# Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents[1–3]

*Johannes B van Goudoever, Eric J Sulkers, Harrie N Lafeber, and Pieter JJ Sauer*

**ABSTRACT**

**Background:** Currently available preterm formulas with energy contents of 3350 kJ (800 kcal)/L promote weight and length gain at rates at or above intrauterine growth rates but disproportionately increase total body fat.

**Objective:** The objective of this study was to determine whether fat accretion in formula-fed, very-low-birth-weight (VLBW) infants could be decreased and net protein gain maintained by reducing energy intakes from 502 kJ (80 kcal)$\cdot kg^{-1}\cdot d^{-1}$ [normal-energy (NE) formula] to 419 kJ (100 kcal)$\cdot kg^{-1}\cdot d^{-1}$ [low-energy (LE) formula] while providing similar protein intakes (3.3 g$\cdot kg^{-1}\cdot d^{-1}$).

**Design:** The study was a randomized, controlled trial enrolling 20 appropriate-for-gestational-age (AGA) and 16 small-for-gestational-age (SGA) VLBW infants (mean birth weight: 1.1 kg; mean gestational age: 31 wk); energy expenditure and nutrient balance were measured at 4 wk of age and anthropometric measurements were made when infants weighed 2 kg.

**Results:** The percentage of fat in newly formed tissue was significantly lower in AGA infants fed the LE formula ($n$ = 9) than in those fed the NE formula ($n$ = 10) (9% compared with 23%; analysis of variance, $P$ = 0.001). Energy expenditure was higher in AGA infants fed the NE formula than in those fed the LE formula. Skinfold thickness was markedly lower in AGA infants fed the LE formula than in those fed the NE formula, resulting in a lower estimated percentage body fat (8.0 ± 1.9% and 10.8 ± 3.5%, respectively; $P < 0.05$). Three of 6 SGA infants fed the LE formula were excluded during the study because of poor weight gain.

**Conclusions:** Body composition can easily be altered by changing the energy intakes of formula-fed VLBW infants. Energy intakes in these infants should be >419 kJ (100 kcal)$\cdot kg^{-1}\cdot d^{-1}$. *Am J Clin Nutr* 2000;71:816–21.

**KEY WORDS** Preterm infants, preterm formula, indirect calorimetry, body composition, body fat, skinfold thickness, average-for-gestational-age infants, small-for-gestational-age infants, very low birth weight

## INTRODUCTION

The intrauterine growth rate remains the standard by which the efficacy of preterm infant formulas is judged. The currently available preterm infant formulas with energy contents of 3350 kJ (800 kcal)/L promote weight and length gain at rates equaling or exceeding intrauterine growth rates when infants are fed ≥150 mL$\cdot kg^{-1}\cdot d^{-1}$. However, several studies (1–6) showed that the administration of ≥500 kJ (120 kcal)$\cdot kg^{-1}\cdot d^{-1}$ results in a deposition of body fat that exceeds the intrauterine value (7). Kashyap et al (8) showed clearly that energy intake is the major determinant of fat accretion and that differences in the ratio of protein to energy do not result in differences in fat accumulation.

Lowering energy intake by feeding infants human preterm milk generally yields weight-gain rates that are lower than those of infants fed formula; however, infants fed human preterm milk still have weight-gain rates approximating intrauterine rates (2, 9–13), with, usually, less fat accretion than in infants fed preterm formula (2, 9). Even so, the nitrogen, calcium, and phosphorus contents of human preterm milk are insufficient to maintain the intrauterine accretion rates of these nutrients (12).

We designed a study specifically to investigate the effect of lowering energy intake on short-term growth and body composition in small-for-gestational-age (SGA) and average-for-gestational-age (AGA) preterm infants. Our hypothesis was that it was feasible to significantly reduce fat-deposition rates without jeopardizing weight gain. Anthropometric measurements, a balance study, and substrate-use measurements were performed to assess differences in growth, body composition, and fuel metabolism of the infants.

## SUBJECTS AND METHODS

### Subjects

Infants with a birth weight <1500 g on admission to the neonatal intensive care unit of the Academic Hospital Rotterdam/Sophia Children's Hospital, Netherlands, were included in the study. Infants were eligible for inclusion if they were free of major metabolic problems or congenital malformations and if

[1] From the Department of Pediatrics, Academic Hospital Rotterdam/Sophia Children's Hospital, Rotterdam Netherlands.

[2] Supported by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek. The formulas were provided by Nutricia, Zoetermeer, Netherlands.

