ALINEA

NUTRITION

JULY 2021

www.alineanutrition.com

TABLE OF

CONTENTS

Rosqvist F, Kullberg J, Ståhlman M, Cedernaes J, Heurling K, Johansson HE, Iggman D, Wilking H, Larsson A, Eriksson O, Johansson L, Straniero S, Rudling M, Antoni G, Lubberink M, Orho-Melander M, Borén J, Ahlström H, Risérus U. Overeating Saturated Fat Promotes Fatty Liver and Ceramides Compared With Polyunsaturated Fat: A Randomized Trial. J Clin Endocrinol Metab. 2019 Dec 1;104(12):6207-6219.

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

While we definitely know that energy balance is the primary determinant of the accumulation of fat in the liver, recent evidence has demonstrated that fatty acid composition may influence the magnitude of liver fat increases or decreases [(1)]. In a previous Deepdive, we saw how overfeeding by 1,000kcal/d from saturated fats, unsaturated fats, and simple sugars, resulted in a hierarchical effect of increasing liver fat: intra-hepatic triglycerides [IHTG] increased by 55% on the saturated fat diet, 33% in response to simple sugars, and 15% in response to unsaturated fats. [(2)].

However, the unsaturated fat group in the above-mentioned study was primarily monounsaturated fats, rendering it impossible to distinguish any distinct effects of unsaturated fat types. There has long been interest in the effects of polyunsaturated fat [PUFA] on post-prandial metabolism, as research in 2001 showed that 5-weeks on a weight-maintenance PUFA-rich diet led to significant decreases in abdominal fat and increased insulin sensitivity, compared to an SFA-rich diet [(3)]. However, liver fat was not measured in that study.

In the HEPFAT trial, Bjermo et al. compared the effects of weight-maintenance diets containing 15% omega-6 PUFA vs. 20% SFA over 10-weeks in lean, healthy participants, and found that while the n-6 PUFA diet resulted in a 9% decrease in liver fat content, while the SFA diet resulted in a 7% increase [(4)]. The present study aimed to investigate the effects of overfeeding targeting a weight gain of 3% initial bodyweight in overweight participants, comparing omega-6 PUFA vs. SFA.

The Study

The LIPOGAIN-2 study was a randomised, double-blind, parallel-group [both groups ran at the same time] intervention conducted over 12-weeks in free-living participants. Participants were randomised to one of two conditions:

- Overfeeding with omega-6 linoleic acid PUFA [delivered in muffins baked in sunflower oil]
- Overfeeding with the 16-carbon [C16:0] saturated fatty acid palmitic acid [delivered in muffins baked in palm oil]

Other than the fatty acid composition, both muffins were identical in macronutrient profile with 51% total fat, 44% carbohydrates, and 5% protein. The muffins were added to the habitual diet of the participants, who were advised to consume the muffins at any time of day. Participants attended a weekly in-patient visit for weight monitoring, and muffin intake was adjusted to meet bodyweight targets.

