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Metabolic effects in neonates receiving intravenous medium-chain triglycerides

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Angsten G, Boberg M, Cederblad G, Meurling S, Stiernström H. Metabolic effects in neonates receiving intravenous medium-chain triglycerides. Acta Pædiatr 2002; 91: 188–197. Stockholm. ISSN 0803-5253

The effects of two lipid emulsions, one with 50% each of medium-chain and long-chain triglycerides, and a long-chain triglycerides lipid emulsion as a control, were evaluated for lipid and carnitine metabolism and respiratory quotient when given to neonates after major surgery during a short period of total parenteral nutrition. Each group included 10 neonates, and all tolerated the total parenteral nutrition well. The relative contents of linoleic acid and α-linolenic acid increased in all lipid esters in plasma and adipose tissue in both groups, indicating that the content of these fatty acids is sufficient even in the medium-chain triglycerides emulsion. The serum concentration of ketones was within normal limits. Free fatty acids in plasma did not increase in either group. The total plasma carnitine concentration decreased in both groups but the distribution of free carnitine and acylcarnitine did not change. The total muscle carnitine did not change significantly but the ratio of acylcarnitine to free carnitine tended to increase in muscle in the treatment group, probably an effect of the medium-chain triglyceride supplementation.

Conclusions: The two groups displayed the same fatty acid pattern in plasma and adipose tissue and the same respiratory quotient during the treatment period. Regarding carnitine status, essentially the same changes were seen in the two groups. However, discrete changes were seen in muscle tissue in the treatment group.

Key words: *Medium-chain triglycerides, muscle and plasma carnitine, neonates, respiratory quotient*

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Total parenteral nutrition (TPN) has been a major advance in the treatment of seriously ill neonates, for example those undergoing oesophago-gastrointestinal surgery. Lipid emulsion is one of the most useful constituents of TPN, as it is a source of energy, provides essential fatty acids, acts as a carrier of fat-soluble vitamins, and has a low osmotic value and a nitrogen-sparing effect. A number of commercial lipid emulsions contain the two long-chain polyunsaturated fatty acids linoleic acid (LA,18:2n-6) and α-linolenic acid (ALA,18:3n-3), which are essential in supporting cell growth and function (1). Their metabolites arachidonic acid (AA,20:4n-6), dihomo-γ-linolenic acid (DHLA, 20:3n-6), eicosapentaenoic acid (EPA,20:5n-3) and docosahexaenoic acid (DHA,22:6n-3) are also important, AA, EPA and DHLA as eicosanoid precursors (2) and DHA for the development of the retina and brain (3).

Medium-chain triglycerides (MCT) are in general considered to be more readily metabolized to energy than long-chain triglycerides (LCT), since MCT are hydrolysed more rapidly into medium-chain fatty acids (MCFA) on account of the greater bioavailability of the lipolytic enzymes from the capillary endothelium (2, 4). MCFA, which are more soluble in plasma, are directly transported to the liver bound loosely to the albumin molecule (4), and do not first associate with chylomicrons or VLDL particles as do the less plasma-soluble long-chain fatty acids (LCFA). The amount of MCFA deposited in the liver and adipose tissue is small (5). Although the intracellular transport of MCFA from the cytoplasm into the mitochondrion in the liver cell for β-oxidation does not need carnitine, recent investigations have shown that these fatty acids are metabolized in several tissues by carnitine-dependent mechanisms (5). MCFA are metabolized in the liver either to energy and CO_2 or to ketones, which in high concentrations will be toxic, causing acidosis. Metabolism of MCT usually leads to an elevation of the concentration of free fatty acids (FFA) in plasma, and despite the loose association of MCFA with albumin, they are known to displace bilirubin competitively from the albumin molecule, and

under certain conditions cause an increase of unbound bilirubin (4).

The main function of carnitine, 3-hydroxy-4-trimethyl-ammonium butyrate, is to serve as a carrier substance, facilitating the transport of LCFA from the cytoplasm across the inner mitochondrial membrane into the mitochondrion, where they are β-oxidized. Moreover, the formation of acylcarnitine and its efflux from the mitochondrion provides a sink for the acyl moieties when acyl-CoA formation exceeds the rate of CoA recycling inside the mitochondrion. Carnitine may also facilitate the transport of these excess acyl groups from the liver to other tissues for utilization (6).

The aim of this study was to determine whether MCTs are more efficient in producing energy than LCTs without being toxic to the patient. During a short period of TPN to neonates after surgery, we compared the nutritional properties of the lipid emulsions Vasolipid®, containing 50% MCT and 50% LCT, with those of Intralipid®, containing only LCT, and studied the effects of these emulsions on the fatty acid compositions in the plasma and adipose tissue and on the carnitine concentrations in the plasma and muscle. The tolerance to the emulsions and their influence on the energy metabolism were also examined.

