Metabolic effects of intravenous LCT or MCT/LCT lipid emulsions in preterm infants

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Abstract Most lipid emulsions for parenteral feeding of premature infants are based on long-chain triacylglycerols (LCTs), but inclusion of medium-chain triacylglycerols (MCTs) might provide a more readily oxidizable energy source. The influence of these emulsions on fatty acid composition and metabolism was studied in 12 premature neonates, who were randomly assigned to an LCT emulsion (control) or an emulsion with a mixture of MCT and LCT (1:1). On study day 7, all infants received [^{13}C]linoleic (LA) and [^{13}C]α-linolenic acid (ALA) tracers orally. Plasma phospholipid (PL) and triacylglycerol (TG) fatty acid composition and ^{13}C enrichments of plasma PL fatty acids were determined on day 8. After 8 days of lipid infusion, plasma TGs in the MCT/LCT group had higher contents of C8:0 (0.50 ± 0.60% vs. 0.10 ± 0.12%; means ± SD) and C10:0 (0.66 ± 0.51% vs. 0.15 ± 0.17%) than controls. LA content of plasma PLs was slightly lower in the MCT/LCT group (16.47 ± 1.16% vs. 18.57 ± 2.09%), whereas long-chain polyunsaturated derivatives (LC-PUFAs) of LA and ALA tended to be higher. The tracer distributions between precursors and products (LC-PUFAs) were not significantly different between groups. Both lipid emulsions achieve similar plasma essential fatty acid (EFA) contents and similar proportional conversion of EFAs to LC-PUFAs. The MCT/LCT emulsion seems to protect EFAs and LC-PUFAs from β-oxidation.—Lehner, F., H. Demmelmair, W. Röschinger, T. Decsi, M. Szász, K. Adamovich, R. Arnecke, and B. Koletzko. **Metabolic effects of intravenous LCT or MCT/LCT lipid emulsions in preterm infants. *J. Lipid Res.* 2006.** 47: **404–411.**

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For many pediatric patients, parenteral nutrition is an essential and often lifesaving therapy (1). Parenteral nutrition of infants and children depends on the use of lipid emulsions that provide a high energy density in an isotonic solution and supply essential fatty acids (EFAs) and fat-soluble vitamins. In premature infants, EFA body stores are very low, whereas their metabolic requirements are high; therefore, EFA supply is of critical importance (2). Conventional fat emulsions for premature neonates are prepared from long-chain triacylglycerols (LCTs), mostly soybean oil. An emulsion based on physical mixtures of LCT and medium-chain triacylglycerols (MCTs) is widely used for the parenteral nutrition of adult patients and provides an energy source that is rapidly oxidized (3). The MCT/LCT emulsion provides less PUFA than an LCT emulsion and thus has been associated with a lower risk of lipid peroxidation and fewer alterations of membrane structures (4). High amounts of linoleic acid (LA; C18:2n-6) and α-linolenic acid (ALA; C18:3n-3) were reported to inhibit Δ6 desaturation, the initial step in the formation of long-chain polyunsaturated fatty acids (LC-PUFAs) (5). Thus, we hypothesized that a reduced supply of LA and ALA with the MCT/LCT emulsion might enhance LC-PUFA formation.

Because of the fast growth of brain and retina during the perinatal period, an inadequate supply of LC-PUFAs, mainly arachidonic acid (AA; C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3), may have profound effects on the development of brain and visual function in preterm infants (6, 7). Although infants are able to synthesize LC-PUFAs from LA (C18:2n-6) and ALA (C18:3n-3) by desaturation and elongation from the first postnatal week onward, the rate of synthesis is rather low relative to the requirements for tissue incorporation (8, 9).

In human adults, infused MCTs are oxidized faster and to a greater extent than LCTs (10), but data on their metabolism in infants are scarce (1, 11). We hypothesized

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Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; APE, atom percent excess; DGLA, dihomo γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acid; LCT, long-chain triacylglycerol; MCT, medium-chain triacylglycerol; PL, phospholipid; TG, triacylglycerol.

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that in preterm infants the supply of a MCT/LCT emulsion would result in predominant oxidation of MCT, rather than LCT, as a major energy source; thus, via decreased LCT oxidation, the lower LCT intake might be partly compensated for by a higher availability of LCT for structural functions and for conversion into LC-PUFAs.

In this randomized study in preterm infants who received total parenteral nutrition for 8 days, we compared the effects of a MCT/LCT-based emulsion and of a LCT emulsion on the fatty acid composition of plasma phospholipids (PLs) and triacylglycerols (TGs). For a more detailed description of the metabolism of EFAs and their LC-PUFA derivatives, we applied ^{13}C-labeled fatty acid tracers, which allow for the comparison of endogenous LC-PUFA synthesis under different conditions (12, 13).

SUBJECTS AND METHODS

Premature neonates were recruited in the Division of Neonatology at the University of Pécs. Inclusion criteria for the study were as follows: gestational age between 25 and 37 weeks; birth weight < 3,000 g; the indication for total parenteral feeding (expected enteral feeding < 20% of daily energy intake) for at least 8 days; and the intention to supply a lipid emulsion within 48 h after birth. Infants with known metabolic diseases were excluded.

In this randomized, double-blind trial, premature infants were assigned either to the control group, receiving a conventional 20% LCT fat emulsion (soybean oil; Lipofundin N 20%®), or to the MCT/LCT group, which received a 20% MCT/LCT emulsion [a physical mixture (1:1 by weight) of soybean oil and coconut oil; Lipofundin MCT 20%] (**Table 1**). The parenteral nutrition also provided 10% glucose, amino acids, electrolytes (sodium chloride, potassium chloride, calcium gluconate), trace elements (Ped-el®; Pharmacia, Budapest, Hungary), and water-soluble vitamins (Soluvit®; Baxter, Deerfield, IL).

On day 7 of the study, 10 mg/kg body weight of uniformly ^{13}C-labeled (98%) LA and 2 mg/kg body weight of uniformly ^{13}C-labeled (98%) ALA (Martek Bioscience Corp., Columbia, MD) were given orally to the infants, dissolved in a small volume of human milk. Blood samples were obtained on day 1 (before introducing the study emulsions), on day 7 (before tracer application), and on day 8. Biochemical safety parameters were measured on each day of the study (data not shown). The study protocol was approved by the ethical committee of the University of Pécs, and written informed consent was obtained from the parents of each infant before study entry.