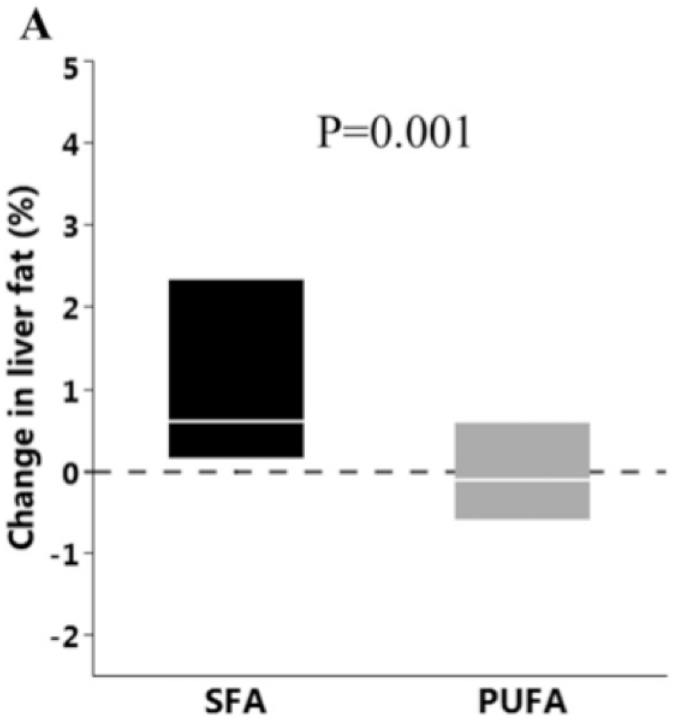
Both diets targeted a 3% weight gain in participants over 8-weeks. Following the 8-week period of overfeeding, participants were then placed on a 4-week 800kcal/d liquid diet. The present paper reported on weight changes, fat depots [liver fat, pancreatic fat, visceral fat], pathways of fat uptake, cholesterol and metabolic markers, and levels of ceramides*.

*Geek Box: Sphingolipids

Lipids - fats - are one of the most complex groups of compounds in the body, and in the diet. Given that ceramides will be a term you read a lot in this Deepdive, it pays to expand on what exactly sphingolipids and ceramides are. So, let us begin. Sphingolipids are synthesised in the body [in the endoplasmic reticulum of cells, to be precise]. They are used as structural lipids, i.e., they help to form cell membranes and form lipoproteins. Sphingolipids are a dizzying array of compounds: there are over 200 sphingolipid species. They are also used as signalling compounds, and used to synthesise bioactive compounds involved in lipid metabolism. Ceramides are an important precursor in the synthesis of different sphingolipids [remember how many there are!]. Ceramides constitute around 3% of circulating plasma sphinoglipids, and ceramides are found on VLDL, LDL, and HDL. There appears to be a relationship between atherogenic lipoproteins, which express apolipoprotein-B [apoB], and ceramide levels. For example, people with a rare condition known as abetalipoproteinemia have no circulating apoB-containing lipoproteins, and have been shown to have up to 80% less circulating ceramides. The relevance of ceramides for the liver is that it is thought that liver cells may produce ceramides to deliver to other tissues, although for what purpose this is not yet known. However, to date it would appear that the increase in ceramide production in the liver may precipitate de novo lipogensis of triglycerides in the liver, providing a potential explanation for the increases in liver fat levels which have been shown to correspond with increasing levels of circulating ceramides.

Results: 60 participants [30 in each group] completed the 8-week overfeeding period, and 51 [26 and 25 in the SFA and PUFA groups, respectively] completed the 4-week calorie restricted phase. Of the 60 participants, 23 were female. Groups were matched for age [~42yrs], BMI [~28], and total weight gain was similar between groups: 2.31kg and 2.01kg in SFA and PUFA, respectively.

- ***Liver Fat:*** Increased by 53% on the SFA group, compared to a 2% decrease in the PUFA group, despite similar weight gain between groups. The liver enzyme ALT, which is released into circulation when the liver is damaged, increased by 18% in the SFA group but remained unchanged in the PUFA group.



Box plot *from paper illustrating the differences between diets for changes in liver fat, expressed as a percentage of liver cells in which triglycerides have accumulated. Bear in mind that >5% of liver cells filled with fat is the diagnosis threshold for non-alcoholic fatty liver disease [NAFLD].*

- ***Total Fat, Visceral Fat, & Pancreatic Fat:*** Changes in total body fat, visceral fat, and pancreatic fat, did not differ significantly between groups. Pancreatic fat increased by 0.49% and 0.46% in the SFA and PUFA groups, respectively. Total fat increased by 2.22L vs. 1.77L and visceral fat by 0.37L vs. 0.26L in the SFA and PUFA groups, respectively.