[3] Address reprint requests to JB van Goudoever, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 1100 Bates Street, Houston, TX 77030. E-mail: jbv@bcm.tmc.edu.

Received May 20, 1999.

Accepted for publication August 17, 1999.

**TABLE 1**
Composition of normal-energy (NE) and low-energy (LE) formulas (per L)

| | NE formula | LE formula |
|---|---|---|
| Energy content | | |
| (kJ) | 3350 | 2810 |
| (kcal) | 800 | 670 |
| Carbohydrates (g) | 80 | 65 |
| Lactose:maltodextrins (%) | 50:50 | 50:50 |
| Fat (g) | 44 | 35 |
| Medium-chain triacylglycerols (%) | 6 | 6 |
| 12:0 (%) | 17 | 17 |
| 18:1 (%) | 36 | 36 |
| 18:2 (%) | 17 | 17 |
| Protein (g) | 22 | 22 |
| Casein:whey (%) | 40:60 | 40:60 |

they were clinically stable and breathing room air at the introduction of oral feedings. Infants were included only after written, informed consent was obtained from at least one parent. The study protocol was approved by the medical ethics committee of the Academic Hospital Rotterdam/Sophia Children's Hospital. The subjects were randomly assigned before the introduction of oral feeding to receive either a preterm formula containing 3350 kJ (800 kcal)/L [normal energy (NE)], as is used routinely in neonatal intensive care units, or a formula containing 2800 kJ (670 kcal)/L [low energy (LE)], with an energy content resembling that of human milk. In each group, the target protein intake was 3.3 $g \cdot kg^{-1} \cdot d^{-1}$. We planned to recruit 10 SGA and 10 AGA infants to each feeding group. However, after the enrollment of 6 SGA infants who were assigned to the LE formula group, we stopped including SGA infants in this feeding group because weight gain in 3 of the 6 infants was $< 5\ g \cdot kg^{-1} \cdot d^{-1}$. We considered it unethical to include more SGA infants in the LE formula group.

All infants received total parenteral nutrition during their first postnatal week. Enteral feedings were gradually introduced from postnatal day 7 onward. Continuous feeding by nasogastric tube was given until a full enteral intake of 150 $mL \cdot kg^{-1} \cdot d^{-1}$ was reached at postnatal day 16–19. The composition of the formulas is given in **Table 1.** The 2 formulas (Nutricia, Zoetermeer, Netherlands) were of equal composition except for the amounts of carbohydrate and fat, which accounted for the different energy values of the formulas. The same batch of each formula was used throughout the study. Patient characteristics are summarized in **Table 2.** A 72-h balance study was performed between 3 and 7 d after the infants started receiving full-strength enteral feedings at an intake of 150 $mL \cdot kg^{-1} \cdot d^{-1}$. Indirect calorimetric measurements were performed twice, once during the balance study period and once within a week of the balance study period.

### Anthropometric measurements

Head circumference and length were measured at the time of the balance study and when the infants weighed 2 kg. Weight was measured daily until the infants reached 2 kg. Infants were excluded from the study if they gained an average of $< 5\ g \cdot kg^{-1} \cdot d^{-1}$ over a period of 1 wk and if no clinical reason for this poor weight gain was found other than a low energy intake. The average weight gain in $g \cdot kg^{-1} \cdot d^{-1}$ over the week that encompassed the balance study and indirect calorimetric measurements was taken as the basis for the calculation of the composition of the weight gain.

Skinfold-thickness measurements were performed in triplicate by using a Harpenden caliper (British Indicators, St Albans, United Kingdom) at 3 different sites: triceps, subscapula, and midthigh. The following lengths and circumferences were also measured in triplicate and averaged: crown-heel and crown-rump lengths, arm length (from the acromial process to the distal metacarpophalangeal joint), head circumference (at the maximal circumference), chest circumference (at the level of the nipples), midupper arm circumference (midway between the acromial process and the olecranon), midthigh circumference (midway between the inguinal crease and midknee), and calf circumference (at the maximal circumference). The technique of McGowan et al (14), with a slight modification, was used. Briefly, the skin was held gently with the fingers and the jaws of a caliper were applied. After 30 s of stabilization, a reading was taken. The mean of 3 readings was taken for each site. All measurements were performed by the same investigator (JBvG) and the CV was 3.8%. Total body fat was calculated as described by Dauncey et al (15). Briefly, the calculation is based on the assumption that the body is composed of 5 cylinders: 1 trunk, 2 upper limbs, and 2 lower limbs. The volume of subcutaneous fat covering each cylinder is calculated as the product of length, circumference, and thickness of the subcutaneous fat layer of each of the cylinders. The method assumes that most of the total fat mass is subcutaneous fat.