Patients and methods

Patients

Neonates needing surgery for various oesophago-gastrointestinal malformations were assigned before surgery, in a double-blind, randomized manner, to receive TPN with either Vasolipid® (treatment group) or Intralipid® (control group). A total of 20 neonates entered the study, 10 in each group. The inclusion criteria were neonates of both sexes with a gestational age (GA) of 36–41 wk at birth, a birthweight of 2.5–4.0 kg, and a postnatal age of up to 4 d, needing surgical treatment and requiring TPN for a minimum of 5 d. The exclusion criteria were: metabolic disease, renal disease, inflammatory disease, septic syndrome or a haematological disorder. The neonates should not be receiving treatment with catecholamines or have any known malignancy. Furthermore, they should not be participating in any other study. The primary diagnoses in the treatment group were: oesophageal atresia, $n = 3$; oesophageal atresia and anal atresia, $n = 1$; gastroschisis, $n = 3$; omphalocele, $n = 1$; duodenal obstruction, $n = 1$; small bowel atresia, $n = 1$. In the control group the diagnoses were: oesophageal atresia, $n = 4$; gastroschisis, $n = 2$; gastroschisis and small bowel obstruction, $n = 1$; omphalocele, $n = 1$; duodenal obstruction, $n = 1$; small bowel atresia, $n = 1$. Parents received verbal and written information about the study before it began and before they gave their consent. They were able to discontinue their participation at any time. The study was approved by the Ethics Committee, Faculty of Medicine, Uppsala University.

Methods

The heart rate, body temperature, body weight and clinical symptoms were recorded daily. Haematological (haemoglobin, leucocytes and platelets) and biochemical (aspartate aminotransferase, alanine aminotransferase, sodium, potassium, creatinine, alkaline phosphatase, albumin and bilirubin) parameters were measured on day 0, just before the first infusion of TPN started, and after termination of the study. These parameters were measured in the hospital laboratory using routine methods. The acid-base balances were analysed by the ABL™® apparatus system 625 (Radiometer Medical A/S, Copenhagen, Denmark). Triglycerides, cholesterol and glucose concentrations in serum were determined twice a day, just before the fat infusion started and after 7 h of TPN. On each occasion, 30 µL capillary blood was sampled into a heparin tube and the plasma was analysed on a Reflotron® (Roche Diagnostics AB, Bromma, Sweden).

TPN including fat (Intralipid® or Vasolipid®), amino acid (Vaminolac®), vitamins (Soluvit®, Vitalipid® Infant), trace elements (Peditrace®) and electrolytes (Addex®-Natriumklorid and Addex®-Kaliumklorid) was given daily for 18 h d^{-1}. Glucose (Glucose® , Pharmacia) was infused continuously, 24 h a day. The contents of the two fat emulsions are presented in Tables 1 and 2. The total amount of fat infused on the first day was 2 g kg^{-1} and this amount was increased at a daily rate of 0.5 g kg^{-1} d^{-1} until an infusion rate of 3 g $kg^{-1}d^{-1}$ was reached (Table 3).

Before the start of TPN, after 5 d of TPN and 5 h after the fat infusion ended, two blood samples (2.5 ml) were taken for measurement of free and total plasma carnitine concentrations, free fatty acids, fatty acid composition of plasma cholesterol esters, phospholipids, triglycerides, and ketones as β-hydroxybutyrate. The samples were drawn from a peripheral vein into vacutainer tubes. They were centrifuged at + 4°C once for plasma and twice for serum and were frozen at −72°C. A piece

Table 1. Contents of the two fat emulsions per 1000 mL.

	Intralipid®	Vasolipid®
Fractionated soybean oil (LCT)	200 g	100 g
MCT	–	100 g
Fractionated egg phospholipids	12 g	12 g
Glycerol	22 g	25 g
pH adjusted with sodium hydroxide	8	–
pH adjusted with sodium oleate	–	7.5
Final volume (water added)	1000 mL	1000 mL
kJ/1000 mL	8400	8100
Ratio of long-chain:medium-chai n fatty acids (mass)	100:0	50:50

Table 2. The relative concentrations of fatty acids in Intralipid® and Vasolipid®.

	Intralipid®		Vasolipid®	
Analysed by	Firm	Our laboratory	Firm	Our laboratory
Fatty acids %				
C 8:0	–	–	26	16.2
C 10:0	–	–	21	29.2
C 12:0	–	–	1.5	–
C 16:0	10.8	11.5	5	6.5
C 18:0	4.2	4.3	2	2.1
C 18:1n-9	22.2	23.8	12	11.7
C 18:2n-6	52.9	47.3	27	24.2
C 18:3n-3	6.9	7.8	4	3.1
Other	–	1.4*	1.5**	1***;

Other = * C 16:1n-7, 20:0, 20:4n-6 and 22:6n-3.
Other = ** C 6:0 and 20:4n-6.
Other = *** C 6:0, 12:0 and 20:4 n-6.

of adipose tissue weighing approximately 15 mg and a piece of muscle weighing approximately 30 mg were taken during surgery as an open biopsy with a scalpel, when the patients were only receiving glucose infusion, and after 5 d of TPN under local anaesthesia with a Biopty-cut® needle (Bard Biopty Biopsy Instrument, Stockholm, Sweden). The pieces were placed in a mixture of alcohol and dry ice. All samples were kept at −72°C until the analyses were performed, after completion of the study.

Plasma lipids were extracted into methanol-chloroform. The plasma lipid fractions of cholesterol esters, phospholipids and triglycerides were separated by thin layer chromatography. The adipose tissue biopsies were homogenized and extracted into methanol-chloroform. After transmethylation, the fatty acid methyl esters in the plasma lipid fractions and in adipose tissue were determined by capillary gas-liquid chromatography, as described (7, 8). Serum free fatty acids were measured by an enzymatic colorimetric method (Waco Chemicals, Neuss, Germany).