- ***Ceramides:*** The SFA group increased total ceramide levels, and specific classes of ceramide. Conversely, omega-6 PUFA was associated with decreased ceramide levels across the board. After adjusting for ceramides containing C16:0 palmitate [the saturated fatty acid used in overfeeding], there was no longer any significant difference between diets on liver fat: this indicates that the increase in liver fat was directly associated with increased C16:0-derived ceramides from saturated fat overfeeding.

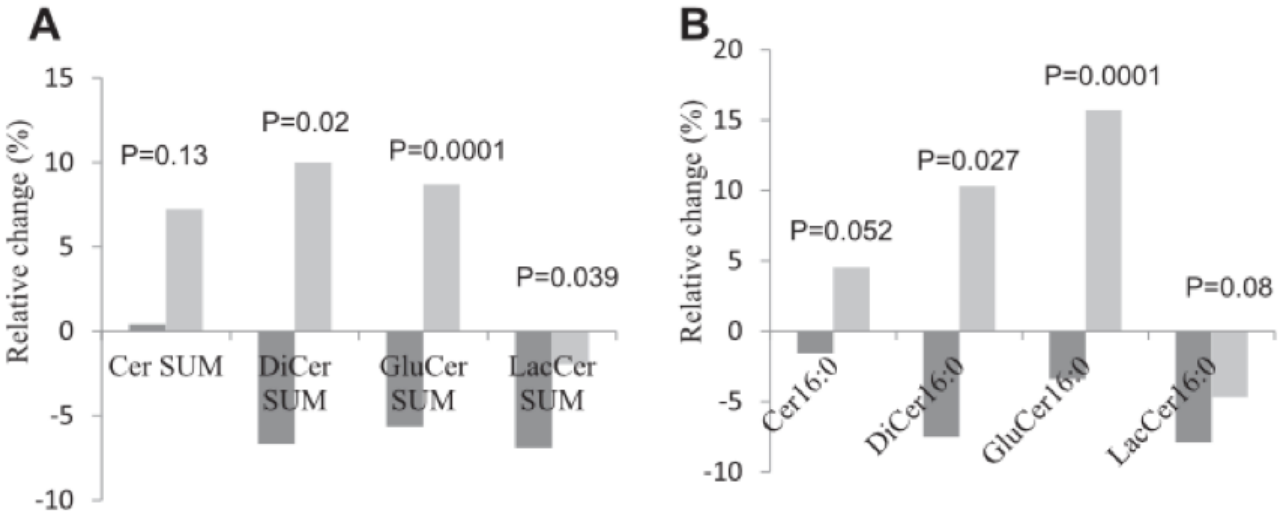


Figure *from paper illustrating the changes in ceramides from saturated fat [light grey bars] vs. polyunsaturated fat [dark grey bars]. In Figure* ***A****, from left to right, is total plasma ceramides and then different ceramide types. In Figure* ***B****, is ceramides derived from C16:0 palmitate, the saturated fatty acid used to enrich the muffins with saturated fat in that intervention group. As can be seen clearly with* ***B****, nearly all C16:0 derived ceramides significantly increased in the SFA group. This increase was directly associated with the increase in liver fat shown in the SFA group.*

- ***Inflammation & Oxidative Stress:*** There were no significant differences in inflammatory markers [C-reactive protein (CRP) and others], endothelial function markers, or lipid peroxidation markers, as a result of omega-6 PUFA overfeeding vs. SFA.
- ***Calorie Restriction:*** Weight loss over the 4-week hypocaloric phase of the intervention was similar between groups, 4.55kg and 4.48kg in the SFA and PUFA groups, respectively. All of the differences observed between diets during the overfeeding phase were abolished by the weight loss phase.

