Fish oil — How does it reduce plasma triglycerides?☆☆☆

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Abstract

Long chain omega-3 fatty acids (FAs) are effective for reducing plasma triglyceride (TG) levels. At the pharmaceutical dose, 3.4 g/day, they reduce plasma TG by about 25–50% after one month of treatment, resulting primarily from the decline in hepatic very low density lipoprotein (VLDL-TG) production, and secondarily from the increase in VLDL clearance. Numerous mechanisms have been shown to contribute to the TG overproduction, but a key component is an increase in the availability of FAs in the liver. The liver derives FAs from three sources: diet (delivered *via* chylomicron remnants), *de novo* lipogenesis, and circulating non-esterified FAs (NEFAs). Of these, NEFAs contribute the largest fraction to VLDL-TG production in both normotriglyceridemic subjects and hypertriglyceridemic, insulin resistant patients. Thus reducing NEFA delivery to the liver would be a likely locus of action for fish oils (FO). The key regulator of plasma NEFA is intracellular adipocyte lipolysis *via* hormone sensitive lipase (HSL), which increases as insulin sensitivity worsens. FO counteracts intracellular lipolysis in adipocytes by suppressing adipose tissue inflammation. In addition, FO increases extracellular lipolysis by lipoprotein lipase (LpL) in adipose, heart and skeletal muscle and enhances hepatic and skeletal muscle β-oxidation which contributes to reduced FA delivery to the liver. FO could activate transcription factors which control metabolic pathways in a tissue specific manner regulating nutrient traffic and reducing plasma TG. This article is part of a Special Issue entitled Triglyceride Metabolism and Disease.

Highlights

► Pharmaceutical long chain omega-3 fatty acids effectively reduce plasma triglyceride levels. ► Fish oil reduces the rate of VLDL synthesis in the liver. ► Reduced NEFA delivery to the liver is a likely locus of action for fish oils. ► Fish oil counteracts the lipolytic release of NEFA from adipose tissue by suppressing inflammation. ► Fish oil increases FA uptake and β-oxidation in adipose, heart and skeletal muscle.

Abbreviations: 4-HDoHE, 4-hydroxydocosahexaenoic acid; ATM, adipose tissue macrophages; CoA, coenzyme A; COX, cyclooxygenase; CYP, cytochrome P450; DGAT, diacylglycerol acyltransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FXR, farnesoid X receptor; FA, fatty acid; FO, fish oils; FCR, fractional catabolic rate; HNF4α, hepatocyte nuclear factor-α; HSL, hormone sensitive lipase; HTG, hypertriglyceridemic; IDL, intermediate density lipoprotein; LOX, lipoxygenase; LpL, lipoprotein lipase; LXRα, liver X receptor alpha; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; RXRα, retinoid X receptor alpha; SREBP, sterol regulatory element binding protein; TG, triglyceride; T2D, type II diabetes; VLDL, very low density lipoprotein

Keywords: Fish oil; Omega-3; Plasma triglyceride; Lipolysis; NEFA; Eicosapentaenoic acid; Docosahexaenoic acid

Article Outline

1. Introduction

Understanding how fish oil (FO) reduces plasma triglycerides (TGs) is important not only because elevated TG is a cardiovascular risk factor, but also because it informs our view of basic lipid biology and can help in the development of new or improved pharmacologic approaches to treating hypertriglyceridemia. This review updates progress in understanding the FO mechanism of action with an emphasis on kinetic modeling and new information that has appeared regarding VLDL production. It focuses on how FO and its active ingredients, eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA) contribute to improved fatty acid (FA) trafficking; it addresses intricacies of the TG-lowering effect of FO such as how reduced hepatic production of TG-rich very low density lipoprotein (VLDL) occurs without causing steatosis.

2. FO effects on plasma TG

Omega-3 FAs have long been known to lower plasma TG [[1], [2] and [3]] along with variety of other drugs such as fibrates, statins, thiazolidinediones, niacin, and metformin. Although the TG-lowering effects of FO are not evident at intakes typical of Western diets (about 130 mg/day) [4] they manifest at “pharmacologic” doses (*i.e.*, > 3 g/day of EPA + DHA) [[5], [6], [7], [8], [9], [10], [11], [12] and [13]]. This is similar to another dietary component, niacin (vitamin B3), that is also lipid-lowering at supra-nutritional intakes [14]. The pharmaceutical preparation of omega-3 acid FAs (Lovaza, GlaxoSmithKline) provides EPA and DHA as acid ethyl esters, and the approved dose is 4, 1-g capsules per day which provides 1860 mg of EPA and 1500 mg of DHA for a total of 3.4 g omega-3 FAs/day. The TG-lowering effect of EPA + DHA has been demonstrated in numerous trials and 3–4 g/day of omega-3 FAs decrease plasma TG by about 30% (range 16–45%) [15]. The effect is dose dependent [16] and [17] with the minimal effective dose being > 2 g/day [7]. EPA and DHA appear to be equally potent in lowering plasma TG [5] and are effective in multiple

settings including type II diabetes (T2D) [6], metabolic syndrome [8], non-alcoholic fatty liver disease (NAFLD) [9], HIV-dyslipidemia [10], nephrotic syndrome [11] and hemodialysis [12] and [13]populations.