TABLE 1. Composition of the two fat emulsions used (data as provided by the manufacturer)

Composition	LCT-Based Emulsion (Soybean Oil, Control)	MCT/LCT-Based Emulsion
Saturated fatty acids (%, w/w)		
C6:0	–	<1
C8:0	–	27
C10:0	–	21
C12:0	–	1.5
C16:0	12	5
C18:0	4.5	2
Monounsaturated fatty acids (%, w/w)		
C18:1n-9	24	12
Polyunsaturated fatty acids (%, w/w)		
C18:2n-6	50	27
C18:3n-3	7	4
Other fatty acids (%, w/w)	2.5	–
Emulsifier (g/l)	12	12
Glycerin (g/l)	25	25
Phosphate (mmol/l)	13	13
α-Tocopherol (mg/l)	227	208

LCT, long-chain triacylglycerol; MCT, medium-chain triacylglycerol.

Blood samples

Blood samples were obtained by venipuncture and collected in EDTA tubes. Plasma and red blood cells were separated by centrifugation at 1,500 *g* for 5 min. An aliquot was used to measure plasma lipids (cholesterol, total TG, total PL, and total nonesterified fatty acids) and 3-hydroxybutyrate. The enzymatic analyses were performed in Pécs with standard clinical chemistry methods (Lipid-Kits from Boehringer Mannheim, Mannheim, Germany).

Plasma free carnitine and acylcarnitines were determined by electrospray tandem mass spectrometry (14, 15), and α- and γ-tocopherol were determined by high-performance liquid chromatography from 100 μl of plasma (16).

For the analysis of plasma fatty acids, an internal standard was added to the samples (trinonanoin for the quantification of C8:0, C10:0, and C12:0 in TG, dipentadecanoylphosphatidylcholine and tripentadecanoin for the quantification of fatty acids with 14 or more carbon atoms; Sigma, St. Louis, MO). Samples were extracted with hexane-isopropanol, as described previously (17). Plasma PLs and TGs were isolated by thin-layer chromatography (18). For transesterification, TGs were dissolved in a methanolic HCl/hexane mixture, whereas for the transesterification of PL, pure methanolic HCl was used. After cooling of the mixture, water was added to TG samples to achieve phase separation and an aliquot of the organic phase was used for gas chromatography analysis. Evaporation steps were omitted in the preparation of FA methylesters from TG, to avoid losses of volatile medium-chain fatty acids. After cooling of the PL samples, the reactant mixture was neutralized with a dry carbonate buffer. Methylesters were extracted twice into hexane, dried under nitrogen, and taken up in hexane containing 2,6-di-tert-butyl-4-kresol for gas chromatographic analysis (19).

Gas-liquid chromatography and mass spectrometry

An HP 5890 II gas chromatograph (Hewlett-Packard, Waldbronn, Germany), equipped with a split/splitless injector, a flame ionization detector, and a BPX70 column (length, 60 m; inner diameter, 0.32 mm; SGE, Weiterstadt, Germany), was used for quantitative analyses. The temperature program started at 130°C and increased at 3 K per minute up to 210°C. Fatty acid methylesters were identified by comparison of the retention times with those of authentic standard compounds.

^{13}C contents in plasma PL fatty acid methylesters were determined by gas chromatography-combustion-isotope ratio mass spectrometry using an HP 5890 II gas chromatograph and a Finnigan MAT delta S isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) (9). Samples were analyzed in duplicate, and further calculations were performed with the means of the δ^{13}C values (‰) obtained.

Calculations

The δ^{13}C (‰) value is the deviation of the $^{13}C/^{12}C$ ratio (R_{FA}) of a sample from the $^{13}C/^{12}C$ ratio (R_{PDB}) of the PDB (Pee Dee Belmnite) standard in relation to the ratio of the international PDB standard (20):

$$\delta^{13}C_{FA}[‰] = [(R_{FA} - R_{PDB})/R_{PDB}] \times 1{,}000$$

TABLE 2. Clinical characteristics and anthropometric data [mean (SD)] of infants at birth and at study start, showing no significant differences between groups

Characteristics	LCT (n = 6)	MCT/LCT (n = 6)
Male/female	3/3	6/–
Birth		
Gestational age (weeks)	33.2 (1.0)	31.4 (1.6)[a]
Weight (g)	1,781.7 (290.3)	1,573.3 (169.8)
Z-score weight	−0.33 (0.65)	−0.81 (1.22)
Study start		
Weight (g)	1780.0 (297.5)[b]	1575.0 (183.4)[b]
Z-score weight	−1.16 (1.42)[b]	−0.35 (0.91)[b]
Height (cm)	41.3 (2.9)	39.8 (1.0)
Day 8		
Weight (g)	1791.7 (296.6)	1490.0 (145.1)
Z-score weight	0.79 (1.20)	0.78 (0.70)[a]

[a] $n = 5$.
[b] $n = 3$.

The atom percent ^{13}C (AP_{FA}; %) of the fatty acid methylester was calculated as the percentage content of ^{13}C relative to total C:

$$AP_{FA} = R_{FA}/(1 + R_{FA}) \times 100$$

^{13}C contents on day 7 were used as baseline values and subtracted from the values on day 8 to obtain tracer-induced atom percent excess (APE_{FA}):

$$APE_{FA} = AP_{FA} \text{ day } 8 - AP_{FA} \text{ day } 7$$

Absolute tracer concentrations in plasma PL fatty acids were calculated as μmol of ^{13}C derived from the tracer in each fatty acid per liter of plasma:

$$\text{tracer} - {}^{13}C_{FA}[\mu\text{mol/l}] = \mu\text{Mol}_{FA} \times (\text{number of carbon atoms of FA} + 1) \times APE_{FA}/100$$

Statistical analyses

Statistical analyses were performed using SAS (SAS System for Windows, release 6.12; SAS Institute, Inc., Cary, NC). Results are given as means ± SD. Differences between the two feeding groups were examined by the *U*-Mann-Whitney-Wilcoxon test. Significant differences over time within one feeding group were examined by paired *t*-tests. Statistical significance was assumed at $P < 0.05$.

