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Green CJ, Pramfalk C, Charlton CA, et al. Hepatic de novo lipogenesis is suppressed and fat oxidation is increased by omega-3 fatty acids at the expense of glucose metabolism. BMJ Open Diabetes Research & Care. 2020;8(1):e000871.

What We Know, Think We Know, or Are Starting to Know

What we know is the non-alcoholic fatty liver disease [NAFLD] is a major concern as a cardiometabolic disease, as the presence of fatty liver increases risk for cardiovascular disease [the 'cardio' part] and type-2 diabetes [the 'metabolic' part] [(1)].

We know that NAFLD is characterised by accumulation of triglycerides in hepatocytes [liver cells], and intra-hepatic triglycerides [i.e., fat in the liver cells] contribute to the liver becoming insulin resistance, which in turn leads to elevations in circulating free fatty acids, and a vicious cycle of impaired glucose tolerance and fat metabolism [(2)].

We' ve also known for some time that polyunsaturated fats have a beneficial effect on triglycerides, although how this is achieved has been more in the ' think we know' category. An early study from distinguished University of Oxford Emeritus Professor Keith Frayn' s research group in 2001 showed that 5-weeks on a PUFA-rich diet led to significant decreases in abdominal fat and increased insulin sensitivity, compared to an SFA-rich diet [(3)]. Further research from the University of Missouri demonstrated that the marine omega-3 fatty acids, eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], reduced post-prandial [i.e., after a a meal] concentrations of triglycerides, an effect which related to faster clearance of triglycerides from chylomicrons* [(4)].

However, it is also thought that omega-3 fatty acid supplementation may impair glucose tolerance, evident through elevated fasting blood glucose levels [(5)]. While this finding has been inconsistent, it certainly warrants further investigation. In addition, although the finding of reduced circulating triglycerides from marine omega-3 fatty acid supplementation is concretely established, the precise mechanisms through which these fatty acids may reduce intra-hepatic triglycerides is less established.

The present study investigated the influence of omega-3 fatty acid supplementation on both fasting and post-prandial de-novo lipogenesis, fatty acid oxidation, and blood glucose levels.

*Geek Box: Fat Metabolism

Fat metabolism can be complex, and the terminology even more so. So let's run through it. Triglycerides are comprised of three fatty acids bound to a sugar compound, glycerol. Fat consumed through the diet is in the form of triglycerides, and when we store fat in the body it is also in the form of triglyceride: triglycerides can be thought of as storage lipids. When we consume fat, bile acids secreted by the gallbladder help to emulsify the triglycerides from diet, breaking them down into their component parts, which are taken up by intestinal cells and repackaged. Chylomicrons are very large compounds which transport triglycerides absorbed from diet to tissues, where they are broken down by the lipoprotein lipase [LPL] enzyme for energy, or stored. However, we also release fats from storage, in the form of 'free-fatty acids' or 'non-esterified fatty acids' [NEFA]: the liver can synthesise new triglycerides from circulating NEFA, and the liver produces very-low-density lipoprotein [VLDL] in order to transport these triglycerides [and also cholesterol] to tissues. So think of two pathways: exogenous [dietary] pathway with chylomicrons as the transporter for triglycerides, and endogenous pathway with VLDL as the transporter. It is important to note that a measure of triglycerides is not abstract, but a measure of the triglyceride content of chylomicrons, VLDL, and remnant lipoproteins. When the triglycerides in a chylomicron are broken down by LPL, it creates chylomicron remnants. When triglycerides in VLDL are broken down, it creates an 'intermediate density lipoprotein' [IDL], before further breakdown transcends IDL into LDL. Thus, you can see how high triglycerides can influence cardiometabolic disease, by burdening the transport system. It is also why dietary factors that influence post-prandial triglyceride metabolism are important in the overall risk equation.

The Study

The study was conducted as an open-label trial, meaning there was no blinding, and the investigators were comparing the effects of the intervention against a placebo.

47 men with fasting triglyceride concentration of >1.5mmol/L [132.75mg/dL], which is toward borderline high, but otherwise healthy, were randomised to consumed 4g/d omega-3 [combined EPA & DHA] or placebo, for 8-weeks. The omega-3 supplement was a pharmaceutical grade product containing 1.84g eicosapentanoic acid [EPA] and 1.52g docosahexanoic acid [DHA], taken in capsules [4 x 1g per day]. The placebo group consumed olive oil in 4 x 1g capsules per day.

Two studies were undertaken:

1. An in vivo* human study to investigate the effects of omega-3 fatty acid supplementation on lipid and glucose metabolism;
2. An in vitro cellular study to investigate the mechanistic effects of omega-3 fatty acids in liver cells

The in vivo human study consisted of several lines of investigation:

a. The intervention study investigating the effects of omega-3 fatty acid supplementation over 8-weeks;

b. A 1-day metabolic study with the omega-3 intervention group using stable isotope tracers [see ***Key Characteristic***, below] to illustrate the metabolic pathways of triglyceride metabolism and fatty acid oxidation.