β-hydroxybutyrate was measured in the hospital laboratory using an enzymatic method. Aliquots of plasma for carnitine determinations were extracted with chloroform-methanol (3/2, by volume). The muscle specimens were freeze-dried and dissected to remove flakes of connective tissue, blood and fat. The powder was sonicated and aliquots were taken for analysis. Carnitine was assayed using an enzymatic radioisotopic method, before and after alkaline hydrolysis for determining free and total carnitine, respectively (9, 10). The acylcarnitine concentration was calculated as the difference between the total and the free carnitine concentrations. The carnitine content in the muscle tissue was referred to two reference bases, dry weight and non-collagen protein (NCP) (11).

To measure the respiratory quotient (RQ), a Deltatrac® apparatus with a paediatric mixing chamber (Datex Division Intrunetarium Corp., Helsinki, Finland) was used. The measurements were made at different time points before and during the treatment period. All RQ measurements were taken before oral feeding had begun. Urine samples were collected on the first and sixth days for assay of 3-methyl-histidine (3MH) as measures of muscle protein degradation. The samples were analysed with an Alpha Plus amino acid analyser (Pharmacia LKB Biochrome Ltd., Cambridge, United Kingdom), using the lithium buffer system and fluorometric assay, according to the manual.

Statistics

Changes in fatty acids, carnitine, RQ and 3MH within the groups were tested for significance by Wilcoxon's paired test and comparisons between the groups were made using the Mann-Whitney two-sample test. Spearman's rank correlation was used to quantify the correlation between two variables. In cases where values lay below the quantification limit, zero was used as an estimated value.

Results

The study comprised 20 neonates, 0–31 h old, with 10 neonates in the treatment group, mean gestational age of 38 ± 1 wk, mean birthweight 3341 ± 428 g, and 10 in the control group, mean gestational age 36 ± 1 wk, mean birthweight 3073 ± 588 g. The mean postnatal age at the start of the study was 17.4 ± 11.1 h in the

Table 3. TPN schedule.

Study day	Total non-protein energy kJkg^{-1}bw	Fat g kg^{-1}bw (ml)	Amino acids g kg^{-1}bw (ml)	Glucose 10% g kg^{-1}bw (ml)
1	202	2.0	0.95	7.0
		(10)	(15)	(70)
2	239	2.5	1.62	8.0
		(12.5)	(25)	(80)
3–6	294	3.0	2.27	10.0
		(15)	(35)	(100)

TPN schedule for vitamins and trace elements in ml and electrolytes in mmol given per kg body weight (bw)

Soluvit®N	Vitalipid® Infant	Peditrace®	Sodium	Potassium
1	1	1	2–3	2

Table 4. Fatty acid composition of plasma cholesterol esters before and after 5 d of TPN with the two fat emulsions.

	Control group, Intralipid®				Treatment group, Vasolipid®			
Fatty acid	Median Day 0	Min-max	Median Day 5	Min-max	Median Day 0	Min-max	Median Day 5	Min-max
8:0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0
10:0	0.4	0.2–1.0	0.2*	0.2–0.5	0.5	0.3–1.2	0.2**	0.1–0.3
16:0	21.7	16.8–23.2	13.3**	12.2–14.4	21.6	19.2–23.5	14.8**	13.0–16.8
16:1n-7	11.6	7.7–14.8	3.9**	2.2–5.8	10.9	7.3–12.9	4.6**	3.9–6.9
18:0	3.4	1.7–5.7	2.1**	1.3–2.7	3.5	2.6–16.7	2.5	1.2–4.8
18:1n-9	30.5	26.1–41.2	28.4	24.5–33.2	29.1	25.9–32.7	29.6	23.7–31.3
18:2n-6	18.0	12.7–27.9	40.7**	36.9–46.3	18.9	11.9–21.6	34.8**	30.4–40.1
18:3n-6	0.6	0.0–0.9	0.9*	0.6–1.3	0.7	0.5–1.7	0.9	0.7–1.7
18:3n-3	0.0	0.0–1.4	1.4**	1.1–2.3	0.0	0.0–0.5	1.0**	0.7–2.0
20:3n-6	0.9	0.0–1.2	0.5	0.5–0.7	1.2	0.0–1.4	0.8	0.6–0.9
20:4n-6	9.2	4.7–11.1	5.7**	3.6–7.4	10.5	7.4–14.3	7.8*	5.7–11.3
20:5n-3	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.7	0.7*#	0.4–1.3
22:6n-3	0.0	0.0–1.4	0.2	0.0–0.9	0.0	0.0–1.4	0.7	0.0–0.9
X	0.8	0.3–2.0	0.5***	0.2–0.8	1.2	0.5–2.0	0.4***	0.2–1.1

Results are given as a percentage of the sum of the fatty acids presented (median and range).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant change within the groups.
# $p < 0.05$ for difference between the groups.
X (12:0, 14:0, 22:4n-6, 22:5n-3) = sum of the remaining fatty acids analysed.

treatment group and 10.8 ± 8.3 h in the control group. Gestational age differed significantly between the groups ($p < 0.05$). No significant differences in the other variables were found between the two groups. All neonates in both groups tolerated the TPN well and thus the TPN schedule could be followed exactly. The haematological and biochemical values, values of acid-base balance and the serum concentration of β-hydroxybutyrate were all within normal limits. The unbound serum bilirubin did not increase, but in both groups some patients with physiological icterus needed treatment with UV radiation.

