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The addition of medium-chain triglycerides to a purified fish oil-based diet alters inflammatory profiles in mice

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ABSTRACT

Objective. Parenteral nutrition associated liver disease (PNALD) is a deadly complication of long term parenteral nutrition (PN) use in infants. Fish oil-based lipid emulsion has been shown in recent years to effectively treat PNALD. Alternative fat sources free of essential fatty acids have recently been investigated for health benefits related to decreased inflammatory response. We hypothesized that the addition of medium-chain triglycerides (MCT) to a purified fish oil-based diet would decrease the response to inflammatory challenge in mice, while allowing for sufficient growth and development.

Materials/methods. Six groups of ten adult male C57/Bl6 mice were pair-fed different dietary treatments for a period of twelve weeks, varying only in fat source (percent calories by weight): 10.84% soybean oil (SOY), 10% coconut oil (HCO), 10% medium-chain triglycerides (MCT), 3% purified fish oil (PFO), 3% purified fish oil with 3% medium-chain triglycerides (50:50 MCT:PFO) and 3% purified fish oil with 7.59% medium-chain triglycerides (70:30 MCT:PFO). An endotoxin challenge was administered to half of the animals in each group at the completion of dietary treatment.

Results. All groups demonstrated normal growth throughout the study period. Groups fed MCT and HCO diets demonstrated biochemical essential fatty acid deficiency and decreased IL-6 and TNF-α response to endotoxin challenge. Groups containing PFO had increased inflammatory response to endotoxin challenge, and the addition of MCT to PFO mitigated this inflammatory response.

Conclusion. These results suggest that the addition of MCT to PFO formulations may decrease the host response to inflammatory challenge, which may pose potential for optimized PN formulations. Inclusion of MCT in lipid emulsions given with PN formulations may be of use in therapeutic interventions for disease states resulting from chronic inflammation.

Abbreviations: EFA, essential fatty acid; PUFA, polyunsaturated fatty acid; ALA, alpha linolenic acid; LA, linoleic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; PN, parenteral nutrition; PNALD, parenteral nutrition-associated liver disease; HCO, hydrogenated coconut oil; MCT, medium-chain tryglycerides; PFO, purified fish oil; IP, intraperitoneal; LPS, lipopolysaccharide; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; EFAD, essential fatty acid deficiency; DHLA, dihomo-gamma-linolenic acid; CRP, C-reactive protein.

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1. Introduction

Essential fatty acids (EFAs), necessary for growth, development, and a variety of biological functions, must be consumed in the diet. Historically, alpha linolenic acid (ALA, n-3 PUFA) and linoleic acid (LA, n-6 PUFA) have been considered the two essential fatty acids. However, recent studies have shown that the downstream metabolic products of ALA and LA, docosahexaenoic acid (DHA) and arachidonic acid (ARA), respectively, are sufficient to sustain growth, development and reproductive function [1–3].

For individuals dependent on parenteral nutrition (PN), EFAs must be provided intravenously as a lipid emulsion. Commercially available lipid formulations in the United States have been exclusively soybean oil-based and contain high levels of LA and lower levels of ALA. Soybean oil-based lipid emulsions are implicated in the development of parenteral nutrition-associated liver disease (PNALD), a progressive and often lethal complication affecting up to 74% of patients dependent on long-term PN [4–7].

Alternative lipid sources have been investigated as a potential strategy to prevent liver disease in PN-dependent individuals. A fish oil-based lipid emulsion, provided at 1 g/kg/day, has been shown to reverse the progression toward liver failure in patients with PNALD [8–10]. However, PN formulations provided with low doses of lipids necessitate higher calories from carbohydrates (dextrose) in order to meet the daily caloric needs of the PN-dependent individual, a consideration that is especially important in developing infants and children. PN formulations high in dextrose predispose patients to hyperglycemia and increased central venous catheter infections, hepatic steatosis, and glycosuria, complications that can lead to significant morbidity and mortality in an already-fragile population [11–13].

