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PARENTERAL STRUCTURED TRIGLYCERIDE EMULSION Safety, tolerance, and effects on nitrogen balance and immunology **Joanna Kruimel**

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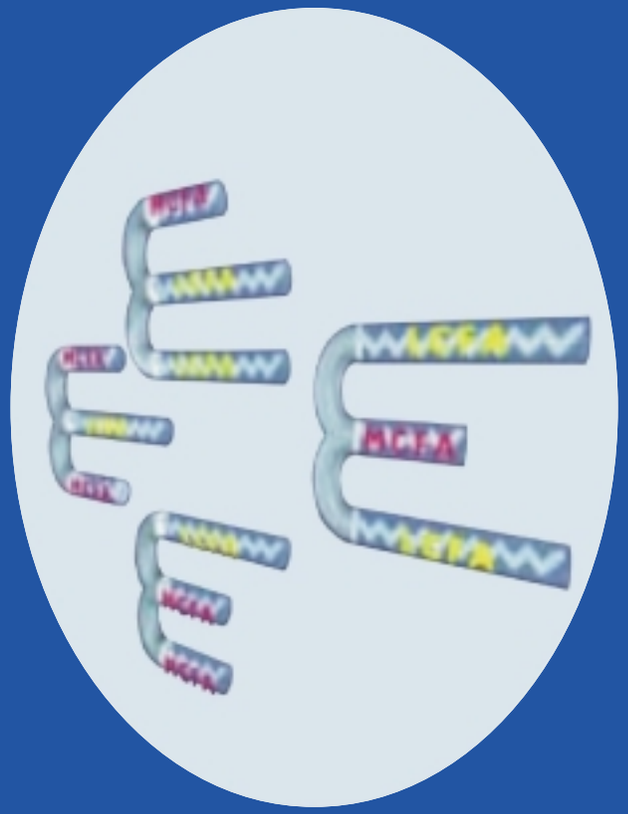

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Een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

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volgens besluit van het College van Decanen
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door

Joanna Wilhelmina Kruimel
geboren op 14 mei 1960 te Amsterdam

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Trefwoorden: total parenteral nutrition; lipid emulsions; long-chain triglycerides; physical mixture of medium- and long-chain triglycerides; structured triglycerides; surgery; nitrogen balance; metabolism; immunology; Mononuclear Phagocyte System function; chemiluminescence; cytokines.

Niet in de scholen, neen, heb ik gevonden,
En van geleerden, och, weinig geleerd;
Wat ons de wijzen als waarheid verkonden,
Straks komt een wijzer, die 't wegredeneert.

Zelf moet gij 't zoeken en zelf moet gij 't vinden,
Mensch, in uw hart, in het Woord, in uw lot;
Anders zoo spelen de wervlende winden,
Mensch, met uw hart, uw geloof en uw God.

1857, P.A. de Génestet (Geschenk van mijn vader, ± 1985)

De Génestet PA: Complete gedichten. Amsterdam, De maatschappij voor goede en goedkoope lectuur, 1910.

Aan mijn moeder,
ter nagedachtenis aan mijn vader,
“For auld lang syne”

ABSTRACT

Parenteral structured triglyceride emulsion: Safety, tolerance, and effects on nitrogen balance and immunology.
PhD thesis by Joanna Kruimel, Department of Gastroenterology and Hepatology, University Medical Center Nijmegen, The Netherlands, October 6th 2004.

Total parenteral nutrition is complete nutrition intravenously administered to patients, incapable of adequate food absorption. An important component in this total parenteral nutrition is the parenteral lipid emulsion. We studied a newly synthesized parenteral lipid emulsion: structured triglycerides. Structured triglycerides consist of triglycerides where the medium-chain and long-chain fatty acids are at random attached to the same glycerol backbone. The aim of our study was to investigate safety, tolerance, and effects on nitrogen balance and immunology of structured triglycerides.

We performed a randomized, double-blind trial on 25 moderately catabolic patients, comparing structured triglycerides with a physical mixture of medium-chain and long-chain triglycerides, as part of 5 days post-operative parenteral feeding. No difference in safety and tolerance between the two emulsions was observed. In the patients who completed the study, the mean cumulative nitrogen balance over the first 5 post-operative days was significantly better in the structured triglyceride group (-8 ± 2 g in 10 patients on structured triglycerides and -21 ± 4 g in 9 patients on the physical mixture, $p = .015$). Structured triglycerides were cleared faster from the blood on the first post-operative day, resulting in lower serum triglyceride levels and lower plasma medium-chain free fatty acid levels, compared with the physical mixture.

The effect of structured triglycerides on immunology was studied by measuring the phagocytosing capacity of the mononuclear phagocyte system and the production of cytokines and oxygen radicals. The phagocytosing capacity of the mononuclear phagocyte system (MPS) was studied in 13 patients by measuring the clearance of 99mTc-S-colloid from the blood and did not change during infusion of structured tiglycerides or the physical mixture. The cytokine production was studied in 18 patients: the effect of surgery overwhelmed the effect of nutrition, making conclusions on the effect of the lipid emulsions on cytokine production impossible. Instead, we demonstrated a downregulation of production and release of pro-inflammatory cytokines during surgery. Finally, in-vitro stimulated polymorphonuclear leukocytes from 10 volunteers pre-incubated with the physical mixture showed higher levels of oxygen radicals ($p < .005$) and faster production of oxygen radicals ($p < .005$) measuring chemiluminescence, compared with polymorphonuclear leukocytes pre-incubated with structured triglycerides or long-chain triglycerides. High levels of oxygen radicals can be detrimental in case

oxygen radicals play a pathogenic role or beneficial when rapid phagocytosis and killing of bacteria is needed.

In conclusion, parenteral structured triglyceride emulsion was a safe and well-tolerated emulsion, and had a better nitrogen balance and was cleared faster from the blood than the physical mixture. In-vivo phagocytosing capacity of the MPS was not impaired by structured triglycerides or the physical mixture. The interaction of the physical mixture with immune cells differed quite strongly from that of structured triglycerides and long-chain triglycerides, with respect to chemiluminescence. Studies on the clinical relevance of these immunologic observations are needed.

CONTENTS

Chapter 1

AIM OF THE THESIS

Total parenteral nutrition is nutrition composed of all the essential nutritional components: lipids, carbohydrates, amino acids, minerals, trace elements and vitamins, and is administered intravenously to patients. This technique is used in the treatment of patients with gastrointestinal diseases and, on a large scale, in the treatment of post-operative patients and patients on Intensive Care Units, in case the gastrointestinal tract is temporarily incapable of adequate food absorption.
Moreover, home parenteral nutrition is administered to patients with longstanding malabsorption or dysmotility of the small bowel. They depend on parenteral nutrition for their intake of calories and nutrients. The most common cause of malabsorption in this group is the short bowel syndrome after partial small bowel resection, characterized by weight loss, dehydration and deficiencies of minerals, trace elements and vitamins.
The aim of this study was to investigate the clinical relevant properties of safety and tolerance, and the effects on nitrogen balance and immunology, of a newly synthesized parenteral lipid emulsion: structured triglycerides. Based on animal experiments, we postulated a positive effect on nitrogen balance with a good tolerance and without severe complications. A positive effect of lipid emulsions on nitrogen balance suggests conservation of muscle mass and organ function, especially important in patients, who are more or less in a catabolic state. A modulating effect of lipid emulsions on immunological functions may have implications for patients, who often have infections and immunological disorders or undergo surgery.

Chapter 2

THE HISTORY OF LIPID EMULSIONS IN TOTAL PARENTERAL NUTRITION

First attempts to use the parenteral route of administration of nutrients

In 1628 William Harvey was the first to describe the general blood circulation (1). This concept leads to studies on the effect of intravenous administration of liquids, like wine and ale in dogs by Christopher Wren in 1665. Wren's study demonstrated that intravenously infused alcohol had the same effect as when alcohol was given orally (2).

In 1712 William Courten was the first to administer native (non-emulsified) olive oil intravenously to a dog. The dog died in severe respiratory distress, probably due to fat embolism in the lungs (3). Because of these side effects, Menzel and Perco infused relatively large amounts of fat subcutaneously into dogs and men in 1869, without detrimental effects (4). However, subcutaneous infusions by Paul Friedrich proved later, around 1900, to be too painful and parenteral infusion of lipids was temporarily discontinued (5).

Nevertheless, other solutions proved to be useful for intravenous treatment. During the cholera epidemic in England, in 1831, Thomas Latta successfully rehydrated patients with intravenously infused salt solution (6) and in 1873 Hodder treated cholera patients with intravenously infused fresh cow's milk with good result (7).

Carbohydrates and proteins

Development of parenteral nutrition continued in the field of carbohydrates and proteins. In 1859 Claude Bernard infused sugar solutions intravenously into animals (8) and in 1896 Biedl and Kraus were the first to administer 10 % glucose solutions intravenously to men (9). Research on carbohydrate infusion in man was continued by Woodyatt et al. in 1915. Glucose proved to be the most efficient carbohydrate and could be infused in doses up to 0.85 g of glucose/kg body weight/hour without glucosuria, an amount twice the normal energy requirement (10).

At the beginning of the twentieth century the importance of proteins for metabolism was understood. However, the first intravenous infusions of proteins caused serious allergic reactions. In 1906 Abderhalden and Rona administered a casein hydrolysate rectally to a boy and reported nitrogen equilibrium (11). And in 1913 Henriques and Andersen were the first to infuse a hydrolysate of beef into a goat, resulting in a positive nitrogen balance (12). But it lasted until 1937, when Robert Elman, known as "the father of intravenous nutrition", successfully infused protein hydrolysates intravenously into men (13).

Although, since the discoveries of Lister and Pasteur, bacteria-free solutions were used, infusions were often accompanied by fever. It lasted until the 1940's when it was discovered that this fever was caused by pyrogens and that pyrogen-free solutions should be administered. Pyrogens, consisting of endotoxins from bacteria, were already discovered in 1923 by Siebert (14).

The starting point of a wide-spread use of parenteral nutrition was the clinical introduction of the central venous catheter by Stanley Dudrick (1968), which made it possible to infuse large amounts of hypertonic glucose and protein hydrolysates into men (15). In the United States, parenteral nutrition consisted for a long time of only carbohydrates and amino acids; governmental permission for the use of lipid emulsions was not given for fear of possible toxic effects. But long-term use of glucose as the main energy source had disadvantages compared to a combination with lipid. Longterm use of glucose alone resulted in essential fatty acid deficiency characterized by growth retardation, poor wound healing, sparse hair growth and dry flaky skin or even scaling eczematoid dermatosis. Moreover, large glucose infusions resulted in hyperglycemia, hyperinsulinemia, increased secretion of catecholamines, hepatic steatosis, and increased formation of carbon dioxide resulting in respiratory problems (16,17,18).

Parenteral long-chain triglyceride emulsions and new lipid emulsions

Until the 1960's experimental intravenous lipid emulsions caused adverse reactions, like nausea, vomiting, back pain, fever and liver function disorders. By trial and error, Schuberth and Wretlind, produced in 1961 a safe intravenous lipid emulsion of soybean oil with egg yolk phospholipids as emulsifier (19,20). This soybean oil emulsion contains triglycerides with only long-chain fatty acids attached to the glycerol backbone and has a high content of essential fatty acids.

Efforts were made to further optimize this lipid emulsion. Research in animals revealed that medium-chain triglycerides have metabolic benefits compared with long-chain triglycerides (see Chapter 3). But long-chain triglycerides contain the essential fatty acids, needed to prevent essential fatty acid deficiency. Therefore, in the 1980's physical mixtures of both medium-chain triglycerides and long-chain triglycerides were introduced.

However, intravenously infused medium-chain triglycerides are metabolized to medium-chain free fatty acids, which may be toxic in higher dose (see Chapter 3). To further improve the safety of the use of medium-chain fatty acids, structured triglycerides were synthesized. Structured triglycerides consist of triglycerides, where the medium-chain and long-chain fatty acids are attached randomly to the same glycerol backbone. Structured triglycerides are the subject of this thesis.

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Chapter 3

STRUCTURED TRIGLYCERIDES

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Introduction

In 1961 Schuberth and Wretlind were the first to produce, by trial and error, a safe intravenous lipid emulsion of soybean oil with egg yolk phospholipids as emulsifier (1). This lipid emulsion contains triglycerides with only long-chain fatty acids (LCFA) attached to the glycerol backbone (Fig. 1, LCT).

In the 1980's an emulsion with a combination of medium-chain triglycerides (Fig. 1, MCT) and long-chain triglycerides was introduced. Intravenously administered triglycerides are hydrolyzed by lipoprotein lipase to free fatty acids and glycerol or monoglycerides. Free fatty acids are taken up by the cells and oxidized in the mitochondria. The uptake of long-chain fatty acids in the mitochondria is carnitine-dependent, but the uptake of medium-chain fatty acids does not require carnitine. This is important because carnitine levels are decreased in critically ill patients, making medium-chain free fatty acids (MCFA) a rapidly accessible energy source for these patients. Medium-chain fatty acids are taken up faster by the mitochondria and are oxidized faster compared with long-chain fatty acids.

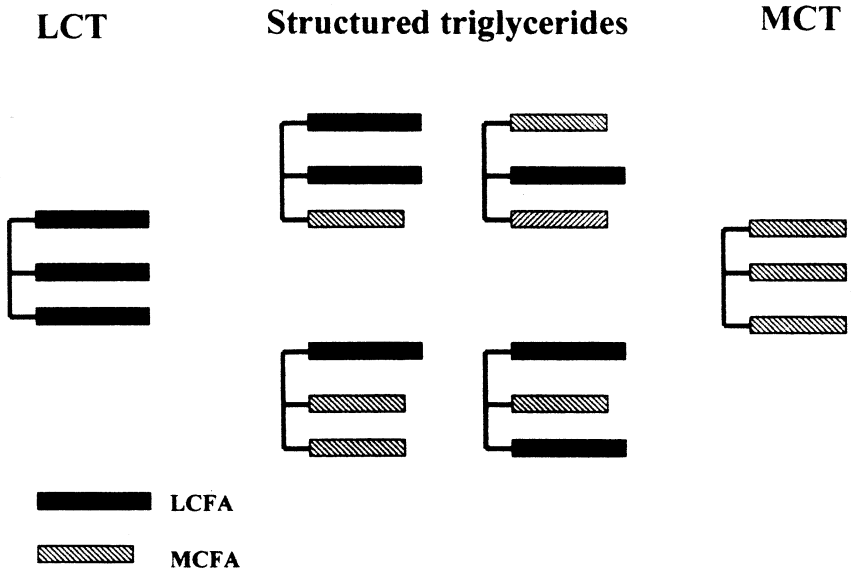

Figure 1. *The molecular structure of long-chain triglycerides (LCT), structured triglycerides, and medium-chain triglycerides (MCT). A physical mixture of medium-chain and long-chain triglycerides consists of triglycerides that contain either three medium-chain fatty acids (MCFA) or three long-chain fatty acids (LCFA). A structured triglyceride emulsion consists of triglycerides where the medium- and long-chain fatty acids are randomly attached to the same glycerol molecule.*

Medium-chain triglycerides

High plasma levels of medium-chain free fatty acids, the metabolic product of medium-chain triglycerides, may cause adverse reactions, such as metabolic acidosis due to higher production of ketone bodies (2) and increased energy expenditure (3). In dogs, high plasma medium-chain free fatty acid levels, in conjunction with high lactate and ketone body concentrations cause serious neurological toxicity (4).

However, previous studies with physical mixtures of medium-chain and long-chain triglycerides suggested that medium-chain triglycerides have metabolic benefits compared with long-chain triglycerides, especially in animals. In humans nitrogen balance improved in 5 out of 9 reported clinical trials comparing physical mixtures with long-chain triglyceride emulsions (5-9); in the other 4 trials no significant difference was found (10-13) (Table I). Medium-chain triglycerides are metabolized

Table I. *Summary of the published controlled clinical trials on nitrogen balance with a physical mixture of medium-chain and long-chain triglycerides (MCT/LCT) versus long-chain triglycerides (LCT).*

MCT/LCT vs. LCT	Patients, number	Effect
Lai	pediatric surgical 38	significant better nitrogen balance from day 3, 14 days
Jiang	surgical patients 12	trend toward better nitrogen balance, 7 days
Ball	critically ill 20	significant better nitrogen balance on 6th and 9th day
Dennison	likely to require TPN for a minimum of 10 days 15	significant better nitrogen balance cross-over, 2 x 5 days
Lünstedt	after elective colon surgery 20	significant better nitrogen balance, 5 days
Nijveldt	critically ill surgical 20	no difference in nitrogen balance, 5 days
Herrmann	after colorectal surgery 29	no difference in protein balance
Ball	malnourished patients 24	no difference in daily nitrogen balance, 6 - 28 days
Calon	head trauma 24	no difference in nitrogen balance, 1 day

more rapidly and stored less in tissues (14). This may be explained by the observation that their uptake by the mitochondria, where oxidation takes place, is mainly independent of carnitine (14).
In the physical mixtures of medium-chain triglycerides (MCT) and long-chain triglycerides (LCT), triglyceride molecules contain three medium-chain fatty acids or three long-chain fatty acids, attached to one glycerol molecule (Fig. 1).

Structured triglycerides

Structured triglycerides were synthesized to improve lipid emulsions by combining medium-chain and long-chain fatty acids in one triglyceride molecule. The newly synthesized structured triglycerides consist of triglycerides where the medium- and long-chain fatty acids are randomly attached to the same glycerol backbone (Fig. 1).
In catabolic animals, parenteral administration of emulsions of structured triglycerides, led to improved nitrogen balance (15,16,17), increased hepatic protein synthesis (16,17) and serum albumin levels (16), and decreased leucine oxidation (15,17), compared with emulsions of a physical mixture of medium- and long-chain triglycerides.

Safety and tolerance

In 1993 Sandström et al. were the first to show that structured triglycerides (Structolipid [R], Pharmacia, now Fresenius-Kabi) are as safe and well-tolerated as a conventional long-chain triglyceride emulsion (Intralipid[R], Fresenius-Kabi) in post-operative patients; in particular there were no signs of keto-acidosis or central nervous system toxicity (18).

Nitrogen balance

The question has been put if structured triglyceride emulsions further improve nitrogen balance compared with physical mixtures of medium-chain and long-chain triglycerides in humans.
In recently published studies in patients, parenteral administration of emulsions of structured triglycerides led to improved nitrogen balance compared with long-chain triglyceride emulsions (19,20).
When we performed our clinical trial in post-operative patients treated with structured triglycerides or with a physical mixture (see Chapter 5), no data were available on nitrogen balance in patients comparing these two lipid emulsions.

Serum triglycerides

In a second study in post-operative patients, Sandström et al. (21) demonstrated lower serum triglyceride levels of structured triglycerides compared with long-chain triglycerides leading to a reduced risk of fat overload. Whole body lipid

oxidation rate of structured triglycerides was increased, suggesting higher utilization of the structured triglycerides. Nordenström et al. (22) showed in healthy volunteers reduced levels of serum triglycerides with structured triglycerides compared with long-chain triglycerides.
No data were available on serum triglyceride levels in patients on structured triglycerides compared with a physical mixture of medium-chain and long-chain triglycerides, when we conducted our clinical trial in post-operative patients with these two lipid emulsions (see Chapter 5) .

Plasma medium-chain free fatty acid levels
A study with structured triglycerides in healthy volunteers showed lower plasma levels of medium-chain free fatty acids and the structured triglycerides were cleared faster from the blood compared with a physical mixture (23,24).
No data were available on plasma levels of medium-chain free fatty acids in patients on structured triglycerides compared with a physical mixture of medium-chain and long-chain triglycerides, during the work on our clinical trial with these two lipid emulsions (see Chapter 5).

Clinical trial
A clinical trial was designed to compare the nitrogen balance in post-operative patients treated with structured triglycerides or with a physical mixture of medium- and long-chain triglycerides. At the same time, serum levels of triglycerides and plasma levels of medium-chain free fatty acids were measured during infusion of the structured triglycerides to get more insight in clearance of medium-chain free fatty acids.

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Chapter 4

INTRAVENOUS LIPID EMULSIONS AND LEUKOCYTE FUNCTION: A REVIEW

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Introduction

Since their introduction in clinical practice, the possible effects of lipid emulsions on the function of the human immune system have remained the subject of debate. In a large study of 395 malnourished patients requiring laparotomy or noncardiac thoracotomy, more infectious complications were seen in the group receiving peri-operative total parenteral nutrition including long-chain triglycerides compared with the control group receiving no peri-operative parenteral nutrition (1). These infections mainly comprised pneumonia or empyema and were not central venous catheter related. The increased rate of infections was confined to borderline or mildly malnourished patients. Severely malnourished patients who received peri-operative parenteral nutrition had fewer non-infectious complications and no increased rate of infectious complications compared with controls. The authors concluded that peri-operative parenteral nutrition should be limited to a selected group of severely malnourished patients. According to this study a possible explanation for the increased rate of infections in the parenteral nutrition group is the effect of the lipid component of parenteral nutrition on leukocyte function.

Lipid emulsions with long-chain triglycerides have been reported to impair leukocyte function (2). Long-chain triglycerides may have a direct effect on leukocytes, but also supply the essential fatty acids. The essential fatty acids are two groups of polyunsaturated fatty acids, ω-3 and ω-6 fatty acids, which cannot be manufactured by human metabolic pathways. They are precursors for inducers of inflammation or immune suppression (prostaglandins, prostacyclins, thromboxanes, leukotrienes) (3). In contrast, the use of lipid emulsions indirectly can also have a beneficial effect on host defence. Lipid emulsions decrease the amount of carbohydrate solutions needed to provide non-protein energy, resulting in a reduced risk of hyperglycemia. Hyperglycemia in itself can increase the susceptibility to infections (4).

Neutrophil functions

When an acute inflammatory reaction starts, leukocytes (firstly polymorpho-nuclear leukocytes and later monocytes) are attracted to the site by chemotactic agents, in a process called chemotaxis. In in-vitro experiments complement C5a, activated serum and fMLP (formylated bacterial peptide) are the most common chemotactic agents used. In contrast, random migration is the motility of leukocytes in the absence of chemotactic agents.

The next step in the inflammatory response is phagocytosis: ingestion of bacteria by leukocytes. This function of cells can be studied in in-vitro experiments by the use of fluorescent polystyrene microspheres, fluorescent yeast particles or bacteria. In-vivo, clearance of bacteria or of radioactively labeled Tc-Sulfur colloid from the blood stream is used to measure the efficiency of phagocytosis.

Using bacteria, bacterial killing by the leukocytes can be measured. Production of oxygen radicals is necessary for adequate bacterial killing. Oxygen radical production can be measured in assays using chemiluminescence or reduction of nitroblue tetrazolium (NBT).

Random migration

Wiernik et al. (5) found that in-vitro incubation of polymorphonuclear leukocytes (PMN's) with Intralipid 20% (20-100 mg/mL) decreased random migration. Incubation of monocytes with Intralipid did not have an effect on random migration. Nordenström et al. (6) could not show a change in random migration of leukocytes after in-vitro incubation with Intralipid 20% in a triglyceride concentration up to 11,5 mmol/L. But in this study also healthy volunteers were infused with Intralipid 20% for 2 hours at a rate of 50 mL/h, 100 mL/h and 200 mL/h; during infusion the leukocytes showed an impairment of random migration and a correlation was obtained between the dose rate and the effect on random migration. Twenty-two hours after infusion the effect on random migration had disappeared. Herson et al. (7) could not find an effect of Intralipid up to 100 mg/mL on neonatal PMN's in-vitro. In-vivo, Intralipid was infused at 1 g/kg over 16 hours to ill neonates; no effect on random migration of PMN's was reported. Kohelet et al. (8) and Bellatini-Pires (9) reported no changes in random migration of PMN's, after in-vitro incubation with long-chain triglycerides. In the last study random migration was decreased, after incubation with a physical mixture of medium- and long-chain triglycerides (9). Usmani et al. (10) showed an increase in random migration of PMN's of neonates after Intralipid infusion.

