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Purified fish oil eliminating linoleic and alpha linolenic acid meets essential fatty acid requirements in rats

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ABSTRACT

This study examined whether purified fish oil (PFO) supplemented to an essential fatty acid deficient (EFAD) diet meets EFA needs in rats. The EFAD diet contained 10% hydrogenated coconut oil (HCO). A similar diet contained 7% HCO and 3% PFO which also provided 2.84% arachidonic acid (AA), 52.50% eicosapentaenoic acid (EPA) and 35.73% docosahexaenoic acid (DHA) but no linoleic acid (LA) or alpha linolenic acid (ALA). A 10% soybean oil control diet provided ample LA and ALA. After 4 weeks of feeding, blood glucose, plasma triglyceride and phospholipid fatty acid profiles, C-reactive protein (CRP), TNF and IL-6 were determined after saline or LPS injection. EFAD developed with the HCO diet with triene:tetraene ratios in plasma phospholipids >.20, which remained <.02 with the control and HCO+PFO diets. Mead acid levels significantly increased by a factor of 10 with the HCO diet compared to the AIN and HCO+PFO diets and were significantly lowest with the HCO+PFO diet. 18:1 n9 levels were significantly higher in plasma phospholipids and triglycerides with the HCO diet. CRP levels were significantly highest with the control diet and significantly lowest with the HCO diet. LPS significantly increased 18:1n9 and cytokines, and decreased AA and plasma glucose in all diets and significantly increased plasma triglycerides and decreased plasma glucose in controls. Providing AA, EPA and DHA in EFAD prevents EFAD over the short-term as reflected in Mead acid production, triene:tetraene ratio, and de novo lipogenesis and may reduce the inflammatory response to LPS.

1. Introduction

Linoleic acid (LA, 18:2n6) and α-linolenic acid (ALA, 18:3n3) are essential fatty acids (EFAs) that must be consumed in the diet. Neither can be synthesized de novo due to the inability in man to desaturate at the n6 or n3 position; however it is their distal elongation and desaturation products, arachidonic acid (AA) (20:4n6), eicosapentaenoic acid (EPA) (20:5n3), and docosahexaenoic acid (DHA) (22:6n3) that serve as precursors for important signaling compounds like eicosanoids (from gamma-linolenic acid, AA and EPA), resolvins (from AA and EPA), and neuroprotectins (from DHA). EFAD in humans rarely occurs through dietary inadequacy but develops occasionally in clinical conditions when there is substantial intestinal malabsorption [1] and was inadvertently produced in the early development of infant formulas [2] and total parenteral nutrition when no fat was provided [3,4]. A triene–tetraene ratio (20:3n9/20:4n6) in plasma phospholipids >0.2 reflecting the ratio of Mead acid (20:3n9), which is produced in replacement of 20:4n6 when 18:2n6 is limiting, to AA

Abbreviations: EFAD, essential fatty acid deficient; AIN, AIN 93 M diet; HCO, hydrogenated coconut oil; PFO, purified fish oil as Lovaza oil; LPS, lipopolysaccharide; LA, linoleic acid; ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CRP, C-reactive protein; TNF, tumor necrosis factor; IL-6, interleukin 6.

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(20:4n6) has been considered pathognomonic for the diagnosis of EFAD, but clinical evidence for EFAD is generally not seen until this ratio rises above 0.4 [5–7]. The minimal recommended dietary LA and ALA to prevent EFAD is estimated at approximately 1% and 0.1%–0.2% of total caloric intake respectively in adults [8,9] although the median intakes in the US are much higher.

More recently, the essentiality of AA, EPA and DHA has been recognized under certain disease conditions. For example, in patients with severe liver diseases provision of sufficient amounts of LA and ALA often does not alter the levels of AA, EPA and DHA in plasma and liver presumably due to impaired hepatic metabolism, suggesting the necessity to directly provide these fatty acids [10,11] at least for the liver. Premature infants [12] and perhaps even term infants [13] also may be unable to produce adequate amounts of AA, EPA, and DHA from LA and ALA through immaturity of similar enzyme systems. Furthermore in healthy populations the consumption of higher amounts of fish oil found particularly in oily fishes is associated with a reduced mortality from principally cardiac disorders [14]. As a consequence fish oil has been promoted and extensively employed for preventive health as well as disease treatment. Although fish oil contains only low levels of LA and AA and essentially no ALA, only recently has an exclusive use of fish oil as the dietary oil source been shown clinically [15] and experimentally in animals [16] to meet essential fatty acid needs. This has been attributed to the more efficient use of the small amount (about 0.5%) of AA in fish oil combined with the also limited LA (about 0.5%–1%) to meet AA needs and the presence of substantial amounts of EPA and DHA to obviate the lack of ALA. Whether purified fish oil provided in similar amounts but devoid of LA and ALA would also prevent EFAD has not previously been tested.

