Effects of quality of energy on substrate oxidation in enterally fed, low-birth-weight infants[1,2]

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ABSTRACT

Background: Carbohydrate and fat may differ in their ability to support energy-requiring physiologic processes, such as protein synthesis and growth. If so, varying the constituents of infant formula might be therapeutically advantageous.

Objective: We tested the hypothesis that low-birth-weight infants fed a diet containing 65% of nonprotein energy as carbohydrate oxidize relatively more carbohydrate and relatively less protein than do infants fed an isoenergetic, isonitrogenous diet containing 35% of nonprotein energy as carbohydrate.

Design: Sixty-two low-birth-weight infants weighing from 750 to 1600 g at birth were assigned randomly and blindly to receive 1 of 5 formulas that differed only in the quantity and quality of nonprotein energy. Formula containing $544 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ with either 50%, 35%, or 65% of nonprotein energy as carbohydrate was administered to control subjects, group 1, and group 2, respectively. Groups 3 and 4 received gross energy intakes of $648 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ with 35% and 65% of nonprotein energy as carbohydrate. Protein intake was targeted at $4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Substrate oxidation was estimated from biweekly, 6-h measurements of gas exchange and 24-h urinary nitrogen excretion.

Results: Carbohydrate oxidation was positively ($r = 0.71, P < 0.0001$) and fat oxidation was negatively ($r = -0.46, P < 0.001$) correlated with carbohydrate intake. Protein oxidation was negatively correlated with carbohydrate oxidation ($r = -0.42, P < 0.001$). Fat oxidation was not correlated with protein oxidation. Protein oxidation was less in infants receiving 65% of nonprotein energy as carbohydrate than in groups receiving 35% nonprotein energy as carbohydrate.

Conclusion: These data support the hypothesis that energy supplied as carbohydrate is more effective than energy supplied as fat in sparing protein oxidation in enterally fed low-birth-weight infants. *Am J Clin Nutr* 2001;74:374–80.

KEY WORDS Low-birth-weight infants, substrate oxidation, oxygen consumption, carbon dioxide production, respiratory quotient, energy expenditure, nonprotein energy

INTRODUCTION

The current goal of nutritional management of preterm infants, as recommended by the American Academy of Pediatrics (1), is to achieve a postnatal growth approximating that of a normal fetus of the same postconceptional age. Although rates of weight gain similar to or greater than the intrauterine rate have been achieved by feeding preterm infants enriched diets (2–6), invariably, these diets lead to a disproportionate increase in body fat. The reasons for this postnatal failure to maintain the high fetal rates of protein synthesis relative to fat deposition are not known. One difference between fetal and neonatal life is the relatively greater dependence of the fetus on carbohydrate rather than fat as a source of energy. If the fetus is relatively more dependent on carbohydrate than fat, then it is possible that the same is true of infants of comparable maturity after birth. There is some evidence that, joule for joule, use of carbohydrate as an energy source is more effective in supporting protein accretion than use of fat (7–10). There are investigators, however, who assert that this effect is transient (11). This hypothesis remains untested in enterally fed, low-birth-weight (LBW) infants. Should there be significant differences in the growth-supporting effects of fat and carbohydrate, these differences might be exploited in the design of formulas for premature infants and human milk supplements.

Many studies have investigated the effect of varying the source of nonprotein energy on substrate use in parenterally fed, newborn infants (12, 13). However, the consequences of variations in the quality of nonprotein energy have not been investigated in enterally fed, preterm infants. In adults, substrate oxidation is dependent on the relative proportions of carbohydrate and fat in the diet (14, 15). The purpose of the present study was to test the hypothesis that LBW infants fed diets containing 65% of nonprotein energy as carbohydrate oxidize relatively more carbohydrate and relatively less protein than do infants fed isoenergetic, isonitrogenous diets containing 35% nonprotein energy as carbohydrate.