RESULTS

Fifteen infants were enrolled and randomized, and 12 (6 per group) completed the study according to the protocol (**Table 2**). Three infants, all assigned to the MCT/LCT group, were excluded because of wrong randomization, breaching of the study conditions, and contraindication against the feeding protocol (one infant each). No dropout was related to any adverse effects of the study medication.

In the control group, birth weights were appropriate for gestational age in five infants and small for gestational age in one, whereas all infants in the MCT/LCT group had birth weights appropriate for gestational age. At baseline, the MCT/LCT group tended to have a lower body weight than the control group, which was preserved until day 8 (Table 2) and day 10 (1,545 ± 140 g vs. 1,802 ± 282 g). Z-scores for body weight were calculated based on data from the longitudinal study of Kramer et al. (21) and showed no group differences (Table 2). Average daily nutrient intakes for days 1–9 are shown in **Table 3**.

TABLE 3. Mean daily energy and nutrient intake [mean (SD)] of the preterm infants studied (no group differences)

Intake	LCT Group (n = 6)	MCT/LCT Group (n = 6)
Intravenous intake		
Energy (kJ/day)	454.0 (164.8)	378.9 (137.1)
Amino acids (g/kg/day)	1.6 (0.7)	1.6 (0,8)
Fat (g/kg/day)	2.3 (1.3)	2.3 (1.2)
Glucose (g/kg/day)	9.1 (1.6)	9.1 (1.7)
Enteral intake		
Energy (kJ/day)	69.9 (30.4)	62.0 (29.7)
Human milk (ml/day)	20.9 (9.0)	20.9 (6.3)
Intake of total liquid (ml/kg/day)	134.4 (43.2)	134.8 (45.2)
Proportion of enteral energy of total energy (%)	13.7 (3.5)	14.4 (5.0)
Total energy intake (kJ/kg/day)	289.6 (115.2)	289.6 (120.4)

TG levels of the two groups were similar (**Table 4**) and within the range of reference values (22), and no hypertriacylglycerolemia was observed. Plasma cholesterol concentrations were within the reference range (22). During the study period, cholesterol levels increased significantly to 3.23 ± 0.83 mmol/l in the MCT/LCT group and to 3.37 ± 0.64 mmol/l in the control group. Nonesterified fatty acid levels were not different between the two groups or between study time points. 3-Hydroxybutyrate concentrations decreased during the study period in the control group. Free carnitine and acylcarnitine concentrations were similar in the two groups. Neither free carnitine nor total carnitine or individual acylcarnitines decreased significantly from day 1 to day 8 in either group.

At study start, the control group had significantly lower levels of γ-tocopherol (0.57 μmol/l vs. not detectable, control vs. MCT/LCT group, respectively), whereas there were no differences in α-tocopherol (10.94 vs. 8.65 μmol/l). In both groups α- and γ-tocopherol levels increased significantly during the study period (at day 8, 54.16 μmol/l α-tocopherol and 17.13 μmol/l γ-tocopherol in controls, 49.48 μmol/l α-tocopherol and 9.55 μmol/l γ-tocopherol in the MCT/LCT group), with no significant group differences on day 8.

There were no significant differences between groups in percentage values of PL fatty acids at baseline, but LA and γ-linolenic acid values on day 8 were significantly higher in the LCT group (**Table 5**). Despite a 50% lower LA (C18:2n-6) and ALA (C18:3n-3) supply with the MCT/LCT emulsion, LA (C18:2n-6) levels increased to a similar 3-fold extent in both groups from day 1 to day 8. ALA (C18:3n-3) levels showed a marked, 13-fold increase in the MCT/LCT group and an 8-fold increase in the LCT group.

On day 8, the MCT/LCT group showed significantly higher levels of medium-chain fatty acids (C8:0 and C10:0). MUFAs and PUFAs in plasma TG were not different on day 1, with the exception of eicosanoic acid (C20:0) (**Table 6**). In both groups, LA (C18:2n-6) and ALA (C18:3n-3) in PL and TG increased significantly from baseline to day 8, whereas AA (C20:4n-6) and DHA (C22:6n-3) decreased. In the MCT/

TABLE 4. Plasma concentrations [mean (SD)] of TGs, cholesterol, PLs, nonesterified fatty acids, 3-hydroxybutyrate, free carnitine, and acylcarnitines of the studied infants before lipid infusion (day 1) and 24 h after the end of lipid infusion (day 8)

	Day 1		Day 8	
Variable	LCT(n = 6)	MCT/LCT (n = 6)	LCT (n = 6)	MCT/LCT (n = 6)
TGs (mmol/l)	0.68 (0.17)	0.72 (0.23)	1.18 (0.53)	0.67 (0.10)
PLs (mg/l)	1,950.7 (352.6)	2,084.7 (733.9)	2,212.5 (329.9)	2,240.8 (605.7)
Cholesterol (mmol/l)	2.12 (1.05)	2.30 (0.89)	3.37 (0.64)[a]	3.23 (0.83)[b]
Nonesterified fatty acids (μmol/l)	466.8 (118.5)	795.0 (446.6)	499.2 (145.2)	746.5 (337.5)
3-Hydroxybutyrate (mmol/l)	73.17 (14.16)	90.33 (63.46)	48.33 (8.24)[a]	53.00 (11.90)
Free carnitine (μmol/l)	18.90 (6.39)	14.62 (9.71)	9.94 (3.11)	10.63 (3.48)
Σ acylcarnitines (μmol/l)	12.79 (5.44)	11.95 (4.52)	9.60 (3.39)	8.45 (1.59)
Σ medium chain (μmol/l)	0.30 (0.20)	0.59 (0.46)	0.53 (0.25)	0.50 (0.14)
Σ long chain (μmol/l)	0.30 (0.20)	0.32 (0.21)	0.14 (0.04)	0.15 (0.02)
Ratio of Σ acylcarnitines to free carnitine	0.68 (0.12)	1.13 (1.02)	1.00 (0.34)	0.84 (0.24)

PL, phospholipid; TG, triacylglycerol.
[a] $P < 0.05$, day 8 versus day 1.
[b] $P < 0.01$, day 8 versus day 1.