Chemotaxis

Nordenström et al. (6) reported decreased chemotaxis of leukocytes after in-vitro incubation with Intralipid 20% in a triglyceride concentration up to 11,5 mmol/L. A linear correlation was observed between triglyceride concentration and chemotaxis. The highest concentration of 11,5 mmol/L Intralipid triglyceride inhibited chemotaxis by 40%. Their infusion studies in healthy volunteers showed decreased leukocyte chemotaxis at dose rates of 50 mL/h to 200 mL/h. Again a correlation between dose rate and inhibition of chemotaxis was demonstrated. Twenty-two hours after infusion the effect on chemotaxis had disappeared. Other authors reported a decrease in chemotaxis of PMN's (5,8,11,12) after incubation with LCT's. Only Palmblad et al. (13) found an increase in chemotaxis of PMN's stimulated by serum of volunteers given 500 mL Intralipid 10% for six hours. Wiernik et al. (5) reported an increase in chemotaxis of monocytes in-vitro incubated with Intralipid 20 to 60 mg/mL. At higher concentrations, of 100 mg/mL, chemotaxis decreased again. Some studies could not find an effect of LCT's

on chemotaxis of PMN's in-vitro (7,9,14,15) or in-vivo (7,14,16,17). Usmani et al. showed an increase of chemotaxis of PMN's of neonates after in-vitro incubation with Intralipid (15) and infusion of Intralipid (10). In one study of Bellatini-Pires (9) chemotaxis was decreased, after incubation with a physical mixture of medium- and long-chain triglycerides.

Phagocytosis and bacterial killing

Wiernik et al. (5) tested the attachment and ingestion of fluorescein labeled yeast particles by leukocytes. After incubation with Intralipid 20% up to 100 mg/mL monocytes in suspension showed a decreased number of attached yeast particles per cell but an increased number of ingested yeast particles per cell. In the same test PMN's in suspension showed the opposite; the number of attached yeast particles per cell was increased and the number of ingested yeast particles was decreased. Bellatini-Pires (18) reported that in-vitro incubation of PMN's with Intralipid reduced phagocytosis and bacterial killing. Jarstrand et al. (19) showed that Intralipid reduced bacterial killing after infusion in patients and in-vitro. Ota et al. (17) evaluated the effect of Intralipid on malnourished patients who received TPN pre-operatively; they found no effect on phagocytosis and bacterial killing of PMN's, using an assay with Staphylococcus aureus. Palmblad et al. (13) gave Intralipid 10% 500 mL in 6 hours to volunteers and saw no effect on PMN bacterial killing also using an assay with Staphylococcus aureus.

Chemiluminescence

PMN's respond to various stimuli with a "metabolic burst" of oxygen radical production, necessary for bacterial killing. By measuring chemiluminescence oxygen radical production can be assessed. PMN's are incubated with a stimulant (concanavalin A for nonphagocytic stimulation and opsonized Zymosan for phagocytic stimulation) and Luminol, which produces light emission in the presence of oxygen radicals. The samples are then counted in a scintillation spectrophotometer.

In the experiments of Palmblad et al. (13) an increase in chemiluminescence of PMN's of healthy volunteers was observed after intravenous infusion of long-chain triglyceride emulsions. In the study of Robin (20), pre-incubation of whole blood from healthy volunteers with Intralipid up to a triglyceride concentration of 400 mg/L did not adversely influence chemiluminescence. However when chronically ill patients were given 500 mL Intralipid in 4 to 6 hours chemiluminescence of PMN's was decreased. Jarstrand et al. (21) showed that in-vitro incubation of PMN's with Intralipid decreased chemiluminescence. Usmani et al. showed that in-vitro incubation of PMN's of neonates with Intralipid had no effect on chemiluminescence (15); after infusion of Intralipid in neonates, chemiluminescence was increased (10).

Nitroblue tetrazolium activity

The nitroblue tetrazolium (NBT) test determines the ability of PMN's to generate superoxide, necessary for bacterial killing, by measuring the reduction of NBT. Ingesting phagocytes convert the yellow soluble dye to an insoluble dark blue precipitate in the phagocytic vacuole.

Wiernik et al. (5) reported a significant decrease in NBT activity of PMN's in vitro after incubation with Intralipid 20% 20 mg/mL to 100 mg/mL; the NBT reduction of monocytes did not change significantly in the in-vitro incubation experiments. However the infusion experiments in healthy volunteers (20% Intralipid for 2 hours at a rate of 100 mL/h) showed that the NBT reduction of monocytes "at rest" as well as on stimulation with E. coli increased significantly. Jarstrand et al. showed that Intralipid decreased NBT reduction after infusion in patients (19) and in-vitro (19,21). Bellatini-Pires reported that in-vitro incubation of PMN's with Intralipid did not effect NBT reduction, but incubation with a physical mixture decreased NBT reduction (18). Strunk et al. (22) did not find a change in NBT reduction of peritoneal macrophages from mice treated with Intralipid 5 mg/kg up to two weeks.

In-vivo clearance of bacteria and Technetium-sulfur colloid

Fisher et al. (11) found an impairment of bacterial clearance (group B streptococcus) in mice given intraperitoneal injections of Intralipid. Sobrado et al. (23) found that the capacity of bacterial clearance (Pseudomonas aeruginosa P4) in healthy and burned guinea pigs was unaffected by the quantity of lipids administered intravenously and the type of lipid emulsion (long-chain triglycerides, medium-chain triglycerides, structured triglycerides) given. But when 75% or more of the non-protein calories was given as long-chain triglycerides to burned guinea pigs (which is not a physiological dose), a different pattern of bacterial clearance was observed compared to the guinea pigs given less of their non-protein calories as long-chain triglycerides. The sequestration of bacteria was reduced in liver and spleen and markedly increased in the lungs. When medium-chain triglycerides or structured triglycerides were given in the same dose, this change in pattern of bacterial clearance was not seen. These data were confirmed by Hamawy et al. (24), who showed that rats treated with medium-chain triglycerides were not bacteremic, three days following a septic injury in contrast to the animals treated with long-chain triglycerides. Moreover the medium-chain triglyceride-treated animals sequestered more bacteria in the liver and less in the lung compared to the long-chain triglyceride-treated animals.

Seidner et al. (25) and Jensen et al. (26) studied the effects of total parenteral nutrition (TPN) with long-chain triglycerides in patients at 0,13 g/kg/h over 10 hours for each of 3 days; there was a significant decline in Technetium-sulfur col-

loid (TSC) clearance rate by the mononuclear phagocyte system. This dose of lipid emulsion is rather high. In contrast, in patients given long-chain triglycerides as a continuous infusion or in patients given a physical mixture of medium-chain and long-chain triglycerides intermittently this decline was not seen (26).

Recent developments

In recent years a number of experimental studies have been published, which suggest that lipid structure, and especially carbonchain-length, is of pivotal importance in the interaction of nutritional triglycerides and the various components of the immune system (27). Earlier studies on the effect of lipids on immune function in PMN's are included in this thesis and successively Wanten has continued these studies. Briefly, the effects of emulsions containing medium-chain triglycerides markedly contrast with those of emulsions containing long-chain triglycerides, structured triglycerides, or emulsions based on olive- or fishoil. For instance, it was found that the production of toxic oxygen radicals by human PMN's is induced by medium-chain triglycerides in the absence of any other stimulus, while long-chain triglycerides and structured triglycerides completely lack such effects (28). Also, the expression of cell surface adhesion molecules as well as neutrophil degranulation, which indicate cell activation, are induced by medium-chain triglycerides, but not by long-chain triglycerides or structured triglycerides (29). These observations were done at clinically relevant lipid concentrations well below 5 mmol/L.

That this activation by medium-chain triglycerides might well be an 'inappropriate' stimulus is suggested by the observation that medium-chain triglycerides, but not long-chain triglycerides or structured triglycerides, impair neutrophil locomotion and killing of the fungal pathogen Candida albicans (30,31). These in-vitro data have been corroborated by a study in human healthy volunteers in which intravenous administration of a physical mixture of medium- and long-chain triglycerides, but not long-chain triglycerides or placebo (saline), modulated the production of cytokines by mononuclear cells in a manner that suggests the introduction of an imbalance between various populations of T helper-cell lymphocytes. Such disturbances of the immune response have been implicated in the development of fungal infections. Further evidence for an increased risk of fungal infections was found, when the growth of Candida was shown to be increased in serum of patients after physical mixture administration, while long-chain triglycerides and placebo did not display any effect.

Possible mechanisms behind these effects of medium-chain triglycerides include alterations in membrane fluidity and effects on calcium-mediated cell signaling (32,33,34). Membrane fluidity is a complex feature of biological membranes that refers to the degree of freedom of molecules in the outer cell membrane, which has

implications for the function of cell surface receptors. It was found that medium-chain triglycerides, but not long-chain triglycerides increase neutrophil membrane fluidity, while medium-chain fatty acid-containing structured triglycerides display an intermediate effect. Also, it was found that medium-chain triglycerides, but not long-chain triglycerides or structured triglycerides, influence the kinetics of the release of calcium from intracellular stores in PMN's in response to stimulation by opsonized yeast particles (serum-treated Zymosan) or the bacterial peptide fMLP.

Conclusion

The effect of intravenous administration of the three types of lipid emulsions on leukocyte function is still controversial. In-vitro studies concern mostly long-chain triglyceride emulsions and in general demonstrated inhibition of leukocyte function. Random migration (5), chemotaxis (5,6,8,11,12), phagocytosis (5,18), bacterial killing (18,19) and production of oxygen radicals (5,18,19,21) by PMN's was impaired by long-chain triglycerides. However, other studies could not find any impairment in PMN function by incubation with long-chain triglycerides (7,9,10,13-15,20). In-vitro, a physical mixture of medium- and long-chain triglycerides inhibited chemotaxis (9), random migration (9), phagocytosis (18) and bacterial killing (18); more studies on physical mixtures are necessary.

The results of infusion studies in volunteers and patients are contradictory and also mainly concern long-chain triglyceride emulsions. Nordenström et al. (6) found a dose rate dependent impairment of random migration and chemotaxis of PMN's by long-chain triglycerides in volunteers. Jarstrand et al. (19) and Robin et al. (20) reported a suppression of production of oxygen radicals by long-chain triglycerides, in the first study accompanied by a reduced bacterial killing (19). Other human infusion studies were not able to confirm suppression of PMN function by long-chain triglycerides (10,13,17). One study in volunteers during infusion of a physical mixture of medium- and long-chain triglycerides did not show any impairment of chemotaxis, adherence and phagocytosis by PMN's (35). TPN, with long-chain triglycerides intermittently given, caused a decline in phagocytotic capacity in patients (25,26); TPN, with a physical mixture of medium- and long-chain triglycerides intermittently given, caused no decline in phagocytotic capacity (26).

Wanten et al. found that the effects of emulsions containing medium-chain triglycerides markedly contrast with those of emulsions containing long-chain triglycerides or structured triglycerides: the production of toxic oxygen radicals by human PMN's was induced by medium-chain triglycerides in the absence of any other stimulus, and the expression of cell surface adhesion molecules as well as neutrophil degranulation (which indicate cell activation) were induced by medium-chain triglycerides, but not long-chain triglycerides or structured triglycerides (27).

In conclusion, some in-vitro and in-vivo studies show impairment of leukocyte function by long-chain triglyceride emulsions. Recent data from our group indicate that the interaction of medium-chain triglyceride emulsions with immune cells differs quite strongly from that of emulsions containing long-chain triglycerides or structured triglycerides. More studies on the effects of lipid emulsions on the immune system are needed, especially studies on the clinical relevance of the observations mentioned above.

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Chapter 5

PARENTERAL STRUCTURED TRIGLYCERIDE EMULSION IMPROVES NITROGEN BALANCE AND IS CLEARED FASTER FROM THE BLOOD IN MODERATELY CATABOLIC PATIENTS

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Abstract

Background Most post-operative patients lose net protein mass, which reflects loss of muscle tissue and organ function. Peri-operative parenteral nutrition may reduce the loss of protein, but in general, with conventional lipid emulsions a waste of protein still remains.

Methods We compared the effects on nitrogen balance of an emulsion containing structured triglycerides, a new type of synthesized triglycerides, with an emulsion of a physical mixture of medium- and long-chain triglycerides as part of parenteral feeding in moderately catabolic patients. The first 5 days after placement of an aortic prosthesis patients received total parenteral nutrition (TPN) providing 0.2 g of nitrogen per kg body weight per day; energy requirement was calculated using Harris and Benedict's equation, adding 300 kcal per day for activity. Twelve patients were treated with the structured triglyceride emulsion and 13 patients with the emulsion of the physical mixture of medium- and long-chain triglycerides. The design was a randomized, double-blind parallel study.

Results In the patients who completed the study, the mean cumulative nitrogen balance over the first 5 post-operative days was -8 ± 2 g in 10 patients on the structured triglyceride emulsion and -21 ± 4 g in 9 patients on the emulsion of the physical mixture of medium- and long-chain triglycerides; the mean difference was 13 g of nitrogen (95 % confidence interval 4 to 22, p = .015) in favor of the structured triglyceride emulsion. On the first post-operative day serum triglyceride and plasma medium-chain free fatty acid levels increased less during infusion of the structured triglyceride emulsion than with the physical mixture emulsion.

Conclusions The parenteral structured triglyceride emulsion improves the nitrogen balance and is cleared faster from the blood, compared with the emulsion of the physical mixture of medium- and long-chain triglycerides, in moderately catabolic patients.

Introduction

After major surgery, most patients become catabolic and lose net protein mass, resulting in loss of muscle tissue and organ function. Improvement of protein retention is important for conservation of muscle mass and organ function. Peri-operative parenteral nutrition may reduce the loss of protein (1) and improve outcome in severely malnourished patients (2,3), but with existing regimes a considerable waste of protein still remains.

Lipid emulsions are an important component of total parenteral nutrition (TPN). They supply energy and essential fatty acids. Soybean oil is the usual source of fat in parenteral fat emulsions and consists of long-chain triglycerides. Medium-chain triglycerides have been suggested as an alternative lipid source, because they improve nitrogen balance (4,5), are metabolized more rapidly and stored less in

tissues (6), and are oxidized mainly independently of carnitine (6). However, high plasma levels of medium-chain free fatty acids, the metabolic product of medium-chain triglycerides, may cause adverse reactions, such as metabolic acidosis due to higher production of ketone bodies (7) and increased energy expenditure (8). In dogs, high plasma medium-chain free fatty acid levels caused serious neurological toxicity (9). To reduce the amount of medium-chain triglycerides and to provide the essential long-chain fatty acids, the medium-chain triglycerides are administered together with long-chain triglycerides, as a physical mixture.

To improve the safety of medium-chain triglycerides, so-called *structured triglycerides* were synthesized. Structured triglycerides are produced by hydrolysis of soybean oil and coconut oil. Reesterification results in structured triglycerides with medium- and long-chain fatty acids at random attached to positions within the same glycerol molecule. An emulsion containing these structured triglycerides caused lower plasma levels of medium-chain free fatty acids and was cleared faster from the blood compared with an emulsion of a physical mixture of medium-chain and long-chain triglycerides, in healthy volunteers (10,11).

The question has been asked if this structured triglyceride emulsion improves nitrogen balance. In animals, parenteral administration of emulsions of structured triglycerides leads to improved nitrogen balance (12-14), increased hepatic protein synthesis (13,14) and serum albumin levels (13), and decreased leucine oxidation (12,14) compared with emulsions of a physical mixture of medium- and long-chain triglycerides.

The predefined objectives of the present clinical trial were to study, in post-operative, moderately catabolic patients, the influence on nitrogen balance and the safety and tolerance of intravenous (IV)-infused structured triglycerides (Structolipid) compared with that of a physical mixture of medium- and long-chain triglycerides (Lipofundin MCT/LCT 20%). The structured triglyceride emulsion has been compared with an emulsion of long-chain triglycerides, proven to be as safe and well tolerated (15), and associated with increased whole body fat oxidation in post-operative patients (16).

Materials and methods

Design

The design was a randomized, double-blind, parallel study. We excluded some important confounders. We chose for a homogeneous and stable group of patients (only elective surgery), and enteral nutrition was not permitted until the end of the study period. We included patients operated for placement of an aortic tube or bifurcation prosthesis because of an abdominal aortic aneurysm or atherosclerotic obstruction. Such patients make a homogeneous group, are moderately catabolic (17), and may require post-operative parenteral nutrition. We chose to administer

parenteral nutrition in all of these patients, although in clinical practice not all of these patients would need parenteral nutrition. Probably in some patients nutritional needs could be reached by tube feeding in the duodenum or jejunum.
We calculated the daily cumulative nitrogen balance for those patients who completed the 5-day study period. We assessed safety and tolerance in accordance with standard clinical practice. Serum levels of triglycerides and plasma levels of medium-chain free fatty acids were measured as an estimate of the clearance rate of the lipid emulsions from the blood. IV-administered triglycerides are hydrolyzed by lipoprotein lipase, producing long-chain and medium-chain free fatty acids.

Patients

The study was approved by the Human Ethics Committee of the University Hospital of Nijmegen, The Netherlands. Over a period of 20 months, 61 patients were admitted to the Department of Surgery for elective surgery. Placement of an aortic tube or bifurcation prosthesis, because of an abdominal aortic aneurysm or atherosclerotic obstruction was performed. Thirty-four patients were excluded because of either hypertriglyceridemia (9), treatment with lipid lowering-drugs (8), not giving written consent (7), treatment with corticosteroid hormones (5), renal disease (3), diabetes mellitus (2), age above 80 years (1), severe overweight (1), or fluid restriction (1). Three patients fulfilled two exclusion criteria.
Twenty-seven patients were informed by verbal and written consent, after which they were randomized. In none of the patients was significant weight loss observed before the hospitalization. Pre-operatively the patients were normally active. In all of these patients elective surgery was performed. Two patients dropped out before treatment started: 1 changed her decision to participate, and 1 suffered a myocardial infarction after inclusion in the study. We used a table with random numbers to assign patients to receive either the structured triglyceride emulsion (n = 12) or the emulsion of the physical mixture of medium- and long-chain triglycerides (n = 13).
Four patients were withdrawn after treatment had started for reasons not related to the lipid emulsions: Adult Respiratory Distress Syndrome, aspiration of stomach contents, ventricular fibrillation, and thrombosis in the subclavian vein.
Post-operatively these patients were not on a respiratory ventilator during the nitrogen balance study, and patients were mobilized as soon as possible. After 5 days, when the study was completed, oral intake was started if possible.
Before nitrogen excretion was measured, and still blinded to treatment, one patient was excluded from calculation of the nitrogen balance. He was treated with the physical mixture. The reason for exclusion of this patient was extensive muscle breakdown due to muscle ischemia and compression, which is known to produce high nitrogen excretion. In one patient treated with the structured triglyceride, day 4 urine was lost. This patient was also excluded from calculation of the nitrogen

balance. Therefore nitrogen balance was calculated for 10 patients treated with the structured triglyceride emulsion and for 9 patients treated with the emulsion of the physical mixture who completed the 5-day study period.

Treatment

In all patients parenteral nutrition was started with an administration rate according to nutritional need. Patients were treated with a structured triglyceride emulsion (Structolipid, Fresenius-Kabi, Sweden) or an emulsion of a physical mixture of medium- and long-chain triglycerides (Lipofundin MCT/LCT 20%, B. Braun Melsungen AG, Germany) (Table I). Figure 1 shows the molecular structure of MCT, LCT, and structured triglycerides. Structured triglycerides are produced by hydrolysis of MCT and LCT in glycerol and free fatty acids and subsequent at random re-esterification of glycerol with free fatty acids. 1,3-specific lipase is not used. Both patient groups received an equal dose of lipid emulsion in weight with equal energy content, because we chose to compare the emulsions under energetically equivalent conditions. The patients, treated with the physical mixture-emulsion, received 1.08 times more triglycerides and 1.25 times more medium-chain fatty acids, both on a molar base.

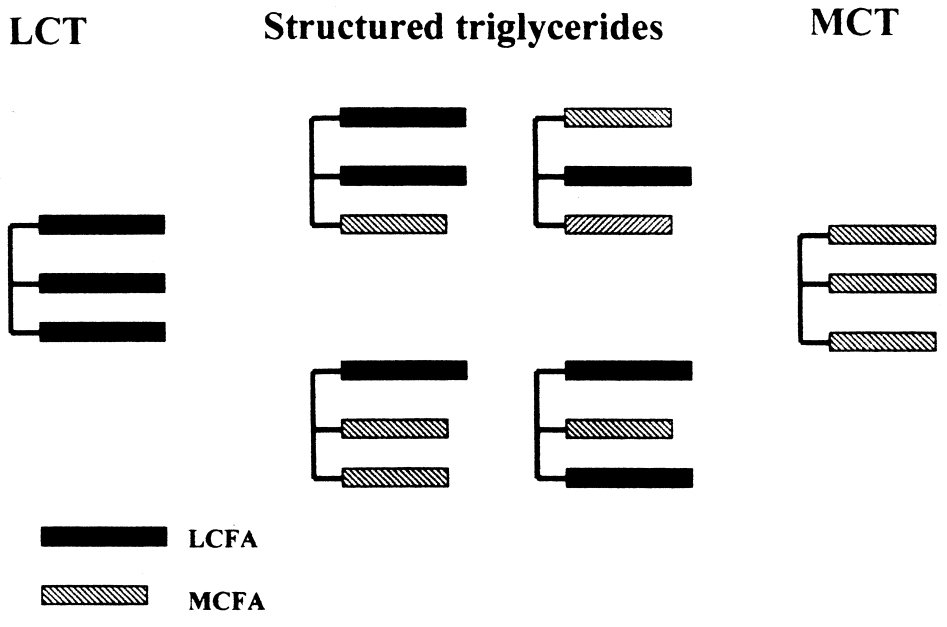

Figure. 1. *The molecular structure of long-chain triglycerides, structured triglycerides, and medium-chain triglycerides.*

Table I. *Composition and characteristics of the structured triglyceride emulsion and of the emulsion of the physical mixture of medium- and long-chain triglycerides*

Composition and characteristics	Structured triglyceride emulsion	Physical mixture emulsion
Structured triglycerides (g/L)	200	
Soy bean oil (g/L)		100
Medium-chain triglycerides (g/L)		100
Mean molecular weight of triglycerides	683	634
Fractionated egg phospholipids (g/L)	12	12
Glycerol (USP) (g/L)	22.5	25.0
Water for injection ad (mL)	1000	1000
pH	8	6.5-8.5
Osmolality (mOsm/kg water)	350	380
Energy content (kcal/L)	1960	1936
Fatty acid composition, % by weight		
Caprylic acid (C8:0)	27	26
Capric acid (C10:0)	10	20
Palmitic acid (C16:0)	7	7
Stearic acid (C18:0)	3	2.5
Oleic acid (C18:1)	13	13
Linoleic acid (C18:2ω6)	33	27
Alpha-linolenic acid (C18:3ω3)	5	3.5
Other	2	1

The patients were treated with TPN for the first 5 days after surgery. We calculated energy requirement in kcal/24 h using Harris and Benedict's equation [males: 66.47 + 13.75 x weight (kg) + 5.0 x height (cm) - 6.76 x age (years); females: 655.10 + 9.56 x weight (kg) + 1.85 x height (cm) - 4.68 x age (years)] and added 300 kcal/24 h for activity. We gave 0.2 g of nitrogen/kg body weight per 24 h (Vamin 18, Fresenius-Kabi, Sweden), which is in accordance to the nutritional needs of this group of patients (18). Two-thirds of the non-protein energy or 53 % of total energy was given as carbohydrates (glucose 40%), one-third of the non-protein energy or 26 % of total energy as lipid emulsion, and 21% of total energy as amino acids. Amino acids and carbohydrates were given daily over 24 hours, throughout the 5-day post-operative period. The lipid emulsions were administered separately, because stability studies of mixtures of the structured triglyceride emulsion, and amino acids and carbohydrates were not available. The lipid emulsions were administered daily from 10 AM to 4 PM, because lipids could disturb the laboratory assessments and baseline blood samples were taken at 8 AM. This intermittent administration of

lipid emulsions allowed us to study the kinetics of the lipid emulsions. All parenteral nutrition was administered through one lumen of a double-lumen subclavian catheter. IV medication was given separately through the other lumen of the subclavian catheter or via a peripheral catheter. Amino acids, carbohydrates and lipid emulsion were given at constant rates using 3 separate infusion pumps (Terufusion STC-503, Terumo, The Netherlands). Oral intake of nutrients was not allowed during the study period.

