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Total Parenteral Nutrition in the Newborn: Energy Substrates and Plasma Total Fatty Acids

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ABSTRACT. Carbohydrate and lipid intakes have both been found to modulate the metabolism of long-chain fatty acids. To define the respective infuence of these two energy substrates on plasma fatty acid concentrations, 32 studies were performed in 16 parenterally fed newborn infants (mean ± SEM, birth wt: 2.15 ± 0.1 kg, age: 10 ± 1 d). In a paired cross-over design, the infants received for a given level of energy (60 *versus* 80 kcal/kg/d) two 6-d isonitrogenous and isocaloric regimens constructed so that the level of fat intake, 1 or 3 g/kg/d varied inversely with that of glucose. Total plasma fatty acid levels did not reflect the composition of the emulsion and varied with energy substrates. Plasma levels of three fatty acids rose inversely to the lipid intake, during the high glucose regimen: 16:1w7, 20:3w9 biologic markers of essential fatty acid deficiency, and 20:3w6 a derivative of 18:2w6. Glucose intake could exert its influence on 20:3w9 and 20:3w6 via insulin, an activator of Δ6 desaturase. Both glucose and fat should be taken into account when evaluating plasma fatty acid profile. (*Pediatr Res* 26:290–293, 1989)

Abbreviations

EFA, essential fatty acid
EFAD, essential fatty acid deficiency
HF, high fat regimen
LF, low fat regimen
LIP, lipid emulsion
NEFA, non esterified fatty acid
PUFA, polyunsaturated fatty acid
TFA, total fatty acid
TG, triglyceride

Premature newborn infants have relatively low fat stores (1). Within a few days on a fat free diet, they can develop EFAD (2). Furthermore, they face a relative inability to handle intravenous fat intake due to their enzymatic immaturity, notably in lipoprotein lipase and lecithin cholesterol-acyl-transferase (3). To support a rapid growth rate, they require high energy regimens consisting of balanced glucose and fat intakes (4). Brain growth is a metabolic priority for the newborn (5), which depends on an adequate supply of nutrients for energy and structural purposes.

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Dietary fatty acids can be converted into long-chain polyunsaturated derivatives and incorporated in structural lipids (6). Modulators of long-chain fatty acids metabolism (7), as well as the low activity of the neonatal desaturase system (8, 9), could affect the metabolism of infused fatty acids during parenteral nutrition. Many factors have been reported to influence this enzyme complex. For example, EFAD, protein intake, and insulin activate the system, whereas the intake of either EFA or carbohydrates, as well as fasting and epinephrine, act as inhibitors (7).

In the course of an ongoing study aimed at defining the optimal proportion of glucose and fat in parenterally fed newborn infants, the effect of different glucose/fat ratios on plasma fatty acids was evaluated.

MATERIALS AND METHODS

The study population, experimental design and intravenous regimens, have been previously described (10). Sixteen appropriate for gestational age newborn infants (mean ± SEM, birth wt: 2150 ± 115 g, age: 10 ± 1 d), were studied in two Latin square cross-over designs under regimens providing either 60 or 80 kcal/kg/d (Fig. 1). Indications for intravenous nutritional support were as follows: necrotizing enterocolitis ($n = 7$), gastroschisis ($n = 4$), esophageal atresia ($n = 2$), duodenal atresia ($n = 2$), intolerance for oral feedings ($n = 1$). In a cross-over fashion, the infants were studied for a given energy intake, over two 6-d isonitrogenous regimens (450 mg/kg/d of nitrogen) differing by the level of fat intake: LF (1 g/kg/d) and HF (3 g/kg/d). These specific fat intakes were chosen to cover EFA requirements and to avoid hypertriglyceridemia. The infused glucose varied inversely with the lipid intake in order to keep the regimens isocaloric (Table 1). To control for the effect of sequence (starting the protocol with LF or HF), half of the patients began with LF (Fig. 1). In this experimental design unpaired comparisons allowed for the evaluation of the respective influence of glucose and fat. The constancy of the infusion rates was assured by an infusion pump for the amino acid-dextrose solution (Travasol 10% blend C, Travenol Laboratories Inc., Deerfield, IL) and a syringe pump for the lipid emulsion (Nutralipid 10%, Pharmacia, Dorval, Quebec, Canada). Blood sampling was done on the 5th day of each period, at steady state (continuous infusion) distal to the infusion site (11). NEFA and TFA were determined on capillary column by gas chromatography following specific methylation (12) and direct transesterification (13), respectively, on 100 μL of plasma. Plasma fat emulsion and TG levels were determined as described previously (10). Approval by the Hospital Human Ethics Committee and parental consent were obtained before the investigation.

