Nutrition 29 (2013) 313–317

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Brain lipid composition in rabbits after total parenteral nutrition with two different lipid emulsions

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ARTICLE INFO

Article history:
Received 16 April 2012
Accepted 24 July 2012

Keywords:
Brain lipids
Lipid emulsions
Total parenteral nutrition

ABSTRACT

Objective: To study the changes occurring in brain lipid composition after the administration of total parenteral nutrition (TPN) by comparing two lipid emulsions, one with long-chain triacylglycerols (LCT) and the other with long-chain and medium-chain triacylglycerols (MCT/LCT 50%/50%).

Methods: We used 21 young New Zealand rabbits divided into three groups of seven animals each. Two groups were subjected to TPN for 7 d, with each group receiving using one of two different lipid emulsions: Intralipid 20% (group LCT) and Lipofundin MCT/LCT 20% (group MCT/LCT). The third control group received an oral diet and underwent the same surgical procedure with the administration of intravenous saline solution. The energy administered in the TPN formulas was non-protein 100 kcal · kg^{-1} · d^{-1}, with 40% corresponding to fats.

Results: There were modest increases in plasma cholesterol and triacylglycerols. In the brain tissue, there was a decrease of phosphatidylcholine in animals with TPN, which was greater in group LCT. There were no significant differences in the overall percentage distribution of brain fatty acids among the groups.

Conclusion: The lipid emulsions administered in TPN, especially those prepared exclusively with LCT, cause changes in the brain lipid polar fractions of young rabbits.

Introduction

The brain has a high lipid content, as much as 60% of its dry weight. The fatty acids (FAs) of the brain phospholipids, especially long-chain monounsaturated FAs, are responsible for maintaining the structure of membranes, whereas polyunsaturated FAs control their fluidity [1]. These FAs are subject to continual renewal. In fact, the changes in maternal dietary lipids in pregnancy or lactation cause changes in the FA profile of the brain of the offspring at birth and in early postnatal development [2,3]. Also, the adult brain can be influenced by specific exogenous FA contributions [4]. These changes involve not only the overall amount of polyunsaturated FAs administered but also the ω-6/ω-3 ratio [5].

Thus, dietary fat intake determines the FA composition of the brain and, hence, may influence neurologic function and learning ability, as has been demonstrated in some animals [6–8] and, less often, in humans [9]. Also, brain lipids may be altered in different pathologies [10,11].

The lipid emulsions used in artificial nutrition, as commonly applied in everyday clinical practice, are also a source of exogenous fat. However, there have been few studies on the changes in brain lipid composition after the use of artificial nutrition, except those by Martínez and Ballabriga [12] in newborns and infants and Maciá et al. [13] on fat overload in adult rabbits using Intralipid. Because parenteral nutrition including lipids is common therapeutic practice, its possible effects on brain

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http://dx.doi.org/10.1016/j.nut.2012.07.020

structure and function need to be better understood. We therefore studied the effects that parenteral nutrition with two types of fat and for a relatively short period might have on brain lipid composition in young rabbits.

Materials and methods

The animals used were 21 New Zealand rabbits (1700 ± 200 g) housed in individual cages at an ambient temperature of 21 ± 2°C, with an air-renewal rate of 14 changes per hour and light/dark cycles of 14/10 h. The animal handling followed the recommendations of the International Guiding Principles for Biomedical Research Involving Animals.

Experimental groups

One group ($n = 7$) received long-chain triacylglycerols (LCTs) and total parenteral nutrition (TPN) for 7 d through a silicone catheter (outer diameter 0.65 cm, inner diameter 0.30 cm; Dow Corning Corporation, Midland, MI, USA) implanted in the internal jugular vein to end in the interscapular zone and connected to the infusion line by a swivel. The lipid source given was Intralipid 20% (Kabi-Pfrimmer, Stockholm, Sweden).

Another group ($n = 7$) received 50% medium-chain triacylglycerols and 50% LCTs and TPN under the same conditions as group LCT, except that the lipid source used was Lipofundin MCT/LCT 20% (Braun Medical SA, Rubi, Barcelona, Spain; group MCT/LCT).

The control ($n = 7$) group underwent the same surgery as groups LCT and MCT/LCT, but a 0.9% NaCl solution instead of TPN was administered through the catheter.

The animals were provided food and water ad libitum, with the feed composition being 15% protein, 2.5% fats, 17% cellulose, 12% ash, 14% starch, and 2.45% minerals (BK Universal G.J. S.L., Tarragona, Spain).

Anesthesia

The animals were anesthetized with ketamine (Ketolar, Parke-Davis Laboratory, Morris Plains, NJ, USA) 25 mg/kg and phenothiazine (Combelen, Laboratories Bayer, Germany) 1 mg/kg, which were administered intramuscularly.