SUBJECTS AND METHODS

Experimental design

LBW infants without gastrointestinal, renal, or severe pulmonary disease and who weighed between 750 and 1600 g at

TABLE 1
Dietary intakes of study groups[1]

Intake	Control group (50% carbohydrate)	Group 1 (35% carbohydrate)	Group 2 (65% carbohydrate)	Group 3 (35% carbohydrate)	Group 4 (65% carbohydrate)
Energy ($kJ \cdot kg^{-1} \cdot d^{-1}$)	544 ± 6.7	552 ± 6.3	540 ± 19.2	648 ± 2.5	640 ± 4.2
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	4.0 ± 0.06	4.2 ± 0.01	4.1 ± 0.11	4.1 ± 0.08	4.0 ± 0.02[2]
Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)	12.9 ± 0.2	9.1 ± 0.09	16.8 ± 1.0	11.2 ± 0.3	20.4 ± 0.1
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	6.1 ± 0.27	7.8 ± 0.20	4.3 ± 0.02	9.5 ± 0.05	5.3 ± 0.03

[1] $\overline{x}$ ± SD. Absolute carbohydrate and fat intakes were significantly different between the groups at both gross energy intakes as per the study design. The groups differed by the percentage of nonprotein energy as carbohydrate.

[2] Significantly different from group 3, $P < 0.05$.

birth were stratified by birth weight into 1 of 3 ranges: 750–1000, 1001–1300, or 1301–1600 g. The infants were then randomly assigned by a blind draw to receive 1 of 4 experimental formulas or a control formula. All formulas were designed to provide protein intakes of 4 $g \cdot kg^{-1} \cdot d^{-1}$ when fed at 180 $mL \cdot kg^{-1} \cdot d^{-1}$. The control formula and formulas 1 and 2 were designed to provide 502 $kJ \cdot kg^{-1} \cdot d^{-1}$ when fed at the same volume. The carbohydrate and fat contents of the formulas were varied to provide 35% of nonprotein energy as carbohydrate in formula 1, 65% of nonprotein energy in formula 2, and 50% of nonprotein energy in the control formula. Formulas 3 and 4 were designed to provide higher energy intakes of 628 $kJ \cdot kg^{-1} \cdot d^{-1}$ when fed at 180 $mL \cdot kg^{-1} \cdot d^{-1}$, with nonprotein energy as 35% and 65% from carbohydrate. All formulas were made specifically for this study by Ross Laboratories (Columbus, OH). Formula bottles were number coded and the investigators were not informed of the code until the study was completed. The protein content of the formulas was modified bovine milk protein with a ratio of whey protein to casein of 60:40. The carbohydrate content of the formulas was composed of equal amounts of lactose and corn syrup solids, and fat was provided as a mixture of coconut, soy, and safflower oils. The electrolyte, mineral, and vitamin contents of the formulas were similar and met recommended dietary intakes (1). The measured energy content of the formulas were slightly higher than what was planned in the study design. The actual macronutrient intakes of the 5 groups are shown in **Table 1**. Protein concentrations in the specially designed formulas varied, which resulted in a slightly lower absolute protein intake by the infants randomly assigned to group 4 than in infants in group 3.

The study was approved by the Institutional Review Board of the College of Physicians and Surgeons of Columbia University. Written, informed parental consent was obtained before infants were enrolled in the study.

Experimental protocol

Shortly after enrollment and as soon as medically permitted, the infants were transferred to the General Clinical Research Center, where they remained until discharge. The assigned formula was administered as soon as enteral feedings could be tolerated by the infant. The volume of the formula was increased as tolerated until the desired intake of 180 $mL \cdot kg^{-1} \cdot d^{-1}$ was achieved. This volume was then maintained throughout the study until the infant's weight reached 2200 g or until the infant was discharged from the hospital. During this time, infants were cared for in servo-controlled, single-walled incubators under thermoneutral conditions. The formulas were administered either by orogastric tube or, if tolerated, by nipple. Vitamin E [25 mg (25 IU)] and a mixture of vitamins A [450 μg (1500 IU)], C

(35 mg), and D [10 μg (400 IU)] were administered daily once feedings were established. The volume of formula intake, nitrogen excretion, oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were monitored serially from the time full feeds were tolerated until discharge. The study period ranged from 13 to 54 d ($\overline{x}$ ± SD: 26.1 ± 10.2 d) for the control group, from 13 to 47 d (25.9 ± 10.3 d) for group 1, from 14 to 48 d (25.3 ± 10.3 d) for group 2, from 13 to 37 d (25.4 ± 7.2 d) for group 3, and from 14 to 37 d (21.9 ± 7.7 d) for group 4.