Population size different than eight was due to nonavailability of plasma. In one patient, 20:5w3 was not detectable at 60 kcal/kg/d, for analytical purposes. The data were evaluated by

ANOVA for repeated and independent measures. In the case of heterogeneity of variances a t test on the mean of the differences was performed for paired data and a Welch t test (14) for unpaired results. The level of significance was set at 0.05.

RESULTS

The macronutrient intakes are presented in Table 1. The fatty acid composition of the fat emulsion is shown in Table 2. The metabolic response to lipid infusions was monitored by plasma level of the fat emulsion, TG (10), and fatty acids; values were within normal ranges (15, 16). Despite the tight linear correlation between the plasma levels of fat emulsion (LIP, g/L) and TG (mmol/L) (TG = 0.85 LIP + 0.05, $r = 0.86$, $p < 0.001$, $n = 19$) the dispersion was important (SE: 0.27 mmol/L) confirming that TG could not be reliably predicted from nephelometry (17). However, with plasma fat emulsion levels <1.5 g/L, TG values were lower than the suggested upper limit of 1.7 mmol/L (18). NEFA (LF: 776 ± 65 *versus* HF: 1584 ± 145 μmol/L, $n = 16$) and TFA (LF: 7025 ± 404 *versus* HF: 13137 ± 571 μmol/L, $n = 13$) rose significantly with intake.

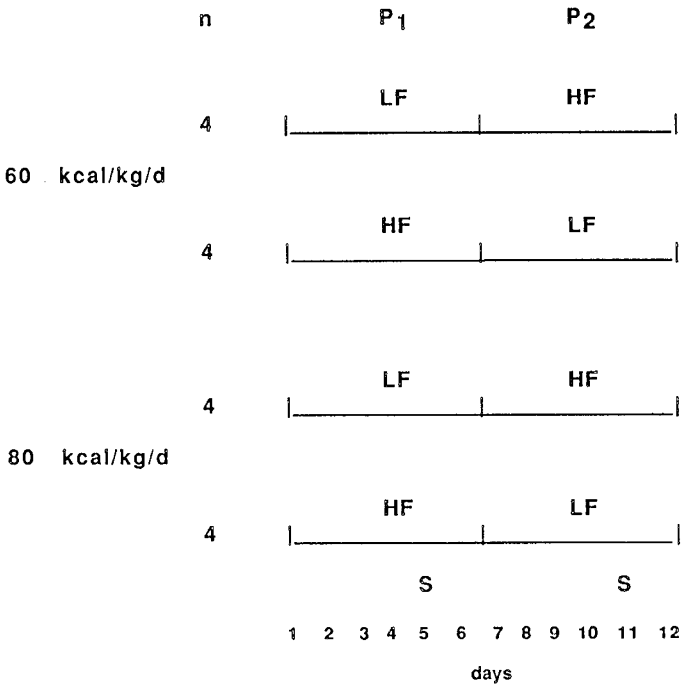

Fig. 1. The experimental design was a Latin square cross-over. For a given level of energy (60 or 80 kcal/kg/d) the infants served as their own control for two 6-d periods (P_1 and P_2) of isonitrogenous infusions differing only by the source of calories: HF and LF. To allow for the evaluation of the sequence of administration, half of the patients started with the high fat regimen. Blood sampling (S) was performed on the 5th d of each period.