Assessments

The following baseline characteristics of the patients were determined: sex, race, age, nutritional status, duration of surgery, blood loss during surgery, and Acute Physiology and Chronic Health Evaluation (APACHE) II score (19). Nutritional status was assessed by measuring height and weight, and calculating body mass index [weight per square height]. Furthermore, skinfold thickness according to Durnin and Womersley (20) was measured at 4 sites (biceps, triceps, subscapular and supra-iliac), giving the body fat content of the patient; the percentage of ideal body fat content was calculated. Also, width of condyle of femur was measured according to de Wijn (21), giving the ideal weight of the patient; the body weight was measured and the percentage of ideal body weight was calculated.

Blood pressure, heart rate, respiratory frequency, body temperature, and adverse events of each patient were monitored, each post-operative day at 8 AM and 4 PM. The patients especially were monitored for infectious complications, allergic reactions, nausea, chills and neurological symptoms. In addition, the APACHE II score was evaluated on days 1, 3 and 6 at 8 AM.

We calculated the nitrogen balance per 24 hours from the amounts of nitrogen administered parenterally and the nitrogen excreted in the urine. We assumed a daily loss of 2 g of nitrogen via other routes (18). During the first 5 days post-operatively nitrogen was only provided by the parenteral nutrition because oral intake of nutrients was not allowed. Under close supervision urine was collected daily and pooled in 24-hour aliquots from 6 AM until 6 AM the following day, and the urine was transported every morning to the laboratory at 8 AM. Urinary nitrogen content was analyzed according to Kjeldahl (22).

Serum levels of triglycerides and plasma levels of medium-chain free fatty acids were measured on the first and fifth day after surgery before and at the end of lipid infusion, i.e. at 8 AM and 4 PM; one more sample was taken at the end of the study, on the sixth day at 8 AM. Serum triglycerides were measured by the Böhringer Mannheim Kit for triglyceride analysis without free glycerol. Plasma medium-chain free fatty acids were analyzed according to Tsuchiya et al. (23). The free fatty acids were separated by reversed-phase high-performance liquid chromatography (HPLC) on a C18 column after a derivatization reaction with a fluorescent reagent,

4-bromomethyl-7-acetoxycoumarin. The free fatty acid derivatives were detected by fluorescence detection.
Blood hemoglobin, white blood cell count with differential count and platelet count, serum sodium, potassium, urea, creatinine, total bilirubin, alkaline phosphatase, aspartate aminotransferase, glucose, and ß-hydroxybutyrate were measured before surgery, at 8 AM on day 1 before the start of parenteral nutrition, and at 8 AM on days 3 and 6. Bèta-hydroxybutyrate was determinated on a centrifugal analyzer (Multistat III) (24).

Statistical analysis
The primary end point for comparison between the groups was the daily cumulative nitrogen balance calculated for those patients who completed the 5-day study period. Two secondary end points were tested: changes in serum levels of triglycerides and changes in plasma levels of medium-chain free fatty acids. All results were expressed as mean ± SEM. Changes in the structured triglyceride-emulsion group were compared with changes in the physical mixture-emulsion group, using Student's *t* test, and the 95 % confidence level was calculated.

Results
Baseline characteristics of the two groups were similar with respect to sex, race, age, nutritional status, duration of surgery, and APACHE II score. After treatment had started, 6 patients dropped out for calculation of the nitrogen balance over the first 5 days. Baseline characteristics of the 2 groups remained similar when these 6 patients were excluded. Blood loss during surgery was a little smaller in the structured triglyceride-emulsion group (1420 ± 680 mL) than in the group treated with the emulsion of the physical mixture of medium- and long-chain triglycerides (2130 ± 880 mL] (Table II). The difference in blood loss did not influence the nitrogen balance study, because the nitrogen balance started the day after surgery.
The mean cumulative nitrogen balance was calculated for those patients who completed the 5-day study period. The mean cumulative nitrogen balances of the 2 study groups became significantly different on the fourth post-operative day: -5 ± 2 g of nitrogen for the 10 patients receiving the structured triglyceride emulsion and -16 ± 4 g of nitrogen for the 9 patients receiving the emulsion of the physical mixture of medium- and long-chain triglycerides. The mean difference in cumulative nitrogen balance over the 4 day-period was 11 grams of nitrogen (95 % confidence interval 2 to 19, $p = .02$). After 5 days the mean cumulative nitrogen balance was -8 ± 2 g for the structured triglyceride emulsion and -21 ± 4 g for the emulsion of the physical mixture; the nitrogen balance was thus 13 g less negative for the structured triglyceride emulsion than for the emulsion of the physical mixture (95 % confidence interval 4 to 22, $p = .015$) (Table III, Fig. 2a and 2b).

Table II. *Baseline characteristics of patients treated with the structured triglyceride emulsion or with the physical mixture emulsion*

Baseline characteristics	Structured triglyceride emulsion	Physical mixture emulsion
Men/women (number)	10/2	10/3
Caucasian/noncaucasian (number)	12/0	12/1
Age (years)	67 ± 6	69 ± 7
Height (m)	1.68 ± 0.09	1.66 ± 0.09
Weight (kg)	73 ± 10	69 ± 13
Body Mass Index (kg/m^2)	26 ± 3	25 ± 3
% of ideal body fat content	106 ± 5	105 ± 5
% of ideal body weight	108 ± 12	106 ± 9
Duration of surgery (min)	150 ± 70	160 ± 40
Blood loss during surgery (mL)	1420 ± 680	2130 ± 880
APACHE II score	7 ± 1	7 ± 1

Values are numbers or means ± SD.

Table III. *Cumulative nitrogen balance over the first five days after surgery for 10 patients treated with the structured triglyceride emulsion and 9 patients treated with the physical mixture emulsion, who completed the 5-day nitrogen balance*

Structured triglyceride emulsion	day 1	day 2	day 3	day 4	day 5
Nitrogen parenterally administered (g)	+15±1	+30±1	+45±1	+60±2	+74±2
Urinary nitrogen excretion (g)	-14±1	-30±2	-44±3	-57±3	-72±4
Assumed nitrogen loss via other routes (g)	- 2	- 4	- 6	- 8	-10
Cumulative nitrogen balance (g)	- 1±1	- 4±1	- 5±2	- 5±2*	- 8±2**
Physical mixture emulsion	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**
Nitrogen parenterally administered (g)	+14±1	+27±2	+41±2	+55±3	+68±4
Urinary nitrogen excretion (g)	-14±1	-31±3	-46±5	-63±6	-79±7
Assumed nitrogen loss via other routes (g)	- 2	- 4	- 6	- 8	-10
Cumulative nitrogen balance (g)	- 2±1	- 8±1	-11±3	-16±4*	-21±4**

Values are means ± SEM.
** mean difference 11 g (95% confidence interval 2; 19, p = .02).*
***mean difference 13 g (95% confidence interval 4; 22, p = .015).*

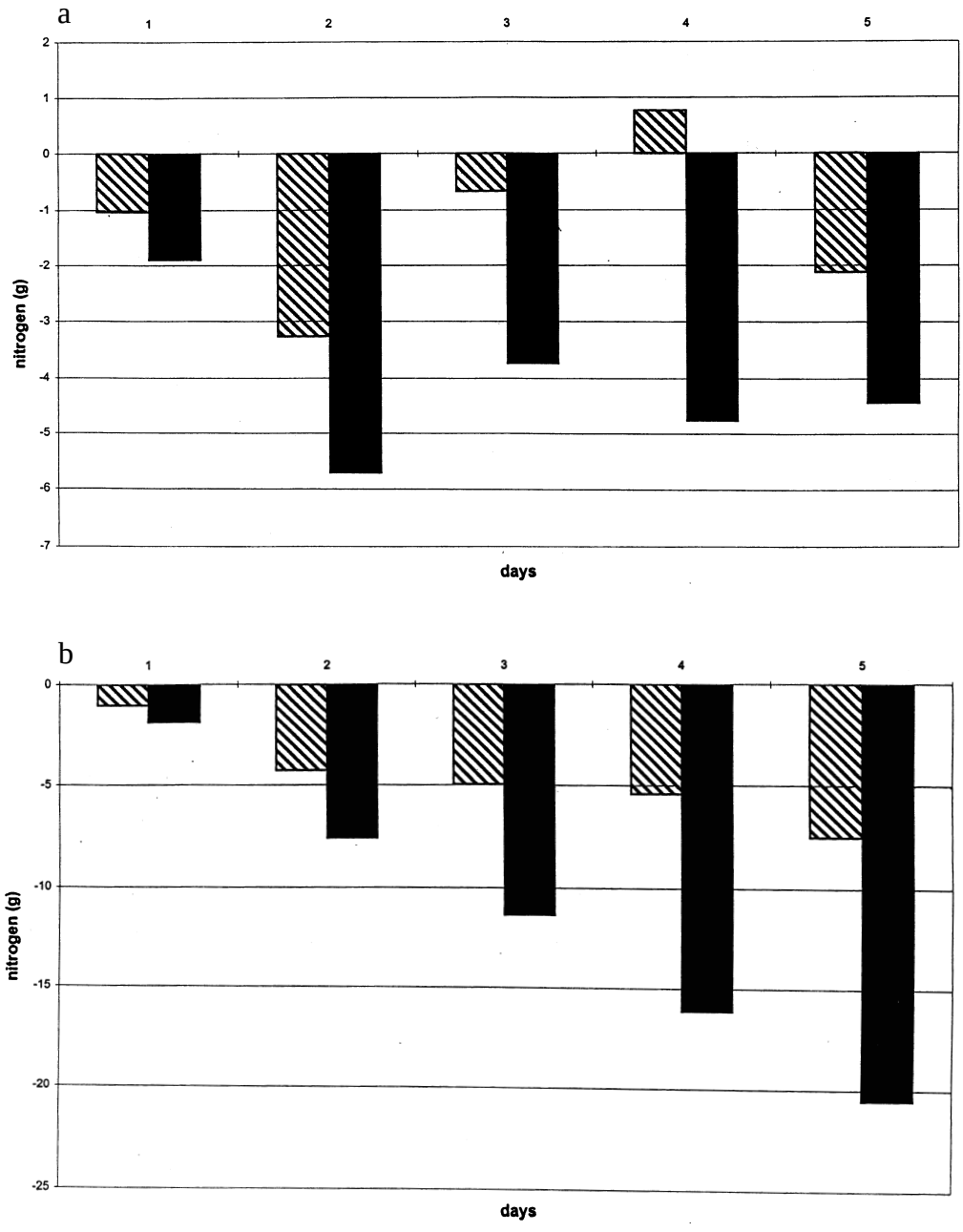

Figure 2. *(a) Daily nitrogen balances and (b) cumulative nitrogen balances over the first five days after surgery for 10 patients treated with the structured triglyceride emulsion (hatched bars) and 9 patients treated with the physical mixture emulsion (solid bars), who completed the 5-day nitrogen balance.*

On the first post-operative day, serum levels of triglycerides and plasma levels of medium-chain free fatty acids increased less during the 6 hours of lipid infusion with the structured triglyceride emulsion compared with the physical mixture of medium- and long-chain triglycerides (the difference in change for triglycerides between treatment with the structured triglyceride emulsion and the physical mixture emulsion was 1.10 mmol/L, 95% confidence interval 0.70 to 1.50, $p <$ .00005, and for medium-chain free fatty acids 145 μmol/L, 95% confidence interval 99 to 161, $p = .00001$, Table IV). These differences were higher than expected from

Table IV. *Changes in mean serum levels of triglycerides (mmol/L) and plasma levels of medium-chain free fatty acids (MCFFA,μmol/L) on day 1 post-operatively, from 8 AM, before the start of the lipid infusion, until 4 PM, at the end of lipid infusion*

Time	Variables	Structured triglyceride emulsion	Physical mixture emulsion
Day 1, 8 AM	triglycerides	0.86 ± 0.07	0.74 ± 0.07
	MCFFA	12 ± 4	7 ± 3
Day 1, 4 PM	triglycerides	1.44 ± 0.12	2.42 ± 0.16
	MCFFA	149 ± 13	289 ± 18
Change day 1,	triglycerides	0.58 ± 0.10	1.68 ± 0.17*
4 PM - 8 AM	MCFFA	137 ± 14	282 ± 18 **

Values are means ± SEM.

* *On day 1 the difference in change between treatment with the structured triglyceride emulsion and the physical mixture emulsion was for triglycerides 1.10 mmol/L, 95% confidence interval 0.70 to 1.50, p < .00005.*

** *On day 1 the difference in change between treatment with the structured triglyceride emulsion and the physical mixture emulsion was for medium-chain free fatty acids 145 μmol/L, 95% confidence interval 99 to 161, p = .00001.*

the difference in emulsion composition on a molar base. Both groups received an equal dose of lipid emulsion in weight; thus, the physical mixture-emulsion group received 1.08 times more triglycerides and 1.25 times more medium-chain fatty acids, both on a molar base. However, serum triglycerides and plasma medium-chain free fatty acids concentrations increased 2 times more with the physical mixture-emulsion group. On the fifth post-operative day after infusion of the lipid emulsions, changes in serum triglycerides and plasma medium-chain free fatty acids concentrations were, although lower in the group receiving the structured triglyceride emulsion, not significantly different for the 2 patient groups (triglycerides 0.27 mmol/L, 95% confidence interval -0.19 to 0.73; medium-chain free fatty acids 70 μmol/L, 95% confidence interval 2 to 138, $p =.07$, Table V).

Table V. *Changes in mean serum levels of triglycerides (mmol/L) and plasma levels of medium-chain free fatty acids (MCFFA,µmol/L) on day 5 post-operatively, from 8 AM, before the start of the lipid infusion, until 4 PM, at the end of lipid infusion*

Time	Variables	Structured triglycerides emulsion	Physical mixture emulsion
Day 5, 8 AM	triglycerides	1.12 ± 0.20	1.10 ± 0.04
	MCFFA	11 ± 3	4 ± 3
Day 5, 4 PM	triglycerides	1.99 ± 0.24	2.24 ± 0.17
	MCFFA	168 ± 25	231 ± 25
Change day 5,	triglycerides	0.87 ± 0.17	1.14 ± 0.16 [ns]
4 PM - 8 AM	MCFFA	157 ± 22	227 ± 26 [ns]

Values are means ± SEM.

[ns] *On day 5 the difference in change between treatment with the structured triglyceride emulsion and the physical mixture emulsion was for triglycerides 0.27 mmol/L, 95% confidence interval -0.19 to 0.73, not significant; and for medium-chain free fatty acids 70 µmol/L, 95% confidence interval 2 to 138, not significant (p = 0.07).*

Adverse events, especially infectious complications, blood pressure, heart rate, respiratory frequency, body temperature, and APACHE II score did not show significant differences between both groups. Blood hemoglobin, white blood cell count with differential count and platelet count, serum sodium, potassium, urea, creatinine, total bilirubin, alkaline phosphatase, aspartate aminotransferase, glucose and ß-hydroxybutyrate were also similar in both groups at 8 AM on days 1, 3 and 6.

Discussion

Nitrogen balance was measured to compare the efficacy of an emulsion containing "structured" triglycerides - i.e. triglycerides having medium-chain and long-chain fatty acids within the same molecule - and an emulsion of a physical mixture of medium-chain and long-chain triglycerides. A less negative nitrogen balance indicates conservation of protein mass, including muscle mass. Nitrogen balance studies are widely used as an index of effectiveness of nutrition support (18); a better index is not yet available (25).

An emulsion of a physical mixture of medium- and long-chain triglycerides is known to improve nitrogen balance in patients compared with long-chain triglyceride emulsions (4,5). We found that parenteral administration of a "structured" triglyceride emulsion improved nitrogen balance in patients after a large vascular operation compared with an emulsion of a physical mixture of medium- and long-chain triglycerides. Daily urinary nitrogen excretion was higher in the

physical mixture-emulsion group, starting from the second day after surgery (Table III, Fig. 2a and 2b). The mean difference in nitrogen balance between the 2 treatment groups was 13 g of nitrogen in 5 days in favor of the structured triglyceride emulsion, which means that 80 g of protein or - in case this protein was completely derived from muscle - approximately 500 g of muscle mass was saved over 5 days by using the structured triglyceride emulsion. The daily amount of nitrogen administered to the patients was a little higher in the structured triglyceride-emulsion group, but based on the weight of the patients and calculated as 0.2 g of nitrogen/kg body weight per 24 h. Indeed the weight of the patients in the structured triglyceride-emulsion group was not significantly higher than the weight of the patients in the physical mixture-emulsion group. Patients with a higher weight have in a steady state condition a higher nitrogen loss. We found that the patients in the structured triglyceride-emulsion group with a higher weight had a lower nitrogen loss, indicating that the nitrogen balance is improved by the treatment with the structured triglyceride emulsion. The difference in blood loss during surgery between the 2 groups could not explain the difference in nitrogen balance because it was too small to affect the calculations. Moreover, the nitrogen balance study started the day after surgery. Our results agree with findings in laboratory animals: structured triglyceride emulsions improved protein retention compared with emulsions of a physical mixture of medium-chain and long-chain triglycerides (12-14).

We also studied serum triglycerides and plasma medium-chain free fatty acids and observed that the levels of serum triglycerides and plasma medium-chain free fatty acids increased less during infusion of the structured triglyceride emulsion than with the physical mixture emulsion. Both groups received an equal dose of lipid emulsion in weight; thus, the physical mixture-emulsion group received 1.08 times more triglycerides and 1.25 times more medium-chain fatty acids, both on a molar base. However, this does not explain that serum triglycerides and medium-chain free fatty acids increased 2 times more with the physical mixture-emulsion group. The smaller increase of serum triglycerides and plasma medium-chain free fatty acids during infusion of the structured triglyceride emulsion may be due to a faster use of the structured triglycerides than of the triglycerides in the physical mixture-emulsion. On day 5 the differences between the 2 lipid emulsions were not significant, possibly because patients were less stressed on day 5 than on day 1. Another explanation to the decrease in changes of serum triglyceride levels on day 5 may be due to the increase in basal levels. This increase in basal levels is most likely due to a too short infusion free period, so the triglycerides were not fully removed from the circulation at the sampling time. The lack of difference in changes in plasma medium-chain free fatty acid concentrations may be due to the small group size: there is a tendency to difference and the changes are similar to the one

observed on day 1. These findings are in agreement with findings in healthy volunteers: structured triglyceride emulsions caused lower plasma levels of medium-chain free fatty acids and were cleared faster from the blood than emulsions of a physical mixture of medium- and long-chain triglycerides (10,11).

One could speculate on the mechanism by which the structured lipids improve nitrogen balance. The present study demonstrates a faster clearance of structured lipid compared with the emulsion of medium- and long-chain triglycerides. A study in patients comparing the administration of structured triglycerides and long-chain triglycerides demonstrated a faster clearance and an increase in oxidation rate (16). This suggests that structured triglycerides are hydrolyzed and oxidized faster.

This is also suggested by the study of Hultin et al. (26). The hypothesis in this study was that the positional specificity of lipoprotein lipase should lead to differences in metabolism of a long-chain fatty acid in the 2-position of triglycerides, compared with one in the 1,3-position. In-vitro experiments have shown that in physical mixtures of long-chain and medium-chain triglycerides, the medium-chain triglycerides are hydrolyzed more rapidly, and the remnant particles become enriched in long-chain triglycerides (27). Also, the lipoprotein lipase shows specificity to the 1,3-position, resulting in the products fatty acids and 2-monoglycerides (28). Monoglycerides recirculate in the plasma to a lesser degree than fatty acids (29). According to these observations, a structured triglyceride with a medium chain fatty acid-long chain fatty acid-medium chain fatty acid (MLM) structure should be cleared faster, compared with long-chain triglycerides or a physical mixture of medium-chain and long-chain triglycerides. In this study a MLM structured triglyceride was compared with long-chain triglycerides and a physical mixture. The hypothesis was confirmed. The clearance and oxidation of MLM was faster, but oxidation was faster only in fasted unanaesthetized rats. The differences were small. In this study no other molecular forms of structured triglycerides were studied. The study of Hultin explains only in part the observations in our study.

The second question is the relation between the faster clearance and oxidation of structured triglycerides and the influence on the protein metabolism. This faster oxidation of structured triglycerides indicates a preference for structured triglycerides as an energy source. Structured triglycerides influence the nitrogen metabolism and may induce a protein saving effect by an increase in protein production or decrease in protein breakdown. Animal studies demonstrated an increase in hepatic protein synthesis (13,14) and decreased leucine oxidation (12,14). The process that regulates this change in protein metabolism, is not yet fully studied.

In conclusion, in moderately catabolic patients the parenteral structured triglyceride emulsion improves the nitrogen balance and is cleared faster from the blood, compared with a physical mixture of medium- and long-chain triglycerides.

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Chapter 6

MEDIUM-CHAIN AND STRUCTURED TRIGLYCERIDE EMULSIONS DO NOT IMPAIR THE MONONUCLEAR PHAGOCYTE SYSTEM IN POST-OPERATIVE PATIENTS

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Abstract

Background Studies gave indications that parenteral lipid emulsions suppress host defense mechanisms. A previous study showed that intravenous administration of a long-chain triglyceride emulsion impaired the phagocytosing capacity of the mononuclear phagocyte system, determined by measuring the clearance of ^{99m}Technetium-Sulfur- (^{99m}Tc-S-) colloid from the blood, in contrast to an emulsion of a physical mixture of medium- and long-chain triglycerides.

Methods In a randomized, double-blind, parallel study, we investigated the influence of intravenously administered triglyceride emulsions containing medium-chain fatty acids on the ^{99m}Tc-S-colloid clearance from the blood in 13 patients after major vascular surgery. Seven patients received an emulsion of a physical mixture of medium- and long-chain triglycerides and 6 patients a structured triglyceride emulsion, in a dose of 0.11 g/kg/h over 6 hours daily. Structured triglycerides consist of triglycerides where the medium- and long-chain fatty acids are attached to the same glycerol molecule.

Results The ^{99m}Tc-S-colloid clearance from the blood was the same in the two study groups, before the administration of lipid emulsion, as well as after 5 days of treatment, and did not change during treatment.

Conclusions Parenteral lipid emulsions with medium-chain fatty acids do not impair the mononuclear phagocyte system. In this respect there is no difference between the effects of an emulsion of a physical mixture of medium- and long-chain triglycerides and a structured triglyceride emulsion.

Introduction

Conventional lipid emulsions in total parenteral nutrition contain long-chain triglycerides, emulsified by phospholipids. Besides long-chain triglyceride emulsions, medium-chain triglyceride emulsions are used, because they improve nitrogen balance (1,2) and because they are utilized more rapidly (3). Medium-chain triglycerides are given together with long-chain triglycerides as a physical mixture emulsion, in order to supply essential long-chain fatty acids and to reduce side effects as induced by high levels of medium-chain free fatty acids, such as metabolic acidosis (4). Recently, structured triglyceride emulsions were synthesized. They consist of triglycerides where the medium- and long-chain fatty acids are attached to the same glycerol molecule (Fig. 1). Such structured triglycerides further reduce plasma levels of medium-chain free fatty acids and do improve nitrogen balance (5). All these parenteral lipid emulsions are sterile and pyrogen-free.