Measurement of gaseous metabolism

Once infants had been receiving full enteral feedings for ≥72 h, gaseous metabolism was measured every 2 wk until discharge. Each study began immediately after the feeding at 0800, was interrupted for the 1100 feeding, and then continued until the infant was ready for the 1400 feeding. Data from ≥150 consecutive minutes were collected during each interfeed period.

$\dot{V}O_2$ and $\dot{V}CO_2$ were measured in a whole-body respirometer by use of flow-through indirect calorimetry. The oxygen concentration of dried gas exiting the respirometer was measured with a Servomex OA 1100 paramagnetic oxygen analyzer (Sybron, Norwood, MA). The concentration of carbon dioxide in the exiting gas was measured with a Beckman LB-2 infrared carbon dioxide analyzer (SensorMedics, Yorba Linda, CA). Air flow through the system was measured with a linear mass flow meter (Matheson, East Rutherford, NJ). Analog outputs from all sensors were digitized at 25 Hz with the use of a Labmaster analog-to-digital converter (Scientific Solutions, Solon, Ohio) and logged to a dedicated AST 286 PC microcomputer (AST, Hong Kong). During each study, the respirometer was warmed indirectly by an overhead radiant heater, servo-controlled to maintain an abdominal skin temperature of 36.5 °C. Electrical and gas standard calibrations were performed during each study. The performance of this system was validated (16) and the bench accuracy established at ± 2.2% for both $\dot{V}CO_2$ and $\dot{V}O_2$.

Offline processing with special purpose software was used to correct the raw digital data for drift, transform the data using the appropriate calibration standards, correct measurements to standard temperature and pressure, correct the flow for differences in respiratory exchange, and integrate the signals on a continuous minute-to-minute basis. Measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were then normalized to body weight and logged as means for each minute. Mean measurements were then calculated for each feeding interval and for each study.

Studies were conducted every 2 wk. Of the 62 infants, 46 participated in 2 studies, 11 participated in 3 studies, and 5 infants participated in 1 study. Measurements for each infant were then

TABLE 2
Characteristics of the study population[1]

	Control group (n = 14)	Group 1 (n = 11)	Group 2 (n = 12)	Group 3 (n = 14)	Group 4 (n = 11)
Birth weight (g)	1259 ± 242	1294 ± 206	1195 ± 265	1251 ± 220	1311 ± 244
Gestational age (wk)	30.3 ± 2.2	30.4 ± 1.7	29.4 ± 3.1	30.4 ± 2.6	31.8 ± 2.1
Age at full formula intake (d)	14.6 ± 5.1	14.2 ± 5.0	18.9 ± 7.5	15.4 ± 6.8	13.3 ± 3.7
Time to recovery of birth weight (d)	11.9 ± 4.2	12.9 ± 3.4	14.6 ± 5.6	12.9 ± 6.4	10.5 ± 3.6

[1] $\overline{x}$ ± SD. There were no significant differences between the groups.

averaged to yield a single estimate of $\dot{V}O_2$, $\dot{V}CO_2$, and a respiratory quotient (RQ) for the entire period of full enteral intake.

Urinary nitrogen excretion

Seventy-two–hour urine collections spanning the measurements of gaseous metabolism were obtained every 2 wk. The methods used for collecting and analyzing urinary nitrogen excretion were described previously (3). The mean urine volume was slightly but significantly less in the infants receiving the higher-energy diets (111 ± 7.3, 110 ± 7.6, and 108 ± 10.4 mL·kg^{-1}·d^{-1} in the control group and groups 1 and 2, and 102 ± 11.3 and 102 ± 6.5 mL·kg^{-1}·d^{-1} in groups 3 and 4, respectively). The variability in the urine volume within groups ranged from 6.5% to 11%. Sex did not influence the urine volume collected.

Calculation of substrate oxidation

Net substrate oxidation was calculated by using the classic theory and equations of Lusk (17, 18). The $\dot{V}O_2$ and $\dot{V}CO_2$ in the combustion of glucose and fat can be computed accurately from their stoichiometry by assuming the fatty acid composition of the triacylglycerols. Comparable although slightly less accurate values for $\dot{V}O_2$ and $\dot{V}CO_2$ by oxidizing protein were estimated empirically by Magnus-Levy (19). The errors that result from the assumptions about the composition of fatty acids and the quality of protein are thought to be small when the composition of dietary fat and protein does not vary appreciably (20, 21). Infants fed the study formula, unlike free-feeding adults, received similar amounts of substrate within each study group. In general, the equations were nearly exact for the calculation of net lipid, glucose, and protein oxidation rates and for the calculation of net lipid synthesis from glucose.