Plasma levels of individual fatty acids are reported as absolute values (μmol/L) as well as percent of total fatty acids (Table 3), which could lead to wide differences in data interpretation. For instance the significant drop in percent of 20:4w6 during HF regimens is in keeping with previously published data expressed in percent of TFA (19), but is in contradiction with absolute values expressed in μmol/L; the same holds true for 24:1w9 at 60 kcal/kg/d. We considered absolute values as they reflect more closely the metabolic activities than percentages; levels expressed in percent are also more sensitive to the dilutional effect of the lipid infusion.

Plasma levels of infused fatty acids rose significantly with the increased lipid intake of the HF regimens (Table 3). Conversely, three derivatives of infused fatty acids in the emulsion, namely 16:1w7, 20:3w9, 20:3w6, rose with the LF regimens (Table 3). For a given level of fat intake increasing the glucose intake in an unpaired fashion (HF 60 *versus* HF 80 kcal/kg/d) had a significant effect on 20:3w9. But for a given glucose intake of 11 g/kg/d, increasing the fat intake from LF 60 kcal/kg/d to HF 80 kcal/kg/d produced a significant drop in plasma levels of 16:1w7 (Table 3).

The Mead acid/arachidonic acid (20:3w9/20:4w6) ratio >0.025 has been used to biochemically characterize EFAD (20). This ratio decreased significantly with the HF regimens (60 kcal/kg/d, LF: 0.012 ± 0.001 *versus* HF: 0.007 ± 0.004, $n = 6$, $p < 0.05$; 80 kcal/kg/d, LF: 0.036 ± 0.008 *versus* HF: 0.015 ± 0.002, $n = 7$, $p < 0.05$). But with a constant LF intake, the Mead acid/arachidonic acid ratio increased significantly at the higher energy level; the difference in energy intake being achieved by an increased glucose intake. There was no effect of glucose intake on plasma TG, NEFA, TFA, and w6 and w3 PUFA concentrations.

DISCUSSION

Parenterally fed newborn infants exhibited a good tolerance to fat intakes, varying from 1 to 3 g/kg/d, as circulating lipid levels were within normal ranges. While on continuous infusion, the plasma TFA did not reflect the composition of the emulsion. Changes in intravenous diet resulted in TFA patterns not proportionally related to the dose of fatty acids infused.

This discrepancy between the rate of infusion and the plasma levels was most striking for 16:1w7, 20:3w9, and 20:3w6, three derivatives that rose during the LF regimens (Table 3). Elevated

Table 1. *Parenteral intakes* of paired isocaloric and isonitrogenous regimens differing by source of nonprotein energy (LF, HF)*†

	60 kcal/kg/d ($n = 8$)		80 kcal/kg/d ($n = 8$)	
	LF	HF	LF	HF
Energy (kcal/kg/d)	59 ± 1	59 ± 1	75 ± 1	79 ± 1
Amino acids (g/kg/d)	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
Glucose (g/kg/d)	10.9 ± 0.3	5.4 ± 0.2	15.6 ± 0.2	11.1 ± 0.3
% of nonprotein energy as glucose	76 ± 1	38 ± 1	83 ± 1	54 ± 1
Lipid (g/kg/d)	1.04 ± 0.02	2.75 ± 0.08	1.02 ± 0.05	2.81 ± 0.05
% of nonprotein energy as lipid	24 ± 1	62 ± 1	17 ± 1	46 ± 1

* Values represent mean ± SEM.

† For the sake of clarity Table 1 does not show the cross-over between LF and HF, in which half of the studies started by HF.