### Indirect calorimetric measurements

Metabolic rate and substrate use were measured in a closed-circuit indirect calorimetric system. Continuous measurements were performed for 6–8 h as described previously (16). In this system, an air mixture devoid of carbon dioxide enters the incubator. In the air leaving the incubator, the carbon dioxide concentration is measured at one side of a differential infrared meter (Unor 6N; Maihak, Hamburg, Germany), whereafter all carbon dioxide is

**TABLE 2**
Birth weight, gestational age, postnatal age, and study weight of 3–4-wk-old preterm infants[1]

| | NE formula | | LE formula | |
|---|---|---|---|---|
| | AGA ($n$ = 5 M, 5 F) | SGA ($n$ = 5 M, 5 F) | AGA ($n$ = 5 M, 5 F) | SGA ($n$ = 2 M, 4 F) |
| Birth weight (kg) | 1.2 ± 0.2 | 1.0 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.2 |
| Gestational age (wk) | 30 ± 1 | 32 ± 1 | 30 ± 2 | 33 ± 2 |
| Postnatal age (d)[2] | 29 ± 8 | 25 ± 5 | 28 ± 9 | 22 ± 2 |
| Study weight (kg)[2] | 1.6 ± 0.2 | 1.3 ± 0.1 | 1.6 ± 0.2 | 1.3 ± 0.2 |

[1] $\bar{x}$ ± SD. NE, normal energy; LE, low energy; AGA, average for gestational age; SGA, small for gestational age.
[2] At day 2 of balance study period.

**TABLE 3**
Anthropometric measurements in 3–4-wk-old preterm infants[1]

| | NE AGA ($n$ = 10) | NE SGA ($n$ = 10) | LE AGA ($n$ = 9) |
|---|---|---|---|
| Weight gain ($g \cdot kg^{-1} \cdot d^{-1}$)[2] | 16 ± 2 | 16 ± 3 | 16 ± 2 |
| Intake ($mL \cdot kg^{-1} \cdot d^{-1}$)[2] | 150 ± 6 | 150 ± 3 | 150 ± 2 |
| Time from 1.5 to 2 kg (d) | 18 ± 2 | 18 ± 4 | 20 ± 4 |
| Arm SF thickness (mm)[3] | 4.3 ± 0.8 | 3.9 ± 0.4 | 3.7 ± 0.6 |
| Leg SF thickness (mm)[3] | 4.7 ± 0.7 | 4.2 ± 0.4 | 4.0 ± 0.6[4] |
| Subscapular SF thickness (mm)[3] | 4.1 ± 0.5 | 3.8 ± 0.4 | 3.7 ± 0.6[4] |
| Percentage body fat (%)[5] | 10.8 ± 3.5 | 8.3 ± 2.2 | 8.0 ± 1.9[4] |
| Length (cm)[3] | 43.5 ± 1.3 | 43.0 ± 2.0 | 43.9 ± 1.3 |
| Head circumference (cm)[3] | 32.0 ± 1.0 | 33.0 ± 1.0 | 32.5 ± 0.5 |

[1] $\bar{x}$ ± SD. NE AGA, average-for-gestational-age infants fed normal-energy formula; NE SGA, small-for-gestational-age infants fed normal-energy formula; LE AGA, average-for-gestational-age infants fed low-energy formula; SF, skinfold thickness.
[2] Measured during balance study.
[3] Measured at a weight of 2 kg.
[4] Significantly different from NE AGA, $P < 0.05$ (ANOVA).
[5] Calculated by using the method of Dauncey et al (15) at a weight of 2 kg.

filtered out by a soda-lime filter. Carbon dioxide is then injected again into the airflow by a mass-flow injector system at such a rate that the same concentration of carbon dioxide is reached at the other site of the differential infrared meter. The amount of carbon dioxide injected equals the amount of carbon dioxide produced by the infant. The amount of oxygen consumed is equal to the amount of oxygen that has to be injected into the system to keep the oxygen tension constant, as measured by polarographic oxygen cells (type 6223771; Beckman Instruments, La Brea, CA).

Metabolic rate and protein, fat, and carbohydrate use were calculated from nitrogen excretion (measured during the balance study period), oxygen consumption, and carbon dioxide production (17). In the presence of lipogenesis, the apparent rate of carbohydrate use is the sum of rates of carbohydrate use for oxidation and lipogenesis at the same time (18). Likewise, fat use represents fat oxidation minus fat formed from glucose through lipogenesis. Therefore, fat storage, calculated as metabolizable fat intake minus fat use, also includes the amount of fat derived from lipogenesis.