LCT group, there was a trend toward higher LC-PUFA contents in TG and PL, with significantly higher levels of C20:2n-6 and DHA (C22:6n-3) in TG.

At 24 h after tracer administration, infants in the MCT/LCT group had significantly higher ^{13}C APE values and ^{13}C tracer concentrations in plasma PL fatty acids than infants receiving the LCT emulsion (**Figs. 1**, **2**).

DISCUSSION

This study shows that infants receiving the MCT/LCT lipid emulsion, with half the EFA supply than in the LCT emulsion, have similar EFA contents in plasma PL and TG, whereas LC-PUFA levels tend to be higher. Thus, MCTs seem to enhance EFA and LC-PUFA incorporation into circulating lipids.

Both lipid emulsions were well tolerated in the infants that we followed, similar to observations made in previous studies (23). Compared with the control group, the MCT/LCT group tended toward a lower body weight at baseline (NS), showed a greater weight loss, and regained birth weight later, although the extent of parenteral nutrition and caloric intake was comparable in the two groups. Postnatal weight loss during the first days of life reflects primarily the loss of relatively expanded fetal water com-

TABLE 5. Fatty acid composition of plasma PLs in preterm infants before (day 1) and after (day 8) LCT (control) or MCT/LCT emulsion

	Day 1		Day 8	
Composition	LCT (n = 6)	MCT/LCT (n = 6)	LCT (n = 6)	MCT/LCT (n = 6)
Saturated fatty acids				
Σ saturated fatty acids	49.82 (1.45)	48.60 (0.95)	47.56 (0.70)	46.64 (0.86)
Monounsaturated fatty acids				
C18:1n-9	10.06 (1.90)	10.49 (2.27)	13.48 (1.46)[a]	14.32 (0.73)[b]
Σ MUFA	17.09 (2.62)	18.68 (2.66)	18.29 (0.93)	19.09 (0.88)
Polyunsaturated fatty acids				
C20:3n-9	0.62 (0.31)	0.79 (0.46)	0.16 (0.10)[b]	0.19 (0.06)[a]
n-6 PUFAs				
C18:2n-6	6.64 (0.86)	5.41 (1.24)	18.57 (2.09)[b]	16.47 (1.16)[b,c]
C18:3n-6	0.05 (0.04)	0.07 (0.03)	0.20 (0.04)[b]	0.13 (0.02)[b,c]
C20:2n-6	0.26 (0.05)	0.24 (0.05)	0.40 (0.04)[b]	0.41 (0.04)[b]
C20:3n-6	2.70 (0.56)	2.64 (0.44)	1.78 (0.56)[b]	1.88 (0.23)[a]
C20:4n-6	17.34 (1.72)	17.97 (2.89)	8.81 (2.10)[b]	9.99 (0.85)[b]
C22:2n-6	0.10 (0.09)	0.04 (0.01)	0.11 (0.02)	0.10 (0.02)[b]
C22:4n-6	0.56 (0.10)	0.62 (0.11)	0.40 (0.06)[b]	0.43 (0.02)[a]
C22:5n-6	0.71 (0.28)	0.79 (0.20)	0.59 (0.09)	0.65 (0.04)
n-3 PUFAs				
C18:3n-3	0.02 (0.05)	0.00 (0.00)	0.15 (0.03)[b]	0.13 (0.05)[b]
C20:3n-3	0.11 (0.06)	0.15 (0.04)	0.07 (0.03)[a]	0.08 (0.02)[b]
C20:5n-3	0.10 (0.06)	0.12 (0.06)	0.20 (0.06)[b]	0.17 (0.02)
C22:5n-3	0.08 (0.03)	0.08 (0.04)	0.24 (0.04)[b]	0.25 (0.03)[b]
C22:6n-3	3.39 (0.50)	3.48 (0.86)	2.32 (0.36)[b]	2.61 (0.28)[a]
Σ PUFA	32.80 (1.99)	32.28 (3.38)	33.67 (1.33)	33.79 (0.94)

Σ PUFA = C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6 + C22:5n-6 + C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. Values shown are means (SD) (%, w/w).
[a] $P < 0.05$ day 8 versus day 1 within one group.
[b] $P < 0.01$ day 8 versus day 1 within one group.
[c] $P < 0.05$ MCT versus control.

TABLE 6. Fatty acid composition of plasma TGs in preterm infants before (day 1) and after (day 8) LCT (control) or MCT/LCT emulsion