Table 2. *Fatty acid composition of parenteral emulsion, nutralipid 10%, expressed as absolute value, and as % of total fatty acids (mean ± SEM)*

Fatty acids	μmol/dL	% of TFA
16:0	4489	12
18:0	1621	4.33
16:1w7	50	0.13
18:1w7	473	1.26
18:1w9	6569	17.55
18:2w6	19710	52.67
18:3w6	133	0.36
20:3w6	174	0.47
20:4w6	116	0.31
18:3w3	3557	9.51
22:6w3	416	1.1
Others	<80	<0.25
TFA	37419	

Table 3. *Plasma fatty acid concentration (mean ± SEM) expressed as absolute value (μmol/L) and as % of total fatty acid (%)*

	60 kcal		80 kcal	
	LF*	HF	LF	HF
16:0	2163 ± 175 (28.42 ± 0.52)	3919 ± 126† (27.43 ± 0.68)	1819 ± 141 (27.53 ± 0.88)	3261 ± 268† (26.59 ± 0.39)
18:0	676 ± 53 (8.9 ± 0.32)	1379 ± 49† (9.67 ± 0.35)	608 ± 53 (9.32 ± 0.22)	1207 ± 114† (9.8 ± 0.26)
16:1w7	218 ± 29 (2.83 ± 0.25)	141 ± 12† (0.99 ± 0.08)	177 ± 22 (2.70 ± 0.26)	111 ± 11† (0.91 ± 0.06)†
18:1w9	1653 ± 118 (21.95 ± 1.03)	3202 ± 33† (22.44 ± 0.13)	1338 ± 134 (20.32 ± 0.63)	2654 ± 278† (21.4 ± 0.60)
20:3w9	6.3 ± 1.8 (0.08 ± 0.02)	3.9 ± 0.4 (0.03 ± 0.003)†	16.1 ± 5.1 (0.26 ± 0.08)	8.8 ± 1.8† (0.07 ± 0.02)
24:1w9	54.2 ± 7.1 (0.70 ± 0.06)	72.4 ± 4.0† (0.51 ± 0.03)†	61.4 ± 5.7 (0.96 ± 0.09)	81.5 ± 6.4† (0.67 ± 0.04)
18:2w6	1772 ± 85 (23.64 ± 0.86)	3991 ± 145† (27.96 ± 0.92)†	1443 ± 176 (21.6 ± 1.13)	3285 ± 268† (26.76 ± 0.82)†
20:3w6	98.9 ± 12.0 (1.30 ± 0.12)	69.3 ± 3.2† (0.49 ± 0.03)†	107 ± 11.7 (1.66 ± 0.12)	84.8 ± 8.2† (0.70 ± 0.07)†
20:4w6	470 ± 79 (5.97 ± 0.70)	573 ± 51 (4.03 ± 0.37)†	407 ± 40 (6.37 ± 0.57)	539 ± 34 (4.49 ± 0.32)†
18:3w3	101 ± 8.8 (1.33 ± 0.05)	283 ± 25† (1.99 ± 0.17)†	82 ± 15 (1.21 ± 0.14)	230 ± 30† (1.85 ± 0.18)†
20:5w3	21.7 ± 5.7 (0.28 ± 0.07)	25.8 ± 3.3 (0.18 ± 0.02)	30.3 ± 3.8 (0.48 ± 0.07)	34.8 ± 3.6 (0.3 ± 0.04)†
22:6w3	177 ± 13 (2.34 ± 0.06)	406 ± 19† (2.84 ± 0.12)†	172 ± 12 (2.67 ± 0.12)	398 ± 24† (3.31 ± 0.19)†
TFA	7591 ± 552	14273 ± 195†	6536 ± 553	12259 ± 952†

* Half of the studies started by HF.

† $p < 0.05$ for comparison between LF and HF at a given level of energy intake. At 60 kcal, $n = 6$ except for 20:5w3 ($n = 5$); at 80 kcal, $n = 7$.

levels of 16:1w7 and 20:3w9 have been proposed as biologic markers of EFAD (20). It is surprising that despite the continuous lipid infusion these markers would be found in plasma after only 9 to 21 d on a low lipid intake [age at study (10) plus 5 days on LF regimens].