These equations must be interpreted differently whenever net lipogenesis from carbohydrate is known to occur (20–25). When nonprotein respiratory quotients are >1.0, ie, when net lipogenesis is thought to occur, fat oxidation is a negative value and thus, measured carbohydrate oxidation and true carbohydrate oxidation differ by the glucose equivalent of the fat synthesized.

Under these conditions, true carbohydrate oxidation is less than measured carbohydrate oxidation (22, 25). It is also known that lipogenesis can occur in some tissues at a nonprotein RQ of <1.0 (22–25). Thus, the calculated carbohydrate oxidation rate represents the true carbohydrate oxidation rate, the amount of glucose converted to fat during lipogenesis, and an unknown but negligible contribution from gluconeogenesis. The equations used in the calculations (22) are listed below:

$$\text{Glucose oxidation (g·kg}^{-1}\text{·d}^{-1}) = [(4.55 \times \dot{V}CO_2) - (3.21 \times \dot{V}O_2)] - (2.87 \times \text{urinary N}) \quad (1)$$

where $\dot{V}CO_2$ and $\dot{V}O_2$ are in L·kg^{-1}·d^{-1} and urinary N is in g·kg^{-1}·d^{-1}.

$$\text{Fat oxidation (g·kg}^{-1}\text{·d}^{-1}) = [(1.67 \times \dot{V}O_2) - (1.67 \times \dot{V}CO_2)] - (1.92 \times \text{urinary N}) \quad (2)$$

$$\text{Protein oxidation (g·kg}^{-1}\text{·d}^{-1}) = \text{urinary N} \times 6.25 \quad (3)$$

where urinary N is equal to nitrogen excretion in g·kg^{-1}·d^{-1} and 6.25 is the conversion factor for converting nitrogen to protein.

Data analysis

Mean values for all variables were computed from all data obtained from each infant. Effects of the quality of energy intake on metabolic gas exchange and substrate oxidation rates were then analyzed by group. Data were analyzed using analysis of variance. When the analysis of variance was significant, post hoc analyses were performed with specific contrasts incorporated into the model (26). The comparisons were limited to outcomes at high compared with low carbohydrate intake at both gross energy intakes.

To determine the relation of carbohydrate and fat intake with carbohydrate and fat oxidation, regression analysis of oxidation rates against intake was performed. The correlation between carbohydrate and fat oxidation and protein oxidation was also esti-

TABLE 3
Energy balance data of the study groups[1]

Energy variable	Control group (n = 14)	Group 1 (n = 11)	Group 2 (n = 12)	Group 3 (n = 14)	Group 4 (n = 11)
			kJ·kg^{-1}·d^{-1}		
Gross energy intake	544 ± 6.7	552 ± 6.3	540 ± 19.2	648 ± 2.5	640 ± 4.2
Excretion (stool and urine)	57.7 ± 25.1	76.1 ± 22.6	34.7 ± 9.2[2]	108 ± 30.1	42.7 ± 9.6[3]
Metabolizable energy intake	486 ± 22.2	475 ± 22.2	506 ± 17.6[2]	541 ± 28.9	596 ± 24.1[3]
Expenditure	264.8 ± 18.4	262.8 ± 22.6	266.9 ± 11.7	271.1 ± 19.7	289.1 ± 14.2[4]
Energy balance	221.3 ± 33.9	212.5 ± 24.3	238.9 ± 23.8[5]	269.9 ± 30.5	306.7 ± 17.2[3]

[1] $\overline{x}$ ± SD.
[2,5] Significantly different from group 1 (ANOVA): [2] $P < 0.01$, [5] $P < 0.05$.
[3,4] Significantly different from group 3 (ANOVA): [3] $P < 0.01$, [4] $P < 0.05$.