### Balance study

Urine was collected in plastic adhesive bags over 3 d. Urinary nitrogen concentration was measured in a pooled sample by combustion in an automatic nitrogen analyzer (Carla Erba, Milan, Italy). The nitrogen concentration of the feces was estimated to be 10% of intake. Fat excretion in the feces was measured by using the method of Jeejeebhoy et al (19) with the modification of adding twice as much hydrochloric acid (1).

### Statistics

Data are presented as means ± 1 SD. Differences between groups were tested by using analysis of variance. Differences in body-composition variables between each group and the reference fetus (7) were tested by using a one-sample $t$ test. Results with $P$ values $<0.05$ were considered significant. Power calculations (with estimated SDs used for these calculations in parentheses) before the start of the study showed that we could detect differences of ≥20% in total body fat mass (3.0), fat storage (1.0), protein storage (0.2), and weight gain (2.5) with a 90% confidence rate at this $P$ value with 20 infants included in each feeding group.

## RESULTS

### Anthropometric measurements

Three infants [mean gestational age: 32.4 wk; mean birth weight: 1045 g; mean energy expenditure: 304 kJ (72.6 $kcal \cdot kg^{-1} \cdot d^{-1}$)] did not meet the minimum weight-gain requirement in their second and third weeks of receiving full enteral LE formula, although they gained weight at the intrauterine rate during the first week of full enteral feeding ($\bar{x}$: 15 $g \cdot kg^{-1} \cdot d^{-1}$). Thus, we stopped enrolling SGA infants in the LE formula group and present only the data for SGA infants receiving the NE formula and AGA and SGA infants receiving the NE and LE formulas.

One AGA infant (gestational age, 28 wk; birth weight, 1065 g) fed the LE formula was excluded from the study just before the balance study and indirect calorimetric study period. His weight gain was $<5$ $g \cdot kg^{-1} \cdot d^{-1}$ for >1 wk despite an intake of 180 $mL \cdot kg^{-1} \cdot d^{-1}$ [502 kJ (120 kcal) $\cdot kg^{-1} \cdot d^{-1}$; 4.0 g protein $\cdot kg^{-1} \cdot d^{-1}$]. He was fed the NE formula at an intake of 180 $mL \cdot kg^{-1} \cdot d^{-1}$ and gained weight at a rate $>15$ $g \cdot kg^{-1} \cdot d^{-1}$.

Postnatal age and study weight at the time of the indirect calorimetric and balance studies were not significantly different between the groups. Additionally, there was no significant difference in postnatal age between the 2 groups of AGA infants at the time they reached 2 kg (44 ± 10 d with the NE formula compared with 45 ± 14 d with the LE formula). SGA infants fed the NE formula had a postnatal age of 48 ± 11 d at a weight of 2 kg.

Weight gain at the time of the balance study, the number of days required to grow from 1.5 to 2 kg, skinfold-thickness measurements, and an estimate of total body fat at a weight of 2 kg, calculated according to Dauncey et al (15), are shown in **Table 3**. For the AGA infants, significant differences between feeding groups were found in 2 skinfold-thickness measurements and in percentage body fat at a weight of 2 kg. Percentage body fat at 2 kg is just <7% in the reference fetus (8). A one-sample $t$ test showed that infants fed the NE formula had significantly more total body fat than did the reference fetus ($P < 0.005$), whereas we could not detect a difference in the infants fed the LE formula.

### Indirect calorimetric measurements

Energy expenditure was significantly higher in AGA infants fed the NE formula than in AGA infants fed the LE formula, but we did not find a significant difference between AGA and SGA infants. Carbohydrate utilization was significantly lower and fat utilization was significantly higher in AGA infants fed the LE formula than in those fed the NE formula (**Table 4**).

### Balance study

There was no significant difference in protein intake (3.3 $g \cdot kg^{-1} \cdot d^{-1}$) between the groups. Urinary nitrogen excretion was significantly lower in SGA infants ($n$ = 15) than in AGA infants ($n$ = 20): 112 ± 16 and 144 ± 32 mg $N \cdot kg^{-1} \cdot d^{-1}$, respectively ($P$ = 0.001). There was no significant effect of formula type on nitrogen excretion.