EPA and DHA belong to a unique group of nutri-pharmaceutical agents that are already present in tissues prior to use. In fact, the endogenous level of EPA and DHA (*e.g.*, red blood cells EPA + DHA, or the omega-3 index [18]) is itself a biomarker for cardiovascular disease risk [19]. No studies to date have examined whether the magnitude of TG-reduction is a function of the baseline omega-3 index. If the response to therapy indeed depends on the baseline omega-3 index, it would suggest that omega-3 intervention studies should stratify for baseline omega-3 levels, or better, subjects should be titrated to an optimal omega-3 index instead of using a single dosing regimen.

In addition to their TG lowering effects, FO also have strong evidence for risk reduction for a number of CVD-related endpoints including primary prevention of major coronary events [20], secondary prevention of death or non-fatal MI [21], and all-cause mortality in heart failure subjects [22]. The role of TG lowering in risk reduction is not clear, however. In the Japan EPA–lipid intervention study [20], statin-treated subjects with high TG (> 150 mg/dL) and low HDL cholesterol (< 40 mg/dL) were at 71% greater risk for major coronary events compared to patients without this pattern. Monotherapy with EPA resulted in a 5% reduction in plasma TG but a 53% reduction in risk for major coronary events in EPA group compared to control group meaning that the reduction in risk occurred independent of a reduction in TGs [23]. While the link between FO-induced risk reduction vs. TG reduction is not clearly established, there remains a strong epidemiological link between elevated TGs and coronary artery disease [24]and [25] and although not yet unambiguously demonstrated in a randomized trial, it seems likely that the TG-lowering has cardiovascular benefit.

3. The underlying causes of hypertriglyceridemia and the effects of FO on lipoprotein metabolism

TGs are transported through the body by all classes of lipoprotein particles, with VLDL and chylomicrons being the primary TG-bearing lipoproteins. Concentrations of plasma lipoproteins are established by the balance between their rate of appearance in plasma, or production, and by their rate of removal from the plasma, or clearance. Thus, elevations in plasma TG could represent either an increase in VLDL-TG production that overcomes peripheral clearance or from a decrease in clearance without a compensatory decrease in production. The former has been demonstrated in genetic dyslipidemias such as familial hypercholesterolemia [26] and [27], and familial combined hyperlipidemia [28] and [29], but also in more common contexts such as obesity [30] and insulin resistance [[31], [32], [33] and [34]] with overproduction being confined primarily to $VLDL_1$ (the largest VLDL particles). Among subjects undergoing hemodialysis, elevated TG is also caused by overproduction of VLDL apoB [35], however in the presence of albuminuria, *i.e.* the nephrotic syndrome, high TGs result from reduced clearance [36]. Thus, while increased production is the most common cause of hypertriglyceridemia, reduced clearance is sometimes involved. Therefore, tracer kinetic studies of lipoprotein metabolism in humans are important to understanding the effects of FO on plasma TG concentrations.

The studies to date are summarized in Table 1. Collectively the results of tracer kinetic studies indicate that omega-3 FAs reduce VLDL-TG production and likely increase TG clearance.

Table 1. Human tracer kinetic studies of the effects of FO treatment on lipoprotein synthesis and clearance rates.

Study	N	Tracer	Omega-3	Duration	Baseline	% chan	Kinetic parameter (% change)

			(g/d)	(weeks)	plasma TG (mg/dL)[a]	ge in fasting plasma TG	Production rate	p-value	FCR	p-value
Change-from-baseline studies										
Nestel [37]	6	^{125}I apoB	19–29[b]	2–3.5	277 +/− 131[b]	− 59%[b]	− 65%[b]	0.002[b and c]	26 %[b]	0.09[b and c]
	5	^{3}H-glycerol	19–23[b]	2–3.5	314 +/− 153[b]	− 68%[b]	− 68%[b]	0.005[b and c]	78 %[b]	0.50[b and c]
Sanders [38]	5	^{3}H-glycerol	4.6[d]	4	1136 +/− 532	− 43%[b]	− 39%[b]	0.03[b and c]	6%[b]	0.36[b and c]
Harris [39]	10	^{3}H-glycerol	10–17	3–5	442 +/− 99	− 66%	− 45%	< 0.005	65 %	< 0.005
Bordin [40]	10	^{13}C-leucine	3	4	97 +/− 11	− 22%	− 29%	0.012	14 %	0.09
Fisher [41]	5	^{3}H-leucine	~ 9	1	591 +/− 188	− 66%	− 43%	0.01	5%[e]	n.s.
Randomized, controlled trials										
Park [42]	33	^{3}H-triolein	4[f]	4	82 +/− 5[b]	− 9%	n.a.	n.a.	~ 30 %[g]	< 0.05
Chan [43]	48	^{2}H-leucine	3.4	6	177 +/− 30	− 28%[h]	− 22%[h]	0.002	− 4%[h]	n.s.
						Conversion from VLDL to IDL:			55 %[h]	0.002
Chan [44]	24[i]	Cholesteryl ^{13}C-oleate	3.4	6	177 +/− 30	− 25%	− 32%	< 0.05	− 4%	n.s.
				Conversion from VLDL to IDL:					49 %	< 0.05

Abbreviations: FCR — fractional catabolic rate; IDL — intermediate density lipoprotein; VLDL — very low density lipoprotein; n.a. — not applicable; n.s. — not significant; TG — triglyceride.

[a] Mean +/− SEM.

[b] Re-calculated from original tables.

[c] Paired ANOVA on lg-transformed data.

[d] EPA + DHA.