Heart rate, body temperature and respiratory rate showed no significant changes in either of the groups and no significant differences between the two groups. In both groups, body weight was measured daily and showed a decrease during the first 3 d followed by an increase during the rest of the study. These changes were not statistically significant, nor were the differences in body weight between the two groups on each day or the total changes in the two groups during the study period.

Fatty acids

The relative proportions of different fatty acids in plasma lipid esters and adipose tissue are recorded in Tables 4–7. In plasma, the relative contents of LA and ALA increased, whereas those of palmitic acid (16:0) and palmitoleic acid (16:1n-7) decreased, in all lipid esters in both groups. The changes in LA were more pronounced in phospholipids and triglycerides in the Intralipid® group. A decrease in capric acid (CA,10:0) in all plasma lipid esters was noted in the treatment group, but only in cholesterol esters in the control group. Stearic acid (18:0) increased in phospholipids and decreased in triglycerides in both groups. Stearic acid also decreased in cholesterol esters in the control group. The relative concentration of oleic acid (18:1n-9) decreased only in the triglycerides in the two groups. γ-linolenic acid (GLA,18:3n-6) increased significantly in phospholipids and triglycerides in the treatment group and in all lipid fractions in the control group. DHLA decreased in phospholipids in both groups, as did AA in cholesterol esters and phospholipids. The relative concentration of EPA increased in cholesterol esters in the treatment group and that of DHA decreased in phospholipids in both groups (Tables 4–6).

The relative concentrations of LA and ALA increased in adipose tissue in both groups, and likewise the proportion of CA in the treatment group and of oleic acid in the control group. Palmitic acid decreased significantly in both groups and palmitoleic acid in the control group. No changes were detected in long-chain polyunsaturated fatty acids in either group, even though ALA increased more markedly in the Intralipid® group (Table 7).

Free fatty acids tended to decrease in the treatment group and to increase in the control group, but the changes were not statistically significant (Table 8).

Carnitine

Initially, the plasma carnitine values did not differ significantly between the groups, although acylcarnitine showed a tendency towards high content in the control group (Table 9). In the muscle tissue, total and acylcarnitine contents were lower in the control group than in the treatment group. However, the degree of acylation did not differ either in plasma or in muscle

Table 5. Fatty acid composition of plasma phospholipids before and after 5 d of TPN with the two fat emulsions.

	Control group, Intralipid®				Treatment group, Vasolipid®			
Fatty acid	Median Day 0	Min-max	Median Day 5	Min-max	Median Day 0	Min-max	Median Day 5	Min-max
8:0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0
10:0	0.02	0.0–0.5	0.1	0.0–0.2	0.3	0.1–0.6	0.1*	0.0–0.3
16:0	35.3	34.3–36.4	30.9**	29.3–33.8	34.0	32.3–36	30.1**	29.2–32.3
16:1n-7	2.2	1.3–3,8	0.6**	0.3–0.9	1.8	1.2–3.3	0.6**	0.5–1.1
18:0	14.3	12.9–15.1	15.8*	14.4–17.0	14.9	13.5–16.3	16.8**	15.5–19.9
18:1n-9	14.9	13.9–19.8	15.3	11.8–17.4	14.2	12.2–15.5	13.9	12.5–15.9
18:2n-6	8.9	6.7–12.0	20.7**	19.9–26.3	8.9	7.3–10.8	17.3** ##	14.8–21.0
18:3n-6	0.0	0.0–0.2	0.2*	0.0–0.3	0.0	0.0–0.2	0.2*	0.0–0.2
18:3n-3	0.0	0.0–0.0	0.3**	0.2–0.5	0.0	0.0–0.0	0.2*	0.0–0.4
20:3n-6	3.7	3.1–4.8	2.0**	1.4–2.6	3.9	2.0–5.7	3.2* #	2.2–4.2
20:4n-6	13.3	10.5–18.1	8.5**	6.4–11.2	14.5	13.0–17.9	11.4** #	9.2–14.4
20:5n-3	0.5	0.0–0.9	0.4	0.3–0.7	0.3	0.0–1.6	0.7	0.3–0.9
22:6n-3	4.8	3.7–7.2	3.5**	2.1–4.4	5.7	4.2–7.2	4.3**	3.2–4.4
X	0.3	0.1–0.6	0.2***	0.1–0.4	0.4	0.2–0.9	0.2***	0.1–0.4

Results are given as a percentage of the sum of the fatty acids presented (median and range).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant change within the groups.
# $p < 0.05$, ## $p < 0.01$ for difference between the groups.
X (12:0, 14:0, 22:4n-6, 22:5n-3) = sum of the remaining fatty acids analysed.

tissue. During the treatment period, the total and free plasma carnitine decreased in both groups. Comparison between the groups on day 5 showed that acylcarnitine and the degree of acylation were higher in plasma in the control group. Some patients showed very low plasma carnitine values. Five patients showed initial total carnitine values below 10 µmol/L and free carnitine values below 6 µmol/L, and a few more had such low values on day 5. No significant changes were found in the muscle tissue during the treatment period, although the acylcarnitine and the ratio of acylcarnitine to free carnitine tended to increase in the treatment group. The higher acylcarnitine content in muscle tissue remained in the treatment group compared with the control group during the treatment period. There was no significant relationship between the total carnitine concentration in plasma and that in muscle tissue on day 0 ($n = 16$).