TABLE 4
Metabolic gas exchange and urinary nitrogen excretion of the study groups[1]

Variable	Control group (n = 14)	Group 1 (n = 11)	Group 2 (n = 12)	Group 3 (n = 14)	Group 4 (n = 11)
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot d^{-1}$)	8.84 ± 0.56	8.94 ± 0.81	8.81 ± 0.41	9.16 ± 0.71	9.41 ± 0.46
$\dot{V}CO_2$ ($mL \cdot kg^{-1} \cdot d^{-1}$)	8.25 ± 0.82	7.76 ± 0.61	8.58 ± 0.57[2]	8.18 ± 0.59	9.70 ± 0.76[3]
RQ	0.934 ± 0.063	0.870 ± 0.041	0.976 ± 0.066[4]	0.895 ± 0.043	1.031 ± 0.072[3]
Urinary N ($mg \cdot kg^{-1} \cdot d^{-1}$)	144.0 ± 19.7	168.6 ± 34.9	136.7 ± 21.0[2]	137.4 ± 23.1	96.3 ± 22.5[3]

[1] $\bar{x}$ ± SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RQ, respiratory quotient.
[2,4] Significantly different from group 1 (ANOVA): [2] $P < 0.01$, [4] $P < 0.001$.
[3] Significantly different from group 3, $P < 0.001$ (ANOVA).

mated by linear regression of the rate of protein oxidation against the rates of carbohydrate and fat oxidation.

RESULTS

Gaseous metabolism was measured in 62 of the 68 infants randomly assigned. Two infants were withdrawn from the protocol because they developed a patent ductus arteriosus requiring fluid restriction (one infant each from groups 1 and 4) and 2 were withdrawn after developing necrotizing enterocolitis (one infant each from groups 2 and 3). One infant's formula (from group 1) was inadvertently changed before the infant completed the protocol, and another infant (from group 4) completed other aspects of the study but was not measured for gaseous metabolism. All of the formulas were well tolerated by the infants. Characteristics of the study groups were not significantly different (**Table 2**).

Data that summarize the bioenergetics of the study populations are shown in **Table 3**. As anticipated, the mean energy balances (energy stored) of infants in groups 3 and 4, ie, the high energy intake groups (648 $kJ \cdot kg^{-1} \cdot d^{-1}$), were greater than the energy balances of infants in groups 1 and 2, the lower energy intake groups (544 $kJ \cdot kg^{-1} \cdot d^{-1}$). Despite the higher rate of energy expenditure, the infants in group 4 who received 65% of their nonprotein energy as carbohydrate retained more energy than did the infants in group 3 who received 65% of their nonprotein energy intake as fat. At the lower energy intake there was no difference in energy expenditure between the groups. However, the infants on the high carbohydrate diet had a higher energy balance than those on the high fat formula. The groups consuming high intakes of fat (groups 1 and 3) lost more energy via excreta than did the infants with high intakes of carbohydrate (groups 2 and 4) and the infants in the control group.

As anticipated, the mean $\dot{V}CO_2$ and the RQ were significantly higher in infants consuming high intakes of carbohydrate at both gross energy intakes (**Table 4**). There was no significant difference in $\dot{V}O_2$ between the groups. Mean urinary nitrogen excretion of infants in groups consuming high amounts of carbohydrate was significantly less than that of infants consuming the high-fat formulas.

The results of the substrate oxidation calculations are shown in **Table 5**. As expected, carbohydrate oxidation increased with carbohydrate intake. Thus, groups receiving 65% of the nonprotein energy as carbohydrate, ie, groups 2 and 4, oxidized carbohydrate at greater rates than did the groups receiving only 35% of nonprotein energy as carbohydrate (groups 1 and 3). Likewise, fat oxidation varied inversely with carbohydrate intake ($r = 0.46$, $P < 0.001$). Consistent with the hypothesis, protein oxidation at both gross energy intakes was less in groups receiving 65% of nonprotein energy as carbohydrate. The protein oxidation of the control group at an energy intake of 544 $kJ \cdot kg^{-1} \cdot d^{-1}$ was intermediate.