[e] Most prominent clearance pathway [R(16,19)] reported.

[f] EPA or DHA ethyl esters.

[g] Estimated from slope.

[h] Adjusted effect size applied to FO group.

[i] Substudy of reference [43].

3.1. Change from baseline studies

Tracer studies have consistently demonstrated that FO reduces VLDL production. Early studies employed large FO doses, included normotriglyceridemic with hypertriglyceridemic (HTG) subjects, and employed bolus injections of radiolabeled tracers. While they captured a large range of subject types, randomized controlled designs were not employed. Nestel et al. treated five normal and two HTG subjects, with FO at high dose of ~ 30% of daily energy needs and found a 65–68% reduction in VLDL production rate measured by either the plasma kinetics of ^{125}I-ApoB or ^{3}H-glycerol tracers [37]. The estimated increase in fractional catabolic rate (FCR; the fraction of the pool cleared per unit of time) of 78% was not significant and was attributed to the smaller apoB pool. When measuring FCR, complications can occur such as here, where even a large effect size is not significant indicating difficult detection and large variance. Further, a decreased pool size due to reduced production can itself increase FCR, making FCR increases a secondary consequence of reduced production. In such cases, reductions in production rate lower plasma TG concentrations below levels which saturate clearance and so FCR is also increased while absolute clearance is unchanged. Sanders et al. traced glycerol-labeled TG in 5 severely HTG males (mean baseline TG = 1136 mg/dL) given 4.6 g EPA + DHA/day and showed a decrease in TG production rate but no change in FCR [38]. Harris et al. used the same approach in 10 HTG subjects (mean baseline TG = 442 mg/dL) receiving > 10 g omega-3 FA/day (which lowered plasma TG by 66%) found a 45% decrease in TG-production rate along with a 65% increase in FCR [39]. Bordin et al. traced apoB in 10 normotriglyceridemic subjects given 3 g omega-3 FA/day and found apoB production was decreased by 29% [40]. They also observed a 14% increase in FCR, however concluded (with a $p = 0.09$) it was not increased. Finally, in a study tracing apoB production in 5 subjects with T2D and mean TG levels of 591 mg/dL using approximately 9 g omega-3 FA/day [41], reductions of 66% in plasma TG were observed. This study using 5 subjects, employed a complex model of apolipoprotein transfer to conclude that apoB synthesis was greatly reduced by FO, primarily by a shifting the initial appearance of apoB in plasma from the VLDL to the IDL and LDL fractions.

3.2. Randomized controlled trials

More recently, two larger randomized controlled trials using doses at or near the approved pharmaceutical dose have improved the parameter estimates of FO effects on VLDL and chylomicron TG kinetics. In the first trial, Park et al. [42] measured the effects of 4 g/day EPA or DHA on postprandial TG in 33 normotriglyceridemic males tracing ^{3}H-triolein administered as a lipid emulsion. This study demonstrated a 15% reduction in chylomicron TG half-life which was associated with a 30% increase in circulating (*i.e.*, *not* heparin released) lipoprotein lipase (LpL) activity. In another randomized, placebo controlled trial, Chan et al. measured the effect of 3.4 g/day of EPA + DHA and atorvastatin (40 mg/day) in a 2 × 2 factorial trial in 48 obese, insulin resistant males with mean plasma TG of 177 mg/dL. Using an apoB tracer (^{2}H-leucine) they demonstrated a 22% decrease in VLDL-apoB synthesis [43] and no change in the FCR of VLDL apoB. They did, however, report a 1.5-fold increase in the fraction of VLDL converted to IDL (from 0.29 to 0.43) as opposed to direct clearance from the plasma. Since this study traced apoB, not TG directly, transfer from VLDL to intermediate density lipoprotein (IDL) corresponds to improved rates of lipolytic delipidation of the apoB-containing lipoprotein particle. A subset of subjects in the Chen et al. study, specifically the placebo and FO groups, also received cholestryl ^{13}C-oleate tracer of VLDL [44], and like the apoB tracer, this sub-study showed a significant reduction in VLDL production rate with no change in FCR and greater fractional transfer to IDL.

In summary, regardless of the cause of hypertriglyceridemia, the number of subjects, the tracer used, or the methodology used to model the effects, reduced hepatic VLDL-TG production is consistently demonstrated as a cause for the reduction of plasma TGs by FO. In studies using a dose of 3–4 g EPA/DHA per day, the size of the FO-induced reduction in synthesis is about − 30%, which is large enough to explain most of the TG-lowering effect in these subjects with mild HTG. This represents an important action of FO since most hypertriglyceridemias are largely caused by increased hepatic secretion of VLDL-TG. Secondarily, FO may also induce an increase in clearance which would contribute to the FO effect.

4. Effects of FO on VLDL production

As described above, a reduced rate of FA incorporation into VLDL is a major effect of FO. In this regard, recent advances in our understanding of how hepatocytes obtain FAs for VLDL production are useful in identifying those effects which are physiologically relevant and where FO may be acting in a tissue specific manner. Hepatic VLDL production coordinates apolipoprotein synthesis with lipid synthesis in a multistep process. The FAs used in hepatic TG synthesis can be derived from at least three sources: 1) the diet (*i.e.* chylomicron/remnant); 2) *de novo* lipogenesis; and 3) circulating non-esterified FAs (NEFAs).