Fat metabolism data are shown in **Table 5**. Fat excretion and percentage of fat absorbed were not significantly different among groups. However, there were clear differences in fat use as measured by indirect calorimetry between AGA infants fed

**TABLE 4**
Indirect calorimetric measurements in 3–4-wk-old preterm infants[1]

| | NE AGA ($n$ = 10) | NE SGA ($n$ = 10) | LE AGA ($n$ = 9) |
|---|---|---|---|
| Nonprotein $\dot{V}O_2$ ($L \cdot kg^{-1} \cdot d^{-1}$) | 11.1 ± 0.7 | 11.8 ± 0.9 | 10.5 ± 1.0 |
| Nonprotein $\dot{V}CO_2$ ($L \cdot kg^{-1} \cdot d^{-1}$) | 10.5 ± 0.7 | 10.9 ± 0.9 | 9.6 ± 0.6[2] |
| Nonprotein RQ | 0.946 ± 0.027 | 0.926 ± 0.029 | 0.916 ± 0.050 |
| Carbohydrate utilization ($g \cdot kg^{-1} \cdot d^{-1}$) | 11.9 ± 1.7 | 11.6 ± 1.8 | 9.6 ± 1.6[3] |
| Fat utilization ($g \cdot kg^{-1} \cdot d^{-1}$) | 1.1 ± 0.6 | 1.6 ± 0.6 | 1.7 ± 1.0[4] |
| Metabolic rate | | | |
| ($kJ \cdot kg^{-1} \cdot d^{-1}$) | 260 ± 12 | 268 ± 21 | 243 ± 17[4] |
| ($kcal \cdot kg^{-1} \cdot d^{-1}$) | 62 ± 3 | 64 ± 5 | 58 ± 4 |

[1] $\bar{x}$ ± SD. NE AGA, average-for-gestational-age infants fed normal-energy formula; NE SGA, small-for-gestational-age infants fed normal-energy formula; LE AGA, average-for-gestational-age infants fed low-energy formula; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RQ, respiratory quotient.
[2–4] Significantly different from NE AGA (ANOVA): [2] $P = 0.01$, [3] $P < 0.005$, [4] $P < 0.05$.

the NE formula and those fed the LE formula. Because of the different fat intakes, there were even more marked differences in the amount of fat stored by the infants fed NE formula and those fed the LE formula. The influence of this on the composition of the weight gain is shown in **Figure 1**. In the infants fed the NE formula, each gram of newly deposited tissue contained 22% fat (23% and 21% for AGA and SGA infants, respectively), compared with 9% in the AGA infants fed the LE formula ($P = 0.001$). The intrauterine value at a postconceptional age of 35–36 wk is 14.8% (7). No significant difference was found in protein accretion among groups (13–15%) and protein accretion was not significantly different from the percentage accretion in the reference fetus (13.5%).

## DISCUSSION

The most important finding of the study was that a lower energy intake resulted in a markedly lower fat accumulation rate, even lower than intrauterine accretion rates, but did not affect protein deposition. This result, taken together with the poor weight gain in some infants fed 419 kJ (100 kcal) $\cdot kg^{-1} \cdot d^{-1}$, led us to conclude that the energy intakes of formula-fed VLBW infants should be >419 kJ (100 kcal) $\cdot kg^{-1} \cdot d^{-1}$.

Although AGA infants fed formula containing 419 kJ (100 kcal) $\cdot kg^{-1} \cdot d^{-1}$ were shown to gain weight at a rate approximating the intrauterine growth rate (8, 20), no studies, to our knowledge, examined the effects of such an LE formula on SGA infants. Although the number of SGA infants included in our study may have been too few, we found it unethical to proceed with the use of LE formula in the SGA infants. Half of the SGA infants had a growth rate ≤5 $g \cdot kg^{-1} \cdot d^{-1}$ for >1 wk and the other half were gaining weight at a lower rate than the intrauterine rate; it took them 59 ± 4 d to reach 2 kg. A possible explanation for the lower weight gain was the relatively higher metabolic rate of SGA infants than of AGA infants, which leaves less energy for growth. The higher metabolic rate of the SGA infants was probably caused by the relatively higher proportion of metabolic active tissue/kg body wt. We conclude that SGA infants usually need >419 kJ (100 kcal) $\cdot kg^{-1} \cdot d^{-1}$ to gain weight at rates matching those in utero.

In contrast, we detected no significant differences in growth rate between the AGA infants fed the LE formula and those fed the NE formula, although one AGA infant fed the LE formula was excluded from the study because of poor weight gain. Nevertheless, the fat gain not only was markedly lower in the LE group but was even lower than intrauterine values.