The regression relations of carbohydrate intake with both carbohydrate and fat oxidation are shown in **Figure 1**. As carbohydrate intake increased, carbohydrate oxidation increased ($r = 0.71$, $P < 0.0001$) and fat oxidation decreased. In this study, by design, carbohydrate and fat intake were closely related. Carbohydrate intake was a superior predictor of carbohydrate and fat oxidation. Inclusion of fat intake in the regression did not improve the regression relation. As noted above, at nonprotein RQs >1, carbohydrate oxidation included true carbohydrate oxidation and carbohydrate used in fat synthesis.

The correlations between protein oxidation and both carbohydrate and fat oxidation are shown in **Figure 2**. Protein oxidation was inversely correlated with carbohydrate oxidation but was not correlated with fat oxidation.

DISCUSSION

Measurements of metabolic gas exchange and 72-h urinary nitrogen excretion obtained prospectively in a double-blind, ran-

TABLE 5
Macronutrient oxidation rates of the study groups[1]

Variable	Control group (n = 14)	Group 1 (n = 11)	Group 2 (n = 12)	Group 3 (n = 14)	Group 4 (n = 11)
			$g \cdot kg^{-1} \cdot d^{-1}$		
Carbohydrate oxidation	12.2 ± 2.71	9.0 ± 2.16	14.0 ± 2.44[2]	10.8 ± 2.36	16.5 ± 1.61[3]
Fat oxidation	1.14 ± 1.32	2.52 ± 1.06	0.29 ± 1.36[2]	2.11 ± 1.06	−0.87 ± 1.61[3]
Protein oxidation	0.90 ± 0.12	1.05 ± 0.22	0.85 ± 0.13[4]	0.86 ± 0.14	0.61 ± 0.15[3]

[1] $\bar{x}$ ± SD.
[2,4] Significantly different from group 1 (ANOVA): [2] $P < 0.001$, [4] $P < 0.01$.
[3] Significantly different from group 3, $P < 0.001$ (ANOVA).
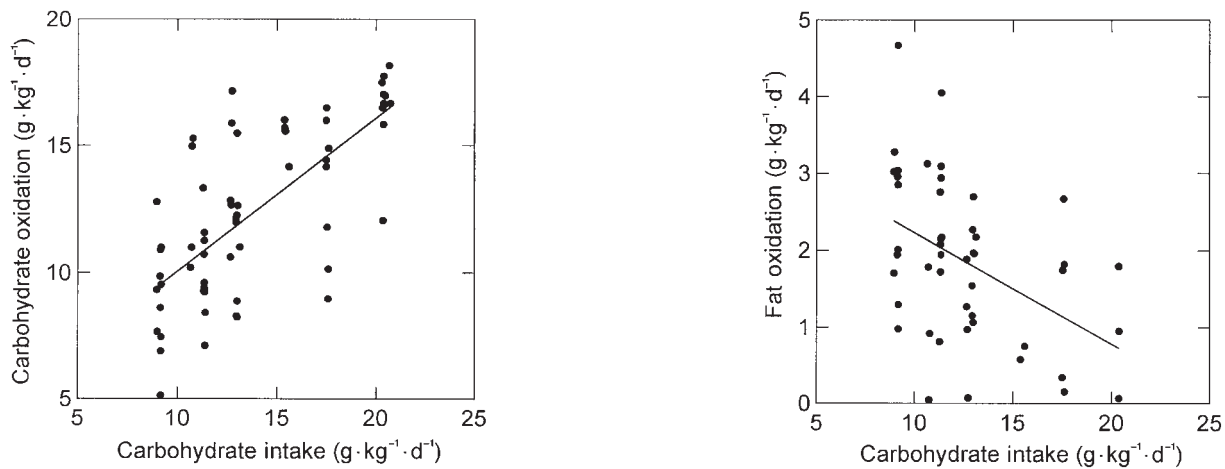
FIGURE 1. Regression relation of carbohydrate oxidation ($n = 62$; $y = 4.064 + 0.601x$; $r = 0.71$; $P < 0.0001$) and fat oxidation ($n = 46$; $y = 3.676 -0.145x$; $r = -0.46$; $P < 0.001$) with carbohydrate intake. At a respiratory quotient >1, the measured carbohydrate oxidation includes true carbohydrate oxidation and carbohydrate used in fat synthesis (negative fat synthesis).

domized study of healthy, growing LBW infants were used to estimate the rates of carbohydrate, fat, and protein oxidation. The results indicate that the rate of protein oxidation is less when 65% of nonprotein energy is supplied as carbohydrate and 35% is supplied as fat than when the proportions are reversed. This was true at gross energy intakes of 544 and 648 kJ·kg⁻¹·d⁻¹. In the infants studied, carbohydrate intake was noted to have a significant, positive relation with carbohydrate oxidation and a negative relation with fat oxidation. Protein oxidation decreased significantly as carbohydrate oxidation increased, but was not related to fat oxidation. Considered together, these data support the hypothesis that, in healthy, enterally fed LBW infants, carbohydrate is more effective than is fat in reducing the oxidation of protein.