	Day 1		Day 8	
Composition	LCT (n = 6)	MCT/LCT (n = 6)	LCT (n = 6)	MCT/LCT (n = 6)
Saturated fatty acids				
C8:0	0.11 (0.03)	0.08 (0.03)	0.10 (0.12)	0.50 (0.60)[a,b]
C10:0	0.15 (0.06)	0.19 (0.09)	0.15 (0.17)	0.66 (0.51)[a,b]
C12:0	0.56 (0.13)	0.48 (0.10)	0.61 (0.27)[a]	0.89 (0.43)
C14:0	1.76 (0.22)	1.71 (0.24)	1.58 (0.62)	1.94 (0.34)
C16:0	34.69 (4.61)	33.21 (3.21)	25.21 (3.71)[a]	25.84 (2.31)[c]
C17:0	0.36 (0.10)	0.31 (0.07)	0.37 (0.07)	0.37 (0.05)
C18:0	6.16 (1.33)	4.66 (1.14)	3.34 (0.54)[c]	3.65 (0.37)
C20:0	0.06 (0.01)	0.04 (0.01)[b]	0.06 (0.07)	0.23 (0.45)
Σ SFA	44.52 (4.64)	40.02 (2.19)	32.09 (4.91)	33.45 (3.09)
Monounsaturated fatty acids				
C18:1n-9	32.13 (5.90)	33.56 (4.84)	26.43 (3.02)[a]	27.24 (2.33)[a]
Σ MUFA	45.47 (7.46)	50.07 (4.15)	32.14 (4.72)	35.70 (2.70)
Polyunsaturated fatty acids				
C20:3n-9	0.35 (0.12)	0.42 (0.11)	0.25 (0.19)	0.32 (0.10)[c]
n-6 PUFA				
C18:2n-6	4.37 (1.85)	4.12 (1.67)	29.57 (9.22)[c]	23.37 (3.29)[c]
C18:3n-6	0.12 (0.06)	0.11 (0.06)	1.0 (0.57)[a]	0.85 (0.13)[c]
C20:2n-6	0.17 (0.02)	0.16 (0.02)	0.26 (0.06)[a]	0.33 (0.07)[b,c]
C20:3n-6	0.84 (0.22)	0.79 (0.27)	0.69 (0.28)	0.89 (0.09)
C20:4n-6	1.68 (1.10)	1.69 (0.73)	1.64 (0.87)	2.28 (0.49)
C22:4n-6	0.20 (0.13)	0.25 (0.07)	0.18 (0.08)	0.24 (0.05)
C22:5n-6	0.47 (0.24)	0.62 (0.12)	0.23 (0.15)	0.34 (0.09)[c]
n-3 PUFA				
C18:3n-3	0.04 (0.03)	0.04 (0.03)	1.39 (1.11)[a]	0.97 (0.25)[c]
C20:3n-3	n.d.	n.d.	0.01 (0.01)	0.04 (0.03)
C20:5n-3	0.02 (0.02)	0.03 (0.04)	0.11 (0.05)[a]	0.13 (0.04)[a]
C22:5n-3	0.01 (0.03)	0.03 (0.03)	0.03 (0.03)	0.05 (0.04)
C22:6n-3	0.78 (0.56)	1.13 (0.64)	0.27 (0.14)	0.47 (0.13)[b]
Σ PUFA	9.17 (3.53)	9.30 (2.78)	35.27 (8.77)	30.63 (3.62)

n.d., not detected. Σ PUFA = C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6 + C22:5n-6 + C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. Values shown are means (SD) (%, w/w).

[a] $P < 0.01$ day 8 versus day 1 within one group.

[b] $P < 0.05$ MCT/LCT versus control.

[c] $P < 0.05$ day 8 versus day 1 within one group.

partments and is proportionally greater in infants with lower birth weight (24, 25). The postnatal weight loss in the MCT/LCT group (11.4%) is within the expected range of 7.3–14.5% (26). Weight gain can best be compared with Z-scores for weight related to gender and age, which do not differ between the two groups, suggesting that under the conditions of our study the choice of the lipid emulsion did not affect weight gain.

The observed plasma TG concentrations are within the accepted reference range, and the results for PL and nonesterified fatty acids are similar to previous reports for premature neonates (27, 28). The increase in cholesterol levels during the first days of life has been described in previous studies after parenteral and enteral nutrition (29, 30). In agreement with previous studies, we found no appreciable differences in plasma free carnitine and acylcarnitine values between the two groups (28). A significant effect of MCT in the diet or of carnitine-free parenteral nutrition might become apparent after longer periods, but during short periods of parenteral nutrition carnitine supplementation has no demonstrable benefits (31).

The control group showed lower γ-tocopherol levels on day 1 but a trend toward higher values on days 7 and 8 (NS), reflecting the higher content of γ-tocopherol in the LCT emulsion with its higher content of soybean oil (32). The concentrations of α-tocopherol, the most important lipid-soluble antioxidant, were similar with both emulsions (Table 2) and well above the 12.4 μmol/l considered a threshold for vitamin E sufficiency in premature neonates (33).

TGs from emulsions containing a MCT/LCT mixture are hydrolyzed faster than those containing exclusively LCT, apparently because of physicochemical effects of MCT incorporated into the surface of emulsion particles (34). The liberated medium-chain fatty acids are rapidly oxidized largely independent of carnitine (35), but only minor amounts are incorporated into plasma TG (Table 6). In contrast to medium-chain fatty acids, long-chain fatty acids are preferentially activated in the cytosol and esterified into TG and PL, and only a small proportion enters the mitochondria via the carnitine cycle (35). It has been suggested that during MCT/LCT ingestion, carnitine palmitoyltransferase I is inhibited by the production of malonyl-CoA, and consequently oxidation of long-chain fatty acids is reduced and their incorporation into complex lipids is enhanced (36). Indeed, we recently found reduced LA oxidation in preterm infants fed an enteral formula with 40% of fat provided by MCT compared with a 100% LCT formula (37). Even though the LCT emulsion provided approximately twice the amounts of palmitic acid (C16:0),

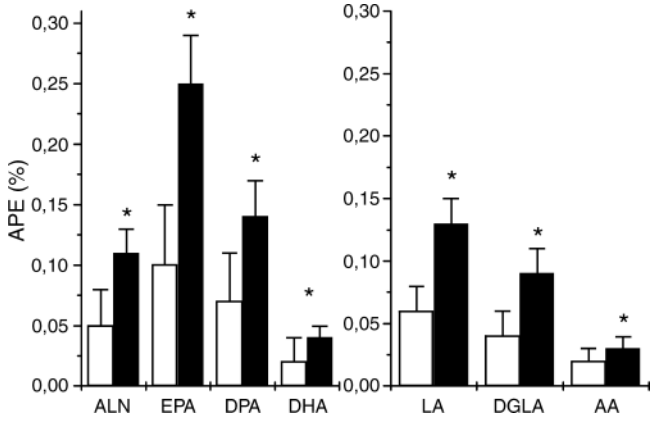

Fig. 1. ^{13}C atom percent excess (APE) in plasma phospholipid (PL) fatty acids of preterm infants after 7 days of intravenous lipid infusion 24 h after tracer intake. Open bars, control group, soybean oil; closed bars, medium-chain triacylglycerol/long-chain triacylglycerol (MCT/LCT) group. Values shown are means ± SD. * $P < 0.05$ between groups. AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.

stearic acid (C18:0), oleic acid (C18:1n-9), LA (C18:2n-6), and ALA (C18:3n-3) as the MCT/LCT emulsion, plasma levels of these fatty acids were rather similar in both groups, except for a slightly higher LA (C18:2n-6) value in plasma PL on day 8 in the LCT group (Table 5). This striking effect may result in part from a decreased long-chain fatty acid oxidation in the MCT/LCT group and thus incorporation of a larger proportion of the long-chain fatty acids supplied into plasma lipids. The trend toward slightly higher values of palmitic (C16:0), stearic (C18:0), and oleic (C18:1n-9) acids but slightly lower LA (C18:2n-6) and ALA (C18:3n-3) in the MCT group, compared with the LCT group, might reflect the greater oxidation of polyunsaturated fatty acids than saturated fatty acids (38). Alternatively, long-chain saturated fatty acids and oleic acid (C18:1n-9) might have been synthesized in the MCT/LCT group from readily available medium-chain fatty acids (39).