FAs derived from *de novo* lipogenesis and/or diet are first stored in hepatocyte lipid stores (as TGs) whereas NEFAs can be directly incorporated into VLDL-TG [45]. Vedala et al. used stable isotopes to measure the relative contributions of these three FA sources to overall VLDL-TG in lean normolipidemic, non-diabetic obese, and diabetic obese subjects [46]. They demonstrated that NEFAs are the major source of FAs for VLDL-TG regardless of metabolic state and the contribution of each FA source to the VLDL-TG pool differs in patients with type-2 diabetes versus simple obesity. This study offers an opportunity to evaluate the maximal contribution that each source could, in theory, make to the FO effect of reducing TG production. Other studies confirm the primary contribution of NEFA to hepatic TG production [47], emphasizing that changes in NEFA flux to the liver would be necessary to reduce production of VLDL-TG. In Table 2, we estimate the TG levels that would result if the contribution of each component to VLDL-TGs were eliminated using the parameters given by Vedala et al. (independent of changes in clearance mechanisms). Notably, completely eliminating the contributions of *de novo* lipogenesis or diet would reduce TGs by no more than 13% even among diabetics with HTG. Therefore, FO interference with these two pathways could not realistically contribute to reduced synthesis in the short term, regardless of changes in the biology or regulation of enzymes, and the best explanation for why FOs reduce VLDL-TG production is *via* an effect on NEFA delivery to the liver.

Table 2. Effect of eliminating FA sources on plasma TG.

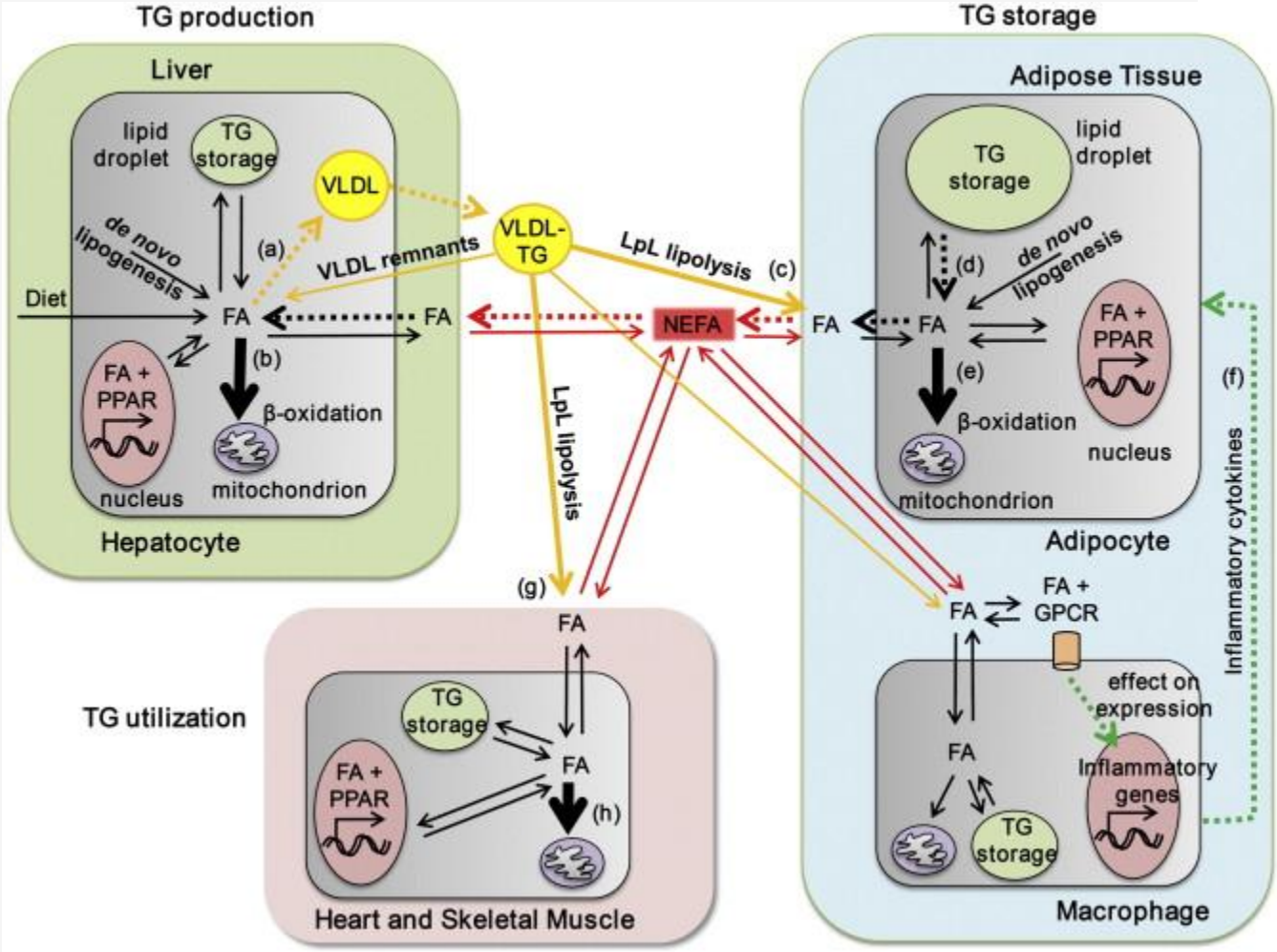
TG precursor	**Control**		**HTG**		**HTG + T2D**	
	TG (mg/dL)	**% reduction**	**TG (mg/dL)**	**% reduction**	**TG (mg/dL)**	**% reduction**
All sources	62	–	179	–	191	–
Eliminate:						
NEFA	3	− 95%	32	− 82%	43	− 78%
De novo lipogenesis	60	− 3%	153	− 15%	171	− 10%
Diet	60	− 3%	173	− 3%	167	− 13%

Vedala et al. [46] studied the relative contribution of various FA sources to VLDL-TG. They report the plasma TG and the absolute contribution of each FA source to VLDL-TG synthesis, which was measured during tracer kinetics study and reported in g/day. We used the reported parameters to estimate the reduction in serum TG that would correspond to the elimination of each FA source, assuming no change in TG clearance.