Artificial nutrition formulas

The two nutritional formulas administered to groups LCT and MCT/LCT contributed non-protein energy 100 kcal · kg^{-1} · d^{-1}, with 60% corresponding to glucose (Glucoibys 40%, Ibys, Madrid, Spain) and 40% to lipids. The lipid source used in group LCT was an emulsion of LCTs (Intralipid 20%, Kabi-Pfrimmer), and that in group MCT/LCT was a 50%/50% emulsion of LCTs and MCTs (Lipofundin MCT/LCT 20%, B. Braun Medical S.A.). The composition of the two lipid emulsions is presented in Table 1. The TPN administered to these two groups also contained amino acids 3 g · kg^{-1} · d^{-1} (Trophamine 6%, Farmiberia S.A., Madrid, Spain) plus standard amounts of electrolytes, trace elements, and vitamins. The final osmolality was 650 mOsm/kg. The infusion rate was 5 mL/kg of weight during the first 24 h and 10 mL/kg of weight during the next 7 d.

Biological controls

All animals were weighed at the beginning and at the end of the experiment. Daily evaluations were made of the animals' clinical status, and glycemia was quantified using test strips (Reflolux, Boehringer, Mannheim, Germany).

Processing of animals

At the end of the seventh day of the artificial nutrition, the animals were anesthetized, and a midline laparotomy was performed. Samples of blood 10 mL were collected by direct puncture of the vena cava. The brain tissue and cerebellum were extracted through a medial craniotomy and stored at −80°C until processing.

Laboratory analyses

A Hitachi 705 autoanalyzer (Hitachi Ltd, Osaka, Japan) was used to determine the plasma levels of glucose, urea, creatinine, total protein, albumin, cholesterol, triacylglycerols, total and direct bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, gamma glutamil transpeptidase, sodium, potassium, chloride, and lactate dehydrogenase.

Brains were homogenized in an OmniMixer apparatus (Omni, Waterbury, CT, USA), and lipids were extracted using the method of Folch et al. [14]. The extract was separated into polar and neutral lipids as described by Bitman et al. [15] using Sep-Pak Silica cartridges (Waters Chromat, Dic Millipire Co., Billerica, MA, USA).

Table 1
Composition of Intralipid 20% and Lipofundin MCT/LCT 20%

	Intralipid	Lipofundin MCT/LCT
Soy seed oil (g/100 mL)	20	10
MCTs (g/100 mL)	–	10
Egg yolk phospholipids (g/100 mL)	1.2	1.2
Lecithin (g/100 mL)	2	1.2
Glycerol (g/100 mL)	2.25	2.5
Phosphorus (mmol/L)	15	15
Water (mL)	100	100
Osmolarity (mOsm/L)	330	380
Energy (kcal/L)	2000	1908
Fatty acids (%)		
Caproic acid (C-6)	–	0.076
Caprylic acid (C-8)	–	25.71
Capric acid (C-10)	–	20
Lauric acid (C-12)	–	1.14
Myristic acid (C-14)	0.07	–
Palmitic acid (C-16)	21.31	7.14
Palmitoleic acid (C-16:1)	0.22	–
Stearic acid (C-18)	9.64	2.38
Oleic acid (C-18:1)	29.42	13.33
Linoleic acid (C-18:2 ω-6)	32.97	25.71
Linolenic acid (C18:3 ω-3)	4.21	3.81
Arachidic acid (C-20)	0.1	–
Arachidonic acid (C-20:4 ω-6)	0.1	–

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

Polar lipid analysis

The dry polar lipid extract was re-dissolved in chloroform and analyzed by thin-layer chromatography using as the mobile phase a chloroform–methanol–water–acetic acid (60:30:6:1) mixture for 60 min. The plates were developed using a 10% molybdic acid solution, with oven heating at 120°C for 30 min. The spots were semiquantified by laser densitometry using a Shimadzu DR-13 CS-9000 plate reader and integrator (Shimadzu, Kyoto, Japan). Assays were made of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and palmitoyl cerebroside.

FA brain analysis

The brain FAs were extracted and methylated according to the technique of Lapage and Roy [16], a method of extraction, methylation, and esterification in one step. The methyl esters extracted were assayed using a Carlo Erba GC 6000 Vega Series gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a 50-m capillary column of polyamide-coated fused silica of 0.22 mm internal diameter (50QC2/BP×70, SGE International Pty Ltd, Victoria, Australia) and using heptadecanoic acid (C-17) as the internal standard. The data were autoprocessed in an HP-3394A integrator (Hewlett Packard, Avondale, PA, USA). The chromatographic conditions used were an injector temperature of 250°C, a detector temperature of 260°C, and an oven temperature programmed with an initial isothermal of 195°C for 5 min, followed by a gradient of 3°C/min to 250°C that remained at this temperature for 10 min. Assays were made of myristic, palmitic, palmitoleic, stearic, oleic, vaccenic, linoleic, linolenic, arachidic, icosatrienoic, dihomo-γ-linolenic, arachidonic, behenic, docosatetraenoic, docosahexaenoic, nervonic, and cerotic acids.

Statistical analyses

The means and standard deviations of all parameters were determined. The non-parametric two-way analysis of variance using the Friedman rank sums was used to compare within-group final and initial values of weight and blood biochemistry parameters. For intergroup comparisons of brain fat among the LCT, MCT/LCT, and control groups, the non-parametric Kruskal–Wallis was used and differences among the groups were calculated with the Dunn approximation [17,18]. $P < 0.05$ was set as the criterion for statistically significant differences.

Results

The mean weight loss of the groups receiving TPN was not significant compared with that of the control group (Table 2).