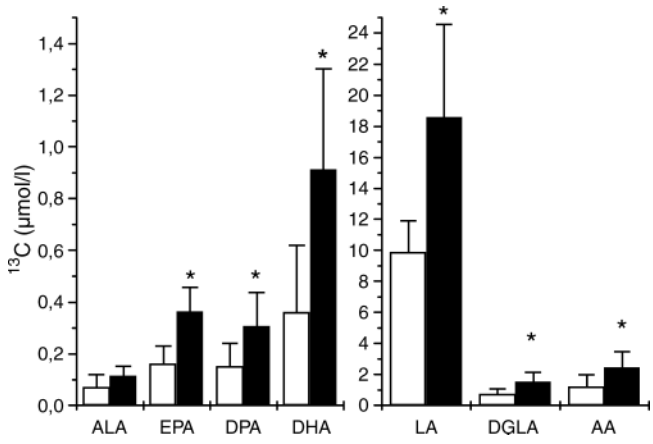

Fig. 2. Tracer concentrations in plasma PL fatty acids of preterm infants after 7 days of intravenous lipid infusion 24 h after tracer intake. Open bars, control group, soybean oil; closed bars, MCT/LCT group. Values shown are means ± SD. * $P < 0.05$ between groups.

Over the course of the postnatal study period, EFAs increased in plasma lipids, whereas LC-PUFAs decreased. The observed values and their changes were similar to those found in enterally fed preterm neonates and reflect the change from placental fatty acid transfer, with a preferential supply of LC-PUFAs (40), to a feeding regimen supplying predominantly EFAs (9).

Polyunsaturated fatty acid turnover was assessed in this study with ^{13}C-labeled tracer fatty acids given orally with small volumes of human milk. Plasma APE values of LA (C18:2n-6) and ALA (C18:3n-3) in the MCT/LCT group were significantly higher, presumably because plasma LA (C18:2n-6) and ALA (C18:3n-3) pools were similar in the groups and the tracer intake was higher in this group relative to the infused amounts of LA (C18:2-6) and ALA (C18:3n-3). Thus, there was less dilution of the tracer before incorporation into PLs or conversion to LC-PUFAs, which would also explain the higher concentrations of ^{13}C-labeled LA (C18:2n-6), ALA (C18:3n-3), and their labeled derivatives in the MCT/LCT group than in the control group.

Excessive availability of LA (C18:2n-6) or ALA (C18:3n-3) may inhibit desaturase activity, which would influence tracer distribution between precursor and product fatty acids at 24 h after tracer intake, when samples were obtained (41, 42). Based on lower DHA (C22:6n-3) incorporation in plasma PLs in preterm infants fed formulae with MCT (43), it has been postulated that MCT might interfere with the conversion of docosapentaenoic acid (DPA; C22:5n-3) to DHA (C22:6n-3), which involves peroxisomal chain shortening of a 24 carbon intermediate (44). However, the observed distribution of the tracer amounts between precursors and products was very similar between groups for the n-6 series [LA, 82.5 ± 4.2% vs. 84.8 ± 7.8% (MCT/LCT vs. LCT); dihomo γ-linolenic acid (DGLA), 6.7 ± 1.3% vs. 5.8 ± 2.3%; AA, 10.8 ± 3.0% vs. 9.5 ± 5.6%] and for the n-3 fatty acids [ALA, 7.3 ± 3.2% vs. 12.1 ± 8.9%; eicosapentaenoic acid (EPA), 22.0 ± 5.1% vs. 25.0 ± 8.7%; DPA, 17.7 ± 3.2% vs. 19.6 ± 4.9%; DHA, 52.9 ± 9.5% vs. 43.3 ± 12.7%]. Also, the ^{13}C APE ratios between product and precursor fatty acids were similar between the two groups (AA/DGLA, 0.31 ± 0.08 vs. 0.30 ± 0.003; DGLA/LA, 0.69 ± 0.11 vs. 0.70 ± 0.10; DHA/DPA, 0.24 ± 0.09 vs. 0.29 ± 0.08; DHA/EPA, 0.16 ± 0.09 vs. 0.16 ± 0.06). Thus, the relative conversion of EFAs to LC-PUFAs is not influenced by the intake of MCT or the intake of LA (C18:2n-6) and ALA (C18:3n-3) under the conditions of this study. Also, the MCT supply with the lipid emulsion seems not to influence the relative incorporation of the different n-6 and n-3 fatty acids into plasma PLs. For the n-6 series, most of the tracer is found in LA (C18:2n-6), whereas in the n-3 series, the distribution is skewed toward DHA (C22:6n-3), reflecting the fact that LA (C18:2n-6) is the most abundant n-6 fatty acid, whereas DHA shows the highest percentage contribution of the n-3 fatty acids. Of importance, the tracer distribution depends on the relative incorporation of individual fatty acids in PLs but does

not indicate a higher relative LC-PUFA synthesis in the n-3 series.

Given no detectable differential effect of the emulsions on the relative conversion of precursors to LC-PUFAs, the trend toward higher LC-PUFA values in the MCT/LCT group appears to result from reduced LC-PUFA oxidation. In animal studies, dietary LC-PUFAs are oxidized to a limited extent but are preferentially incorporated into structural lipids and oxidized to a lower extent than other fatty acids (saturated, monounsaturated, LA, ALA) (45, 46). We presume that the parenteral MCT supply has effectively reduced LC-PUFA oxidation and, thereby, induced the trend toward increased LC-PUFA contents in plasma lipids.