5. FA trafficking

FAs are transported throughout the body in esterified and non-esterified forms. FA trafficking (the production, transport and delivery of FAs throughout the body) is a regulated process that distributes FA between different tissues compartments. VLDL-TG and NEFA are each important components of FA trafficking *in vivo* (illustrated

inFig. 1). VLDL are essentially targeted, one-way transporters of FAs to tissues which consume large amounts of FAs by expressing LpL — for energy storage in the case of adipocytes, and for energy generation in the case of skeletal muscle and the heart. On the other hand, NEFAs distribute into a single plasma pool which exchanges FAs with all cell types. The FA transporters generally control facilitated uptake of NEFA by different tissues [48] and [49] however small amounts can cross cell membranes by slow diffusion termed ‘flip-flop’ [50]. NEFA concentrations play an important role in establishing plasma TG levels [51] and three factors contribute to NEFA concentrations in plasma: 1) the rate of NEFA sequestration by adipose and other tissues; 2) the rate of FA release from the adipocyte TG stores by hormone sensitive lipase[1] (HSL); 3) and the FAs released from circulating lipoproteins by LpL localized to the vascular endothelium, commonly termed ‘spillover’ [47] and [52].



High-quality image (969K)

Fig. 1.

FA transport and FO induced changes. FAs are transported throughout the body in two major forms: esterified in TG carried by VLDL (VLDL-TG) and non-esterified carried by serum albumin (NEFA). From these two pools FA are distributed to different tissues depending on energy requirements and hormonal status. In the tissues, FA are used

for ATP production *via* β-oxidation, re-esterification in TG for energy storage, incorporation into lipids composing cell membranes, and production of signaling molecules. Non-essential FA can also be *de novo*synthesized from other carbon sources. The liver (top left) acts as a major site for FA distribution, processing FAs from all sources: diet, *de novo* lipogenesis, circulating NEFA and VLDL remnants, and producing VLDL-TG which are secreted into the circulation. Adipose tissue (right) is the main storage depot for FA (as TG storage in lipid droplets). Adipocyte uptake of FAs from the circulation occurs *via* LpL lipolysis of VLDL-TG. Adipocytes largely contribute to the plasma NEFA pool by regulated release of FA from lipid droplets *via* HSL-lipolysis. Adipose tissue macrophages (bottom right) regulate intracellular lipolysis in adipocytes by secreting inflammatory cytokines. Heart and skeletal muscle (bottom left) obtain FAs from the NEFA pool and from the VLDL-TG pool *via* LpL-lipolysis. β-oxidation plays a major role in FA disposal in the heart and skeletal muscle. In hepatocytes, FO (a) down-regulates VLDL production and (b) up-regulates β-oxidation; in adipocytes, FO (c) increases FA uptake from LpL lipolysis of plasma TG, (d) decreases intracellular lipolysis in adipocytes, and (e) increases β-oxidation; FO also (f) reduces the secretion of pro-inflammatory cytokines from adipose tissue macrophages; in heart and skeletal muscle, FO (g) up-regulates LPL lipolysis of plasma TG and (h) β-oxidation. Pathways enhanced by FO are indicated by bold arrows, and those reduced by FO are indicated by dotted arrows. Abbreviations: FA — fatty acid, GPCR — g-protein coupled receptor; LpL — lipoprotein lipase; NEFA — non-esterified fatty acid; PPAR — peroxisome proliferator-activated receptor; VLDL — very-low density lipoprotein.

6. Fish oil and NEFA

We were able to identify only one randomized controlled trial examining the effects of FO on NEFA as a primary endpoint [53]. In this study of 20 healthy medical students, large amounts of FO (30 g/day) were administered in the diet over 7 days. Based on their mean HOMA score of 1.8 (calculated from reported glucose and insulin values), the subjects were insulin sensitive, and FO had no effect on either fasting glucose or insulin. While the subjects were normotriglyceridemic at baseline (84 mg/dL), they had a 36% decrease in TG, and their fasting NEFA decreased by practically the same amount, 37%, an amount sufficient to reduce VLDL-TG synthesis. Using Vedala's model [46], and assuming no change in clearance and that NEFA levels are proportional to production rates, we calculated that the 37% reduction in NEFA would translate into a 31% reduction in plasma TG which is nearly that observed. Unfortunately, the large FO dose (corresponding to ~ 9 g/day omega-3 FA) and the type of subjects studied (healthy, insulin sensitive, and normotriglyceridemic) do not allow for straightforward extrapolation to insulin resistant, HTG subjects given pharmaceutical doses of omega-3 FAs (*e.g.* 3.4 g/day). In obese or insulin resistant subjects, a reduction of only 13% in NEFA levels would produce a 20% decrease in plasma TG, and given the relatively high coefficient of variation for NEFA (15–30% depending on the study population [54] and [55] large sample sizes would be required to detect a meaningful effect of omega-3 FAs on NEFA. Thus, either larger trials powered on a NEFA endpoint, or more sensitive measures of NEFA metabolism are required to determine if changes in NEFA flux are the primary contributor to the omega-3 FAs induced reduction in VLDL-TG synthesis.