Glucose and lipids in plasma

In both groups the plasma glucose concentration remained within the normal range during the study period (Fig. 1). Triglycerides in plasma returned essentially to baseline every day after a period without

Table 6. Fatty acid composition of plasma triglycerides before and after 5 d of TPN with the two fat emulsions.

	Control group, Intralipid®				Treatment group, Vasolipid®			
Fatty acid	Median Day 0	Min-max	Median Day 5	Min-max	Median Day 0	Min-max	Median Day 5	Min-max
8:0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0
10:0	0.3	0.1–1.4	0.4	0.1–0.9	0.6	0.0–1.3	0.2* ##	0.1–0.3
16:0	31.8	27.6–35.8	21.6**	17.4–27.4	32.1	29.4–35.4	25.7** ##	19.6–28.1
16:1n-7	8.2	4.3–12.3	3.2**	1.5–5.4	8.3	6.2–10.9	4.4**	3.4–6.7
18:0	6.9	3.6–10.2	4.7*	4.0–6.6	7.0	5.5–7.7	4.6*	3.9–7.6
18:1n-9	39.3	35.0–47.5	30.7**	26.8–33.6	37.7	29.7–43.2	31.8**	26.3–35.9
18:2n-6	5.4	2.4–9.2	32.0**	21.6–36.6	6.4	3.7–8.0	24.0** ##	19.7–29.3
18:3n-6	0.1	0.0–0.4	0.6**	0.3–1.3	0.0	0.0–0.2	0.8**	0.4–1.7
18:3n-3	0.0	0.0–0.1	2.0**	1.2–2.9	0.0	0.0–0.2	1.8**	1.2–3.3
20:3n-6	0.5	0.0–2.0	0.5	0.0–0.7	0.6	0.0–1.2	0.7	0.5–0.8
20:4n-6	1.9	0.9–3.9	2.3	1.4–3.2	2.4	1.4–5.1	2.4	1.4–5.2
20:5n-3	0.0	0.0–0.2	0.0	0.0–0.4	0.0	0.0–0.2	0.3	0.2–0.8
22:6n-3	2.7	0.0–6.0	1.9	0.7–2.6	3.1	0.0–6.2	1.9	1.3–2.9
X	1.4	0.2–2.5	0.7**	0.1–1.3	1.4	0.2–2.4	0.9***	0.1–1.8

Results are given as a percentage of the sum of the fatty acids presented (median and range).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant change within the groups.
# $p < 0.05$, ## $p < 0.01$ for difference between the groups.
X (12:0, 14:0, 22: n-6, 22:5n-3) = sum of the remaining fatty acids analysed.

Table 7. Fatty acid composition of adipose tissue before and after 5 d of TPN with the two fat emulsions.

	Control group, Intralipid®				Treatment group, Vasolipid®			
Fatty acid	Median Day 0	Min-max	Median Day 5	Min-max	Median Day 0	Min-max	Median Day 5	Min-max
8:0	0.1	0.0–0.2	0.1	0.0–1.0	0.1	0.0–0.2	0.3	0.0–0.6
10:0	0.1	0.0–0.2	0.1	0.0–1.0	0.1	0.1–0.2	1.0**	0.4–2.0
16:0	45.2	40.8–49.1	42.4**	35.5–45.5	45.0	39.4–47.9	42.6**	40.0–44.9
16:1n-7	13.9	11.5–16.2	12.2**	9.7–15.4	13.2	11.9–16.0	12.8	11.2–15.9
18:0	3.9	3.1–7.1	4.3	3.1–5.6	4.0	2.8–4.8	4.0	3.1–4.6
18:1n-9	28.4	25.2–32.2	31.2*	28.6–32.6	28.9	25.1–32.8	29.5	26.4–32.1
18:2n-6	1.7	1.0–3.2	4.2**	3.6–10.3	2.2	1.5–3.7	4.0**	2.6–5.7
18:3n-6	0.1	0.0–0.2	0.1	0.0–0.2	0.1	0.1–0.2	0.1**	0.1–0.1
18:3n-3	0.1	0.0–0.2	0.5**	0.3–1.1	0.1	0.0–1.0	0.3** ###	0.2–0.4
20:3n-6	0.1	0.0–0.5	0.1	0.0–0.3	0.1	0.0–0.2	0.1	0.0–0.3
20:4n-6	0.6	0.5–2.9	0.6	0.4–1.6	0.7	0.5–1.2	0.7	0.5–1.5
20:5n-3	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0	0.0	0.0–0.0
22:6n-3	0.4	0.0–1.0	0.4	0.3–0.9	0.5	0.4–1.0	0.5**	0.3–0.8
X	2.0	0.2–5.6	1.5***	0.1–4.7	1.9	0.2–5.4	1.9	0.2–4.9

Results are given as a percentage of the sum of the fatty acids presented (median and range).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant change within the groups.
$p < 0.001$ for difference between the groups.
X (12:0, 14:0, 22:4n-6, 22:5n-3) = sum of the remaining fatty acids analysed.