The periods of lipid infusion (LF, HF) of our study were relatively short compared to membrane phospholipid fatty acid turnover (21). However, Farrell *et al.* (22) recently suggested that, in the premature infant, tissue linoleate responded as early as 7 d to exogenous fat intake. Although plasma phospholipids or cholesterol esters might have been a better indicator of the effect of diet on endogenous fat metabolism (23), others (24) found that lipid fractionation does not provide additional information to justify the extra step. This procedure requires more blood and is also time consuming. Furthermore, Lepage *et al.* (25) found that the plasma total fatty acid pattern was representative of phospholipid red blood cells and platelets fatty acid profiles in EFA deficient children with cystic fibrosis.

Monitoring of plasma lipids is most frequently performed after interruption of the infusion because of the concern that infused TG might change plasma ratios of endogenous and exogenous fatty acids. Therefore plasma levels of fatty acids infused in large amounts such as 18:2w6, cannot be interpreted. However, even in hyperlipemic patients, the total plasma fatty acid pattern was shown to provide a reliable assessment of EFA status (26). In our study, despite the continuous lipid intake, 20:3w9, a noninfused fatty acid, increased during the LF regimen. As in the study from Farrell *et al.* (22), premature infants on a fat free or low fat diet rapidly showed elevated plasma levels of 20:3w9 accompanied by normal levels of 20:4w6. This fatty acid profile seems to be characteristic for this population early after birth. The provision of an adequate linoleic acid intake promptly corrected the deficient state in plasma and tissue (22), as in newborn infants tissue response to changes in dietary fat profile is very rapid compared to adults (27). It remains to be demonstrated whether these changes in specific plasma fatty acids are an early indication of EFAD.

Provision of linoleic acid representing 4% of total energy intake has been recommended to cover essential fatty acid requirements in the newborn (28, 29). More recently Farrell *et al.* (22) recommended even larger amounts of linoleic acid (1.19 g/kg/d) for premature infants. The present protocol provided a minimum of 1 g/kg/d of soy bean oil emulsion, or 8 to 10% of energy as 18:2w6. Mead acid/arachidonic acid (20:3w9/20:4w6) ratio is a widely used index of EFAD. The highest ratios were found during the low fat intakes. Noteworthy is that with the LF 80 kcal regimen this ratio was in a range associated by Siguel *et al.* (2) with EFAD. For a given low fat intake, the ratio was significantly greater at LF 80 than at LF 60 kcal/kg/d, suggesting an influence of glucose or insulin. However, the interpretation of this ratio can be misleading; only the variations in the numerator 20:3w9 were responsible for these results because the arachidonic acid levels remained normal, as reported by others (22).

Among the dietary, environmental, and hormonal factors known to influence long-chain fatty acid metabolism glucose has been reported to exert an inhibitory effect, whereas insulin is an activator of the Δ6 desaturase (30). Despite the wide range of glucose intakes (5 to 17 g/kg/d) the infants remained remarkably normoglycemic, but insulinemia rose significantly (8.6 ± 1.0 to 14.5 ± 1.5 μU/mL) when changing from the 60 to 80 kcal/kg/d energy intakes (10). The increase in circulating insulin levels could explain the statistically significant influence of glucose intake on the levels of 20:3w9. The insulin-induced increase in Δ6D activity could also account for the trend toward higher plasma levels of 20:3w6 and 20:5w3 found at 80 kcal/kg/d despite constant lipid intakes (Table 3). In contrast to studies measuring the effect of glucose during refeeding of fasted rats (31) or after a glucose-rich diet (32), our observations were made in patients receiving carbohydrates at commonly used levels of intake.

These results show that plasma total fatty acids are influenced by variations in both glucose and lipid intakes. With as little as 100 μL of plasma, changes in uninfused PUFA can be detected as early as 5 d after switching energy substrates. In a study designed more specifically to document EFAD, it remains to be verified if circulating levels of 16:1w7 and 20:3w9 are early plasma indicators of later tissue deficiencies.

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