In interpreting the results of these studies in a clinical sense, the key question is, What is the optimal body composition of premature infants? Although the body composition of the reference fetus is often considered the standard, the data used by Ziegler et al (7) were collected from studies published from 1902 to 1963 and included only 22 fetuses. Although these investigators did not observe a secular trend, we acknowledge that it may not be appropriate to extrapolate data from fetuses examined ≈40–100 y ago to the present population of preterm infants. However, in the absence of more recent data, these old data must be used for reference purposes.

In addition, it might be questioned whether intrauterine values should be compared directly with values measured after exposure to the extrauterine environment. We believe that the optimal goal is to achieve fat accretion rates at or slightly above intrauterine rates. This means that, on the basis of our results, an enteral intake of 419 kJ (100 kcal) $\cdot kg^{-1} \cdot d^{-1}$, even when providing 3.3 g protein $\cdot kg^{-1} \cdot d^{-1}$, does not provide sufficient energy to maintain an adequate fat accretion rate. The provision of 502 kJ (120 kcal) $\cdot kg^{-1} \cdot d^{-1}$, on the other hand, resulted in markedly higher fat accretion than that of the reference fetus. However, the influence of early nutritional intervention on lipid metabolism in later life remains controversial. Both the size and the number of adipocyte cells are influenced by diet. The gain in

**TABLE 5**
Fat metabolism as measured by indirect calorimetry in 3–4-wk-old preterm infants[1]

| | NE AGA ($n$ = 10) | NE SGA ($n$ = 10) | LE AGA ($n$ = 9) |
|---|---|---|---|
| | | $g \cdot kg^{-1} \cdot d^{-1}$ | |
| Fat intake | 6.6 ± 0.2 | 6.6 ± 0.2 | 5.3 ± 0.2 |
| Fat excretion | 1.9 ± 0.8 | 1.7 ± 0.2 | 1.9 ± 0.8 |
| Metabolizable fat intake | 4.7 ± 0.6 | 4.8 ± 0.2 | 3.4 ± 0.8[2] |
| Fat utilization | 1.1 ± 0.6 | 1.6 ± 0.6 | 1.7 ± 1.0[3] |
| Fat retention | 3.6 ± 0.9 | 3.2 ± 0.6 | 1.5 ± 1.4[4] |

[1] $\bar{x}$ ± SD. NE AGA, average-for-gestational-age infants fed normal-energy formula; NE SGA, small-for-gestational-age infants fed normal-energy formula; LE AGA, average-for-gestational-age infants fed low-energy formula.
[2–4] Significantly different from NE AGA (ANOVA): [2] $P < 0.005$, [3] $P < 0.05$, [4] $P < 0.001$.

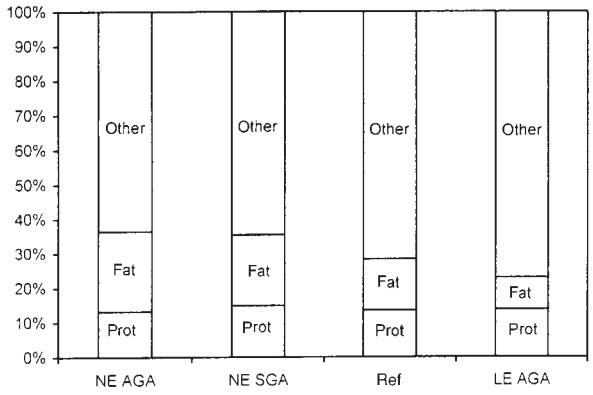


**FIGURE 1.** Composition of newly gained tissue compared with the reference fetus (Ref) (7), measured in 3–4-wk-old preterm average-for-gestational-age (AGA) and small-for-gestational-age (SGA) infants receiving 502 kJ · kg$^{-1}$ · d$^{-1}$ [normal energy (NE)] or 281 kJ · kg$^{-1}$ · d$^{-1}$ [low energy (LE)]. Prot = protein gain/g newly formed tissue. Fat = fat gain/g newly formed tissue. Other = percentage gain accounted for by components other than protein or lipids.

fat mass during infancy is accompanied mostly by an increase in the size of the adipocytes (21). Brook (22) suggested a sensitive period of adipocyte development in early life. Kramer (23) showed retrospectively that infants who were breast-fed, but not those who were bottle-fed, were protected from later obesity. It is likely that the breast-fed infants had lower energy intakes than the bottle-fed infants. One might argue, therefore, that lowering the energy intake in the first few months of life reduces the incidence of obesity. Clearly, follow-up studies are needed, but it seems feasible to accomplish a significant reduction in total body fat in the first few months of life with, for instance, an intake of 460 kJ (110 kcal) · kg$^{-1}$ · d$^{-1}$ while maintaining a weight gain > 15 g · kg$^{-1}$ · d$^{-1}$.