Suppression of the function of the mononuclear phagocyte system by parenteral long-chain triglyceride emulsions has been reported (6). The mononuclear phagocyte system comprises all highly phagocytic mononuclear cells as well as their precursors (7). The function of the mononuclear phagocyte system can be evaluated

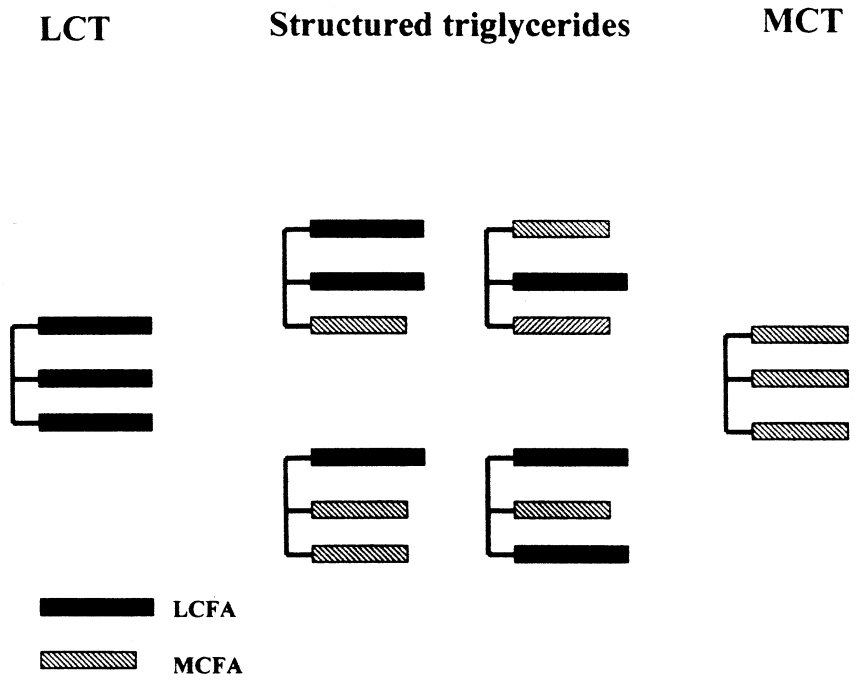

Figure 1. *The molecular structure of long-chain triglycerides, structured triglycerides, and medium-chain triglycerides.*

in-vivo by measuring the clearance of ^{99m}Tc-S-colloid from the blood (8,9). Seidner et al. reported that 3 days of parenteral administration of a long-chain triglyceride emulsion to patients reduced the ^{99m}Tc-S-colloid clearance (8). In contrast, in a patient study by Jensen et al., 3 days parenteral administration of an emulsion of a physical mixture of medium- and long-chain triglycerides did not change the ^{99m}Tc-S-colloid clearance from the blood (9). No studies have been performed as yet investigating the effect of a structured triglyceride emulsion on ^{99m}Tc-S-colloid clearance.

Our objective was to study in post-operative patients the influence of parenteral infusion of an emulsion of a physical mixture of medium- and long-chain triglycerides or a structured triglyceride emulsion on the mononuclear phagocyte system. For that purpose we used the in-vivo ^{99m}Tc-S-colloid clearance test and compared the change in pharmokinetics of ^{99m}Tc-S-colloid after lipid administration, between the two patient groups, as described by Seidner et al. (8).

Methods

Patients

In this randomized, double-blind, parallel study we included patients electively operated for placement of an aortic tube or bifurcation prosthesis because of an abdominal aortic aneurysm or atherosclerotic obstruction. Such patients make a homogeneous group, are moderately catabolic, and may require post-operative parenteral nutrition.

Exclusion criteria for this study were age above 80 years, diseases requiring fluid restriction, unstable cardiac disease or myocardial infarction, renal or liver disease, sepsis, severe malnourishment or overweight, diabetes mellitus, hypertriglyceridemia or treatment with lipid lowering-drugs, steroid hormones, chemotherapy or radiotherapy.

All patients were informed orally and in writing, after which they gave their written consent and were randomized. Thirteen patients, 11 men and 2 women, were included; 6 patients were in the structured triglyceride group and 7 patients in the physical mixture group. Their mean age was 66.8 years (range 51 - 76 years) and their mean weight 75 kg (range 52 - 91 kg). The study belongs to category I (up to 0.5 mSv) of the WHO classification and was approved by the Human Ethics Committee of the University of Nijmegen, The Netherlands.

Treatment

Total parenteral nutrition was administered for the first 5 days after surgery. We calculated energy requirement in kcal/24 h using Harris and Benedict's equation [males: 66.47 + 13.75 x weight (kg) + 5.0 x height (cm) - 6.76 age (years); females: 655.10 + 9.56 x weight (kg) + 1.85 x height (cm) - 4.68 x age (years)] and added 300 kcal/24 h for activity. We gave 0.2 g of nitrogen/kg body weight per 24 hours or 21 energy % as amino acids (Vamin 18, Fresenius-Kabi, Sweden). Two thirds of the remaining non-protein energy or 53 energy % was given as carbohydrates (glucose 40%) and one third or 26 energy % as lipid emulsion. The lipid emulsion was a structured triglyceride emulsion (Structolipid, Fresenius-Kabi, Sweden) or an emulsion of a physical mixture of medium- and long-chain triglycerides (Lipofundin MCT/LCT 20%, B. Braun Melsungen AG, Germany) (Table I). The dose of lipid emulsion was 0.11 g/kg/h over 6 hours daily. During the first 5 days after surgery, amino acids and carbohydrates were given daily over 24 hours. The lipid emulsions were administered daily from 10 AM to 4 PM. Amino acids, carbohydrates and lipid emulsion were given at constant rates using three separate infusion pumps (Terufusion STC-503, Terumo, The Netherlands). All parenteral fluids were sterile and pyrogen-free. Oral intake of nutrients was not allowed during the study period.

Table I. *Composition and characteristics of the structured triglyceride emulsion and of the emulsion of the physical mixture of medium- and long-chain triglycerides*

Composition and characteristics	Structured triglyceride emulsion	Physical mixture emulsion
Structured triglycerides (g/L)	200	
Soy bean oil (g/L)		100
Medium-chain triglycerides (g/L)		100
Mean molecular weight of triglycerides	683	634
Fractionated egg phospholipids (g/L)	12	12
Glycerol (USP) (g/L)	22.5	25.0
Water for injection ad (mL)	1000	1000
pH	8	6.5-8.5
Osmolality (mOsm/kg water)	350	380
Energy content (kcal/L)	1960	1936
Fatty acid composition, % by weight		
Caprylic acid (C8:0)	27	26
Capric acid (C10:0)	10	20
Palmitic acid (C16:0)	7	7
Stearic acid (C18:0)	3	2.5
Oleic acid (C18:1)	13	13
Linoleic acid (C18:2ω6)	33	27
Alpha-linolenic acid (C18:3ω3)	5	3.5
Other	2	1

Preparing of Antimone-Sulfur-pre-colloid

Hundred mg of $K(SbO)C_4H_4O_6$-$0.5H_20$ (Merck, Germany) was dissolved in 5 mL of water. This was slowly added to 50 mL of boiling water, saturated with H_2S (F.I.L. Hoekloos, The Netherlands). The colloid was stabilized with 240 mg poly-vinylpyrrolidon (K25) (Fluka, Switzerland) and the solution was heat sterilized.

Labeling and preparing of ^{99m} Tc-S-colloid

Two mL of the pre-colloid, 1 mL 0.1 M HCL (Merck, Germany) and 740 MBq ^{99m}Tc-pertechnetate (Mallinckrodt, The Netherlands) was heated for 15 minutes at 120 °C. The radiochemical purity of the ^{99m}Tc-S-colloid was determined by instant thin layer chromatography (ITLC) on Gelman-ITLC-SG strips (Gelman Laboratories, MI) with sterile saline 0.9% as mobile phase: more than 99% of the ^{99m}Tc-S-colloid was found at the origin of the chromatogram. One mL of 0.15 M Na-acetate buffer (pH 7.0) (Merck, Germany) was added to neutralize the colloid and the colloid was sterilized by leading the solution over a 0.22 μm millipore filter.

Finally, the solution was diluted with sterile saline 0.9% to a concentration of 20 MBq in 5 mL.

Assessment

A bolus of 20 MBq of ^{99m}Tc-S-colloid was administered intravenously in 5 mL, using a peripheral venous catheter in one arm. To guarantee equal intravascular distribution, the bolus was flushed immediately with 10 mL of sterile saline 0.9%. By a winged infusion set, put into a vein of the other arm, serial blood samples of 2 mL each were drawn into heparinized tubes. Blood samples were taken at 1.5, 3, 4.5, 6, 9, 12, 15, 30 and 60 minutes after injection and centrifuged at 2600 rpm. Plasma samples of 0.5 mL were counted in a Wizzard-3-1480 Wallac Gamma Counter and compared with a standard solution, prepared from the injection dose. The plasma activity was expressed in percents of injected activity per mL plasma, as a function of time.

A two-compartment model as described by Seidner et al. (8) was used to assess ^{99m}Tc-S-colloid clearance from the blood by the mononuclear phagocyte system. The plasma data were analyzed by nonlinear least-squares fit, using a bi-exponential model and plasma clearance was calculated in mL per minute, using the following formula:

$$Cl\ (mL/minute) = \frac{K_A * K_B * D}{A_0K_A + B_0K_B}$$

Cl = clearance of ^{99m}Tc-S-colloid from the plasma (mL/minute)
K_A = regression coefficient of the fast phase (minute^{-1})
K_B = regression coefficient of the slow phase (minute^{-1})
D = injected activity (counts)
A_0 = fraction of injected activity in plasma of the fast phase (counts/mL)
B_0 = fraction of injected activity in plasma of the slow phase (counts/mL)

Statistics

The two-tail probation test was used to calculate differences in ^{99m}Tc-S-colloid clearance from the blood between the two treatment groups, before the start of total parenteral nutrition on the first post-operative day, and after 5 days of total parenteral nutrition (10). The same test was used to calculate differences in change of ^{99m}Tc-S-colloid clearance from the blood between the treatment groups after 5 days of total parenteral nutrition (difference between ^{99m}Tc-S-colloid clearance before treatment and after 5 days of treatment). All results were expressed as mean

± SD. The number of patients in the study was calculated using the differences seen during administration and using the data of Seidner et al. (8).

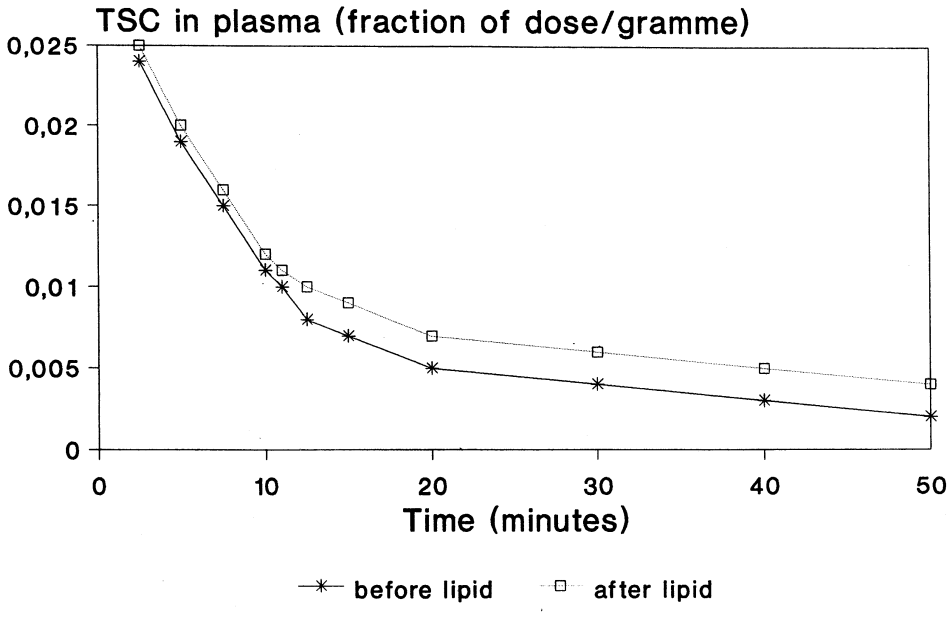

Figure 2. *The activity of 99mTc-S-colloid in percents of injected activity per mL plasma in blood samples before treatment and after 5 days of intravenous administration of a structured triglyceride emulsion (STG). Plasma activity is expressed as a function of time. No significant differences between samples before and after treatment were observed (results of one patient). The same results were obtained with intravenous administration of an emulsion of a physical mixture of medium- and long-chain triglycerides (not shown).*

Results

Figure 2 shows graphically the results of one patient. We expressed the activity of ^{99m}Tc-S-colloid in percents of injected activity per mL plasma, as a function of time. This plasma activity was measured in blood samples before the start of total parenteral nutrition, on the first post-operative day, and after 5 days of total parenteral nutrition. No significant differences between samples before and after administration of total parenteral nutrition were observed.

For both treatment groups, before administration of total parenteral nutrition, on the first post-operative day, the clearance of ^{99m}Tc-S-colloid from the blood was the same (192 ± 66 mL/min in the structured triglyceride group, and 202 ± 34 mL/min in the physical mixture group, $p > .05$) (Table II). Two patients in the physical

Table II. *^{99m}Tc-S-colloid (TSC) clearance from the blood (mL/minute) before treatment and after 5 days of parenteral administration of a structured triglyceride emulsion or an emulsion of a physical mixture*

	Structured triglycerides n = 6	Physical mixture n = 7
TSC clearance before treatment	192 ± 66	202 ± 34
TSC clearance after treatment	206 ± 62	183 ± 94
Change in TSC clearance	+14 ± 74	-20 ± 57

Values are means ± SD.

mixture group did not complete the study, for reasons not related to the lipid emulsion or the study: aspiration of stomach contents and repeated ventricular fibrillation. After 5 days of total parenteral nutrition, the ^{99m}Tc-S-colloid clearance from the blood was not changed significantly (+14 ± 74 mL/min in the structured triglyceride group, n=6, and -20 ± 57 mL/min in the physical mixture group, n=7, $p > .05$) and still the same for both groups (206 ± 62 mL/min in the structured triglyceride group, n=6, 183 ± 94 mL/min in the physical mixture group, n=7, $p > .05$) (Table II).

Discussion

Our results in patients show that the clearance of ^{99m}Tc-S-colloid from the blood is unchanged after 5 days of total parenteral nutrition with an emulsion of a physical mixture of medium- and long-chain triglycerides or a structured triglyceride emulsion, in a dose of 0.11 g/kg/h over 6 hours daily. This indicates that emulsions of both a physical mixture and structured triglycerides, containing triglycerides with medium-chain fatty acids, do not suppress the mononuclear phagocyte system function.

Earlier, Seidner et al. (8) reported in a patient study, that 3 days of parenteral administration of a long-chain triglyceride emulsion, at 0.13 g/kg/h over 10 hours daily, reduced the clearance of ^{99m}Tc-S-colloid from the blood. One day of parenteral treatment with a long-chain triglyceride emulsion did not affect the ^{99m}Tc-S-colloid clearance (8) or the ^{125}I-microaggregated human serum albumin clearance (11). In another study by Jensen et al. (9) the same daily dose of long-chain triglyceride emulsion, 1.3 g/kg/day, was given continuously over 3 days, and did not impair the mononuclear phagocyte system. These findings suggest that lower total dose, shorter infusion period and lower dose rate of infusion of long-

chain triglyceride lipid emulsions cause less impairment of the mononuclear phagocyte system.

In accordance to our results, Jensen et al. (9) reported in the same study that 3 days parenteral administration of an emulsion of a physical mixture of medium- and long-chain triglycerides, at 0.13 g/kg/h over 10 hours daily, did not change the ^{99m}Tc-S-colloid clearance from the blood.

In-vitro and animal studies on phagocytosis are scarce, have conflicting results and concern mainly long-chain triglyceride emulsions. In-vitro incubation with a long-chain triglyceride emulsion impaired phagocytosis by murine peritoneal macrophages (12), but improved chemotaxis and phagocytosis by human monocytes (13). In two animal experiments, intraperitoneal administration of a long-chain triglyceride emulsion impaired bacterial clearance from the peritoneal cavity (12) and the blood (14). In another animal study intravenous administration of a long-chain triglyceride emulsion did not change bacterial clearance from the blood (15).

Interestingly, the last animal study showed a different pattern of bacterial organ sequestration by intravenous administration of a long-chain triglyceride emulsion, compared with a medium-chain triglyceride or structured triglyceride emulsion (15). In contrast to a medium-chain triglyceride or structured triglyceride emulsion, the long-chain triglyceride emulsion reduced bacterial uptake in the liver and increased bacterial uptake in the lungs, suggesting overload of the mononuclear phagocyte system in the liver with increased bacterial sequestration in the lungs. Hamawy et al. (16) reported increased bacterial clearance from the blood in animals treated with intravenous administration of a physical mixture emulsion, compared with a long-chain triglyceride emulsion or controls without lipid emulsion. The same pattern of organ sequestration was observed: liver uptake of bacteria was reduced with the long-chain triglyceride emulsion and enhanced with the physical mixture emulsion, compared to controls without lipid emulsion; lung uptake of bacteria was enhanced with the long-chain triglyceride emulsion and reduced with the medium-chain triglyceride emulsion, compared with controls without lipid emulsion. Pscheidl et al. (17) compared the effects of intravenous administration of a structured triglyceride emulsion with an emulsion of a physical mixture on bacterial organ sequestration in animals. Bacterial sequestration in the animals treated with the structured triglyceride emulsion was higher in the liver and spleen and lower in the lungs compared with the animals treated with the emulsion of the physical mixture, implicating a further improvement of mononuclear phagocyte function by structured triglyceride emulsions.

The mechanism by which parenteral long-chain triglyceride emulsions impair mononuclear phagocyte function is still not clear. One of the explanations is the process of engulfment of lipid particles by macrophages, after which further phagocytosis of bacteria is impaired (11). Other explanations involve alterations in

membrane composition by the infused lipids, which can change the qualities of the membrane such as deformability and fluidity, and expression of receptors (11,13).
In conclusion, in contrast to a long-chain triglyceride emulsion, an emulsion of a physical mixture of medium- and long-chain triglycerides and a structured triglyceride emulsion, both containing triglycerides with medium-chain fatty acids, do not impair the mononuclear phagocyte system in patients.

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Chapter 7

DEPRESSION OF PLASMA LEVELS OF CYTOKINES AND EX-VIVO CYTOKINE PRODUCTION IN RELATION TO THE ACTIVITY OF THE PITUITARY-ADRENAL AXIS, IN PATIENTS UNDERGOING MAJOR VASCULAR SURGERY

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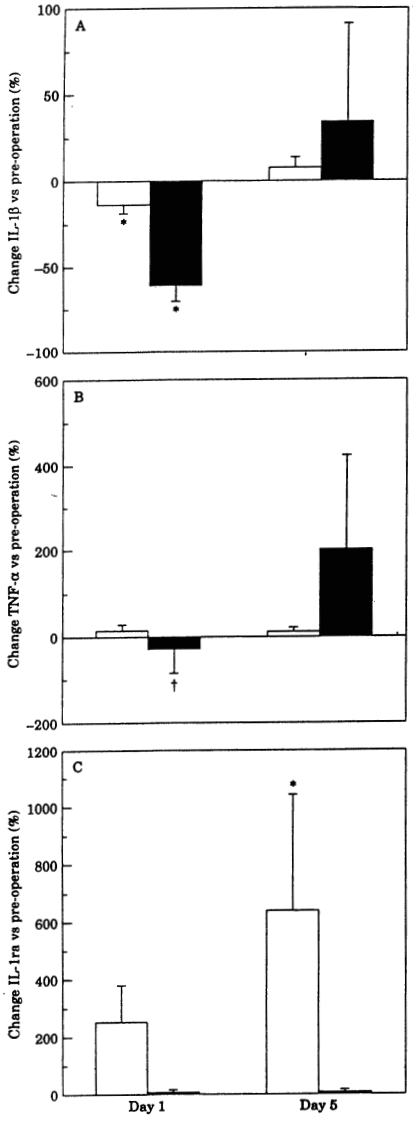
Abstract
The relation between the immune and neuroendocrine response during surgery was studied. In 18 patients undergoing major vascular surgery, circulating interleukin (IL)-1ß, and ex-vivo production of IL-1ß and tumor necrosis factor (TNF)-α were lower on day 1 after surgery compared to pre-operation values (-14 ± 5%, $p<0.05$; -62 ± 9%, $p<0.05$; and -31 ± 54%, $p<0.005$, respectively). Circulating IL-1 receptor antagonist (IL-1ra) was higher on the 5th day post-operatively compared to pre-operation values (mean +640% ± 400, $p<0.05$).
In a more detailed study in 6 patients, the ex-vivo production of IL-1ß and TNF-α started to decrease at induction of general anesthesia and dropped to under 10% of initial values at the end of surgery. Circulating IL-1ra and ex-vivo production of IL-1ra started to increase at the end of surgery and remained elevated up to 6 days post-operatively. Plasma antidiuretic hormone (ADH) and adrenocorticotropic hormone (ACTH) increased during surgery, but cortisol remained unchanged.
We demonstrate a depression of circulating pro-inflammatory IL-1ß and an increase of circulating anti-inflammatory IL-1ra during surgical stress. The ex-vivo production of IL-1ß and TNF-α was suppressed, indicating a downregulation of the production of these cytokines. This paralleled the hormonal reaction with high ADH and ACTH, but not of cortisol, suggesting that glucocorticoid is not the key-factor in downregulation of production and release of pro-inflammatory cytokines.

Introduction

Surgery elicits a physiological response (1,2) characterized by suppression of the immune system (3) and stimulation of the neuroendocrine system (4). The immune response during surgery involves almost every component of the immune system, such as function of phagocytes and lymphocytes (5-8). The neuroendocrine response during surgery leads to an increased secretion of adrenocorticotropic hormone (ACTH), growth hormone, prolactin and antidiuretic hormone (ADH) from the pituitary. Secondary changes include stimulation of cortisol and aldosterone production and inhibition of insulin and somatomedin secretion (4). At the same time the equilibrium between the sympathetic and parasympathetic autonomic nervous systems is shifted towards the sympathetic system leading to the release of noradrenaline from presynaptic sympathetic fibers and adrenaline from the adrenal medulla (4).The neuroendocrine and the immune system are intimately linked and involved in bidirectional communication (9). Both systems share the same set of signal molecules (hormones and cytokines) and their receptors. Glucocorticoids inhibit LPS-induced increase in interleukin (IL)-1ß mRNA transcription and selectively inhibit the stability of IL-1ß mRNA (10). ACTH inhibits directly interferon-γ production by T-lymphocytes in vitro (11). On the other hand, chronic administration of IL-1 induces a dose-dependent and long-term increase of plasma levels of ACTH and

corticosterone in rats (12), possibly by an action on the hypothalamus and pituitary gland. Thus, classical hormones can inhibit immunological cell function and products of immune cells can stimulate the hypothalamic-pituitary-adrenal axis.
We addressed the question to what extent cytokine production and release, and plasma concentrations of ACTH, cortisol and ADH were affected by surgery, and searched for a time relation between the cytokine and neuroendocrine response during surgery.