In the experimental work in animals [16], mice were pair-fed on diets containing 1%, 5% and 10% conventional fish oil by weight for 9 weeks which showed that EFAD only occurred in the 1% fish oil group, appeared marginal in the 5% fish oil group, and was not present at all in the 10% fish oil group. In the 10% fish oil group, it is estimated that the 0.39% of energy was provided by the LA contained in the diet [16], approximately one half the conventionally recommended 1% as LA. It was also noted that 0.24% of energy was provided by the AA present in the 10% fish oil. Thus, it seems that the amounts of LA and AA in 10% fish oil might be enough to meet n6 fatty acid requirements and prevent the development of EFAD. The aim of the present study was to determine if a purified fish oil with LA and ALA removed and containing principally AA, EPA, and DHA, could prevent essential fatty acid deficiency when provided at amounts found in 10% of conventional fish oil intake by weight which is approximately 25% of total energy. If so, this should provide evidence that neither LA nor ALA may be required in the diet when adequate levels of AA, EPA, and DHA are provided. A second aim was to determine whether the relatively lower AA levels in plasma phospholipids seen when feeding conventional fish oil would also be found, which might be expected to reduce inflammation in a manner characteristic of conventional fish oil feeding.

Thus this study examined the effects of an EFAD diet, when supplemented by PFO containing AA, EPA, and DHA in amounts found in conventional fish oil when provided at 10% by weight, on the triene–tetraene ratio in plasma phospholipids. In addition, the inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor (TNF), and interleukin 6 (IL-6), and plasma glucose response were also measured after exposure to saline or lipopolysaccharide (LPS). As a source of PFO, commercial omega-3 acid ethyl esters, (Lovaza, GlaxoSmithKline, Research Triangle Park, NC) which contain 2.84% of AA, 52.50% of EPA and 35.73% of DHA, but no LA and ALA (Table 1) were used. It is calculated that 3% of Lovaza oil provides the same amount of AA as found in 10% of fish oil by weight and somewhat more EPA and DHA. A classical EFAD diet containing HCO as the oil source was used to induce EFAD to which PFO at 3% by weight was used to supplement HCO at 7% as the experimental diet. AIN93M diet with 10% soybean oil as the fat source was used as the control EFA-sufficient diet.

2. Material and methods

2.1. *Animals and diets*

Based on our previous study [16], the sample size of 5 animals in fish oil diet and AIN control groups would provide 80% power to detect a mean difference of 0.05 in the triene–tetraene ratio between groups. Therefore, in this study, a total of 42 weanling male Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY) and housed in a 12:12-h light–dark photoperiod room at 24 °C to 26 °C for 4 days before the experiments. During this period, animals had free access to standard laboratory rodent diet (5008, Purina, Ralston, Purina, St. Louis, MO). When their body weights reached 80–90 g, all animals were randomly divided into three groups: one consumed a modified AIN93 M diet which contains 100 g soy bean oil/kg (10% of soybean oil by weight) as a diet sufficient in LA and ALA (AIN); one consumed a modified AIN93 M diet which contains 100 g HCO/kg (10% of

Table 1 – Fatty acid compositions (%) of different oils.

Fatty acids	Soy bean oil	Hydrogenated coconut oil	Lovaza oil
10:0	0.00	8.64	0.00
12:0	0.00	52.51	0.00
14:0	0.30	18.66	0.00
16:0	12.09	9.22	0.00
16:1n7	0.41	0.00	0.00
18:0	3.85	7.42	0.00
18:1n9	23.55	3.06	0.00
18:2n6	52.56	0.46	0.00
18:3n3	7.24	0.03	0.00
20:0	0.00	0.00	2.02
20:4n6	0.00	0.00	2.84
20:5n3	0.00	0.00	52.50
22:0	0.00	0.00	2.06
22:4n6	0.00	0.00	0.38
22:5n6	0.00	0.00	0.83
22:5n3	0.00	0.00	3.64
22:6n3	0.00	0.00	35.73

The values are from 3 samples.