	6.1
10	7.79 (6)	8.30	7.10	9.49	14.4	9.05 (2)	8.96	7.10	10.74	20.3	2.36 (3)	2.36	1.86	2.93	22.7	1.08	.87	1.28	19.0	2.54	2.33	2.76	8.4
11	10.93 (6)	11.06	10.02	11.93	8.6	10.77 (6)	10.36	9.33	11.24	9.2	2.33 (3)	2.34	1.91	2.83	19.7	0.97	.85	1.02	8.8	3.27	3.12	3.43	4.7
12	7.51 (6)	7.71	6.88	8.45	10.2	7.95 (6)	7.70	6.90	8.50	10.4	3.12 (3)	3.10	2.75	3.50	12.1	1.00	.89	1.10	10.5	2.31	2.19	2.45	5.6
13	5.73 (6)	6.01	5.18	6.79	13.4	7.01 (6)	6.73	5.96	7.45	11.1	3.27 (3)	3.24	2.92	3.58	10.2	1.12	.98	1.26	12.5	1.86	1.73	1.99	6.9
Mean	7.90	8.21	7.22	9.11	11.7	8.29	8.02	7.04	8.98	12.1	2.94	2.93	2.52	3.37	15.1	0.98	.86	1.10	12.4	2.46	2.30	2.61	6.3
SD	1.19	1.15	1.11	1.18	1.8	1.14	1.12	1.02	1.26	3.1	0.79	0.78	0.79	0.78	4.2	0.08	.06	0.10	2.6	0.36	0.35	0.37	1.0
SEM	0.33	0.32	0.31	0.33	0.5	0.34	0.34	0.31	0.38	0.9	0.22	0.22	0.22	0.22	1.2	0.02	.02	0.03	0.8	0.11	0.11	0.11	0.3

* $\dot{V}CO_2$ data from two infants were eliminated from analysis because of technical difficulties.

† Average measured value of (n) determinations per patient.

‡ Best estimate of the true mean value from the linear regression graph (see Fig. 1).

§ Estimated values of the measurement error bounds from inverse transformed data. $\alpha = 0.1$ when 90% of the measurements will be within the measurement error bounds of the true mean value (18).

‖ Bandwidth, % = [(upper limit − lower limit)/2] [100%]/$\bar{X}$.

¶ Estimated from large sample approximations (19).

consecutive days. The coefficient of variation of $\dot{V}O_2$ of the infants they studied was just outside the accuracy (±4%) expected during routine measurement of respiratory exchange (29). Furthermore, they claimed that estimation of 24-h energy expenditure from measurements made during 1½–3 h was valid because the coefficient of variations for measurements performed over 24 h were low. Other whole-body calorimetry studies confirm this finding (3, 13).

According to recent observations (1, 9, 12, 30, 31), the respiratory quotient of healthy growing premature infants may be greater than 1.00. When glucose is converted into palmitate, the adipose tissue consumes 27 carbon atoms as glucose to store 16 carbon atoms as palmitate with the remaining 11 carbon atoms appearing as CO_2 (32). The ratio of CO_2 output to oxygen usage during palmitate synthesis from glucose can be given as:

$$4.5\ C_6H_{12}O_6 + 40_2 \rightarrow C_{16}H_{32}O_2 + 11\ CO_2$$

$$+ 631\ \text{kcal where NPRQ} = 11/4 = 2.75.$$

Consequently, under conditions of lipogenesis, the amount of carbon dioxide excreted through the lungs was greater than oxygen uptake so that the respiratory quotient could rise to values >1.00 (33, 34). Under these conditions the calculation of metabolic rate using the respiratory gas equations remains valid (17). Of interest was the respiratory quotient data from patient 13 with the highest carbohydrate intake (in the form of rice cereal added to the formula) was associated with the highest respiratory quotient values.