Results

In 18 patients undergoing major vascular surgery plasma levels of circulating interleukin (IL)-1ß, tumor necrosis factor (TNF)-α and IL-1 receptor antagonist (IL-1ra), and ex-vivo production of IL-1ß, TNF-α and IL-1ra were measured. To compare data results are expressed as percentage of pre-operative values. Figure 1 shows that the plasma level of circulating IL-1ß was significantly lower (-14 ± 5%, $p<0.05$) on the first post-operative day compared to pre-operative values (0.10 ± 0.003 ng/mL). On the 5th day after operation the level had returned to initial values. Plasma levels of circulating TNF-α and IL-1ra had not changed significantly

Figure 1. *Plasma levels of circulating (open bars) and ex-vivo production (closed bars) of IL-1ß (A), TNF-α (B) and IL-1ra (C) in 18 patients, on the first day and the fifth day post-operatively. Data are expressed as percentage of pre-operative values. On the left side of the figure are the results on day 1 and on the right side the results on day 5. Open bars are plasma levels of circulating IL-1ß, TNF-α and IL-1ra respectively; closed bars are ex-vivo production of IL-1ß, TNF-α and IL-1ra respectively. * indicates statistical significance at $p < 0.05$ and † at $p < 0.005$.*

one day after operation (pre-operative values 0.1 ± 0.01 ng/mL and 0.3 ± 0.04 ng/mL, respectively). On the 5th post-operative day however, the plasma level of circulating IL-1ra was found to be significantly increased (mean +640% ± 400, $p<0.05$) compared to the pre-operative level.
The ex-vivo production of IL-1ß and TNF-α was significantly lower after surgery (from pre-operatively to the first day post-operatively mean -62% ± 9, $p<0.05$ and -31% ± 54, $p<0.005$, respectively), whereas the production was increased, although not significantly, on day 5 after operation (pre-operative values 6.0 ± 0.68 ng/mL, 4.1 ± 0.69 ng/mL, respectively). Compared to pre-operative values (10.9 ± 0.50 ng/mL), the ex-vivo production of IL-1ra remained unchanged on day 1 and day 5 after operation (Fig. 1).
To obtain more insight in the patterns of cytokine release and production, in 6 patients 14 blood samples were taken peri-operatively with short intervals (Table I).

Table I. *Time schedule for collecting blood samples in the subgroup of 6 patients.*

During operation:
1. Pre-induction, 10 min after insertion of a radial artery catheter
2. 5 min after intubation
3. 5 min after skin incision
4. 20 min after skin incision
5. 40 min after skin incision
6. 60 min after skin incision
7. 120 min after skin incision
8. end of surgery
Post-operative:
9. post-operatively day 1 at 8 AM
10. post-operatively day 2 at 8 AM
11. post-operatively day 3 at 8 AM
12. post-operatively day 4 at 8 AM
13. post-operatively day 5 at 8 AM
14. post-operatively day 6 at 8 AM

As shown in Figure 2 plasma levels of circulating IL-1ß and TNF-α showed no remarkable changes. The plasma level of circulating IL-1ra, however, started to increase from 0.2 ng/mL at 60 minutes after skin incision to 0.6 ng/mL at the end of surgery, and even further increased to 0.8 ng/mL at the first day post-operatively. Thereafter circulating IL-1ra plasma levels sharply decreased, but remained elevated on a level of approximately 0.5 ng/mL for at least 6 days.
The basal ex-vivo cytokine production values ranged between 2.9 - 19.0 ng/mL (IL-1ß), 0.6 - 6.9 ng/mL (TNF-α) and 10.5 - 13.5 ng/mL (IL-1ra) respectively. Again,

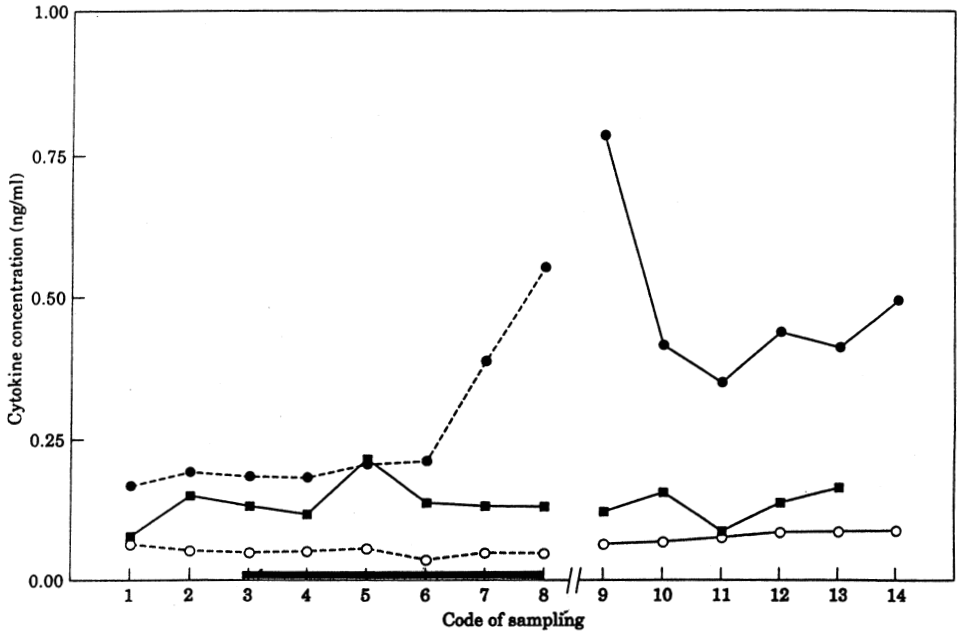


Figure 2. *Time course of plasma levels of circulating cytokines in six patients. IL-1ß, open circles; TNF-α, closed squares; and IL-1ra, closed circles (ng/mL): sampling codes 1 to 8 signify sampling during operation; sampling codes 9 to 14 signify post-operative sampling. Detailed explanation for the time schedule for collecting blood samples is given in Table 1. The horizontal bar indicates the period of surgery.*

for reasons of comparison, ex-vivo production data are expressed as percentage of pre-operative values (Fig. 3). The ex-vivo production of IL-1ß and TNF-α started to decrease immediately after intubation and skin incision and decreased to 9 % and 6% of initial values at the end of surgery. In the post-operative period the production returned to baseline values. The ex-vivo production of IL-1ra on the other hand showed only a slight decrease during surgery (-25 % of initial value at 40 min after skin incision), and already during surgery the production started to rise. After surgery IL-1ra production further increased and remained elevated up to the 6th day post-operatively (Fig. 3).

Five of these patients were randomly selected to measure also ACTH, cortisol and ADH (Fig. 4). Plasma ACTH concentrations showed a peak of 10 pmol/L at 20 min after skin incision and a second much larger peak of 30 pmol/L at the end of surgery. Post-operatively ACTH concentrations rapidly decreased, and again were slightly elevated (10 pmol/L) on the sixth day after operation. Plasma cortisol concentrations did not significantly change during the surgical procedure.

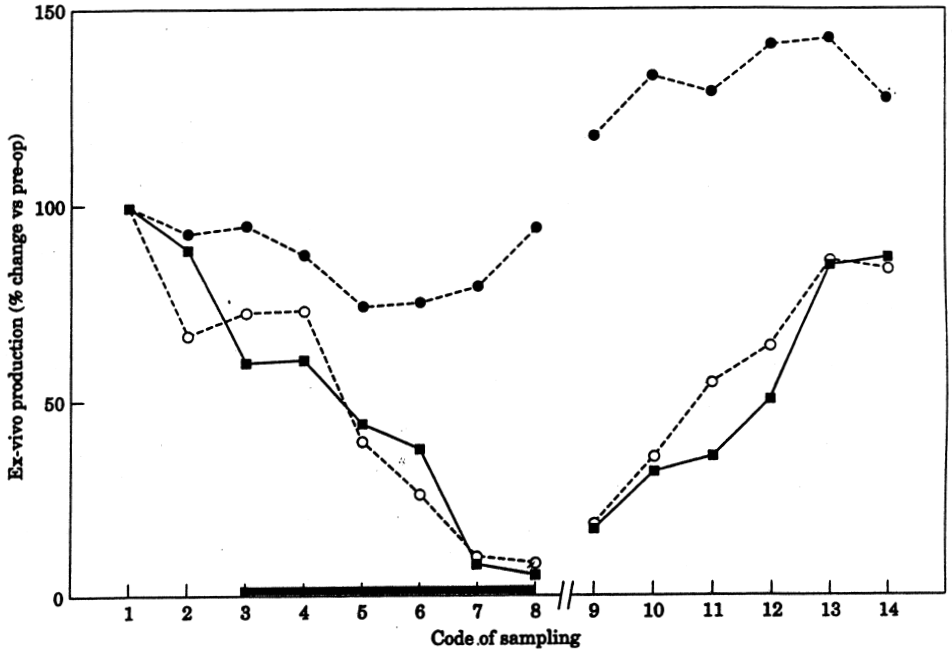


Figure 3. *Time course of ex-vivo production of cytokines in six patients.*
IL-1ß, open circles; TNF-α, closed squares; and IL-1ra, closed circles.
Data are expressed as percentage change versus pre-induction values (code of sampling 1). Sampling codes 1 to 8 signify sampling during operation; sampling codes 9 to 14 signify post-operative sampling. Detailed explanation for the time schedule for collecting blood samples is given in Table 1. The horizontal bar indicates the period of surgery.

On the first day after surgery, however, cortisol concentrations increased to 0.6 µmol/l and remained elevated up to the end of the study period. Plasma levels of ADH showed a prominent peak of approximately 30 pmol/L at 5 min after skin incision and a second peak also of approximately 30 pmol/L, at the end of surgery. Post-operatively ADH plasma values returned to baseline levels.
To investigate whether the observed effect was a result of blood loss, hemodilution or decrease of amount of mononuclear cells during surgery, hemoglobin concentration and mononuclear cell count were determined in the blood specimens. Hemoglobin concentration was measured in five patients and decreased only slightly at the end of surgery. Blood leukocyte count was performed in two patients and showed a small decrease during surgery and a small rise after surgery.

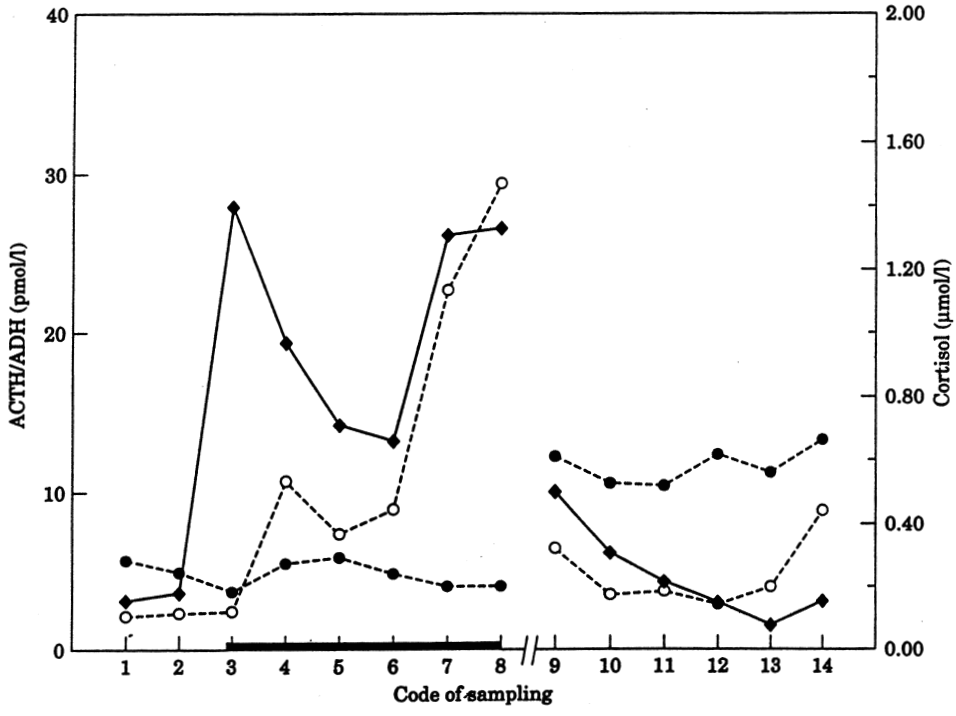


Figure 4. *Time course of hormonal measurements in five patients.*
ACTH (pmol/L), open circles; ADH (pmol/L), closed squares; and cortisol (µmol/l), closed circles. For explanation of code of sampling see Table 1.

Discussion

We studied plasma levels of circulating cytokines and ex-vivo cytokine production during the surgical stress response and observed in a group of 18 patients a small, but significant, depression of circulating pro-inflammatory IL-1ß level on day 1 post-operatively, and an increase in circulating anti-inflammatory IL-1ra level, on day 5 post-operatively. On day 1 post-operatively the ex-vivo production of IL-1ß and TNF-α was depressed. In the group of 6 patients for determination of a more detailed cytokine pattern, we could not detect the above-mentioned small depression of plasma levels of circulating IL-1ß, which we found in the larger group of 18 patients. The number of patients was probably too small and the difference only of borderline significance. In both studies blood mononuclear cell count and hemoconcentration changed only slightly, and blood loss was small, and therefore could not explain for the major changes in cytokine production and release.

Depression of circulating and ex-vivo production of pro-inflammatory cytokines in combination with elevation of circulating anti-inflammatory cytokines like IL-1ra

has been described during the acute phase of typhoid fever (13), meningococcal infection (14) and *Pneumocystis carinii* infection (15). Studies with isolated peripheral blood mononuclear cells or tissue macrophages showed low production capacity of cytokines in post-operative infection (16), sepsis (17-19) and attacks of familial Mediterranean fever (20,21).

The ex-vivo production of IL-1ß and TNF-α started to decrease already from the moment of insertion of the radial artery catheter, whereas plasma levels of circulating IL-1ra, ACTH and ADH increased during surgery. The plasma level of cortisol did not change during surgery, but was increased in the post-operative phase. The depression of ex-vivo IL-1ß and TNF-α production preceded the rise in the stress hormone cortisol and its regulator ACTH. In animal experiments, it has been shown that the neuroendocrine system and the immune system are intimately linked and involved in bidirectional communication (9). Both systems share the same set of signal molecules (hormones and cytokines) and their receptors. Thus, although a direct relation between production of cortisol and cytokines has been reported in literature, the late cortisol response in the present study strongly suggests that this immunosuppressive glucocorticoid is not a primary key player in the downregulation of synthesis and release of the pro-inflammatory cytokines during the surgical stress response.

Our findings support the hypothesis that the depression of IL-1ß and TNF-α production and the increase of circulating IL-1ra level, is caused by a switch of cytokine-producing cells from a mainly pro-inflammatory to an anti-inflammatory repertoire, and not by an exhaustion of productive capacity of the immune cells or an enhanced lysis of cytokine-producing cells (13,14). The present study shows that not only in infectious disease or fever these cytokine changes are present, but also during surgery. Therefore, this switch in cytokine pattern could be part of a general physiological stress response with a common mechanism of cytokine changes in stress situations like surgery, febrile disease and sepsis. Interestingly, Keuter et al. (13) found a lower cytokine production in patients with complicated typhoid fever than in patients with uncomplicated disease, suggesting that the degree of injury or stress is correlated with the degree of changes in cytokine pattern.

Pro-inflammatory cytokines are thought to be important for an adequate host defence. Depression of these pro-inflammatory cytokines and an increase of anti-inflammatory cytokines during surgery can cause suppression of the immune response and an increase in the risk of infectious complications. More knowledge on the mechanism of changes in cytokines and hormones and their mutual relation in the surgical stress response, may be important in the prevention of post-operative infectious complications.

Materials and methods

The study was approved by the Human Ethics Committee of the University of Nijmegen Academic Hospital. All patients were informed orally and in writing after which they gave their written consent.

Patients

In 18 patients (14 men and 4 women; age 53 to 78 years) undergoing elective implantation of an aortic bifurcation or tube prosthesis because of an abdominal aortic aneurysm or atherosclerotic obstruction, blood was drawn pre-operatively, on the first day post-operatively and on the fifth day post-operatively, for measurement of cytokines. Patients with endocrine or immune diseases were excluded from the study.

In six patients undergoing the same type of operation and not treated with glucocorticoids, 14 blood samples were collected starting 10 min after insertion of a radial artery catheter, prior to induction of general anesthesia, up to 6 days post-operatively (Table I), for determination of a more precise cytokine pattern. In five of these patients plasma levels of ACTH, cortisol and ADH were also measured.

Blood collection and handling

Blood for measurement of ACTH, ADH and cortisol was collected in pre-chilled EDTA-K3 tubes (Vacutainer Systems, Becton and Dickinson, Rutherford, NJ) and centrifuged for 10 min at 1500 x ***g*** (4 °C). The plasma obtained was aliquoted in polystyrene tubes containing 25 µL Trasylol^{R} (aprotinin, 10 000 kallikreine inactivating units (KIU)/mL; Bayer, Leverkusen, Germany). Blood for measurement of circulating cytokines was drawn in 4 mL EDTA-K_3 tubes containing 250 µL Trasylol^{R}. The tubes were centrifuged immediately at 2000 x ***g*** for 10 min and the resulting plasma at 15 000 x ***g*** for 5 min to obtain platelet-poor plasma. Aliquots were stored at -20 ^{0}C until assay. The ex-vivo production of cytokines was measured in whole blood using two 4 mL EDTA-K_3 tubes containing 250 µL Trasylol^{R} (22). Fifty microlitres LPS (final concentration 10 µg/mL; E. coli serotype 055:b5; Sigma, St. Louis, USA) was added under sterile conditions to one tube; the other tube was incubated without LPS. After 24 h incubation at 37 ^{0}C, both tubes were centrifuged and the plasma handled as described above.

RIA for cytokines

IL-1ß, TNF-α and IL-1ra were measured in duplicate by radioimmunoassay (RIA) as described previously by Drenth et al. (23). The sensitivity of the assays was 0.060 ng/mL (IL-1ra), 0.040 ng/mL (IL-1ß) and 0.020 ng/mL (TNF-α), respectively.

Hormone measurement

ACTH

Extraction from plasma. ACTH was extracted and concentrated from plasma by addition of 25 mg Vycor^{R}-glass powder (Corning Glass Works, New York, NY) in 250 µL bidistilled water to 500 µL aliquots of plasma. ACTH was eluted from the glass pellet with acetone/bidistilled water (50/50). The acetone fractions were evaporated to dryness and the residues were reconstituted in 250 µL of a solution containing 0.9% NaCl and 0.25% BSA (pH 3.5). Standard curves were prepared by spiking ACTH-free human plasma with ACTH (MRC 74/555). The standard samples were subjected to the same extraction procedure as the unknowns. The recovery was approximately 70%. All samples were extracted in duplicate.

RIA. ACTH-immunoreactivity in the supernatants was measured by RIA using a commercial ACTH antibody (IgG Corporation, Nashville, USA) and [^{125}I]ACTH$^{1-39}$ as tracer. The ACTH RIA was performed as follows: 100 µL rabbit ACTH antiserum (final dilution 1:30 000 in RIA-buffer (phosphate buffer containing 13 mM EDTA, 0.02% sodium azide, 0.25% BSA (ORHD 20/21, Behring), 0.1% Triton X-100 and 250 kallikreine inactivating units (KIU) Trasylol/mL)) was added to 100 µL sample or standard. The mixture was preincubated for 3 days at 4 ^{0}C with antiserum. Then, tracer (approximately 7000 dpm/100 µL) was added and incubation was continued for another day. Bound and free ACTH were separated by a second antibody system (10% sheep anti-rabbit IgG and 0.01% rabbit IgG). The antibody complex was precipitated by addition of 1 mL 7.5% PEG 6000 (Merck). The sensitivity of the assay system was 1 pmol/L, and the within- and between-assay coefficients of variation of the procedure were 9.1% and 15.3% respectively (12).

Cortisol

Plasma cortisol levels were measured by RIA using an antiserum raised in rabbit against a cortisol-21-hemisuccinate-bovine serum albumin conjugate. Standard and samples (50 µL) were diluted 1:100 with ethanol/water (5/95, v/v) and corticosteroid binding globulin was denatured by incubation at 70 ^{0}C for 1 h. Subsequently, tracer ([1,2,6,7-^{3}H]cortisol) and antibody were added, and bound and free hormone were separated by dextran-coated charcoal after incubation for 18 h at 4 ^{0}C. After centrifugation the supernatants were decanted and radioactivity measured. The sensitivity of the assay was 0.02 µmol/L. The within- and between-assay coefficients of variation were 4.5% and 6.6% at 0.21 µmol/L.

ADH

Extraction from plasma. Extraction of ADH was performed over Sep Pak C18 columns (Waters) with 1 mL acidified plasma aliquots. The column was washed

with 10 mL 4% acetic acid, 2 mL chloroform, and 10 mL 4% acetic acid, respectively. ADH was eluted with 4 mL 100% methanol. The recovery was approximately 80%. Eluates were dried overnight in a Speedvac and the residues redissolved in RIA buffer.

RIA ADH-immunoreactivity in the eluates was measured by RIA using an ADH antibody (P31080, kindly provided by dr. D. Swaab, NIH, Amsterdam). ADH (MRC77/501) was used as standard and [^{125}I]ADH as tracer. The ADH RIA was performed as follows: 50 µL rabbit ADH antiserum (final dilution 1:60 000) was added to 50 µL sample or standard. The mixture was preincubated for 3 days at 4 ^{0}C with antiserum. Then, tracer (approximately 7000 dpm/50 µL) was added and incubation was continued for another day. Separation of bound and free ADH was identical to that of the ACTH RIA. The sensitivity of the assay system was 0.8 pmol/L, and the within- and between-assay coefficients of variation of the procedure were less than 8% (24).

Blood loss and mononuclear cell counting

To estimate blood loss and hemodilution the hemoglobin concentration was measured in five patients. Mononuclear cell count (monocytes and lymphocytes) in peripheral blood of two patients was measured. Hemoglobin concentration and mononuclear cell count was determined using an automated hematological analyzer Sysmex NE-8000 (Charles Goffin Medical Systems bv, Tiel, The Netherlands).

Statistics

For the group of 18 patients, differences in cytokine values between pre-operative values and values on the first day post-operatively, and differences in cytokine values between the first day post-operatively and the fifth day post-operatively, were tested by Wilcoxon signed rank test.

The results of the additional study in the group of six patients, each giving 14 blood samples, are presented in Figure 2, 3 and 4. In all six patients plasma levels of cytokines were measured; in five of these patients plasma levels of ACTH, cortisol and ADH were also determined. Data are presented as mean ± SEM.

Acknowledgments

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Addendum

A secondary aim of this study was to compare the influence of two parenteral lipid emulsions, structured triglycerides and a physical mixture of medium- and long-chain triglycerides, on cytokine production and neuroendocrine response during surgery. At random, total parenteral nutrition with structured triglycerides or the physical mixture was administered for the first 5 days after surgery as described in Chapter 5. No differences were observed between the results of the group of patients treated with structured triglycerides or with the physical mixture. The results of both groups were combined.

The lack of difference between the results of the two lipid emulsions is most likely explained by the overwhelming influence of surgery on cytokine production and neuroendocrine response.

Chapter 8

WITH MEDIUM-CHAIN TRIGLYCERIDES, HIGHER AND FASTER OXYGEN RADICAL PRODUCTION BY STIMULATED POLYMORPHONUCLEAR LEUKOCYTES OCCURS

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Abstract

Background Parenteral lipid emulsions are suspected of suppressing the immune function. However, study results are contradictory and mainly concern the conventional long-chain triglyceride emulsions.

Methods Polymorphonuclear leukocytes were preincubated with parenteral lipid emulsions. The influence of the lipid emulsions on the production of oxygen radicals by these stimulated leukocytes was studied by measuring chemiluminescence. Three different parenteral lipid emulsions were tested: long-chain triglycerides, a physical mixture of medium- and long-chain triglycerides, and structured triglycerides. Structured triglycerides consist of triglycerides where the medium- and long-chain fatty acids are attached to the same glycerol molecule.