To conduct the metabolic study, participants consumed a stable istope-labelled water to measure fasting and post-prandial hepatic *de-novo lipogenesis* [DNL]. On the morning of the study day, participants consumed an additional stable istope-labelled chocolate drink, to trace the metabolic fate of the fatty acids consumed with breakfast [40g fat].

For the in vitro study, human liver cells were grown under laboratory conditions, and were treated with mixtures of fatty acids [oleic, palmitic, and linoleic acid: OPL], or OPL with EPA and DHA, to determine the effects of EPA+DHA on DNL and TGs.

Outcome measures were fasting and post-prandial triglycerides [TGs], hepatic de novo lipogenesis [DNL; the synthesis of new triglycerides], intra-hepatic triglycerides [IHTG], fatty acid [FA] oxidation, blood glucose and insulin levels, and insulin sensitivity. The respiratory exchange ratio [RER], which indicates macronutrient oxidation, was also measured. For hepatic DNL, IHTG, and FA oxidation, the omega-3 group was compared against its baseline levels, not compared to the placebo group.

*Geek Box: Experimental Terminology

Spend any time reading research and you will come across terms that describe the environment in which an experiment was conducted. 'In vitro' comes from the Latin meaning, "within the glass", and refers to experiments that are conducted outside of a living organism, for example in a cell culture petri-dish, or in a test tube. The issue with such studies is that the controlled environment does not necessarily replicate the biological environment occurring in the living organism. 'In vivo', meaning "within the living", means the experiment is conducted in the whole living organism; a human, or a mouse, for example. The limitation of in vivo research is we may not gain insight into biological mechanisms, depending on the type of experiment or what exactly it is we want to know. You may also see 'ex vivo', or "outside the living": ex vivo studies are where experiments are conducted on a tissue taken from an organism, where the structural and biological properties of the tissue are maintained. The limitation here is that, although ex vivo may be considered to better replicate internal conditions than in vitro experiments, ex vivo studies still may differ in response from the whole-organism. All lines of research have their place, particularly for biological sciences where we study complex organisms that are multi-cellular, and have various levels of cells, tissues, organs, and glands.

Results:

- ***Intervention study:*** Over 8-weeks, fasting TG concentration decreased significantly in the omega-3 group, compared to placebo. Compared to baseline levels, IHTG decreased by 18.1% after 8-weeks of omega-3 supplementation. Omega-3 supplementation led to a 30% decrease in fasting and post-prandial DNL. Compared to baseline, blood glucose levels increased significantly by 4.2% in the omega-3 group. However, there was no difference in insulin levels or in insulin sensitivity.
- ***Metabolic tracer study:*** Omega-3 supplementation resulted in a significant 20% decrease in chylomicron-TGs. Both hepatic fat oxidation and whole-body fat oxidation increased significantly after omega-3 supplementation. Post-prandial RER in response to the test meal decreased significantly after 8-weeks, compared to baseline, reflecting post-prandial fat oxidation increasing significantly, while post-prandial carbohydrate oxidation decreased significantly, following omega-3 supplementation [more under ***Interesting Finding***, below].
- ***In vitro study:*** EPA+DHA resulted in a significant decrease in intracellular TG content. EPA+DHA suppressed DNL from glucose, reducing the proportion of fatty acids derived from glucose.

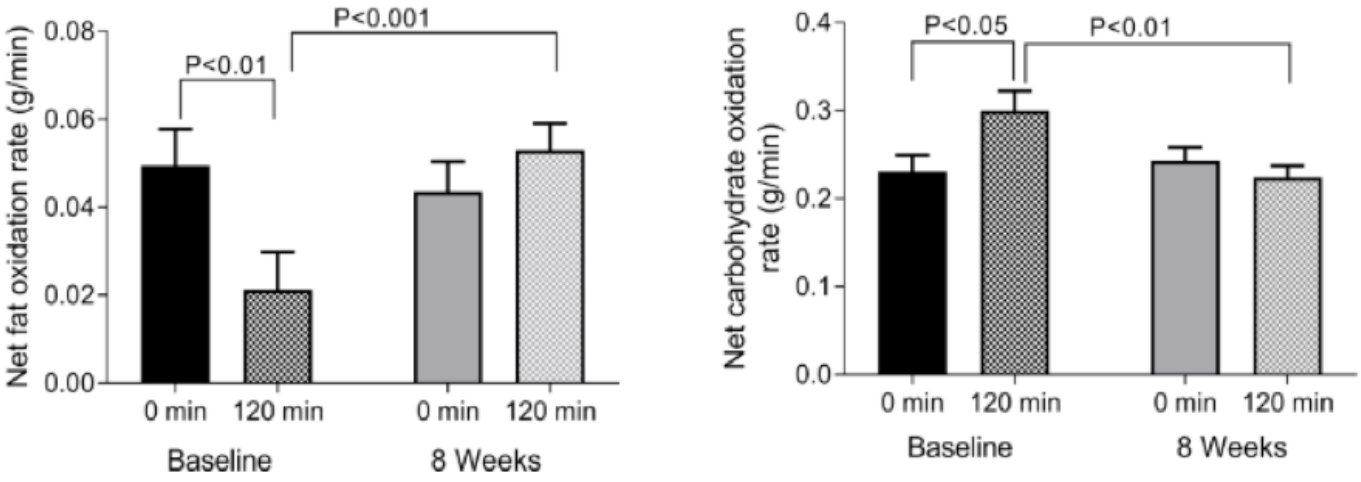

Figure *from study illustrating the response to the test meal at baseline and after 8-weeks of omega-3 supplementation: at baseline, fat oxidation decreased and carbohydrate oxidation increased, while the reverse was observed at 8-weeks.*