On the basis of numerous studies, Kashyap et al (8) computed an equation predicting weight gain on the basis of energy and protein intakes and birth weight. According to this equation, weight gain should have been 18 g · kg$^{-1}$ · d$^{-1}$ in infants fed the LE formula and 20 g · kg$^{-1}$ · d$^{-1}$ in infants fed the NE formula. The weight-gain rates we found were lower than those predicted with this equation but were in accordance with the rates found in infants in other studies who were fed formula with a similar protein-to-energy ratio and had similar energy intakes (20, 24).

Fat absorption was low in the infants in our study, possibly because of the analytic process or the type of formula used. Fat absorption was measured by subtracting the amount of fat recovered in the feces from fat intake. The amount of fat recovered in the feces is more likely to have been an underestimate than an overestimate. Thus, we think that the low fat absorption in our study was probably due to the type of formula used. We used a formula that contained 6% medium-chain triacylglycerols. In an earlier study, we obtained similar values by using a similar formula; however, use of a formula with a fat blend containing 50% medium-chain triacylglycerols resulted in much higher (88%) fat absorption rates (1).

Fat use was lower and carbohydrate use was higher in the infants fed the NE formula than in the infants fed the LE formula; this could have been the result of higher lipogenesis from glucose, lower fat oxidation, or both. Either of these conditions would result in a higher amount of fat laid down in the newly formed tissue, a result that was confirmed by the higher skinfold-thickness measurements of the infants fed the NE formula at a weight of 2 kg.

As expected, energy balance was different between the 2 feeding groups because of the difference in energy intakes. Mean metabolizable energy intake was 427 kJ (102 kcal) · kg$^{-1}$ · d$^{-1}$ in the AGA infants fed the NE formula and 343 kJ (82 kcal) · kg$^{-1}$ · d$^{-1}$ in the AGA infants fed the LE formula. Subtracting energy expenditure from metabolizable energy intake resulted in a markedly different energy storage rate. Energy storage in infants fed the NE formula was 167 kJ (40 kcal) · kg$^{-1}$ · d$^{-1}$, whereas in infants fed the LE formula it was 100 kJ (24 kcal) · kg$^{-1}$ · d$^{-1}$. This difference did not result in a lower rate of weight gain in the group fed the LE formula during the balance study or in the period thereafter. However, as shown in Figure 1, the amount of fat stored, as a percentage of newly formed tissue, was only 9%, compared with 23% for AGA infants fed the NE formula. Thus, the energy density of the newly formed tissue was much lower in the infants fed the LE formula, whereas the energy storage per gram gain in the infants fed the NE formula was in accordance with the results cited previously (25).

The low energy density of the newly formed tissue in the infants fed the LE formula agrees with the lower skinfold thickness of these infants at a weight of 2 kg and the calculated percentage body fat. Although some criticism may be raised about the value of calculating total body fat by using the method of Dauncey et al (15), the method gives a good estimate of total body fat because most fat in preterm infants is subcutaneous. We found strong agreement between the differences in body fat at a weight of 2 kg in the infants fed the LE formula compared with the group fed the NE formula as estimated from skinfold-thickness, length, and circumference measurements (56 g) and that extrapolated from the nutrient balance study and indirect calorimetric measurements (61 g). The latter value was calculated by assuming that both groups of infants had a similar fat percentage at the start of the study, and the difference in fat retention and in time to grow from 1.5 to 2 kg was taken into account. Considering the 2 completely different methods, it is remarkable that we found such strong agreement.

In summary, AGA infants fed 419 kJ (100 kcal) · kg$^{-1}$ · d$^{-1}$ stored less fat than did AGA infants fed 502 kJ (120 kcal) · kg$^{-1}$ · d$^{-1}$, irrespective of whether fat storage was estimated by using nutrient balance and indirect calorimetry or by using skinfold-thickness measurements. No influence of energy intake was seen on protein gain, which matched the intrauterine accretion rate. Because the composition of the newly formed tissue showed less fat accretion in both AGA and SGA infants fed 419 kJ (100 kcal) · kg$^{-1}$ · d$^{-1}$ than was observed in utero, we conclude that formula-fed preterm infants need > 419 kJ (100 kcal) · kg$^{-1}$ · d$^{-1}$ to maintain an adequate body composition.

We thank Leslie Loddeke for her expert contribution.