Results Stimulated polymorphonuclear leukocytes preincubated with the physical mixture of medium- and long-chain triglycerides showed higher levels of oxygen radicals ($p < .005$) and faster production of oxygen radicals ($p < .005$) compared with polymorphonuclear leukocytes preincubated with long-chain triglycerides or structured triglycerides. Additional studies indicated that differences in results of various lipid emulsions were not caused by differences in emulsifier. The overall production of oxygen radicals was significantly lower after preincubation with the three lipid emulsions compared with controls without lipid emulsion.

Conclusions A physical mixture of medium- and long-chain triglycerides induced faster production of oxygen radicals, resulting in higher levels of oxygen radicals, compared with long-chain triglycerides or structured triglycerides. This can be detrimental in cases where oxygen radicals play a pathogenic role or beneficial, when rapid phagocytosis and killing of bacteria is needed. The observed lower production of oxygen radicals by polymorphonuclear leukocytes in the presence of parenteral lipid emulsions may result in immunosuppression by these lipids.

Introduction

Total parenteral nutrition (TPN) is nutrition administered directly into the bloodstream of patients who are unable to meet their nutritional needs by using the normal enteral route. The triglycerides in TPN are emulsified by phospholipids. These emulsions are sterile and pyrogen-free. In a large study, more infectious complications were observed in borderline and mildly malnourished patients, who were peri-operatively treated with TPN, compared with controls not receiving peri-operatively TPN (1). The infectious complications were mainly pneumonia and wound infection. The authors suggested that the long-chain triglyceride emulsion in the parenteral nutrition was a possible cause of this increased rate of infections.

Conventional parenteral lipid emulsions are long-chain triglyceride emulsions. These emulsions are made of soybean oil and consist of long-chain fatty acids. In

addition to long-chain triglycerides, medium-chain triglycerides are used in parenteral lipid emulsions, because in some studies they improve nitrogen balance (2,3) and are utilized more rapidly (4). Medium-chain triglycerides consist only of medium-chain fatty acids. Medium-chain triglycerides are always given together with long-chain triglycerides as a physical mixture, in order to provide the essential long-chain fatty acids and to reduce side effects such as metabolic acidosis (5) and neurological toxicity (6) caused by high levels of medium-chain free fatty acids (Fig. 1). Recently, so-called structured triglycerides were synthesized to reduce the risk of toxicity by medium-chain free fatty acids further. Structured triglycerides consist of triglycerides where the medium- and long-chain fatty acids are attached to the same glycerol molecule (Fig. 1). In our clinical trial on 25 moderately catabolic patients after major vascular surgery, structured triglycerides caused lower plasma concentrations of medium-chain free fatty acids (7) and improved nitrogen balance, compared with the physical mixture of medium- and long-chain triglycerides (8).
The influence of various lipid emulsions on leukocyte function is still unclear. The results of in-vitro studies, and in-vivo studies, both in volunteers and in patients, are contradictory and concern mainly the conventional long-chain triglyceride emulsions (9-26). Studies on the effects of the physical mixture on leukocyte function are rare and inconclusive: in-vitro, the physical mixture impaired leukocyte

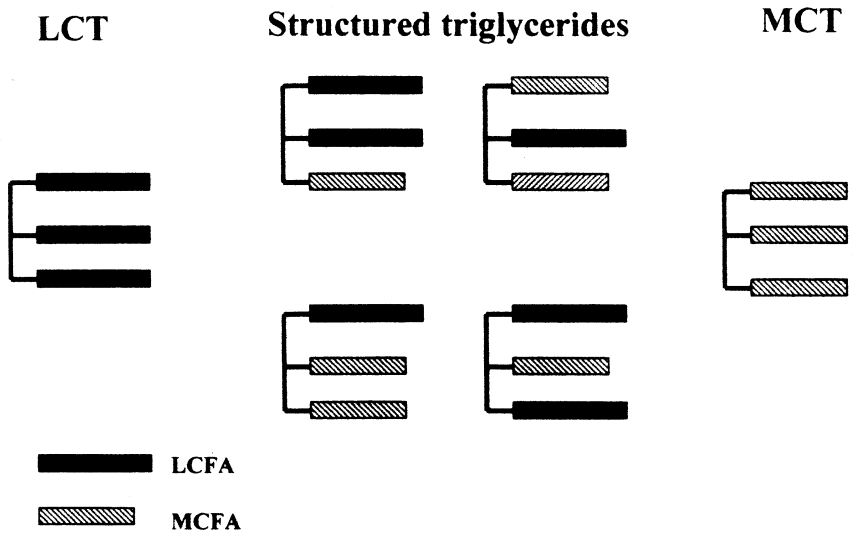

Figure 1. *A physical mixture of medium- and long-chain triglycerides consists of triglycerides that contain either three long-chain fatty acids or three medium-chain fatty acids. A structured triglyceride emulsion consists of triglycerides where the medium- and long-chain fatty acids are randomly attached to the same glycerol molecule.*

function (19,20) but infusion in volunteers and patients did not have any negative effect on leukocyte function (27,28). Data on the effects of structured triglycerides on leukocyte function are scarce, consisting of only two animal studies on the function of the mononuclear phagocyte system with conflicting results (29,30).
Our goal was to study the in-vitro effects of lipid emulsions containing fatty acids with different chain length on the production of oxygen radicals by polymorphonuclear leukocytes. These oxygen radicals are important for bacterial killing. Three parenteral lipid emulsions used in clinical practice were tested: long-chain triglycerides, a physical mixture of medium-chain and long-chain triglycerides, and a structured triglyceride emulsion. We studied the production of oxygen radicals by measuring chemiluminescence of phagocytosing polymorphonuclear leukocytes. Chemiluminescence is the emission of light by a chemical reaction and occurs during the production of oxygen radicals (31).

Materials

Volunteers and handling of venous blood samples

Venous blood samples were obtained from 30 healthy volunteers, 15 men and 15 women, aged 22 to 46 years. Their laboratory tests, including blood hemoglobin and white blood cell count with differential count, and serum total bilirubin, alkaline phosphatase, aspartate aminotransferase, and fasting cholesterol and triglycerides were normal. Fasting serum triglycerides ranged from 0.39 to 1.60 mmol/L. The blood was collected into twelve 4 mL-draw sterile vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing 4.5 mg lyophilized EDTA(Na_2).

Opsonized Zymosan

Zymosan A particles (Sigma Chemical Company, St.Louis, MO), 300 mg, were washed once in 30 mL phosphate-buffered saline, pH 7.3. For 1 hour, the solution was treated in a sonificator (Bransonic Ultrasonic Cleaner, Model B-2200 E3, Branson Cleaning Equipment Company, Shelton, CT) at 47 kHz, to promote equal particle size. The zymosan particles were opsonized with 30 mL of serum from one donor with blood group antigen AB, during 60 minutes at 37 ^{0}C. Then the particles were washed three times with phosphate-buffered saline, 4 ^{0}C, and centrifuged at 200***g*** for 10 minutes. The pellet of zymosan particles was suspended in 30 mL of Hank's Balanced Salt Solution without phenol red (Life Technologies, Paisley, Scotland) to a final concentration of 10 g/L and stored in portions of 1.4 mL at -70 ^{0}C.

Luminol solution

Luminol (Sigma Chemical Company) was dissolved in dimethyl sulphoxide (Sigma Chemical Company) at a concentration of 10^{-2} M. Before use, the stock solution was further diluted to a concentration of 2 x 10^{-5} M in Hank's Balanced Salt

Solution without phenol red. To this solution, bovine serum albumin (Sigma Chemical Company) was added, 20 g/L, to prevent cell aggregation.

Lipid emulsions

In experiment 1 and 2, we studied the effect of lipid emulsions on leukocyte function. We tested three lipid emulsions: a long-chain triglyceride emulsion (Intralipid; Fresenius-Kabi, Stockholm, Sweden); a physical mixture of medium-chain and long-chain triglycerides (Lipofundin MCT/LCT; B. Braun Melsungen AG, Melsungen, Germany); and a structured triglyceride emulsion (Structolipid, Fresenius-Kabi) (Fig. 1, Table I). The weight ratio of long-chain to medium-chain fatty acids is 50% to 50% in the physical mixture and 64% to 36% in the structured triglyceride emulsion. These three lipid emulsions have egg yolk phospholipids as the emulsifying agent.

Table I. *Composition and characteristics of the long-chain triglyceride (TG) emulsion (Intralipid), the physical mixture of medium- and long-chain triglycerides (Lipofundin MCT/LCT), and the structured triglyceride emulsion (Structolipid).*

Composition and characteristics	Long-chain TG	Physical mixture	Structured TG
Fractionated soy bean oil (g/L)	200	100	0
Medium-chain triglycerides (g/L)	0	100	0
Structured triglycerides (g/L)	0	0	200
Mean molecular weight of triglycerides	865	634	683
Fractionated egg phospholipids (g/L)	12	12	12
Glycerol (USP) (g/L)	22.0	25.0	22.5
Water for injection ad (mL)	1000	1000	1000
pH	8	8	6.5-8.5
Osmolality (mOsm/kg water)	350	380	350
Energy content (kcal/L)	2000	1936	1960
Fatty acids, % by weight			
Caprylic acid (C8:0)	0	26	27
Capric acid (C10:0)	0	20	10
Palmitic acid (C16:0)	13	7	7
Stearic acid (C18:0)	4	2.5	3
Oleic acid (C18:1)	22	13	13
Linoleic acid (C18:2ω6)	52	27	33
Alpha-linolenic acid (C18:3ω3)	8	3.5	5
Other	1	1	2

In experiment 3 we studied the influence of the emulsifier in the lipid emulsions on leukocyte function. Three long-chain triglyceride emulsions were tested. Intralipid and Emulsan (both produced by Fresenius-Kabi) have egg yolk phospholipids as the emulsifying agent, whereas Lipofundin S (B. Braun Melsungen AG) uses soybean phospholipids.
All these lipid emulsions used in parenteral nutrition are sterile and pyrogen-free.

Isolation of polymorphonuclear leukocytes
Venous blood was mixed 1:1 with phosphate-buffered saline. Polymorphonuclear leukocytes were isolated by density gradient centrifugation, using a Percoll gradient (Percoll, Pharmacia, Uppsala, Sweden], at 500***g*** for 20 minutes. To prevent cell aggregation, isolation was done at 4 ^{0}C and the cells were suspended in phosphate-buffered saline, containing 1 g gelatin per liter (Bacto-Gelatin, Difco Laboratories, Detroit, MI). After centrifugation at 500***g*** for 5 minutes, the pellet was resuspended in ammonium chloride solution (8 g/L), pH 7.4, and contaminating erythrocytes were lysed for 10 minutes at 4 ^{0}C. The cells were then centrifuged at 500***g*** for 5 minutes and the pellet washed once in phosphate-buffered saline containing gelatin. The number of cells was counted in a cell counter (Coulter Counter ZM, Coulter Electronics Nederland, Mijdrecht, The Netherlands) and the suspension adjusted to a final concentration of 5 x 10 ^{9} per liter with phospate-buffered saline containing gelatin. At random Cytospin preparations (Shandon, Astmoor, United Kingdom) demonstrated a contamination of polymorphonuclear leukocytes by other cells of less than 10 %.

Incubation of the polymorphonuclear leukocytes with the lipid emulsions
One-milliliter samples of polymorphonuclear leukocyte suspension were incubated for 30 minutes at 37 ^{0}C with lipid emulsion. Suspensions of polymorphonuclear leukocytes in phosphate-buffered saline with gelatin served as a control. During incubation, the suspensions were continuously mixed. After incubation with the lipids, the polymorphonuclear leukocyte suspensions were washed twice in phosphate-buffered saline with gelatin, 4 ^{0}C and centrifuged for 5 minutes at 500***g***. The pellets were resuspended in 1 mL of phosphate-buffered saline with gelatin at room temperature and stored for 45 minutes. The number of cells was counted in the cell counter and the suspensions adjusted to a final concentration of 2 x 10 ^{9} per liter. With trypan blue (0.4 % in 0.85 % saline, Flow Laboratories Inc, McLean, VA), the percentage of viable cells in these suspensions was greater than 95 %.

Measurement of chemiluminescence
The reaction mixtures consisted of 0.2 mL polymorphonuclear leukocyte suspension, 0.1 mL opsonized zymosan, and 0.7 mL Luminol in Hank's Balanced

Salt Solution. Therefore the concentration of polymorphonuclear leukocytes in the reaction mixture was 0.4 x 10^9 per milliliter and the concentration of opsonized zymosan in the reaction mixture was 1 mg/mL. Blanks of 0.2 mL phosphate-buffered saline with gelatin, 0.1 mL opsonized zymosan, and 0.7 mL Luminol were measured simultaneously. The mixtures were prepared in disposable polystyrene cuvettes. Chemiluminescence was measured with an automatic photoluminometer (LKB Wallac Luminometer 1251, Wallac Oy, Turku, Finland), at 37 ^{0}C and recorded as millivolts (mV). The computer calculated the peak light emission (peak height, mV), the time until the peak of the light emission was reached (peak time, s), and the total amount of light emitted during the first 100 minutes (mVs).

Statistical analysis

Wilcoxon signed-rank test was applied to test the differences between the three lipids, and the differences of each lipid with respect to the control (no lipid emulsion). Bonferroni correction was used to determine significance: significance was reached when the *p*-value was smaller than .0166. The parameters were peak height, peak time, and the amount of light emitted.

Experiments

Experiment 1

Polymorphonuclear leukocytes were preincubated with long-chain triglycerides (Intralipid), a physical mixture of medium-chain and long-chain triglycerides (Lipofundin MCT/LCT), or structured triglycerides (Structolipid) in supraphysiologic concentrations of 25 mmol/L, 50 mmol/L, or 100 mmol/L.

Experiment 2

Polymorphonuclear leukocytes were preincubated with the same three lipid emulsions as in experiment 1, but now physiologic concentrations were used of 1.25 mmol/L, 2.50 mmol/L, or 5.00 mmol/L. These physiologic concentrations are obtained in clinical practice during the treatment of patients with TPN (32).

Experiment 3

We investigated the role of the emulsifier. We tested three long-chain triglyceride emulsions, which contain various emulsifiers: Lipofundin S, Intralipid or Emulsan, in one physiologic concentration of 1.00 mmol/L. Intralipid and Emulsan use egg yolk phospholipids as emulsifying agent, whereas Lipofundin S uses soybean phospholipids.

All experiments

In all three experiments, samples that were preincubated without lipid emulsion

were used as control. In each of the three experiments, polymorphonuclear leukocytes from 10 volunteers were tested on 10 different days.

Results

Experiment 1

In all of the supraphysiologic dosages of lipid emulsions, phagocytosing polymorphonuclear leukocytes preincubated with the physical mixture of medium-chain and long-chain triglycerides showed a different pattern of chemiluminescence compared with preincubation with long-chain triglycerides, structured triglycerides, or controls (Table II). The production of oxygen radicals was faster ($p < .005$) and reached a higher peak height ($p < .005$ vs. long-chain triglycerides or structured triglycerides; $p < .02$ vs. controls). Furthermore, the overall production of oxygen radicals was significantly lower with the physical mixture of medium-chain and long-chain triglycerides ($p < .02$) or long-chain triglycerides ($p < .005$) compared with controls. With structured triglycerides, a significant difference was not reached. The production of oxygen radicals was faster with all three lipid emulsions compared with controls, although with long-chain triglycerides or structured triglycerides the difference was not significant. Chemiluminescence results (peak height, peak time and overall production of reactive oxygen metabolites) with long-chain triglycerides and structured triglycerides were equal. Results with 25 mmol/L, 50 mmol/L, or 100 mmol/L of lipid emulsion did not differ and so these results were pooled.

Table II *Chemiluminescence by phagocytosing polymorphonuclear leukocytes, preincubated with various lipid emulsions in supraphysiologic dosages (long-chain triglycerides, physical mixture of medium- and long-chain triglycerides, or structured triglycerides versus controls)*[§].

	Controls		
	Peak height (mV)	Peak time (s)	Overall production (Vs)
Long-chain triglycerides	-3 ± 3	-97 ± 88	-23 ± 6**
Physical mixture MCT/LCT	+12 ± 3*	-618 ± 117**	-38 ± 12*
Structured triglycerides	-1 ± 2	-52 ± 47	-12 ± 7

[§]*Polymorphonuclear leukocytes of 10 volunteers were measured. Supraphysiologic dosages of lipid emulsion were 25 mmol/L, 50 mmol/L, or 100 mmol/L. Results with the three concentrations did not differ and so these results were pooled.*

** $p < .02$ and ** $p < .005$, using Wilcoxon signed-rank test.*

Experiment 2

In physiologic dosages of lipid emulsions, the same different pattern of chemiluminescence was observed with phagocytosing polymorphonuclear leukocytes preincubated with the physical mixture of medium-chain and long-chain triglycerides compared with preincubation with long-chain triglycerides, structured triglycerides, or controls (Table III). Again, the production of oxygen

Table III. *Chemiluminescence by phagocytosing polymorphonuclear leukocytes, preincubated with various lipid emulsions in physiologic dosages (long-chain triglycerides, physical mixture of medium- and long-chain triglycerides, or structured triglycerides versus controls)*[§].

	Controls		
	Peak height (mV)	Peak time (s)	Overall production (Vs)
Long-chain triglycerides	-18 ± 4**	+186 ± 21**	-29 ± 9*
Physical mixture MCT/LCT	+ 7 ± 4	-322 ± 28**	-49 ± 10**
Structured triglycerides	-15 ± 3**	+144 ± 34**	-30 ± 9*

[§]*Polymorphonuclear leukocytes of 10 volunteers were measured. Physiologic dosages of lipid emulsion were 1.25 mmol/L, 2.50 mmol/L, or 5.00 mmol/L. Results with the three concentrations did not differ and so these results were pooled.*
** $p < .02$ and ** $p < .005$, using Wilcoxon signed-rank test.*

radicals was faster ($p < .005$) and reached a higher peak height ($p < .005$) vs. long-chain triglycerides or structured triglycerides (Fig. 2). Only in physiologic dosages was the peak height with the physical mixture of medium-chain and long-chain triglycerides increased, although not significantly compared with controls. Again, the overall production of oxygen radicals was significantly lower with the physical mixture of medium-chain and long-chain triglycerides ($p < .005$), the long-chain triglycerides ($p < .02$) or the structured triglycerides ($p < .02$), compared with controls. In supraphysiologic dosages, the production of oxygen radicals was faster with all three lipid emulsions compared with controls (Table II). However, in physiologic dosages, the production of oxygen radicals was only faster with the physical mixture ($p < .005$) and slower with long-chain triglycerides ($p < .005$) or the structured triglycerides ($p < .005$). Chemiluminescence results with long-chain triglycerides and structured triglycerides were again equal. Results with 1.25 mmol/L, 2.50 mmol/L, or 5.00 mmol/L of lipid emulsion did not differ and so these results were pooled.

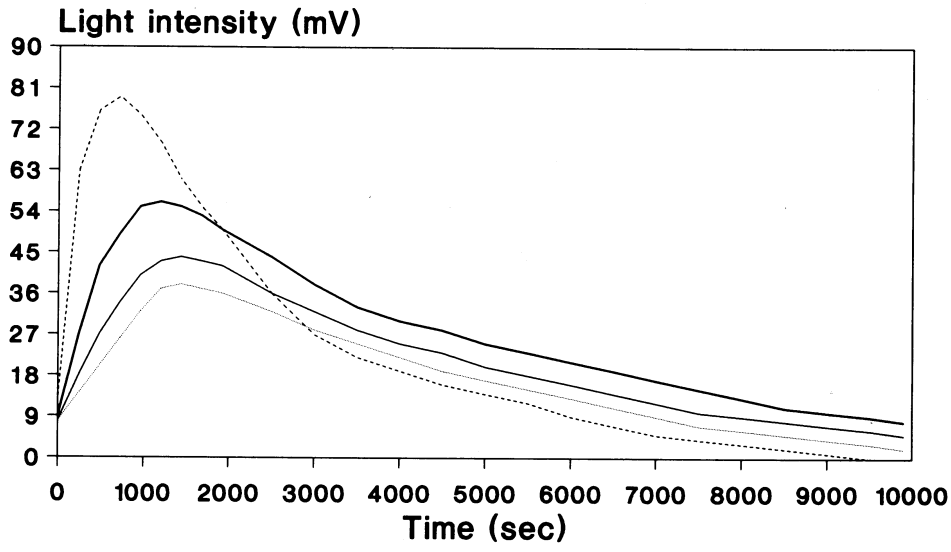

Figure 2. *Luminol-enhanced chemiluminescence of zymosan-stimulated phagocytosis by polymorphonuclear leukocytes of one volunteer. The polymorphonuclear leukocytes were preincubated with lipid emulsion (1.00 mmol/L) or without lipid emulsion (solid, bold line). Preincubation with a physical mixture of medium-chain and long-chain triglycerides (broken line) results in a different pattern of chemiluminescence compared with preincubation with long-chain triglycerides (solid, not bold line) or structured triglycerides (dotted line), characterized by a faster production of oxygen radicals and higher peak levels of oxygen radicals.*

Experiment 3

There were no differences in production of oxygen radicals between the three long-chain triglyceride emulsions Lipofundin S, Intralipid, or Emulsan (results not shown). These lipid emulsions differ in emulsifier: Lipofundin S uses soybean phopholipids and Intralipid and Emulsan use egg yolk phospholipids.

Discussion

Polymorphonuclear leukocytes preincubated with a physical mixture of medium-chain and long-chain triglycerides differed in pattern of light emission, compared with preincubation with long-chain triglycerides, structured triglycerides or controls. This different pattern was seen with supraphysiologic and physiologic dosages of lipid emulsions, and was characterized by higher peak height and shorter peak time of light emission. This indicates, that phagocytosing polymorphonuclear leukocytes preincubated with the physical mixture produced oxygen radicals faster, resulting in higher peak levels of oxygen radicals. The clinical consequences in

patients could be that this pattern of oxygen radical production is detrimental when oxygen radicals either play a pathogenic role (as in multiple organ failure) or a beneficial one as when rapid phagocytosis and killing of bacteria is needed.

Preincubation with long-chain triglycerides, the physical mixture or structured triglycerides reduced the overall production of oxygen radicals, compared with controls without lipid emulsion; only with structured triglycerides in high dosages significance was not reached. Almost all of the studies on the influence of lipid emulsions on oxygen radicals have used only long-chain triglyceride emulsions. These studies also reported a depressed production of oxygen radicals (9,13-15). Only one study has investigated the effect of a physical mixture and showed a depressed production of oxygen radicals (20). The different pattern of chemiluminescence with a physical mixture was not described earlier. Only two in-vivo infusion studies in humans have been performed on oxygen radical production, both with long-chain triglycerides. These two studies confirmed a depression of oxygen radical production in humans after infusion of long-chain triglycerides (14,16).