In the present study, the higher metabolizable energy intake in the high-carbohydrate diet groups confounds the group comparison for the specific effect of quality of energy on protein oxidation. There was too little variation in metabolizable energy to permit within group analysis between metabolizable energy and

protein oxidation. As expected, metabolizable energy intake of the infants correlated significantly with protein oxidation ($r = 0.53$, $P < 0.0001$). However, the effect of carbohydrate intake on protein oxidation was apparent with or without metabolizable energy as a covariate in the analysis of variance.

This decrease in protein oxidation (ie, increase in protein stores) with increasing carbohydrate intake at a constant protein intake is consistent with reports in adults of enhanced protein retention when carbohydrate is the primary energy substrate in both enteral (7, 8) and parenteral diets (9). Although greater nitrogen retention with increasing energy intake was reported in enterally fed (3, 27) and parenterally fed (28) LBW infants, knowledge regarding possible differential effects of carbohydrate compared with fat (quality of nonprotein energy) on nitrogen retention in enterally fed LBW infants has been lacking. Published reports of nonprotein energy effects on nitrogen retention are limited to parenterally fed infants, and results have been inconsistent. Investigators reported increased (10), unchanged
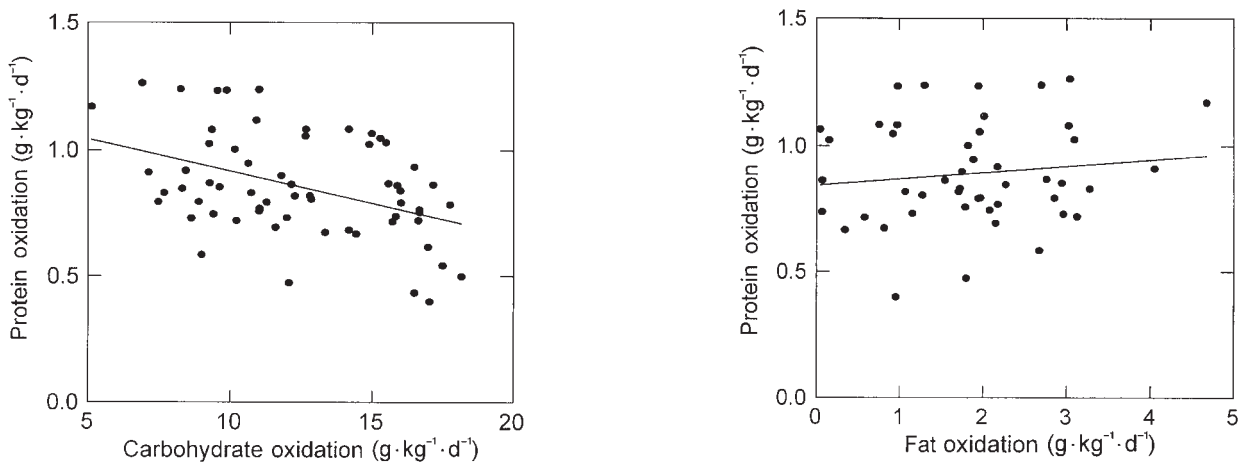
FIGURE 2. Correlation of protein oxidation with carbohydrate and fat oxidation. There was a significant negative correlation between protein and carbohydrate oxidation ($n = 62$; $r = -0.42$; $P < 0.001$), whereas the correlation between protein and fat oxidation was not significant ($n = 46$; $P = 0.39$). Sixteen patients with a respiratory quotient ≥1 were not included when plotting protein against fat oxidation because there was no occurrence of net fat oxidation.