Other trials reporting the effects of omega-3 FAs on NEFA as secondary endpoints in more appropriate populations do exist. While significant reductions are not consistently reported, the observed changes have been large enough to explain the reduced TG levels. For example, in a randomized controlled trial of severely HTG subjects (N = 40) treated with 3.4 g/day of omega-3 FAs for six weeks, Pownal et al. found a mean decrease in NEFA from 0.86 to 0.66 mmol/L. This effect was significant in the FO arm, but not when compared to placebo (0.89 to 0.85 mmol/L). Such an effect size could account for about 75% of the observed reduction plasma TG levels [56], again assuming no change in clearance. Bordin et al. showed a 25% decrease in NEFA from 0.45 to 0.33 mmol/L in a normotriglyceridemic population [40] which, based on their reported kinetic parameters, corresponds to a 22% drop in plasma TG. Healthy men exposed to high NEFA by infusion of intralipid with heparin responded by increasing hepatic VLDL synthesis by 35% and somewhat surprisingly, by increasing intestinal chylomicron synthesis by 70% [57]. Notably, we found two trials in which there were no reported FO induced changes in fasting NEFA concentrations. The OPTILIP trial was

designed to deliver specific ratios of omega-6 and omega-3 FAs and so delivered a sub-pharmacologic dose of EPA and DHA (~ 1.6 g/day). Although this trial found no change in NEFA, the intervention produced only a 15% decrease in fasting TG, and so the corresponding NEFA decrease could have been even smaller [58]. Given the study's coefficient of variation for NEFA (15%), an effect size of this small magnitude was unlikely to be observed. In another trial studying subjects undergoing hemodialysis, there was no decrease in the rate of appearance of NEFA or the NEFA levels, however baseline NEFA levels were very low [13].

7. FO improves TG clearance

Of the tracer-label studies (Table 1), two studies identified an effect on clearance as an increased FCR (*i.e.*, the fraction per unit time leaving the VLDL compartment) [39]and [42] or as fractional transfer from VLDL to IDL [42]. A third, smaller study reported an effect nearing significance [40]. The effect of FO on TG clearance may be enhanced in the post-prandial state. Park et al. traced ^{3}H-triolein-labled chylomicron-like particles in the post-prandial state and found both increased clearance and a corresponding increase in non-heparin-stimulated LpL activity in plasma [42]. The rate limiting step of VLDL-TG clearance is LpL, not only as lipolytic enzyme but also as a ligand to capture and bind VLDL to the endothelial proteoglycans [59] and [60]. LpL is synthesized by mesenchymal cells behind the endothelial barrier and then transported to the luminal endothelial surface and attached to heparan sulfate where it first captures the circulating particle in the glycocalyx [59]. Interestingly, endothelial binding can, in and of itself, nearly normalize clearance even in animal models where the endothelium has no LpL-lipolytic capacity [61]. LpL-mediated lipolysis and cellular uptake of the released FAs follows endothelial binding. CD36 facilitates the uptake of FAs from TG-rich lipoproteins and their remnants following LpL-lipolysis [62]. During the postprandial state, binding of chylomicron-like particles to the endothelium is 60% greater in subjects given 4 g/day EPA or DHA than in those given placebo [63]. Circulating LpL activity is a surrogate for LpL-lipolytic capacity in humans, and it is also higher after EPA or DHA treatment suggesting that the increased binding could be the result of increased bridging activity between particles and LpL [63]. The high NEFA in HTG increases VLDL apoC-III [57], which is an inhibitor of LpL-lipolysis, and FO blocks the accumulation of apoC-III on VLDL [64], potentially increasing LpL-lipolysis.

FO treatment may also direct FAs away from the liver to adipose tissue. In adipose tissue of subjects with atherogenic lipid profiles, LpL mRNA is increased by 55% as is post-heparin lipase, and these changes correlate with an improvement in clearance of post-prandial TG [65]. In fasting rats, FO appears to direct TG towards skeletal muscle (and away from the liver) by increasing the relative expression of skeletal muscle LpL [66]. Adipose tissue is responsible for a large portion of post-prandial TG removal and thus, while increased clearance of TG may contribute less to the overall reduction in TGs than decreased synthesis, it still contributes to a beneficial shift in FA trafficking.

8. Transcription factors regulating FA metabolism and the effects of FO

Changes in transcription of several nuclear receptors are reported to mediate the TG-reducing effects of FO: sterol regulatory element binding proteins (SREBP), liver X receptor-alpha (LXRα), retinoid X receptor alpha (RXRα), farnesoid X receptor (FXR), and peroxisome proliferator-activated receptors (PPARs), and each play prominent roles in controlling lipid metabolism.