The most likely explanation for differences in oxygen radical production between lipid emulsions is the difference in triglyceride composition. Another possible factor that could be of influence is the emulsifier. The emulsifier is a component of every parenteral lipid emulsion and consists of phospholipids. We studied the effect of three long-chain triglyceride emulsions with various emulsifiers. No differences in oxygen radical production between these emulsions were observed. This indicates that the differences in oxygen radical production are caused by differences in triglycerides. Other investigations also indicate the fatty acid composition of the triglycerides as a cause of changes in polymorphonuclear leukocyte function. The long-chain fatty acids, palmitic and oleic acid, present in parenteral lipid emulsions, caused depression of chemotaxis and phagocytosis by polymorphonuclear leukocytes (33). One study described a synergistic stimulatory action of oleic acid and the cytokine tumor necrosis factor-α on the production of oxygen radicals (34). Functional changes of leukocytes were accompanied by morphological alterations on electron microscopic examination (33), with cytoplasmic lipid droplets, and dilations of endoplasmic reticulum with degenerative degranulated cytoplasmic areas. Indeed, after infusion of long-chain triglycerides, cytoplasmic lipid droplets in the polymorphonuclear leukocytes, surrounded by a bilayer membrane were observed (14). This suggests engulfment of lipid particles, which reduces the cell surface and may cause impaired motility and phagocytosis (14). Also, it was hypothesized that decreased deformability of the cells (33), alterations in lipid composition of the cell membrane, changes in membrane fluidity (9), degranulation, and cell injury cause impaired polymorphonuclear function (33). Finally, fatty acids, especially the polyunsaturated essential fatty acids, have a direct immunomodulatory effect (34).

In conclusion, a physical mixture of medium-chain and long-chain triglycerides has a different effect on the production of oxygen radicals by phagocytosing polymorphonuclear leukocytes compared with a long-chain or a structured triglyceride emulsion, causing higher peak levels and a faster production of oxygen radicals. This can potentially be detrimental when oxygen radicals play a pathogenic role or beneficial when rapid phagocytosis is needed. The overall production of oxygen radicals was decreased with all three lipid emulsions, which may explain immunosuppression by parenteral lipid emulsions.

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Chapter 9

OUR RESULTS ON STRUCTURED TRIGLYCERIDES AND NITROGEN BALANCE IN PERSPECTIVE TO OTHER TRIALS AND HYPOTHESES ON THE MECHANISMS

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Introduction

Over fifteen years ago animal studies already indicated that structured triglycerides have beneficial effects on nitrogen balance. In rat experiments it was demonstrated that structured triglycerides improved nitrogen retention and body weight compared to lipid emulsions containing medium-chain triglycerides, long-chain triglycerides or physical mixtures (1,2,3).

Structured triglycerides versus long-chain triglycerides

The first clinical studies on structured triglycerides compared structured triglycerides with long-chain triglyceride emulsions. Sandström et al. published the first clinical trial on structured triglycerides in 1993 (4). They infused lipid emulsions containing structured triglycerides or long-chain triglycerides in post-operative patients and demonstrated that structured triglycerides were safe and well-tolerated.

Two years later the same group studied structured triglycerides again in surgical patients. Patients were randomized to receive structured triglycerides for one day followed by long-chain triglycerides the next day or vice versa for a period of six days. In part one of the study patients were provided 1.0 g fat/kg/day in the presence of 80 % of basal energy expenditure as non-protein calories; in part 2 they received 1.5 g fat/kg/day and 120 % of basal energy expenditure as non-protein calories (5). Structured triglycerides were well-tolerated and increased lipid oxidation rate. In both studies no differences between the two groups were seen concerning nitrogen balance. In the structured triglyceride group, β-hydroxybutyrate concentrations were higher.

Bellantone et al. compared structured triglycerides and long-chain triglycerides in terms of nitrogen balance in patients with colorectal surgery (6). They also demonstrated safety and tolerability of structured triglycerides. The cumulative nitrogen balance on day 5 was significantly better in the structured triglyceride group (10.7 ± 10.5 vs. 6.5 ± 17.9 g nitrogen; p=0.05).

A more recent study was published by Lindgren et al (7). Thirty Intensive Care patients with pancreatitis, ARDS or peritonitis were included and randomized to receive either structured triglycerides or a long-chain triglyceride emulsion. Patients in the structured triglyceride group had a significantly better nitrogen balance compared to the long-chain triglyceride group after 3 days (–0.7 ± -6.0 vs. –16.5 ± -3.9 g nitrogen/day), but not after 5 days. Probably the reason for the loss of significance is the loss of patients during this study.

A safety study with patients on long-term home parenteral nutrition has also been carried out (8). In this double blind controlled cross-over trial, patients with Crohn's disease or a short bowel syndrome received for four weeks a long-chain triglyceride emulsion and the following four weeks an emulsion containing

structured triglyceride, and vice versa. There were no differences in liver and kidney function, plasma lipids, free fatty acids and 3-OH fatty acids. Two patients showed liver function disorders during the administration of the long-chain triglyceride emulsion, which normalized after switch to structured triglycerides.

Structured triglycerides versus physical mixture

Even more interesting as the comparison of structured triglycerides with long-chain triglycerides, is the comparison of structured triglycerides with a physical mixture of medium-chain and long-chain triglycerides, because in animal studies it has been shown that structured triglycerides are even better than physical mixtures with regard to various effects. We carried out a clinical trial, which was designed as a randomized, double-blind parallel study in 25 surgical patients, after elective implantation of an aortic prosthesis (9). The cumulative nitrogen balance after 5 days was significant better in the structured triglyceride group (-8 ± 2 g vs. –21 ± 4 g nitrogen over 5 days; $p = .015$).

Chambrier et al. included 40 surgical patients and provided 50 % of the non-protein calories as lipids (10). The endpoints were the cumulative nitrogen balance and 3-methylhistidine excretion. No different effects of the two lipid emulsions on any of the endpoints could be demonstrated.

Overview structured triglycerides and nitrogen balance

Table I summarizes the results of the fully published clinical trials on structured triglycerides and nitrogen balance. In two of the four fully published trials

Table I. *Summary of the published controlled clinical trials on nitrogen balance with structured triglycerides (STG) versus long-chain triglycerides (LCT), or versus a physical mixture of medium-chain and long-chain triglycerides (MCT/LCT).*

	Patients, number	Effect
STG vs. LCT		
Lindgren	on ICU with sepsis or multiple injury, 30	significant better nitrogen balance, 3 days
Bellantone	colorectal surgery 19	significant better nitrogen balance, 5 days
Sandström, 1993	elective surgery 20	no difference in nitrogen balance/day, 5 to 7 days
Sandström, 1995	elective surgery 19 + 18, cross-over	no difference in nitrogen balance/day, 2 x 3 days
STG vs. MCT/LCT		
Chambrier	abdominal surgery 40	no difference in nitrogen balance, 7 days
Kruimel	major vascular surgery 19	significant better nitrogen balance, 5 days

comparing structured triglycerides with long-chain triglycerides the nitrogen balance was better in the structured triglycerides group. In the second study of Sandström the results can be explained by the study design. In this study the type of lipid emulsion was switched every day, which makes it difficult to reach significant differences between the groups.
In the two fully published papers comparing structured triglycerides with a physical mixture, our study demonstrated improvement of nitrogen balance. We provided 30 % of the calories by the lipid emulsions. In the study by Chambrier 50% of the calories were provided by the lipid emulsions. The nitrogen balance in their study was slightly positive in the structured triglycerides group, but they did not find a significant difference between the groups. The differences in results between the two trials could be explained by the differences in study design.

Hypotheses to explain the effects of the various lipid emulsions

In our study we assessed serum triglycerides, plasma medium-chain free fatty acids and β-hydroxybutyrate. Triglycerides are hydrolyzed by lipoprotein lipase to free fatty acids, monoglycerides and glycerol, which enter the mitochondria and are oxidized. In case the oxidative system is overloaded, only partial oxidation of the fatty acids takes place and intermediates (acetyl-CoA) are converted to β-hydroxybutyrate. In our trial on the first post-operative day, serum triglyceride concentrations were almost doubled during the 6-hour period of lipid infusion in the physical mixture group compared with the structured triglycerides group (Fig. 1). The same picture emerged for the plasma medium-chain free fatty acid concentrations (Fig. 2). Both could not be explained by the slightly higher amount of triglycerides (1.08 times) and medium-chain fatty acids (1.25 times), on a molar base, in the

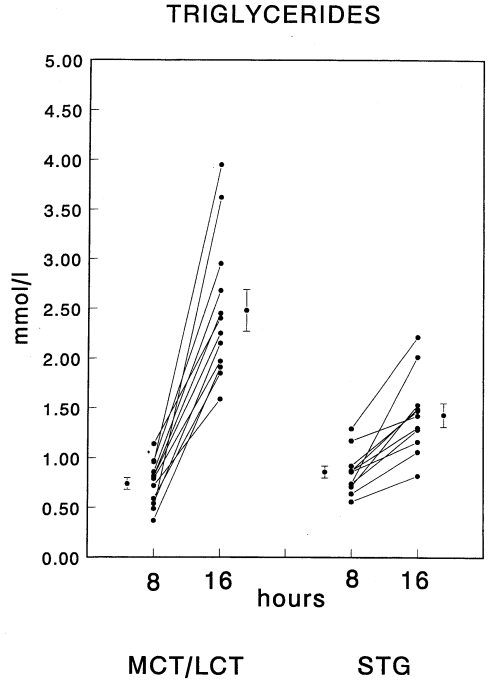

Figure 1. *Concentrations of serum triglycerides during a six hour administration of structured triglycerides (STG) and a physical mixture of medium-chain and long-chain triglycerides (MCT/LCT) (difference in change 1.10 mmol/L, 95 % confidence interval 0.70 to 1.50, p< 0.00005).*

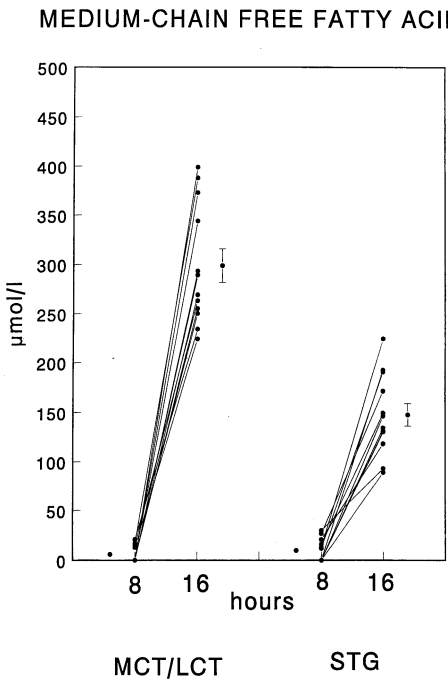

Figure 2. *Concentrations of plasma medium-chain free fatty acids during a six hour administration of structured triglycerides (STG) and a physical mixture of medium-chain and long-chain triglycerides (MCT/LCT) (difference in change 145 µmol/L, 95 % confidence interval 99 to 161, p< 0.00005).*

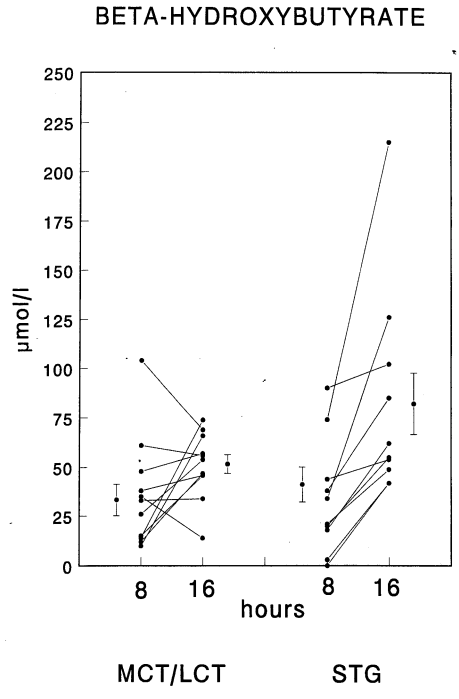

Figure 3. *Concentrations of plasma β-hydroxybutyrate during a six hour administration of structured triglycerides (STG) and a physical mixture of medium-chain and long-chain triglycerides (MCT/LCT) (difference in change 35 µmol/L, 95 % confidence interval 3 to 66, p< 0.05).*

physical mixture group. Contrarily, the concentrations of plasma β-hydroxybutyrate were significantly higher in the structured triglyceride group (Fig. 3). These concentrations did not reach toxic levels.

These results indicates a faster clearance of structured triglycerides, a faster uptake by the cells and a high oxidation rate, because the increase in β-hydroxybutyrate points to the hypothesis that the β-oxidative system has reached the maximum capacity. The high oxidation rate has been demonstrated before. Nordenström et al., who administered structured triglycerides and a physical mixture to healthy volunteers, have shown a faster oxidation with structured triglycerides (11). Thörne et al. demonstrated, in an experimental system using the clamp technique, that structured triglycerides were cleared 36 % faster than long-chain triglycerides (12).

Hultin et al. investigated in an in-vitro model the effect of lipoprotein lipase on long-chain triglycerides (LLL), medium-chain triglycerides (MMM) or structured triglycerides, in which long-chain fatty acids were attached to the middle position in the triglyceride molecule (MLM) (13). They found a fast hydrolysis of structured triglycerides (MLM) with the production of free medium-chain fatty acids and long-chain monoglycerides. The cells took up the long-chain monoglycerides very rapidly, probably because of their lipophilic nature. This could be at least in part the reason for structured triglycerides being hydrolyzed and taken up faster than long-chain triglycerides or physical mixtures. Because only part of the structured triglycerides in our study were structured as MLM this mechanism can not completely explain the highly significant lower triglyceride and medium-chain fatty acid concentrations during the structured triglyceride administration.

Also, the simple fact that more lipid is taken up by the cells and oxidized faster cannot completely account for the better nitrogen balance with structured triglycerides. In our study we found a significant increase of β-hydroxybutyrate concentrations, a ketone body. Ketone bodies may influence the metabolism by induction of insulin release (14). Insulin has a positive effect on the nitrogen balance. Another explanation could be the effect of ketone bodies as an alternative energy source for certain organs.

Magnusson Borg et al. investigated the influence of structured triglycerides on protein metabolism (15). Thirty four partially hepatectomized rats were given long-chain triglycerides or structured triglycerides. The fat emulsions were each provided with or without glucose to measure the effect on acetyl-CoA. Protein synthesis was assessed by ^{14}C-phenylalanine incorporation and protein oxidation was measured by the ^{14}C-leucine oxidation rate. Weight loss was lower in the structured triglycerides group. Protein oxidation was not influenced by the lipid emulsions, but was increased in both groups without glucose administration. In both groups with structured triglycerides the protein synthesis was significantly higher compared with the long-chain triglyceride groups, indicating that structured triglycerides improve protein synthesis and has no influence on protein breakdown.

Conclusion

Parenteral structured triglycerides improve nitrogen balance in most of the studies, and are utilized faster and converted to ketone bodies. The improvement in nitrogen balance could in part be explained by the effect of lipoprotein lipase, resulting in a higher oxidation rate. Also the higher production of ketone bodies increase insuline levels, which may preserve protein mass. This preservation is mainly due to improvement of protein synthesis. Moreover, ketone bodies are an important energy source for various organs. Another explanation for the effect on nitrogen balance could be that structured triglycerides or ketone bodies, by a direct

effect, improve nitrogen balance by interference in the metabolic pathways.

More human data on the influence of structured triglycerides on protein metabolism and triglyceride clearance, and on the effects of the various compositions of structured triglycerides on lipoprotein lipase, are needed. Further human studies are necessary, particularly dose-response studies, bearing in mind the study of Chambrier (10), where the main difference to our own study was the higher lipid dose.

Studies with clinical endpoints like complications and survival rates are not published. The present studies do not indicate detrimental effects on morbidity and mortality rates, and may indicate positive effects by the effects on the metabolic pathways. Studies with real structured triglycerides, in which every fatty acid in each position within the triglyceride molecule is determined, are of great importance.

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Chapter 10

SUMMARY

Total parenteral nutrition is complete nutrition intravenously administered to patients, incapable of adequate food absorption. Total parenteral nutrition consists of lipids, carbohydrates, amino acids, minerals, trace elements and vitamins. This type of nutrition is necessary in the treatment of post-operative patients, patients on Intensive Care Units and patients with gastrointestinal diseases, in case the gastrointestinal tract is temporarily or permanently incapable of adequate food absorption.

A special group of patients with longstanding malabsorption or dysmotility of the small bowel is totally dependent on home parenteral nutrition for adequate intake of calories and nutrients. The most common cause of malabsorption in this group is the short bowel syndrome after partial small bowel resection.

The aim of this study was to investigate safety, tolerance, and effects on nitrogen balance and immunology of structured triglycerides, a newly synthesized parenteral lipid emulsion (Chapter 1). A positive effect of lipid emulsions on nitrogen balance suggests conservation of muscle mass and organ function, especially important in severe catabolic patients. A modulatory effect of lipid emulsions on immunological functions may have implications for patients, who often have infections and immunological disorders or undergo surgery, where wound healing and post-operative infections are important problems.

The first experiments on the use of parenteral lipid emulsions dated back to 1712, when William Courten administered intravenously olive oil to dogs (Chapter 2). It lasted until 1961, when Schuberth and Wretlind, by trial and error, produced the first intravenous lipid emulsion of soybean oil with egg yolk phospholipids as emulsifier, which proved to be not toxic. This first intravenous lipid emulsion was a long-chain triglyceride emulsion, containing mainly triglycerides with only long-chain fatty acids attached to the glycerol backbone. Long-chain triglycerides are hydrolyzed by lipoprotein lipase to free fatty acids and glycerol. Free fatty acids are taken up by the cells and oxidized in the mitochondria. The uptake of long-chain fatty acids in the mitochondria is carnitine-dependent, but the uptake of medium-chain fatty acids does not require carnitine. This is important, because carnitine levels are decreased in critically ill patients.

In the 1980's efforts were made to improve these lipid emulsion: physical mixtures of medium-chain triglycerides and long-chain triglycerides were introduced. In animals medium-chain triglycerides had metabolic benefits compared with long-chain triglycerides; they improved nitrogen balance, were metabolized more rapidly and stored less in tissues, and were oxidized mainly independently of carnitine.

However, intravenously infused medium-chain triglycerides are metabolized to medium-chain free fatty acids, which may be toxic in higher dose mainly due to higher production of ketone bodies resulting in metabolic acidosis. To improve the

safety of the physical mixtures, structured triglycerides were synthesized (Chapter 3). Structured triglycerides consist of triglycerides where the medium-chain and long-chain fatty acids are at random attached to the same glycerol backbone. With the use of structured triglycerides plasma levels of medium-chain free fatty acids were lower in healthy volunteers compared with a physical mixture of medium-chain and long-chain triglycerides; structured triglycerides were cleared faster from the blood compared with a physical mixture. No data were available on plasma levels of medium-chain free fatty acids and serum levels of triglycerides in patients on structured triglycerides, compared with a physical mixture.

Moreover, in animals the structured triglyceride emulsion improved nitrogen balance, increased hepatic protein synthesis and serum albumin levels, and decreased leucine oxidation compared with emulsions of a physical mixture of medium-chain and long-chain triglycerides. Again no data were available on nitrogen balance in patients on structured triglycerides, compared with a physical mixture.

On the immunological effects of intravenous lipid emulsions much controversy exists (Chapter 4). Some in-vitro and in-vivo studies showed impairment of leukocyte function by long-chain triglyceride emulsions. In other studies this could not be confirmed. Studies on the effects of lipid emulsions containing medium-chain triglycerides are scarce, but there are indications of a different effect of these lipids.

We studied in a randomized, double-blind parallel trial on 25 patients, the effects on nitrogen balance of structured triglycerides compared with a physical mixture of medium-chain and long-chain triglycerides, as part of 5 days post-operative parenteral feeding in moderately catabolic patients (Chapter 5). In the patients who completed the study, the mean cumulative nitrogen balance over the first 5 post-operative days was significantly better in the structured triglyceride group (-8 ± 2 g in 10 patients on the structured triglyceride emulsion and -21 ± 4 g in 9 patients on the emulsion of the physical mixture of medium- and long-chain triglycerides, p = .015). On the first post-operative day serum triglyceride and plasma medium-chain free fatty acid levels increased less during administration of the structured triglyceride emulsion compared with the physical mixture emulsion. Therefore, the parenteral structured triglyceride emulsion improved the nitrogen balance and was cleared faster from the blood, compared with the physical mixture. No difference in safety and tolerance between the two emulsions was observed.

In 13 patients of the trial the phagocytosing capacity of the mononuclear phagocyte system, determined by the clearance of ^{99m} Technetium-Sulfur-colloid from the blood, was measured (Chapter 6). This method represents the clearance of bacteria from the blood by the mononuclear phagocyte system. Seven patients received an emulsion of a physical mixture of medium-chain and long-chain

triglycerides and 6 patients a structured triglyceride emulsion. The ^{99m} Technetium-Sulfur-colloid clearance from the blood was the same in the two study groups, before the administration of lipid emulsion, as well as after 5 days of treatment, and did not change during treatment. Therefore, parenteral lipid emulsions with medium-chain fatty acids did not impair the clearance of ^{99m} Technetium-Sulfur-colloid by the mononuclear phagocyte system. In this respect there was no difference between the effects of an emulsion of a physical mixture of medium-chain and long-chain triglycerides and a structured triglyceride emulsion.
We also wanted to study the effect of parenteral lipid emulsions on cytokines in our trial (Chapter 7). No differences were observed. The effect of surgery overwhelmed the effect of nutrition, making conclusions on the effect of the lipid emulsions on cytokine production impossible. Instead the relation between the immune and neuroendocrine response during surgery was studied in 18 patients and we demonstrated a depression of circulating pro-inflammatory IL-1ß and an increase of circulating anti-inflammatory IL-1ra during surgical stress. The ex-vivo production of IL-1ß and TNF-α was suppressed, indicating a downregulation of the production of these cytokines. This paralleled the hormonal reaction with high ADH and ACTH, but not of cortisol, suggesting that glucocorticoid is not the key-factor in downregulation of production and release of pro-inflammatory cytokines.
Finally, in an in-vitro study polymorphonuclear leukocytes of volunteers were pre-incubated with parenteral lipid emulsions and the influence of these lipid emulsions on the production of oxygen radicals by the leukocytes was studied by measuring chemiluminescence (Chapter 8). Stimulated polymorphonuclear leukocytes preincubated with the physical mixture of medium-chain and long-chain triglycerides showed higher levels of oxygen radicals ($p < .005$) and faster production of oxygen radicals ($p < .005$), compared with polymorphonuclear leukocytes pre-incubated with long-chain triglycerides or structured triglycerides. Additional studies indicated that differences in results of various lipid emulsions were not caused by differences in emulsifier. The overall production of oxygen radicals was significantly lower after pre-incubation with the three lipid emulsions, compared with controls without lipid emulsion. Therefore, a physical mixture of medium-chain and long-chain triglycerides induced faster production of oxygen radicals, resulting in higher levels of oxygen radicals, compared with long-chain triglycerides or structured triglycerides. This can be detrimental in case oxygen radicals play a pathogenic role or beneficial when rapid phagocytosis and killing of bacteria is needed. The successive studies by Wanten et al. confirmed that the interaction of medium-chain triglyceride emulsions with immune cells differs quite strongly from that of emulsions containing long-chain triglycerides or structured triglycerides. Studies on the clinical relevance of these observations are needed.
In chapter 9 the results of the present studies are positioned between other studies.

The recently published papers on structured triglycerides are discussed and hypotheses to explain the difference in nitrogen balance between structured triglycerides and a physical mixture of medium- and long-chain triglycerides in moderately catabolic patients are proposed.

Future

Continuously improvement of the fat emulsions in parenteral nutrition is pursued. Triglycerides are especially interesting molecules, because they have nutritive, as well as pharmacological properties. Examples of these pharmacological properties are effects on the immune system, on platelet aggregation, blood viscosity and on microvascular permeability. We studied structured triglycerides where the medium- and long-chain fatty acids are at random attached to positions on the same glycerol molecule. Real structured triglycerides are synthesized molecules in which on demand specific fatty acids are attached to a specific position in the glycerol molecule. This will enables us to make tailor-made fatty acid patterns, adjusted to the needs of specific patient groups using specific properties of the fatty acids. A further possibility for the future is the introduction of other useful nutrients like glutamine, and even drugs, into the structured triglycerides. This will be the ultimate adaptation of the properties of the infused lipid emulsion to the needs of the patient: pharmacological parenteral lipid emulsions.