The Critical Breakdown

Pros: Both intervention and placebo were in the same capsules, taken at the same time [with breakfast]. Compliance assessed via red blood cell fatty acid composition, a robust correlate of dietary intake of EPA and DHA. Weight was stable in the participants to within 0.1%, thus effects may be attributable to dietary intervention. The study used advanced laboratory techniques, in particular stable isotopes, to illustrate metabolic pathways.

Cons: The main limitation is that that the analysis of hepatic DNL, IHTG, and FA oxidation was confined to the omega-3 group, and not compared against the placebo group. Dietary intake was not assessed or controlled, and no data is presented regarding diet; and as a free-living study, habitual diet may have influenced the outcomes. The study was confined to men, and sex differences in lipid metabolism and adipose tissue activity are a factor which prohibits extrapolating the findings to women.

Key Characteristic

The use of stable isotopes gives this study a level of precision in illustrating the metabolic fate of marine omega-3 fatty acids. Stable isotopes have provided a quantum leap for nutrition science as a means of "looking under the hood", and precisely tracing the metabolic fate of macronutrients, micronutrients, minerals and trace elements [(6)].

A 'stable isotope' is a less abundant form of a common chemical element, for example 13-carbon instead of the most common 12-carbon. When the most common form is substituted for the less common form in a molecule, for example a fat or glucose, it creates a 'tracer', and the appearance of this tracer in tissues, or in excreted air [or urine/faeces], provides a means to measure the metabolic fate of the nutrient of interest [(6)].

Most studies measure plasma concentrations of various metabolites, but these measures are often static, and do not reflect the dynamic flux of nutrients in and out of tissues. An analogy is this: plasma measures provide a photographic image, stable isotopes provide a video of the action. The use of stable isotope tracers in the present study allowed for a precise quantification of the metabolism of marine omega-3 fatty acids, and their effect on TGs, DNL, and FA oxidation.

Interesting Finding

The most interesting finding of this study is also the one which may explain the increase in blood glucose levels from omega-3 supplementation. In measuring RER after a mixed-meal, it would be expected to see fat oxidation suppressed and carbohydrate oxidation increased.

In this study, however, the data indicated the opposite: RER in response to the mixed-macronutrient test meal was suppressed from the baseline measure to 8-weeks following omega-3 supplementation. At baseline, as would be predicted, fat oxidation decreased significantly following the meal, while carbohydrate oxidation increased [see figure, below]. The reverse happened after 8-weeks: there was greater oxidation of fat following the test meal, and slightly lower carbohydrate oxidation. The lower carbohydrate oxidation may provide some insight into the higher blood glucose levels from omega-3 supplementation.

Relevance

This study has provided several layers of insight - intervention, metabolic, and *in vitro* - into the role of marine omega-3 fatty acids on fat metabolism and liver fat accumulation.

While previous research has shown a benefit to PUFA generally for visceral fat [3], this study indicates that marine omega-3' s have an effect that is very specific to the liver. It also challenges the status quo in relation to post-prandial metabolism, suggesting that the expected suppression of fat oxidation following a mixed meal may not hold true in all dietary circumstances. In this respect, the study provides mechanistic plausibility to the finding of increased post-prandial fat oxidation following omega-3 supplementation.

Of particular note in this study is that the *in vitro* results corroborated the in vivo effects of reduced TG and DNL in liver cell lines. It may be tempting to label the increase in blood glucose levels as an outright negative, but keep the powder dry on this: insulin levels were unchanged, and there was no evidence of impaired insulin sensitivity. Further research will be needed to tease out whether this increase in blood glucose levels following high-dose omega-3 supplementation is cause for concern, or a red herring.

Ultimately, the significant reduction in intra-hepatic triglycerides, post-prandial TGs, and hepatic DNL, suggest numerous pathways through which omega-3 supplementation may protect against liver fat accumulation.

Application to Practice

It is important to bear in mind that the study, and most interventions using the 4g/d dose of marine omega-3 fatty acids, used a pharmaceutical-grade product, not the "fish oils" you' ll find in Holland & Barrett. From a clinical nutrition perspective, there may be utility in such omega-3 supplemental dosage ranges beyond cardiovascular disease, and for nutritional management of NAFLD. For general advice, the study adds more weight to the importance of polyunsaturated fats, in particular the long-chain, marine-derived EPA + DHA, for cardiometabolic health.

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