The systems of calorimetry used in the clinical studies were very different which may explain the variable results published on the estimation of resting energy expenditure in the low birth weight neonate. A canopy system or metabolic chamber provides a relatively noninvasive method and allows measurements of gas exchange without the use of a face mask. This avoids discomfort, lessens the chance for air leaks, and avoids facial contact, especially with the trigeminal area which, if stimulated, increases tidal volume, minute ventilation, and the minute ventilation/CO_2 production ratio (35). However, the ventilated hood or face mask system does offer the advantage over the metabolic chamber of shorter response time (about 7 s for 90% deflection) and ready access to the infant. Furthermore, a metabolic chamber enclosing the infant limits accessibility, requiring constant supervision of the infant, and careful design of the chamber to minimize access time in case of an emergency. The response time of the metabolic chamber was relatively slow (about 90 s) due to the large volume of dead space and gas flow rates and was thus better suited for energy expenditure studies during sleep. It should be kept in mind that the dynamic responsiveness of any combination of flow meter and gas analyzer should be determined and the compensation for lag and delay time must be considered for the accurate analysis of respiratory gas exchange. Although the methods of respiratory gas exchange measurements are highly system specific, they all require meticulous calibration aimed to validate O_2 uptake and CO_2 production rates of similar magnitude to those encountered in normal calorimetric use (15).

For practical purposes, the clinical factors that contribute appreciably to variation in $\dot{M}R$ over the short-term include activity, temperature, and food intake. In these infants in whom sleep accounted for >90% of the total measurement time, an increase in physical activity was largely due to changes in the distribution of deep and light sleep. The only practical way of assessing the energy cost of sleep-related physical activity in relation to total $\dot{M}R$ was by calorimetry combined with EEG and EOG monitoring. There are relatively few studies, with conflicting data, on premature infants of $\dot{V}O_2$ and $\dot{M}R$ during sleep (*i.e.* using indirect calorimetric systems) that employ EEG monitoring allowing for a comparison of heat production during the different sleep states and a comparison of sleep and waking states. Darnall and Ariagno (22) noted no differences in $\dot{V}O_2$ during rapid eye movement and nonrapid eye movement sleep when infants were at thermoneutrality. In contrast, other investigators (7, 21, 23, 36) have reported a 9.4–16.0% increment in $\dot{V}O_2$ between quiet and active sleep. No EEG or EOG measurements were made in this study so that the present findings do not permit us to quantitate the energy utilization during sleep-related physical activity. On the other hand, by using extended experimental periods to incorporate a comparatively wide range of behavioral states we did obtain longitudinal replicate measurements of $\dot{M}R$ during multiple transitional physiological states.

In summary, during this study, the nutritional and thermal factors known to effect the metabolic rate, were kept constant. Our findings showed that, at least on a short-term basis, the variability in each infants' resting energy expenditure was likely to be largely a result of the variation in individual activity or sleep state. Even though the changes in $\dot{V}O_2$ during sleep states were relatively small when compared to those that occur with short bursts of maximal muscle contraction, their absolute magnitude was such that they can hardly be considered negligible over time. These and related processes add up to the large energy cost of maintenance. It may be possible that continuous monitoring for sleep state variability (EEG, EOG) and body movements by methods based on electronic imaging or using the Doppler principle (17) could be used to delineate further the variability of energy expenditure in neonates. The number of measurements per infant should be maximized as this directly affects the width of the 90% confidence interval of the estimated metabolic rate. As expected, the error bounds decrease with increasing number of measurements. Note, for example, the wide confidence interval for patient 10 in whom $\dot{V}CO_2$, was determined from two samples, compared to the intervals of other patients determined from six samples (Table 2). It was also clear that the investigator must exert considerable judgment in deciding on a minimal level of energy expenditure of infants using indirect calorimetry in the clinical setting. A standardized and rigorous method of testing the apparatus together with the use of the linear regression graph with confidence bands served to ensure that the reported experimental data were accurate as well as providing a quantitative assessment of their uncertainty.

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