Table 8. Serum triglycerides and free fatty acids before and after 5 d of TPN with the two fat emulsions.

	Control group, Intralipid®				Treatment group, Vasolipid®			
	Median Day 0	Min-max	Median Day 5	Min-max	Median Day 0	Min-max	Median Day 5	Min-max
Triglycerides	0.62	0.09–1.93	0.51	0.25–1.20	0.65	0.27–2.22	1.12*	0.59–1.66
Free fatty acids	0.18	0.02–0.95	0.24	0.11–0.41	0.31	0.10–0.79	0.22	0.08–0.81

* $p < 0.05$: significant change during treatment.

Table 9. Concentrations of carnitine in muscle and plasma in control and treatment groups before and after 5 d of TPN with two fat emulsions.

	Control group, Intralipid®						Treatment group, Vasolipid®					
	n	Median day 0	Min-max	*n*	Median day 5	Min-max	*n*	Median day 0	Min-max	*n*	Median day 5	Min-max
Muscle carnitine[a]												
Total	8	4.8	4.0–7.9	7	3.8	2.8–6.1	9	7.1#	2.2–15.9	10	5.5#	3.1–17.8
Free	8	3.3	2.1–5.4	7	2.6	0.8–3.6	8	4.1	1.4–7.7	7	3.3	1.4–11.3
Acyl	8	2.0	0.8–3.5	7	1.5	0.5–3.4	8	3.6(#)	0.8–6.3	7	3.7(*)##	2.0–7.3
Acyl/free	8	0.48	0.22–1.46	7	0.88	0.14–2.50	8	0.73	0.40–2.23	7	1.35(*)	0.51–2.64
Free/total, %	8	68	41–81	7	53	29–88	8	58	31–71	7	42	28–66
Muscle carnitine[b]												
Total	8	6.6	5.4–10.7	7	8.6	4.5–12.7	9	13.4##	4.5–29	10	10.4	5.4–33.0
Free	8	4.5	3.0–7.3	7	4.3	2.4–11.0	8	7.4(#)	2.9–17.7	7	4.1	2.7–20.9
Acyl	8	2.6	1.2–5.9	7	2.3	1.0–9.0	8	6.9#	1.6–11.8	7	6.2#	2.7–12.8
Acyl/free	8	0.48	0.22–1.87	7	0.88	0.15–2.43	8	0.72	0.40–2.19	7	1.38(*)	0.52–2.59
Free/total, %	8	68	41–81	7	53	29–86	8	58	31–71	7	42	28–66
Plasma carnitine[c]												
Total	10	22.3	4.6–53.4	10	14.1*	4.0–43.9	9	13.1	5.4–51.2	10	10.7*	5.1–25.0
Free	10	16.3	3.1–40.7	10	9.3*	2.8–43.0	9	10.6	4.2–46.0	10	7.7(*)	3.6–24.9
Acyl	10	5.6	1.2–12.8	10	3.6	0.9–10.0	9	2.5(#)	1.2–5.1	10	1.9#	0.1–4.2
Acyl/free	10	0.31	0.13–0.46	10	0.47	0.02–1.10	9	0.25	0.06–0.59	10	0.31(#)	0.00–0.47
Free/total, %	10	77	67–84	10	67	48–98	9	80	63–94	10	77#	68–99

(*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$: statistically significant changes in free, acyl and total carnitine within the control and treatment groups.
(#) $p < 0.1$, # $p < 0.05$, ## $p < 0.01$: statistically significant differences between the two groups.
[a] Expressed in μmol/g dry weight (dw); [b] μmol/g noncollagen protein (NCP); [c] Expressed in μmol/L