In contrast to LC-PUFAs, the EFA intermediate γ-linolenic acid (C18:3n-6) showed significantly higher contents in PLs of the control group, which was also found after longer infusion periods in adults (42) but not after 5 days of lipid infusion in neonates (28). We could not determine tracer contents in γ-linolenic acid (C18:3n-6) in the small samples available from these preterm infants and thus can only speculate that a rapid exchange between the relatively large LA (C18:2n-6) pool and the relatively small γ-linolenic acid (C18:3n-6) pool might be the underlying metabolic cause.

We conclude that the use of the MCT/LCT emulsion in parenteral nutrition of preterm infants for a period of 8 days is well tolerated and provides equivalent carnitine, vitamin E, and EFA status compared with the LCT emulsion. The concentration of the functionally important n-3 fatty acid DHA (C22:6n-3) was higher in plasma TG of the MCT/LCT group, and there is also a trend toward higher levels of other LC-PUFAs in TG and PL. Because the availability of LC-PUFAs, and particularly of DHA (C22:6n-3), was shown to be of great functional importance in early life for the development of visual acuity (7) and cognitive development (6), the use of the MCT/LCT emulsion might provide important clinical benefits over the use of a standard soybean oil emulsion in these patients.

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REFERENCES

1. Koletzko, B. 2002. Intravenous lipid infusion in infancy—physiological aspects and clinical relevance. *Clin. Nutr.* **21 (Suppl.):** 53–65.
2. Koletzko, B., C. Agostoni, S. E. Carlson, T. M. Clandinin, G. Hornstra, M. Neuringer, R. Uauy, Y. Yamashiro, and P. Willatts. 2001. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta Paediatr.* **90:** 460–464.
3. Adolph, M. 1999. Lipid emulsions in parenteral nutrition. *Ann. Nutr. Metab.* **43:** 1–13.
4. Halliwell, B., and S. Chirico. 1993. Lipid peroxidation: its mechanism, measurement and significance. *Am. J. Clin. Nutr.* **57 (Suppl.):** 715–724.
5. Spielmann, D., U. Bracco, H. Traitler, G. Crozier, R. T. Holman, M. Ward, and R. Cotter. 1988. Alternative lipids to usual omega 6 PUFAS: gamma-linolenic acid, alpha linolenic acid, stearidonic acid, EPA etc. *J. Parenteral. Enteral. Nutr.* **12 (Suppl.):** 111–123.
6. O'Connor, D. L., R. Hall, D. Adamkin, N. Auestad, M. Castillo, W. E. Connor, S. L. Connor, K. Fitzgerald, S. Groh-Wargo, E. Hartmann, et al. 2001. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics.* **108:** 359–371.
7. SanGiovanni, J. P., S. Parra-Cabrera, G. A. Colditz, C. S. Berkey, and J. T. Dwyer. 2000. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics.* **105:** 1292–1298.
8. Salem, N., B. Wegher, P. Mena, and R. Uauy. 1996. Arachidonic and docohexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Natl. Acad. Sci. USA.* **93:** 49–54.
9. Szitanyi, P., B. Koletzko, A. Mydlilova, and H. Demmelmair. 1999. Metabolism of ^{13}C-labelled linoleic acid in newborn infants during the first week of life. *Pediatr. Res.* **45:** 669–673.
10. Metges, C. C., and G. Wolfram. 1991. Medium- and long-chain triglycerides labeled with ^{13}C: a comparison of oxidation after oral or parenteral administration in humans. *J. Nutr.* **121:** 31–36.
11. Rubin, M., A. Moser, N. Naor, P. Merloh, R. Pakula, and L. Sirota. 1994. Effect of three intravenously administered fat emulsions containing different concentrations of fatty acids on the plasma fatty acid composition of premature infants. *J. Pediatr.* **125:** 596–602.
12. Demmelmair, H., B. Iser, A. Rauh-Pfeiffer, and B. Koletzko. 1999. Comparison of bolus versus fractionated oral applications of [^{13}C]-linoleic acid in humans. *Eur. J. Clin. Invest.* **29:** 603–609.
13. Uauy, R., P. Mena, B. Wegher, S. Nieto, and N. Salem. 2000. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. *Pediatr. Res.* **47:** 127–135.
14. Millington, D. S., N. Kodo, D. L. Norwood, and C. R. Roe. 1990. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J. Inherit. Metab. Dis.* **13:** 321–324.
15. Fingerhut, R., W. Röschinger, A. C. Muntau, T. Dame, J. Kreischer, R. Arnecke, A. Superti-Furga, H. Troxler, B. Liebl, and B. Olgemöller. 2001. Hepatic carnitine palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific. *Clin. Chem.* **47:** 1763–1768.
16. Göbel, Y., C. Schaffer, and B. Koletzko. 1997. Simultaneous determination of low plasma concentrations of retinol and tocopherols in preterm infants by a high-performance liquid chromatographic micromethod. *J. Chromatogr. B.* **688:** 57–62.
17. Kolarovic, L., and N. C. Fournier. 1986. A comparison of extraction methods for the isolation of phospholipids from biological sources. *Anal. Biochem.* **156:** 244–250.
18. Carnielli, V. P., F. Pederzini, R. Vittorangeli, I. H. T. Luijendijk, W. E. M. Boomaars, D. Pedrotti, and P. J. J. Sauer. 1996. Plasma and red blood cell fatty acid of very low birth weight infants fed exclusively with expressed preterm human milk. *Pediatr. Res.* **39:** 671–679.
19. Demmelmair, H., F. Feldl, I. Horvath, T. Niederland, V. Ruszinko, D. Raederstorff, C. De Min, R. Muggli, and B. Koletzko. 2001. Influence of formulas with borage oil or borage oil plus fish oil on the arachidonic acid status in premature infants. *Lipids.* **36:** 555–566.
20. Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta.* **12:** 133–149.
21. Kramer, M. S., R. W. Platt, S. W. Wen, K. S. Joseph, and A. A. Allen. 2001. A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics.* **108:** E35–E41.
22. Behrman, R. E., R. M. Kliegman, and H. B. Jenson. 2000. Nelson Textbook of Pediatrics. W.B. Saunders Company, Philadelphia, PA.
23. Donnell, S. C., D. A. Lloyd, S. Eaton, and A. Pierro. 2002. The metabolic response to intravenous medium-chain triglycerides in infants after surgery. *J. Pediatr.* **141:** 689–694.
24. Shaffer, S. G., C. L. Quimiro, J. V. Anderson, and R. T. Hall. 1987. Postnatal weight changes in low birth weight infants. *Pediatrics.* **79:** 702–705.
25. Shaffer, S. G., S. K. Bradth, and R. T. Hall. 1986. Postnatal changes in total body water and extracellular volume in the preterm infant with respiratory distress syndrome. *J. Pediatr.* **109:** 509–514.
26. Pauls, J., K. Bauer, and H. Versmold. 1998. Postnatal body weight curves for infants below 100 g birth weight receiving early enteral and parenteral nutrition. *Eur. J. Pediatr.* **157:** 416–421.