The SREBPs are transcription factors that regulate expression of lipid synthesizing enzymes, including fatty acid and TG synthesis, and SREBP-1c, the primary hepatic activator of lipogenesis [67]. It is itself under the control of the Liver LXRα which binds to the LXR response element as a heterodimer with RXRα and activates expression of SREBP-1c. FOs prevent activation of SREBP-1c expression by inhibiting LXRα/RXRα binding to the LXR response element located in the promoter regions of SREBP-1c gene [68], and this is reflected in reduced mRNA levels of SREBP-1c in animals fed FO diets [69]. FOs also act directly on SREBP-1c protein to inhibit its maturation [70]. Since

omega-6 FAs have similar effects on SREBP-1c but do not affect plasma TG levels [71], the reduction in SREBP-1c may be a response to the reduced FA flux rather than the cause.

FXR is expressed in the liver and small intestines and forms dimers with the RXR receptor subunits. It controls lipoprotein metabolism by stimulating expression of apolipoproteins, especially apoC-II [72] which is an activator of LpL. Since DHA is an activator of FXR [73], some effects of FO could be mediated through this receptor, however as with the SREBP-1c/LXRα/RXRα axis, omega-6 polyunsaturated FAs (PUFAs) are more potent activators [73] making the omega-3 activation of this pathway a less likely explanation of their TG-lowering effect. Other transcription factors of interest in mediating these adaptations include hepatocyte nuclear factor-α (HNF4α), a central regulator of enzymes controlling lipid metabolism [74] and is decreased by FO [75].

The most consistent effect of FO in animal studies is the activation PPARs [76]. PPARs are ligand-activated transcription factors that regulate the expression of enzymes and proteins involved in energy homeostasis: PPARα increases fatty acid oxidation in the liver, adipose, heart and skeletal muscle; PPARγ promotes adipocyte storage of FAs as TG; PPARβ/δ ubiquitously induces β-oxidation of fatty acids and along with other FAs, EPA and DHA are PPAR ligands [77]. PUFA metabolites, such as eicosanoids and more generally oxylipins, are potent activators of PPARs. Oxylipins are produced *via* the action of cyclooxygenase (COX) [78], lipoxygenase (LOX) [79] and [80] or cytochrome P450 (CYP) [81] and [82] activity on PUFAs, and can be more potent PPAR agonists than their parent FAs [83]. Many are acylated into VLDL-glycerolipids and released by LpL [84], and LpL-lipolysis of VLDL activates PPARα [85] demonstrating that VLDL are a source of non-paracrine PPAR activators. Further, FO therapy increases the plasma levels of the EPA and DHA-derived oxylipins which are analogs of the arachidonate oxylipins in plasma glycerolipids [86] and activation of PPARγ by 4-hydroxydocosahexaenoic acid (4-HDoHE; or 4-HDHA), an oxylipin derived from DHA, has been documented [87]. In many cases, EPA- and DHA-derived oxylipins have greater potency than their arachidonate analogs, especially among epoxygenated fatty acids [88], and such a scenario would provide a basis for greater PPAR activation in tissues with abundant FO.

9. Tissue specific effects of FO and the regulation of TG and FA trafficking

Plasma TG levels are merely a static snapshot of FA trafficking between tissues, so the kinetic contributions of individual tissues must be considered. These effects are discussed below.

9.1. Liver

The most consistent plasma TG-lowering effect of FO is the reduction of VLDL-TG production as demonstrated by all tracer kinetic studies [[37], [38], [39], [40], [41], [42], [43]and [44]] (see discussion above). VLDL assembly is a complex process in which the synthesis of apoB is coordinated with hepatocyte TG synthesis [89]. FO inhibits assembly and secretion of VLDL-TG and apoB100 from cultured hepatocytes (Fig. 1a) [90] and [91]. EPA-coenzyme A (CoA) directly reduces TG synthesis *via* inhibition of the diacylglycerol acyltransferase (DGAT) activity and reduces esterification of 1,2-diacylglycerol in rat liver microsomes [92]. Additionally, synthesis of apoB is affected by dietary omega-3 FA. A recent study showed that chylomicron remnant-like particles enriched with omega-3 PUFAs reduce the expression of HNF-4alpha protein and the expression of mRNA for HNF-4alpha target genes, including apoB and the microsomal TG transfer protein [75]. It has been shown that peroxide derivatives of EPA and DHA can stimulate degradation of apoB-100 thus reducing VLDL-TG secretion [93]. Moreover, omega-3 PUFA up-regulate β-oxidation in hepatocytes [91] and [94] (Fig. 1b) thus reducing the pool of FA available for TG synthesis.

Excessive accumulation of TG in the liver leads to NAFLD, a typical co-morbidity of HTG [95] and [96]. However NAFLD is ameliorated with FO supplementation in humans[97] and [98] and in animal models of liver

steatosis [99] and [100], and these effects of FO are consistent with a reduced intracellular pool of free FAs and increased β-oxidation.

9.2. Adipose tissue

FO affects the primary function of adipose tissue, fat storage. FO supplementation reduces adiposity in animals fed high fat diets [[101], [102], [103], [104] and [105]] despite stimulating FA uptake due to increased expression of LpL (Fig. 1c) and of CD36, the major FA membrane transporter [106] and [107]. Similarly, although FO upregulates postprandial LpL expression in human adipose tissue [108], suggestive of increased FA uptake by adipose, no effect on body weight has been seen in multiple studies. Indeed, a few small clinical trials have reported weight loss [[109], [110] and [111]]. Reduced fat mass, to the extent that it occurs, could be explained mechanistically by mitochondrial biogenesis and increased β-oxidation (Fig. 1e), concurrent with reduced *de novo* lipogenesis in white adipose tissue [102] and [109].