(13, 29, 30), and decreased (12) nitrogen retention with high carbohydrate intakes. A study investigating the administration of a high-fat formula to premature infants with bronchopulmonary dysplasia reported no difference in nitrogen retention of infants receiving a high-fat compared with a high-carbohydrate diet (31). In contrast, we observed higher rates of nitrogen retention (444.1 ± 22.1 compared with 420.2 ± 23.7 mg·kg^{-1}·d^{-1} in groups 2 and 1, respectively, and 459.2 ± 20.4 compared with 438.7 ± 26.3 mg·kg^{-1}·d^{-1} in groups 4 and 3, respectively) and weight gain (23.2 ± 2.9 compared with 20.2 ± 1.8 g·kg^{-1}·d^{-1} in groups 2 and 1, respectively, and 24.9 ± 2.4 compared with 20.7 ± 2.3 g·kg^{-1}·d^{-1} in groups 4 and 3, respectively) in infants fed the high-carbohydrate diets at both gross energy intakes (32). However, infants fed the high-energy, high-carbohydrate diet had increased fat deposition as evidenced by the significantly greater increase in skinfold thickness (1.70 ± 0.47 compared with 0.93 ± 0.38 mm/wk in groups 4 and 3, respectively).

The direct correlation of increasing carbohydrate oxidation with increasing carbohydrate intake observed in the present study of enterally fed LBW infants has also been observed in enterally fed adults (15, 33) and parenterally fed infants (12, 13, 34). The diminished fat oxidation with increasing carbohydrate intake and the enhanced fat oxidation with increasing fat intake observed in this study was also noted in some studies (12, 14) but not in others (35, 36).

The conclusions of the present study must be interpreted in light of the study population, experimental design, experimental methodology, and assumptions made in estimating substrate oxidation rates from metabolic gas exchange and urinary nitrogen excretion. The study subjects were healthy, growing LBW infants who were receiving full enteral intakes. The dietary intervention was meticulously controlled, and the experimental protocol spanned a substantial period of time (study duration was a mean of 25 ± 9 d). We carefully validated the indirect calorimeter and defined the error in the use of 6-h studies to predict total daily gas exchange (16, 37, 38). Multiple serial measurements were obtained in most of these infants to further reduce the sampling error associated with these measurements. Among the limitations of the study design was that the diets were designed with a fixed inverse relation between carbohydrate and fat intake; thus, the carbohydrate and fat intakes were not truly independent of each other.

Although thought to be both theoretically and empirically sound, the interpretation of substrate oxidation values as measured by indirect calorimetry is sensitive to both methodologic errors and erroneous assumptions. The assumptions underlying the classic technique for calculating macronutrient oxidation from gas exchange are thought to be most secure when applied to long-term net oxidation rates (21). However, these assumptions may not hold when experimental conditions are not rigidly controlled. For example, when the sampling period is brief, the effects of potentially confounding variables, such as variations in the quality of the diet, physical activity of the infant, and changes in the physical environment (eg, temperature and humidity), need to be adequately controlled. $\dot{V}CO_2$, $\dot{V}O_2$, and RQ all vary significantly depending on the time elapsed after feeding, the amount of food, and, as shown here, the composition of the food (37, 39). Thus, estimates of oxidation rates made from short periods of measurement without control of the aforementioned variables could be inaccurate. As we and others have shown, inclusion of 2 full feeding periods can eliminate most of the variability related to feeding, physical activity, and sleep state (38, 40).

This randomized, prospective investigation of the effects of carbohydrate compared with fat, when fed at 2 isonitrogenous gross energy intakes, on the substrate oxidation of enterally fed LBW infants showed a significant positive relation between carbohydrate intake and carbohydrate oxidation and an inverse relation between fat oxidation and carbohydrate intake. There was less protein oxidation at both gross energy intakes in groups receiving 65% of nonprotein energy as carbohydrate. Within the limitations of the theory and the methodology, these data support the hypothesis that carbohydrate is more effective than is fat in sparing protein oxidation in enterally fed LBW infants. However, because of the increased metabolic load of carbon dioxide and the lack of evidence for the safety of carbohydrate-enriched dietary intakes in LBW infants, particularly regarding the enhanced release of insulin when such diets are given over prolonged periods of time, recommendations for specific enrichment of preterm infant formulas with carbohydrate cannot be made without further evaluation.

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