The Critical Breakdown

Pros: Method of randomisation was computerised, and stratified according to sex, age, and BMI. The participants were blinded to which muffins they were receiving [SFA or PUFA], and the investigators were blind until completion of the statistical analysis of the primary outcomes. The muffins were baked in a metabolic kitchen at the research groups' facility. Biomarker analysis of plasma fatty acid levels combined with self-reported intakes suggest high compliance with the intervention. In this regard, the weight gain was close to predicted and also suggests good compliance. The study used a range of advance methodologies to investigate body composition, pathways of fat uptake, and circulating biomarkers. The study was adequately powered in numbers for the outcome of liver fat levels. The trial was pre-registered at ClinicalTrials.gov.

Cons: Given the previous study from this research group in lean participants, the present study feels like a rinse-and-repeat insofar as the participants were completely healthy. Mean BMI was 26.5-28.3, and all cardiovascular and metabolic risk factors - including liver fat levels - were all normal. If the intent was to repeat the study in a population sample with different metabolic characteristics, then this fell short of that aim. It would have been far more insightful to, having already conducted a study in healthy individuals, conducted this study in people with fatty liver. Muffins were added to the habitual diet, however, no data on diet characteristics between groups is presented in the paper [pet peeve: diet study, report on diet]. Participants were also free to consume the muffin at any time of day, but fat metabolism may vary across the day so this is just unnecessary leeway on a variable that was relatively straightforward to try and control for.

Key Characteristic

The use of omega-6 linoleic acid as the PUFA of choice, particularly given that the metabolic effects of omega-3's have previously been a more common exposure of interest. As we covered in a previous Deepdive, omega-3 fatty acids reliably triglycerides, which may be one mechanism by which there is a reduction in liver fat with omega-3 supplementation [5]. In that study, omega-3 supplementation was found to lower the triglycerides in chylomicrons [i.e., triglycerides being transported from dietary intake] by 20% [5]. However, the present study only looked at pathways of the saturated fatty acid palmitate uptake, which seems like a missed opportunity to examine potential pathways through which omega-6 linoleic acid may reduce accumulation of fat in the liver. It may be that that omega-6 linoleic acid reduces levels of triglyceride-rich lipoproteins in the postprandial period [6]. However, the potential mechanisms specific to liver fat remain to be teased out: future studies will hopefully use stable isotope labelling to trace the metabolic fate of omega-6 with regard to liver fat reductions.

Interesting Finding

Now that you' re familiar ceramides from the **Geek Box**, above, let' s think about this finding a bit further. This increase in circulating ceramides from saturated fat overfeeding has been demonstrated before [previous Deepdive], with a 49% increase shown in the study by Luukkonen et al. [2]. In that study, the increase in ceramides was only observed from saturated fat, and there was no effect of either unsaturated fats or simple sugars on circulating ceramides [2]. In the present study, omega-6 PUFA and the saturated fatty acid C16:0 palmitate had opposed effects on ceramides: PUFA led to reduced ceramide levels, while SFA increased circulating levels.

Where it gets interesting is in looking at the fatty acid sources of ceramides: adjusting for C16-derived ceramides abolished the difference in effects of PUFA and SFA. This indicates that the pathway of saturated fatty acid C16 palmitate increasing production of ceramides was primarily responsible for the increase in liver fat from saturated fat. As Luukkonen et al. used a more mixed saturated fatty acid composition, they also demonstrated significant increases in ceramides derived from long-chain saturated fatty acids [2]. Taken together, these studies would suggest that the increases in liver fat from saturated fats may be derived from increasing ceramide production, which precipitates increased triglyceride synthesis in the liver.

Relevance

The findings from overfeeding studies, or indeed energy balance studies, are often caveated with "but calorie deficit tho". And certainly from the perspective of liver fat, there is some truth in this: the present study demonstrated that any differences between diets were abolished by the 4-week very low-calorie diet which followed the overfeeding period.

However, whenever the majority of the population is routinely in an energy deficit, be sure to let me know. Studies targeting energy balance are more efficacious at identifying pathways and mechanisms unconfounded by weight loss, while overfeeding studies provide additional important insights into dietary factors that may either exacerbate or attenuate the effects of an overall surplus of energy. The primary finding of this study corroborates a line of evidence building for the past 5yrs or so: that even in otherwise healthy individuals eating at maintenance energy levels or with an energy surplus, saturated fats significantly increase liver fat, while polyunsaturated fats exert protective effects against the accumulation of fat in the liver.