Recently, attention has been given to non-essential fatty acids, so called “EFA-free” lipids, which may be utilized as additives to lipid emulsions in order to augment the total fat calories provided and decrease the requirement for additional dextrose in PN. Ling et al. examined the metabolic effects of combinations of EFAs with hydrogenated coconut oil (HCO), an EFA-free lipid source. Rats fed a diet with HCO as the sole source of calories and then given an endotoxin challenge demonstrated a lower inflammatory response, as measured by serum IL-6 and C-reactive protein, when compared to rats fed HCO supplemented with DHA and ARA [14]. These findings are in keeping with Cook et al., who also demonstrated a decreased systemic inflammatory response in states of essential fatty acid deficiency in rats [15].

Medium chain triglycerides (MCT) are an alternative source of EFA-free lipids. Studies have shown benefits of MCT oil in the prevention of alcohol-induced liver injury in animal models by a mechanism that is not fully understood but may be related to decreased inflammation [16–18]. Others have demonstrated that dietary supplementation of MCT reduces the degree of endotoxin-induced intestinal and hepatic injury in rats [19,20]. In 2012, the American Society of Parenteral and Enteral Nutrition identified MCT oil as a potentially beneficial additive to lipid emulsions containing soybean and/or fish oil and recommended further research in identifying an optimal ratio of n-3 fatty acids, n-6 fatty acids, and MCT [21].

The creation of an optimized lipid formulation, which provides sufficient EFAs to sustain growth and development, while modifying the intensity of inflammation, is of high value to the PN-dependent population. Purified fish oil (PFO) is a rich source of DHA and ARA that meets essential fatty acid requirements in rodent models [2,22] and in humans [23,24]. Herein, we utilize a murine model to study several dietary lipids including soybean oil, PFO, HCO and MCT to determine the effects of these lipid sources on growth, serum EFA levels, liver histology, and inflammatory profiles with and without an endotoxin challenge.

2. Methods

2.1. Animals and diets

Adult male C57/Bl6 mice (Jackson Laboratories, Bar Harbor, ME) were housed five to a cage and maintained in a climate-controlled facility with a 12:12-h light–dark cycle for a period of four days of acclimation prior to experimentation. During this period, animals had free access to water and a standard rodent chow diet. Experimental protocols were approved by the Boston Children’s Hospital Institutional Animal Care and Use Committee. Animals were then assigned by cage to one of the following six dietary treatment groups (10 animals per group) based on fat content by weight: 10.84% soybean oil (SOY), 10% coconut oil (HCO), 10% medium-chain triglycerides (MCT), 3% purified fish oil (PFO), 3% purified fish oil with 3% medium-chain triglycerides (50:50 MCT:PFO) and 3% purified fish oil with 7.59% medium-chain triglycerides (70:30 MCT:PFO). Purified fish oil was derived primarily from sardine oil from which all saturated fat and fats with carbon lengths less than 18 were removed, donated by Pronova BioPharma/BASF (Lysaker, Norway). Medium-chain triglyceride (MCT) oil was purchased from Health and Sport (Amston, CT). All diets were formulated by or purchased from Dyets (Bethlehem, PA) and provided in pelleted form. Equivalent amounts of casein, L-cystine, sucrose, dyetrose (a version of dextrose used by the manufacturer which allows the diet to be easily pelleted), and vitamin and mineral mixtures were included in all diets. Slight variations in corn starch and cellulose were necessary to make the diets as nearly isocaloric as possible. Total kcal/kg was slightly lower in the PFO and 50:50 MCT:PFO diets. Table 1 lists the composition of the six different diets.

2.2. Experimental design

Animals were pair-fed the experimental diets in groups of 5 for a period of 12 weeks. Individual body weight and total cage food intake were recorded every three days for the duration of the study period. Food intake was controlled by the lowest food intake among the six groups at each time to ensure that there were no differences in food intake across the six dietary groups. Blood samples were obtained from each animal prior to initiation of dietary treatment and again at 2, 4, 6, 9, and 12 weeks after diet initiation. At the end of 12 weeks, animals in each dietary group were divided into two subgroups: one group received an intraperitoneal (IP) injection of lipopolysaccharide (LPS) 150 mg in saline (1 mg/mL) and the second group received an IP

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