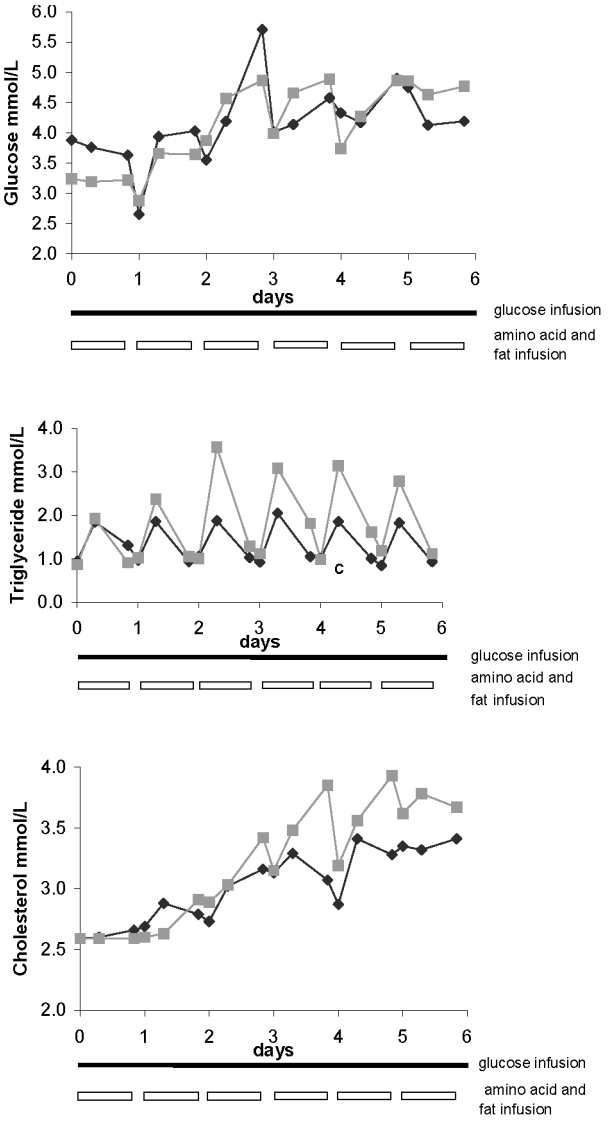

Fig. 1. Serum glucose, triglyceride and cholesterol concentrations (mean values) from day 1 to day 6 in the treatment group, receiving Vasolipid® and the control group, receiving Intralipid®. At the end of the study period the triglycerides had increased from 0.65 to 1.12 mmol/L in the treatment group, whereas no change was found in the control group. –◆– Control. –■– Treatment.

lipid infusion in both groups. At the end of the study period the triglycerides had increased from 0.65 to 1.12 mmol/L in the treatment group ($p < 0.05$), whereas there was no change in the control group. Cholesterol in plasma tended to increase during the study period in both groups (Fig. 1).

Respiratory quotient

The respiratory quotient was measured just before the start of the TPN, 3 h after the start on day 1 and 3 h after the start on day 6. No difference in RQ was observed between the groups before TPN was commenced, when only glucose infusion was given continuously. After 3 h of TPN, the RQ was decreased compared with the pre-TPN value and on day 6, 3 h after the start of TPN, an increase in RQ was noted in both groups compared to the value on day 1. There were no significant differences in RQ between the groups during the study period.

3-methyl-histidine

The quantities of 3MH, reflecting muscle degradation, in a 24-h urine sample showed a significant decrease in both groups after 5 d of TPN. Because of technical problems, it was difficult to obtain accurate 24-h urine collections, especially without an indwelling catheter. The mean value for 3MH in the two groups together was 2.78 ± 1 g 24 h^{-1} on day 1 and 1.82 ± 0.73 g 24 h^{-1} on day 6 (12).

Discussion

In the present study Vasolipid®, a fat emulsion with 50% medium-chain and 50% long-chain triglycerides, was compared with Intralipid®, a long-chain triglycerides fat emulsion with a content of saturated and unsaturated long-chain fatty acids twice as high as that in Vasolipid®. The emulsions have the same phospholipid:triglyceride ratio.

The purpose of having fatty acids in TPN is to provide an adequate energy supply for optimal utilization of protein, to provide essential fatty acids for normal pathways of eicosanoid metabolism, to promote incorporation of fatty acids in cell membranes and to support cell growth and function (13). DHA can only be synthesized in very small quantities in neonates (14, 15), since neonates have a limited capacity to elongate and desaturate long-chain polyunsaturated fatty acids (LCPUFA) on account of their immature liver function (16). A continuous supply of small amounts of DHA is absolutely necessary for normal development of the brain and retina.

In this study the results for all fatty acids are presented as percentages of the sum. DHLA and AA in the plasma phospholipids, and AA in the cholesterol esters decreased in both groups, despite a slight increase in GLA in phospholipids and triglycerides in the plasma. This may indicate, contrary to a previous proposal (1), that delta-6-desaturase might not be the rate-limiting enzyme in this pathway (17). EPA in cholesterol esters also showed a slight increase in the treatment group, in agreement with earlier results (17), but DHA in plasma phospholipids nevertheless decreased in both groups. It is not clear which of the enzymes is the rate-limiting one in the desaturation-elongation steps of LA and ALA (18) or whether a substrate inhibition occurs even with Vasolipid®. Deficiency of DHA and AA in early life will result in

learning difficulties, impaired motor development and visual dysfunction, which will be irreversible (1, 19). Long-term treatment with these TPN compounds in neonates could be hazardous, but this study period of 5 d was too short to evaluate such a possible risk.

The relative concentration of both LA and ALA showed an increase after 5 d in all lipid esters in plasma in both groups, which is in conformity with previous results (17), and in adipose tissue in both groups, indicating that fatty acids in adipose tissue of newborns are rapidly accreted (20), and that the contents of LA and ALA in Vasolipid® are sufficient. On the other hand, the pronounced increase in LA in the plasma lipid esters and adipose tissue in the Intralipid® group could imply a risk of peroxidation and formation of free radicals. The CA concentration increased in the treatment group in adipose tissue, and the serum level of β-hydroxybutyrate remained within normal limits. This might indicate, that the MCT in the cells was not completely oxidized or that the relative concentration of CA in the emulsion (29.2%) was too high (5).

The plasma triglycerides were effectively cleared during the periods without fat infusion, although the fat content in the TPN gradually increased to a maximum of 3 g kg^{-1} d^{-1}. The plasma triglycerides were higher in the Vasolipid® group at the end of the study, as observed in another study (4). One explanation for this could be that although the content of lipids by mass is the same in both lipid emulsions, the MCT/LCT emulsion has a molar triglycerides ratio of approximately 1.4 compared with that of the LCT emulsion.

FFA usually increase in neonates receiving MCT/LCT fat emulsion (21), but in this study the concentration of FFA remained the same after 5 d of TPN in the two groups. β-hydroxybutyrate was within the normal limits in both groups, probably because the energy supply was sufficient. The results presented here show that, in a short period, there is no risk of ketoacidosis or of an increase in unbound bilirubin in neonates supplied with 3 g kg^{-1} d^{-1} of MCT/LCT in equal amounts.