27. Haumont, D., M. Richelle, R. J. Deckelbaum, and Y. A. Carpentier. 1993. Effect of liposomal content of lipid emulsions on plasma lipid concentrations in low birth weight infants receiving parenteral nutrition. *J. Pediatr.* **121:** 759–763.
28. Angsten, G., M. Boberg, G. Cederblad, S. Meurling, and H. Stiernström. 2002. Metabolic effects in neonates receiving intravenous medium-chain triglycerides. *Acta Paediatr.* **91:** 188–197.
29. Rovamo, L., E. A. Nikkila, M. R. Taskinen, and K. O. Raivio. 1984. Postheparin plasma lipoprotein and hepatic lipases in preterm neonates. *Pediatr. Res.* **18:** 1104–1107.
30. Genzel-Boroviczény, O., and R. Hroboticky. 1996. Plasma values of polyunsaturated fatty acids in extremely low birth weight (ELBW) infants fed breast milk or formula very early in life. *Eur. J. Med. Res.* **1:** 495–498.
31. Cairns, P. A., and D. J. Stalker. 2000. Carnitine Supplementation of Parenterally Fed Neonates. The Cochrane Library, Oxford, UK.
32. Souci, S. W., W. Fachmann, and H. Kraut. 2005. Die Zusammensetzung der Lebensmittel. Nährwerttabellen 1989/90. Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany.
33. Kaempf, D. E., and O. Linderkamp. 1998. Do healthy premature infants fed breast milk need vitamin E supplementation: α- and γ-tocopherol levels in blood components and buccal mucosal cells. *Pediatr. Res.* **44:** 54–59.
34. Carpentier, Y. A., C. Simoens, V. Siderova, I. El Nakadi, V. Vanweyenberg, D. Eggerickx, and R. J. Deckelbaum. 1997. Recent developments in lipid emulsions: relevance to intensive care. *Nutrition.* **13 (Suppl.):** 73–78.
35. Bach, A. C., and V. K. Babayan. 1982. Medium-chain triglycerides: an update. *Am. J. Clin. Nutr.* **36:** 950–962.
36. Bach, A. C., Y. Ingenbleek, and A. Frey. 1996. The usefulness of dietary medium-chain triglycerides in body weight control: fact or fancy? *J. Lipid Res.* **37:** 708–726.
37. Rodriguez, M., S. Funke, M. Fink, H. Demmelmair, M. Turini, G. Crozier, and B. Koletzko. 2003. Plasma fatty acids and [^{13}C]linoleic acid metabolism in preterm infants fed a formula with medium-chain triglycerides. *J. Lipid Res.* **44:** 41–48.
38. DeLany, J. P., M. M. Windhauser, C. M. Champagne, and G. A. Bray. 2000. Differential oxidation of individual dietary fatty acids in humans. *Am. J. Clin. Nutr.* **72:** 905–911.
39. Carnielli, V. P., E. J. Sulkers, C. Moretti, J. L. D. Wattimena, J. B. van Goudoever, H. J. Degenhart, F. Zacchello, and P. J. J. Sauer. 1994. Conversion of octanoic acid into long-chain saturated fatty acids in premature infants fed a formula containing medium-chain triglycerides. *Metabolism.* **43:** 1287–1292.
40. Larque, E., H. Demmelmair, B. Berger, U. Hasbargen, and B. Koletzko. 2003. In vivo investigation of the placental transfer of ^{13}C-labeled fatty acids in humans. *J. Lipid Res.* **44:** 49–55.
41. Chern, J. C., and J. E. Kinsella. 1983. The effects of unsaturated fatty acids on the synthesis of arachidonic acid in rat kidney cells. *Biochim. Biophys. Acta.* **750:** 465–471.
42. Martin-Pea, G., J. M. Culebras, L. de la Hoz-Perales, J. P. Barro-Ordovás, R. Catalá-Pizarro, and J. Ruiz-Galiana. 2002. Effect of 2 lipid emulsions (LCT versus MCT/LCT) on the fatty acid composition of plasma phospholipid: a double-blind randomized trial. *J. Parenteral. Enteral.Nutr.* **26:** 30–41.
43. Carnielli, V. P., K. Rossi, T. Badon, B. Gregori, G. Verlato, A. Orzali, and F. Zachello. 1996. Medium-chain triacylglycerols in formulas for preterm infants: effect on plasma lipids, circulating concentrations of medium-chain fatty acids, and essential fatty acids. *Am. J. Clin. Nutr.* **64:** 152–158.
44. Sprecher, H. 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta.* **1486:** 219–231.
45. Leyton, J., P. J. Drury, and M. A. Crawford. 1987. Different oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *Br. J. Nutr.* **57:** 383–393.
46. Leyton, J., P. J. Drury, and M. A. Crawford. 1987. In vivo incorporation of labeled fatty acids in rat liver lipids after oral administration. *Lipids.* **22:** 553–558.