## REFERENCES

1. Sulkers EJ, Van Goudoever JB, Leunisse C, Wattimena JLD, Sauer PJJ. Comparison of two preterm formulas with or without addition of medium-chain triglycerides (MCTs). I: Effects on nitrogen and fat balance and body composition changes. J Pediatr Gastroenterol Nutr 1992;15:34–41.

2. Putet G, Senterre J, Rigo J, Salle B. Nutrient balance, energy utilization, and composition of weight gain in very-low-birth-weight infants fed pooled human milk or a preterm formula. J Pediatr 1984; 105:79–85.
3. Whyte RK, Haslam R, Vlainic C, et al. Energy balance and nitrogen balance in growing low birthweight infants fed human milk or formula. Pediatr Res 1983;17:891–8.
4. Schulze KF, Stefanski M, Masterson J, et al. Energy expenditure, energy balance, and composition of weight gain in low birth weight infants fed diets of different protein and energy content. J Pediatr 1987;110:753–9.
5. Reichman BL, Chessex P, Putet G, et al. Diet, fat accretion, and growth in premature infants. N Engl J Med 1981;305:1495–500.
6. Fairey AK, Butte NF, Mehta N, Thotathuchery M, Schanler RJ, Heird WC. Nutrient accretion in preterm infants fed formula with different protein:energy ratios. J Pediatr Gastroenterol Nutr 1997;25:37–45.
7. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ. Body composition of the reference fetus. Growth 1976;40:329–41.
8. Kashyap S, Schulze KF, Ramakrishnan R, Dell RB, Heird WC. Evaluation of a mathematical model for predicting the relationship between protein and energy intakes of low-birth-weight infants and the rate and composition of weight gain. Pediatr Res 1994;35:704–12.
9. Chessex P, Reichman B, Verellen G, et al. Quality of growth in premature infants fed their own mothers' milk. J Pediatr 1983;102:107–12.
10. Atkinson SA, Bryan MH, Anderson GH. Human milk feeding in premature infants: protein, fat, and carbohydrate balances in the first weeks of life. J Pediatr 1981;99:617–24.
11. Gross SJ. Growth and biochemical response of preterm infants fed human milk or modified infant formula. N Engl J Med 1983;308:237–41.
12. Kashyap S, Schulze KF, Forsyth M, Dell RB, Ramakrishnan R, Heird WC. Growth, nutrient retention, and metabolic response of low-birth-weight infants fed supplemented and unsupplemented preterm human milk. Am J Clin Nutr 1990;52:254–62.
13. Hendrickse WA, Spencer SA, Roberton DM, Hull D. The calorie intake and weight gain of low birth weight infants fed on fresh breast milk or a special formula milk. Eur J Pediatr 1984;143:49–53.
14. McGowan A, Jordan M, MacGregor J. Skinfold thickness in neonates. Biol Neonate 1975;25:66–84.
15. Dauncey MJ, Gandy G, Gairdner D. Assessment of total body fat in infancy from skinfold thickness measurements. Arch Dis Child 1977; 52:223–7.
16. Sauer PJJ, Dane HJ, Visser HKA. Longitudinal studies on metabolic rate, heat loss, and energy cost of growth in low birth weight infants. Pediatr Res 1984;18:254–9.
17. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 1988;47:608–28.
18. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983;55:628–34.
19. Jeejeebhoy KN, Ahmad S, Kozak G. Determinations of fecal fats containing both medium and long chain triglycerides and fatty acids. Clin Biochem 1970;3:157–63.
20. Bell EF, Rios GR, Ungs CA, Johnson KJ, Trapp SA, Ziegler EE. Influence of energy and protein intake on energy utilization and body composition of small premature infants. Pediatr Res 1988;23:479 (abstr).
21. Soriguer Escofet FJC, Esteva de Antonio L, Tinahones FJ, Pareja A. Adipose tissue fatty acids and size and number of fat cells from birth to 9 years of age—a cross-sectional study in 96 boys. Metabolism 1996;45:1395–401.
22. Brook CGD. Evidence for a sensitive period in adipose cell replication in man. Lancet 1972;2:23–9.
23. Kramer MS. Do breast-feeding and delayed introduction of solid foods protect against subsequent obesity? J Pediatr 1981;98: 883–7.
24. Feymond D, Schutz Y, Decombaz J, Micheli JL, Jequier E. Energy balance, physical activity, and thermogenetic effect of feeding in premature infants. Pediatr Res 1986;20:638–45.
25. Bell EF. Diet and body composition of preterm infants. Acta Paediatr Scand 1994;405:25S–8S.