Chapter 11

SAMENVATTING

Totale parenterale voeding is volwaardige intraveneuze voeding, die toegediend wordt aan patiënten, die niet in staat zijn om te eten of om voldoende voedingsstoffen op te nemen uit hun maaltijden of uit vloeibare voeding toegediend via een slangetje gepositioneerd in het maagdarmstelsel. Totale parenterale voeding bestaat uit vetten, koolhydraten, aminozuren, mineralen, sporenelementen en vitamines. Dit soort voeding is noodzakelijk bij de behandeling van postoperatieve patiënten, patiënten op afdelingen Intensieve Zorgen en patiënten met maag- en darmziekten, wanneer het maagdarmkanaal tijdelijk of blijvend niet in staat is voldoende voeding op te nemen.

Een bijzondere groep van patiënten heeft thuis parenterale voeding nodig door langdurige problemen van malabsorptie of motiliteitsstoornis op het niveau van de dunne darm. De belangrijkste oorzaak voor malabsorptie in deze groep is het "short bowel" syndroom, waarbij een deel van de dunne darm door de chirurg moest worden weggenomen door ziekte.

Het doel van deze studie was om de veiligheid, de eventuele bijwerkingen, en de effecten op de stikstofbalans en het immunologisch systeem te onderzoeken van "structured triglycerides", een nieuwe, gesynthetiseerde vorm van parenterale vetemulsie (Hoofdstuk 1). Een positief effect van vetemulsies op de stikstofbalans wijst op behoud van spiermassa en orgaanfunctie, wat bijzonder belangrijk is bij patiënten, bij wie meestal sprake is van eiwitafbraak, dus spierafbraak. Een verandering in de functie van het immunologische systeem door vetemulsies kan eveneens belangrijke gevolgen hebben voor patiënten, die vaak lijden aan infecties en ziekten van het immuunsysteem, of een operatie hebben ondergaan, waarbij wondgenezing en postoperatieve infecties juist een belangrijke rol spelen in het verloop van het herstel.

De eerste, die experimenten uitvoerde met parenterale vetemulsies, was William Courten, die al in 1712 olijfolie intraveneus toediende aan een hond; de hond overleed door longproblemen (Hoofdstuk 2). Pas in 1961 werd door de beroemde Shuberth en Wretlind voor het eerst een veilige intraveneuze vetemulsie voor patiënten samengesteld, bestaande uit sojaboonolie met als emulgator fosfolipiden uit eigeel. Deze eerste intraveneuze vetemulsie bestond met name uit lange-keten vetten, met triglyceriden opgebouwd uit lange-keten vetzuren verbonden met glycerol. Vetzuren worden vrijgemaakt uit triglyceriden door splitsing van triglyceriden in vrije vetzuren en glycerol onder invloed van het enzym lipoproteine lipase. De vetzuren worden opgenomen door de cel en verbrand in de mitochondriëen. De opname van lange-keten vetzuren door de mitochondriëen is afhankelijk van de aanwezigheid van carnitine, in tegenstelling tot de opname van middellange-keten vetzuren die carnitine-onafhankelijk verloopt. Dit is van belang, omdat bij ziekte carnitine vaak deficiënt is.

In de jaren tachtig werden pogingen gedaan om de lange-keten vetemulsie te verbeteren: mengsels bestaande uit middellange-keten vetten en lange-keten vetten werden geïntroduceerd. In dierexperimenten hadden middellange-keten vetten voordelen boven lange-keten vetten; de stikstofbalans verbeterde, hun afbraak was sneller en hun opslag in de weefsels minder, en zij konden onafhankelijk van het carnitinesysteem benut worden.
Intraveneus toegediende middellange-keten vetten worden afgebroken tot middellange-keten vetzuren en glycerol. Deze middellange-keten vetzuren kunnen echter toxisch zijn in hogere dosis door vooral een hogere productie van ketonen waardoor metabole acidose kan ontstaan. Om de veiligheid van de vetemulsies met mengsels van middellange-keten en lange-keten vetten te verbeteren werden recent "structured triglycerides" gesynthetiseerd (Hoofdstuk 3). Structured triglycerides bestaan uit triglyceriden, waarbij middellange-keten en lange-keten vetzuren in willekeurige volgorde zijn verbonden aan glycerol. Bij gebruik van structured triglycerides bij gezonde vrijwilligers waren plasma concentraties van middellange-keten vetzuren lager dan bij het gebruik van de mengsels van middellange-keten en lange-keten vetten; structured triglycerides werden ook sneller opgenomen uit de bloedbaan dan deze mengsels. Er waren tot op heden geen gegevens beschikbaar over plasma concentraties van middellange-keten vetzuren en serum concentraties van triglyceriden bij behandeling van patiënten met structured triglycerides, in vergelijking met de mengsels van middellange-keten en lange-keten vetten. Bovendien werd in dierexperimenten aangetoond, dat structured triglycerides een verbetering gaf in stikstofbalans, eiwitsynthese in de lever en serum concentratie van albumine, en een afname van de afbraak van het aminozuur leucine, in vergelijking met een mengsel van middellange-keten en lange-keten vetten. Gegevens over de stikstofbalans bij behandeling van patiënten met structured triglycerides, in vergelijking met een mengsel van middellange-keten en lange-keten vetten, ontbraken bij de start van ons onderzoek.
Eerdere onderzoeken gaven niet een eenduidig antwoord op de vraag wat het effect is van intraveneuze vetemulsies op het immunologisch systeem (Hoofdstuk 4). Enkele onderzoeken waarbij immunologisch actieve cellen (leukocyten) werden samengebracht met deze vetemulsies in het laboratorium en ook enkele onderzoeken bij gezonde vrijwilligers en patiënten toonden een nadelig effect aan van lange-keten vetemulsies op de functie van leukocyten. Andere studies konden dit echter niet bevestigen. Studies over de effecten van middellange-keten vetten zijn schaars, maar er zijn aanwijzingen, dat middellange-keten vetten een ander effect hebben op het immunologisch systeem.
Wij bestudeerden in een gerandomiseerd, dubbelblind onderzoek met 25 patiënten de effecten van structured triglycerides op de stikstofbalans, in vergelijking met een mengsel van middellange-keten en lange-keten vetten, tijdens 5 dagen

postoperatieve parenterale voeding (Hoofdstuk 5). In de groep patiënten, die de studie konden afmaken, was de stikstofbalans significant beter, wanneer behandeld was met structured triglycerides (-8 ± 2 g bij 10 patiënten uit de structured triglycerides groep en -21 ± 4 g bij 9 patiënten uit de groep behandeld met een mengsel van middellange-keten en lange-keten vetten, p = .015). Op de eerste postoperatieve dag stegen serum concentraties van triglyceriden en plasma concentraties van middellange-keten vetzuren minder tijdens toediening van de structured triglycerides dan tijdens toediening van het mengsel van middellange-keten en lange-keten vetten. Geconcludeerd werd dat de parenterale structured triglycerides emulsie de stikstofbalans verbeterde en sneller opgenomen werd uit de bloedbaan dan het mengsel van middellange-keten en lange-keten vetten. Er werden geen verschillen in het optreden van bijwerkingen geconstateerd tussen beide patiëntengroepen.

Bij 13 patiënten uit het onderzoek werd de fagocytose-capaciteit van het mononucleaire fagocyten systeem onderzocht, door de klaring van ^{99m} Technetium-Sulfur-colloid uit het bloed te meten (Hoofdstuk 6). Fagocytose is het opnemen van schadelijke deeltjes of micro-organismen door immunologisch actieve cellen, bijvoorbeeld door cellen uit het mononucleaire fagocyten systeem. Zeven patiënten werden behandeld met een mengsel van middellange-keten en lange-keten vetten en 6 patiënten met structured triglycerides. De ^{99m} Technetium-Sulfur-colloid klaring uit het bloed was gelijk in de 2 groepen na 5 dagen behandeling en veranderde niet tijdens de behandeling. Geconcludeerd werd, dat beide parenterale vetemulsies met middellange-keten vetzuren (het mengsel van middellange-keten en lange-keten vetten én de structured triglycerides) de functie van het mononucleaire fagocyten systeem niet nadelig beïnvloedden.

Wij trachtten ook het effect van parenterale vetemulsies op cytokines te bestuderen in ons patiëntenonderzoek (Hoofdstuk 7). Er werden geen verschillen geconstateerd. Het onverwachte effect van chirurgie op cytokines bleek dermate groot, dat het onmogelijk was om modulerende effecten van vetemulsies op cytokineproductie te detecteren in dit model. In de plaats hiervan bestudeerde wij de relatie tussen de immuunrespons en de neuroendocriene respons tijdens chirurgie in 18 patiënten en toonden een onderdrukking aan van het circulerende pro-inflammatoire IL-1ß en een toename van het circulerende anti-inflammatoire IL-1ra tijdens chirurgische stress. De ex-vivo productie van IL-1ß en TNF-α was onderdrukt tijdens chirurgie, wat wees op een downregulatie van de productie van deze cytokines. Dit liep parallel met de hormonale reactie met een hoog ADH en ACTH, maar niet met cortisol, wat een aanwijzing vormde, dat glucocorticoïden geen sleutelrol spelen in de downregulatie van productie en vrijmaking van pro-inflammatoire cytokines.

Tot slot werd een studie verricht, waarbij in het laboratorium polymorfonucleaire leukocyten (immunologisch actieve cellen) van gezonde vrijwilligers werden samengebracht met parenterale vetemulsies; de invloed van deze vetemulsies op de productie van zuurstofradicalen (van belang voor het onschadelijk maken van micro-organismen) door de leukocyten werd bestudeerd door chemiluminescentie te meten (Hoofdstuk 8). Gestimuleerde polymorfonucleaire leukocyten samengebracht met het mengsel van middellange-keten en lange-keten vetten toonden hogere niveaus van zuurstofradicalen ($p < .005$) en snellere productie van zuurstofradicalen ($p < .005$), dan polymorfonucleaire leukocyten samengebracht met lange-keten vetten of structured triglycerides. Verdere studies toonden aan, dat dit verschil in resultaat niet werd veroorzaakt door verschillen in de emulgator. De overall productie van zuurstofradicalen was significant lager na behandeling van de leukocyten met de drie vetemulsies, vergeleken met de controles zonder vetemulsie. Geconcludeerd werd, dat het mengsel van middellange-keten en lange-keten vetten leidde tot een snellere productie van zuurstofradicalen met hogere niveaus van zuurstofradicalen, vergeleken met lange-keten vetten of structured triglycerides. Dit kan enerzijds een nadelig effect hebben bij ziekten, waarbij zuurstofradicalen een oorzakelijke rol spelen. Anderzijds kan dit ook een gunstig effect hebben bij ziekten, waarbij het snel onschadelijk maken van bacteriën door zuurstofradicalen noodzakelijk is. De hierna uitgevoerde studies door Wanten et al. bevestigden, dat de interactie van middellange-keten vetten met immunologisch actieve cellen duidelijk verschilt van lange-keten vetten of structured triglycerides. Verdere studies met betrekking tot de klinische relevantie van deze observaties zijn noodzakelijk.
Recent zijn er meer studies met structured triglycerides gepubliceerd. Deze studies worden in Hoofdstuk 9 vergeleken met bovenbeschreven studies. Tevens worden hypotheses geopperd, die het gevonden verschil kunnen verklaren tussen de stikstofbalans bij structured triglycerides en het mengsel van middellange-keten en lange-keten vetten in onze patiëntengroep.

Toekomst

Onderzoekers trachten steeds verder de vetemulsies in parenterale voeding te verbeteren. Vetten zijn bijzonder interessante moleculen, omdat zij enerzijds deel uitmaken van onze voeding, maar anderzijds ook farmacologische eigenschappen hebben. Voorbeelden van deze farmacologische eigenschappen zijn effecten op het immuunsysteem, op de aggregatie van bloedplaatjes, op de viscositeit van bloed en op microvasculaire permeabiliteit. In de structured triglycerides die wij bestudeerden zijn de midellange-keten en lange-keten vetzuren als bij toeval op bepaalde posities aan het glycerol molecuul gekoppeld. Men kan echter specifieke vetzuren op specifieke plaatsen aan het glycerol molecuul koppelen. Dit biedt de

mogelijkheid om vetzuurpatronen samen te stellen, specifiek gericht op de speciale behoeften van bepaalde patiëntengroepen, waarbij de eigenschappen van de verschillende vetzuren optimaal benut worden. Een verdere mogelijkheid voor de toekomst is de introductie van andere nuttige nutriënten, zoals glutamine, en zelfs medicamenten in de structured triglycerides. Dat zal leiden tot de best denkbare aanpassing van de eigenschappen van de parenterale vetemulsie aan de behoeften van de patiënt: farmacologische parenterale vetemulsies.

DANKWOORD

Lieve patiënten, ik dank jullie voor de bereidheid om aan deze klinische trial mee te werken; het legde de basis voor dit proefschrift. Ik maakte jullie mee in een zeer ingrijpende, levensbedreigende periode, waarin jullie steeds grote indruk op mij maakten. Mijn empathie was niet gespeeld, maar berustte op herkenning van jullie situatie en gevoelens. Dit gevoel was tegelijkertijd een bescherming en stelde duidelijke grenzen.

Dr. A.H.J. Naber, beste Ton, mijn stage gastro-enterologie bij Dr. J.H.M. van Tongeren wekte mijn belangstelling op voor gastro-enterologie en toen je me vroeg voor een promotie-onderzoek op deze afdeling was ik meteen enthousiast. Voorop wil ik je danken voor je aanmoedigingen. Steeds gaf je me de vrijheid om testen op te starten en ondersteunde je me met nieuwe ideeën.

Prof. Dr. J.B.M.J. Jansen, beste Jan, jou dank ik voor de gelegenheid die je me gaf om op de afdeling gastro-enterologie dit onderzoek uit te voeren. Prof. Dr. M.B. Katan, beste Martijn, je opmerkingen bij artikelen waren steeds zeer behulpzaam. Prof. Dr. J.W.M. van der Meer, ook u dank ik voor adviezen bij onderzoek en artikelen.

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Een bezoek en later een werkweek in het in leukocytenfunctie gespecialiseerde laboratorium van Prof. Dr. D. Roos (CLB, Amsterdam), begeleid door Dr. Anton Tool, betekende een nieuwe impuls voor ons immunologisch onderzoek, waarvoor mijn dank.

Bij de uitvoering van het onderzoek waren meerdere studenten Geneeskunde, Gezondheidswetenschappen en Humane Voeding betrokken: Johan Vehof, Mira Wenker, Annet Tuinman, Jolanda Hoefnagel en Karen Visser bedankt voor jullie inzet bij het tot stand komen van dit onderzoek.

Bij de uitvoering van de patiëntenstudie was de medewerking van een grote groep mensen nodig: Dr. Buskens en Dr. J.A. van der Vliet opereerden de patiënten; Dr. T. Liem gaf de anesthesie en plaatste de centrale catheter; Dr. Roelof van Dalen, intensivist, en verpleegkundigen op Intensieve Zorg H20 en H35 zorgden direct postoperatief voor de patiënten; en ik voelde mij als internist goed thuis op de chirurgische afdeling A20 bij Charles Wielersen en zijn verpleegkundig team; Mevr. Benneker, apotheekster, en Kitty van Hees zorgden voor de levering van de vetemulsies; en bij Bea van Köhler en mede-analisten op het klinisch-chemische laboratorium van het ziekenhuis leverde ik vele bloedmonsters in.

Meerdere betrokkenen vanuit Pharmacia, later Pharmacia & Upjohn, later Kabi Pharmacia en weer later Fresenius-Kabi volgden elkaar op: Tatjana Romanyk, Christina Rylander, Rob Bakx, Bauke Buwalda en Claes Carneheim. Bedankt voor jullie hulp bij de verwerking van een deel van de gegevens en Claes, thanks for the interesting discussions and advices. Voor de statistiek werd meerdere malen hulp verkregen bij Dr. van 't Hof, waarvoor dank.

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Lieve Adrie, jij bent het maatje waar ik naar verlangde. In de recente jaren verzetten we veel werk, maar beleefden we ook veel geluk. Corien en Jan groeien inmiddels op als twee vrolijke, gezellige kinderen. Ik hoop dat we hun de normen en waarden van onze ouders, maar ook ons onbezorgd genieten kunnen overdragen. Tot slot dank ik je voor je onvoorwaardelijke steun, aansporing en hulp bij de vormgeving, zonder welke dit proefschrift niet voltooid was.

CURRICULUM VITAE

Joanna Kruimel werd op 14 mei 1960 geboren te Amsterdam. In 1978 behaalde zij het gymnasium-ß diploma aan het Vossiusgymnasium te Amsterdam. Omdat in 1978 geen plaatsingsbewijs voor de studie Geneeskunde werd verkregen, volgde zij een studie Scheikunde aan de Gemeente Universiteit Amsterdam en slaagde in 1979 voor het propedeutisch examen Scheikunde.
De studie Geneeskunde kon worden gestart in 1979, eveneens aan de Gemeente Universiteit Amsterdam, en in 1984 slaagde zij voor het doctoraal examen en in 1986 voor het artsexamen. Tijdens deze studie verrichte ze in 1982 gedurende één jaar wetenschappelijk onderzoek op de afdeling Niertransplantaties van het Wilhelmina Gasthuis te Amsterdam (Dr. S. Surachno) naar recidief glomerulopathieën in niertransplantaten.
Van 1986 tot 1987 was zij als AGNIO (assistent-geneeskundige niet in opleiding) werkzaam op de afdeling Longziekten, locatie Dekkerswald, en de afdeling Nierziekten van het Academisch Ziekenhuis Nijmegen. De opleiding tot Internist werd gestart eind 1987 in het Diaconessenhuis Arnhem (Opleider Dr. C. van Gastel) en vanaf 1989 voortgezet in het Academisch Ziekenhuis Nijmegen (Opleider Prof. Dr. A. Van 't Laar, later Prof. Dr. J.W.M. van der Meer). Van 1991 tot 1994 was zij assistent-lid van de Locale en Regionale Opleidingscommissie Interne Geneeskunde Nijmegen. Op 1 juli 1994 volgde registratie als Internist.
Vanaf februari 1992 tot januari 1994 werd haar opleiding tot Internist onderbroken en gestart met onderliggend promotieonderzoek, waarvoor financiële ondersteuning verkregen werd via Fresenius-Kabi en de Universiteit Nijmegen (subsidie Assistentenpool). Zij kreeg in 1996 de 2e prijs bij de Young Investigator Award van de Najaarsvergadering van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), werkgemeenschap Voeding. Veel werk werd met Dr. T. Naber verzet in het schrijven van een aanvraag voor een NWO subsidie voor vervolgonderzoek; deze subsidie werd verkregen en inmiddels leidde dit tot het promotie-onderzoek van Dr. G. Wanten.
De opleiding tot Gastro-enteroloog werd gevolgd in het Academisch Ziekenhuis Nijmegen van 1995 tot 1999 (Opleider Prof. Dr. J.B.M.J. Jansen) en op 1 mei 1999 werd zij geregistreerd als Gastro-enteroloog, met behoud van registratie als Internist. Tijdens haar opleiding werkte zij mee in het Voedingsteam van het ziekenhuis. Het diploma Stralingshygiëne, deskundigheidsniveau 4a voor Medische Specialisten werd behaald in 1999.
Gedurende haar opleiding tot Gastro-enteroloog, ondersteunde zij onder meer met het geven van onderwijs aan studenten Geneeskunde en Gezondheidswetenschappen de leerstoel Voedingsleer van Prof. Dr. M.B. Katan in het Academisch Ziekenhuis Nijmegen van 1995 tot 1998. Ook werden in dit kader colleges gegeven aan studenten Humane Voeding van de Landbouw Universiteit te Wageningen.

Van mei tot december 1999 volgde zij een aanvullende stage Hepatologie in het Academisch Ziekenhuis Rotterdam (Prof. Dr. S.W. Schalm).
Vanaf 26 november 1999 is zij als geneesheer-specialist in de Gastro-enterologie erkend in België, waar zij vanaf 1 januari 2000 werkzaam is als Gastro-enteroloog in het Maria Ziekenhuis Noord-Limburg (campus Lommel en Neerpelt). Voltooiing van de nieuwbouw van dit ziekenhuis te Overpelt is voorzien voor 2005 (348 bedden).
Joanna Kruimel is getrouwd met Adrie Stevens. Samen hebben zij twee kinderen, Corien en Jan.

Stellingen

behorend bij het proefschrift

PARENTERAL STRUCTURED TRIGLYCERIDE EMULSION

Safety, tolerance,
and effects on nitrogen balance and immunology

Joanna Kruimel, oktober 2004

1. Parenterale voeding met een structured triglyceride emulsie verbetert de stikstofbalans en wordt sneller opgenomen uit de bloedbaan, vergeleken met een mengsel van middellange-keten en lange-keten vetten, indien toegediend aan matig katabole patiënten.
   (Dit proefschrift)

2. Middellange-keten vetzuren bevattende parenterale vetemulsies hebben geen nadelig effect op de fagocyterende functie van het mononucleaire fagocyten systeem.
   (Dit proefschrift)

3. Tijdens een operatie wordt de ex-vivo productie van IL-1ß en TNF-α onderdrukt, wat wijst op een downregulatie van de productie van deze cytokines. Dit loopt parallel met de hormonale reactie met een hoog ADH en ACTH, maar niet met cortisol, wat een aanwijzing vormt, dat glucocorticoïden geen sleutelrol spelen in de downregulatie van productie en vrijmaking van pro-inflammatoire cytokines.
   (Dit proefschrift)

4. Vetemulsies, bestaande uit een mengsel van middellange-keten en lange-keten vetten geven aanleiding tot een snellere productie van zuurstofradicalen door polymorfonucleaire leukocyten, met hogere niveaus van zuurstofradicalen, vergeleken met lange-keten vetten of structured triglycerides.
   (Dit proefschrift)

5. De manier, waarop in the New England Journal of Medicine "Lorenzo's oil" aanvankelijk bestempeld werd als waardeloos (N Engl J Med 329:745-752, 801-802,1993), gaat voorbij aan de geweldige prestatie van de ouders Odone, die een veelbelovende behandelingsvorm voor adrenoleukodystrofie ontwierpen.

6. Een vrouw, die een maatschappelijke carrière ambieert, is nu meestal afhankelijk van geëmancipeerde mannen: een geëmancipeerde vader, een geëmancipeerde echtgenoot en een geëmancipeerde werkgever.

7. Een ziekenhuis, waar zwangerschapsverlof van artsen slechts op papier goed geregeld is, maar waar in de praktijk geen enkele vorm van vervanging van zwangere artsen georganiseerd wordt, voert geen goed emancipatiebeleid.

8. In een arbeidssector, zoals de gezondheidszorg, waar steeds meer vrouwen worden opgeleid, zou in toenemende mate aandacht moeten worden besteed aan een goede kinderopvang.

9. Oorlogsslachtoffers geven hun verdriet door aan hun kinderen, omdat het lijden niet vergeten mag worden. Niets kan het lijden uit het verleden zijn zinloosheid ontnemen, alleen kan men trachten in het heden herhaling te voorkomen.

10. Fanatisme bestaat, omdat we behoefte hebben aan simpele antwoorden op gecompliceerde problemen (D.C. Dennett in t.v. programma W. Kayzer, 10-01-1993).

11. De pathofysiologische basis voor verslaving aan een geliefde en "mourir d'amour" is waarschijnlijk te vinden in een door verliefdheid geïnduceerde neuroendocriene stress respons met vrijmaking van endorfines en een immunologische stress repons met verminderde afweer tegen infecties (eigen observatie).