Adipose tissue is the primary source of plasma NEFA, and there is good evidence from animal studies that FO can reduce NEFA output to the circulation by reducing HSL-mediated intracellular lipolysis (Fig. 1d). In a rat study comparing lard to lard + FO diets, FO lowered plasma NEFA (0.15 vs. 0.28 μmol/L) and lowered basal intracellular lipolysis by 50% [112]. Chronic low-grade adipose tissue inflammation in obesity and insulin resistant states is emerging as an important activator of HSL-mediated lipolysis in adipocytes [[113], [114] and [115]]. TNF-α and serum amyloid A secreted in adipose tissue induce intracellular lipolysis [[116], [117], [118] and [119]], while IL-6 appears to increase the lipolytic response to adrenergic stimulus [120]. TNF-α also reduces the activity of LpL in cultured adipocytes [121] and [122], increasing the gradient for FA efflux from adipocytes. *In vitro* studies using cultured adipocytes have shown that FO counteracts the effects of TNF-α and IL-6 on HSL-lipolysis [123], and adipocytes appear to have improved function with DHA treatment as evidenced by increased adiponectin production [124].

Inflammatory cytokines (Fig. 1f) are primarily produced in adipose tissue by infiltrating immune cells, specifically by adipose tissue macrophages (ATM). ATM secrete cytokines into the extracellular space where these inflammatory molecules find their receptors on the surface of adipocytes. Administration of high amounts of oral EPA (1 g/kg/day) in rats prevented TNF-α gene over-expression in adipose tissue induced by high fat diet [125]. The rate of *ex vivo* intracellular lipolysis in adipocytes isolated from these FO-treated and control rats, however, was similar implying that when present, EPA and DHA modify the response of adipocytes to activated ATM. Therefore, up-regulation of HSL-mediated intracellular lipolysis likely requires paracrine factors secreted by other cells residing in adipose tissue. A recent genetic study in mice identified a plausible mechanism for how FO might down-regulate an inflammatory cytokine secretion from ATM [126]. According to the study, DHA can act as extracellular ligand for GPR120, a G-protein coupled receptor up-regulated on ATM by high fat diet. Activation of GPR120 by DHA attenuated the expression of pro-inflammatory cytokines in adipose tissue. The attenuation of pro-inflammatory cytokine expression was abrogated in GPR120 deficient mice. Moreover, the effect of DHA on cytokine expression was intrinsic to immune cells because it was not effective in irradiated wild type mice reconstituted with GPR120-deficient bone marrow. Although adipocyte lipolysis was not reported in this study, it is reasonable to suggest that the reduced expression of proinflammatory cytokines *via* activation of GPR120 might lead to paracrine down-regulation of intracellular lipolysis and the reduction of FA efflux from adipocytes. However, the reported effects of DHA on GPR120 may not be specific to omega-3 PUFAs since palmitoleic acid exhibited nearly the same activity on some metrics. Reduction of NEFA efflux by adipose tissue reduces the availability of FA as a substrate for TG synthesis and VLDL production in hepatocytes.

9.3. Heart and skeletal muscle

Heart and skeletal muscles are the primary sites for FA utilization, and LpL is highly expressed in both tissues [127]. In both tissues, LpL activity is enhanced by FO [128]. These results suggest that increased removal of TG by heart and skeletal muscle contributes to the plasma TG lowering effect (Fig. 1g). The uptake of FAs released from VLDL *via* LpL-lipolysis appears to be more efficient in skeletal muscle than in adipose tissue, suggesting less spillover of FAs into the NEFA pool. FO also increases the expression of genes regulating β-oxidation in skeletal muscle [129] (Fig. 1h). Moreover, the FO-induced increase in plasma adiponectin is likely to have a systemic effect on skeletal and cardiac muscle increasing TG hydrolysis, FA uptake and β-oxidation [130]. As a result cardiac and skeletal muscles could facilitate the TG-lowering effects of FO by upregulating FA uptake and oxidation.

10. Conclusion

There is uniform support for decreased production of TG by FO. The randomized controlled studies also demonstrate an increase in clearance. Based on the fact that NEFAs are by far the primary source of FAs for VLDL-TG production and that FO reduces plasma NEFA, this could be the primary mechanism for the hypotriglyceridemic effect. Plasma NEFAs are largely the result of intracellular HSL lipolysis in the adipocyte which FO counteracts by suppressing adipose tissue inflammation and increasing FA uptake in adipose, heart and skeletal muscle. In addition, a systemic increase in β-oxidation provides a sink for FA disappearance and contributes to decreased hepatocyte availability of FAs for VLDL-TG production.

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[1]It is worth pointing out that within the diabetes literature, the term 'lipolysis' usually refers to intracellular lipolysis, while in the lipoprotein literature it refers to extracellular lipolysis on the vascular endothelium from TG-bearing lipoproteins. These two types of lipolysis have opposing effects. LpL-mediated intravascular (peri-endothelial) lipolysis of lipoprotein TG would increase the local FA concentration, favoring FA flux into LpL-expressing tissues, including adipose. On the other hand, intracellular (*i.e.*, hormone sensitive lipase-mediated in the adipocyte) lipolysis would increase intracellular FAs concentrations, thus promoting FAs efflux from the adipocyte. In the interest of clarity, here we will use two more descriptive terms: extracellular lipolysis refers to the former and intracellular lipolysis refers to the latter.