In the first study from this group, in lean participants, while both groups had gained the same amount of absolute weight of 1.6kg, liver fat increased by 58% on the saturated fat diet compared to only 5% on the n-6 PUFA diet. Interestingly, overfeeding n-6 PUFA caused a greater lean mass increases, and the ratio of lean mass to fat mass gained was 1:1 in the PUFA group, compared to 1:4 in the saturated fat group, in which total body fat and visceral fat both increased in addition to liver fat. Thus, the increase in liver fat was independent of the gain in bodyweight, which was similar between groups. The present study also demonstrated that changes in liver fat were independent of changes in total body weight.

In the prior energy balance study by Bjermo et al. [4], increases in circulating serum omega-6 linoleic acid levels were shown to correspond to the reduction in liver fat in the PUFA group, while increased liver fat positively correlated with greater circulating saturated fatty acids. The present study demonstrated the same relationship, in the context of overfeeding. Thus, it would appear that increasing circulating levels of omega-6 are associated with protective effects against the accumulation of fat in the liver. The exact mechanisms do remain to be further teased out, but one could suggest that the reduced burden of postprandial triglycerides may be one such mechanism. Conversely, the line of evidence suggests that production of ceramides from saturated fatty acid may be one pathway through which saturated fats increase liver fat levels.

Another point worth mentioning given omega-6 was one exposure of interest: there was absolute no effect of omega-6 overfeeding on markers of inflammation or oxidative stress. This is, of course, the status quo in the evidence [7,8]. But one wonders how many nails must go in the "oMeGa-6 tOxIc bRo" coffin. Certainly not enough to nail down all the automatons who actually believe it.

Application to Practice

Check the label on Cuisine de France muffins for palm oil, your liver may thank you. Poor attempts at humour aside, this is another line of evidence that supports a beneficial metabolic effect of PUFA, and in this case omega-6 linoleic acid. For the general population who are not necessarily in a routine hypocaloric condition, it is important to note that the differences between saturated and unsaturated fats are observed in energy balance as well as energy surplus. Given the prevalence of fatty liver in the general population, and the cardio-metabolic consequences of increasing liver fat, dietary modifications that my protect against liver fat accumulation even in the absence of an energy deficit are welcome. PUFA>SFA.

References

1. Green C, Hodson L. The Influence of Dietary Fat on Liver Fat Accumulation. Nutrients. 2014;6(11):5018-5033.

2. Luukkonen P, Sadevirta S, Zhou Y, Ali A, Ahonen L, Lallukka S et al. Saturated fat is more metabolically harmful for the human liver than polyunsaturated fat or simple sugars. Journal of Hepatology. 2018;68:S836.

3. Summers L, Fielding B, Bradshaw H, Ilic V, Beysen C, Clark M et al. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia. 2002;45(3):369-377.

4. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. The American Journal of Clinical Nutrition. 2012;95(5):1003-1012.

5. Green C, Pramfalk C, Charlton C, Gunn P, Cornfield T, Pavalides M 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.

6. Bergeron N, Havel R. Influence of Diets Rich in Saturated and Omega-6 Polyunsaturated Fatty Acids on the Postprandial Responses of Apolipoproteins B-48, B-100, E, and Lipids in Triglyceride-Rich Lipoproteins. Arteriosclerosis, Thrombosis, and Vascular Biology. 1995;15(12):2111-2121.

7. Rett B, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. Nutrition & Metabolism. 2011;8(1):36.

8. Johnson G, Fritsche K. Effect of Dietary Linoleic Acid on Markers of Inflammation in Healthy Persons: A Systematic Review of Randomized Controlled Trials. Journal of the Academy of Nutrition and Dietetics. 2012;112(7):1029-1041.e15.