The values of total and free carnitine in plasma and total carnitine in muscle tissue were essentially similar to those found in other studies of newborns, in muscle specimens obtained at autopsy (22–24). However, the carnitine content in muscle tissue was substantially lower than that found in abdominal muscle in adults, 12.4 ± 3.4 µmol/g dry weight, range 6.9–18.2 (11). Methodological differences, especially in the determination of free and acylcarnitine, exist. The extract of the method used here includes free, short- and long-chain acylcarnitines in contrast to a perchloric acid extraction, in which the supernatant includes acylcarnitines with chain lengths of up to C6–C8. This also means that a change in the distribution of individual acylcarnitines cannot be detected with the present method. The reagent used with the formed CoASH in the radioenzymatic method also influences the proportion of free carnitine and acylcarnitine determined, which contributes to the various results seen in different studies (10). A contributory factor to the finding that the control group had lower muscle carnitine concentration than the treatment group is probably that the control group had significantly lower gestastional age than the treatment group. Several studies have shown a positive correlation of gestational age to total carnitine and acylcarnitine concentrations in muscle and a negative correlation to plasma carnitine concentration (23, 24, unpublished results). These developmental changes of carnitine status might influence the comparison between the two groups but to a lesser degree the changes within each group during treatment. Signs and symptoms related to carnitine deficiency in the newborns are incompletely defined and can be similar to those of other metabolic defects. It is recommended that carnitine levels should be measured in infants at risk of carnitine deficiency (25). Our study confirms the report from a study of critically ill children that abnormal plasma carnitine values are frequently found without any association with secondary carnitine deficiency (26).

Several studies indicate that carnitine is involved in the transport and oxidation of MCFA (5). In healthy adult volunteers, a comparison between the effects of infusion of a mixture of 50% LCT and 50% MCT emulsions and those of an LCT emulsion showed that the former was more ketogenic and caused a larger shift of carnitine from the free form to the short-chain acylcarnitine form (27). In the present study, the plasma total carnitine and free carnitine values decreased during the treatment period, an observation that is made in many studies of newborn children receiving carnitine-free nutrition (23, 28). No change in the degree of esterfication of carnitine in plasma took place. According to a recent review, no evidence has been presented that justifies routine carnitine supplementation of neonates receiving total parenteral nutrition during a short period of treatment (29). The total muscle carnitine content was unchanged during the treatment period in both groups. This finding is in agreement with the observation in other studies that only the muscle carnitine content was maintained after 10 d of TPN, and not the content in other tissues (24). This observation is also compatible with the much slower turnover rate of the muscle carnitine pool, about 8 d, compared to that of other tissue (30). The tendency of an increase of acylcarnitine and the ratio of acyl to free carnitine observed in the treatment group might indicate an influence of the MCT supplementation. *In vitro* studies in rat skeletal muscle have shown that β-oxidation of fatty acids with chain lengths of C 16:0 and C 8:0 are carnitine-dependent (31).

After 3 h of TPN, RQ decreased significantly in both groups, indicating increased fat oxidation. However, there were no differences between the groups, although MCFAs are considered to be more available for oxidation than LCFAs. After 5 d of TPN, 3 h after the

start of TPN on day 6, RQ showed a higher value in both groups, indicating reduced fat oxidation, and this correlated well with the increased contents of LA and ALA in the adipose tissue.

3MH values reflect the protein degradation, and the mean value of 3MH in the two groups combined decreased. This indicates, that the metabolism in neonates was anabolic during the study period.

In conclusion, neonates receiving an MCT/LCT lipid emulsion in TPN for a short period displayed essentially the same fatty acid patterns in plasma and adipose tissue and the same RQ values as those receiving only LCT. The energy supply and concentration of PUFA were sufficient with the MCT/LCT emulsion and there was no increase in β-hydroxybutyrate. The total and free carnitine in plasma decreased during the study, as has been observed in many studies on carnitine-free nutrition. Total carnitine content was unchanged in the muscle tissue during the treatment period in both groups, whereas acylcarnitine and the ratio of acylcarnitine to free carnitine tended to increase in the treatment group. Further studies including long-term TPN are needed to evaluate the clinical significance of the changes seen in carnitine status and LCPUFA, especially of the n-3 fatty acids.

Acknowledgements.—The authors are indebted to P. G. Lindgren, Professor at the Department of Radiology, Uppsala University, for lending us the biopsy needle, Gunnar Ronquist, Associate Professor at the Department of Clinical Chemistry for analysing the β-hydroxybuturate, Gunilla Hjort for analysing 3MH and Elisabeth Pettersson for measuring the RQ values. We also thank the nurses of the neonatal ward for taking care of the neonates and Lisa Wernroth, BA, Statistician, for performing the statistical analyses of the fatty acids. The study was funded by The Crown Princess Lovisa and Axel Thielman Foundation, the Ollie and Elof Ericsson Foundation and the Swedish Medical Research Council (grant no. 7136). B Braun Melsungen AG, Melsungen, Germany is acknowledged for providing us with the Vasolipid® solution.

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Received April 2, 2001; revision received Aug. 2, 2001; accepted Nov. 8, 2001