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NUTRITION

NOVEMBER 2021

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TABLE OF

CONTENTS

Winters-van Eekelen E, Verkouter I, Peters HPF, et al. Effects of dietary macronutrients on liver fat content in adults: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr. 2021;75(4):588-601.

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

The effect of macronutrient manipulations for the treatment of non-alcoholic fatty liver disease [NAFLD] has attracted substantial interest [1]. This is important given that the development in fatty liver is just the first stage toward more serious liver conditions, including liver cirrhosis and carcinoma [2].

There are three main pathways of fat accumulation in the liver in the fed state*. The first two relate to dietary fat metabolism, while the third pathway relates to dietary carbohydrate and the conversion of carbohydrate to fat [1,3]. These pathways mean that both dietary fat and carbohydrate may have a role in generating fat accumulation in the liver.

However, there is also the question of energy balance. Interventions targeting macronutrient manipulations are strongly modified by energy balance. For example, during overfeeding simple sugars will increase liver fat, however, without an energy surplus sugars have little effect on liver fat [4,5]. Conversely, saturated fats may increase liver fat both in overfeeding and during energy balance [6].

Interventions have also suggested the unsaturated fats, polyunsaturated fats in particular, may protect against increasing liver fat levels during overfeeding, and enhance the reduction in liver fat in energy balance conditions [7–9].

These fascinating lines of evidence from intervention studies have been synthesised in a number of reviews [1,10]. However, to date it does not appear that this research area has been assessed by meta-analysis. The present study did exactly that.

*Geek Box: Pathways of Liver Fat Accumulation

In the fasted state, adipose tissue lipolysis (i.e., the breakdown of stored TGs and release of free fatty acids) constitutes the primary endogenous pathway delivering NEFA to the liver. As humans spend most of the day in the fed state, however, it is important to look at the various pathways through which fatty acids may be delivered to the liver from dietary intake. There are three main pathways:

- *chylomicron-spillover NEFA*
- *chylomicron remnants*
- *de novo lipogenesis [DNL]*

The chylomicron pathways are derived from dietary fat. Dietary fat in the form of triglycerides [TGs] enters circulation from the intestines packaged into chylomicrons, large triglyceride-rich lipoproteins which constitute the pathway of dietary fat intake. TGs in chylomicrons are hydrolysed [i.e., broken down] into NEFA by a group of enzymes known as lipases, in particular lipoprotein lipase [LPL].

A proportion of fatty acids mobilised from LPL acting on chylomicron-TGs are not taken up by adipose tissue, and "spillover" into the pool of circulating NEFA which contributes the greatest proportion of fatty acids to intra-hepatic triglycerides [IHTG]. In general, the contribution of systemic NEFA to hepatic fatty acids may be in the region of 45-75%. This pathway of LPL-mediated breakdown of chylomicron-TGs also produces what are known as "remnants", formed when the hydrolysis of chylomicron-TGs results in a smaller lipoprotein, i.e., a chylomicron-remnant. These chylomicron-remnants are taken up by the liver, and the remaining TGs in the remnant particle may be repackaged into VLDL. Over a 24-hr period, the contribution of NEFA derived from chylomicron-remnants has been shown to be greater than the contribution of chylomicron spillover NEFA.

The final exogenous pathway is DNL, where fatty acids are synthesised in the liver from non-fat precursor sources, primarily from excess dietary carbohydrate, in particular free sugars [proteins contribute very little to DNL]. The contribution of DNL to hepatic NEFA in metabolically healthy individuals is relatively small at <5%, however, the presence of fatty liver substantially modifies the rate of DNL, which may be up to ~22-24% in individuals with NAFLD. In addition, insulin resistance strongly modifies post-prandial DNL, which increases in individuals with elevated insulin levels.

In sum, hepatic fat originates from endogenous systemic NEFA derived from adipose tissue and splanchnic lipolysis, exogenous dietary fatty acids derived from chylomicron spillover or chylomicron remnants, and the de novo synthesis of fatty acid from non-fat precursors, in particular carbohydrate. The respective contribution of fatty acids to VLDL-TG have been shown to be in the region of 75-84% from the systemic NEFA pool, 12-39% from dietary fatty acids, and 5-22% from DNL, with the range of contributions reflecting variability due to metabolic health of the individual, in addition to dietary composition.

The Study

The present study was a systematic review and meta-analysis, with the following inclusion criteria:

- Randomised controlled trials
- Evaluated macronutrient composition for effects on liver fat content
- Trials had to have measured liver fat using robust methods [i.e., MRI, liver biopsy]
- If the study was an overfeeding or energy deficit study, if both the intervention and control groups had to be matched for energy intake
- Trials lasted a minimum of 7-days
- Trials provided participants with study foods

Relevant databases were searched, with no limitation on language or year of publication up to 2020.

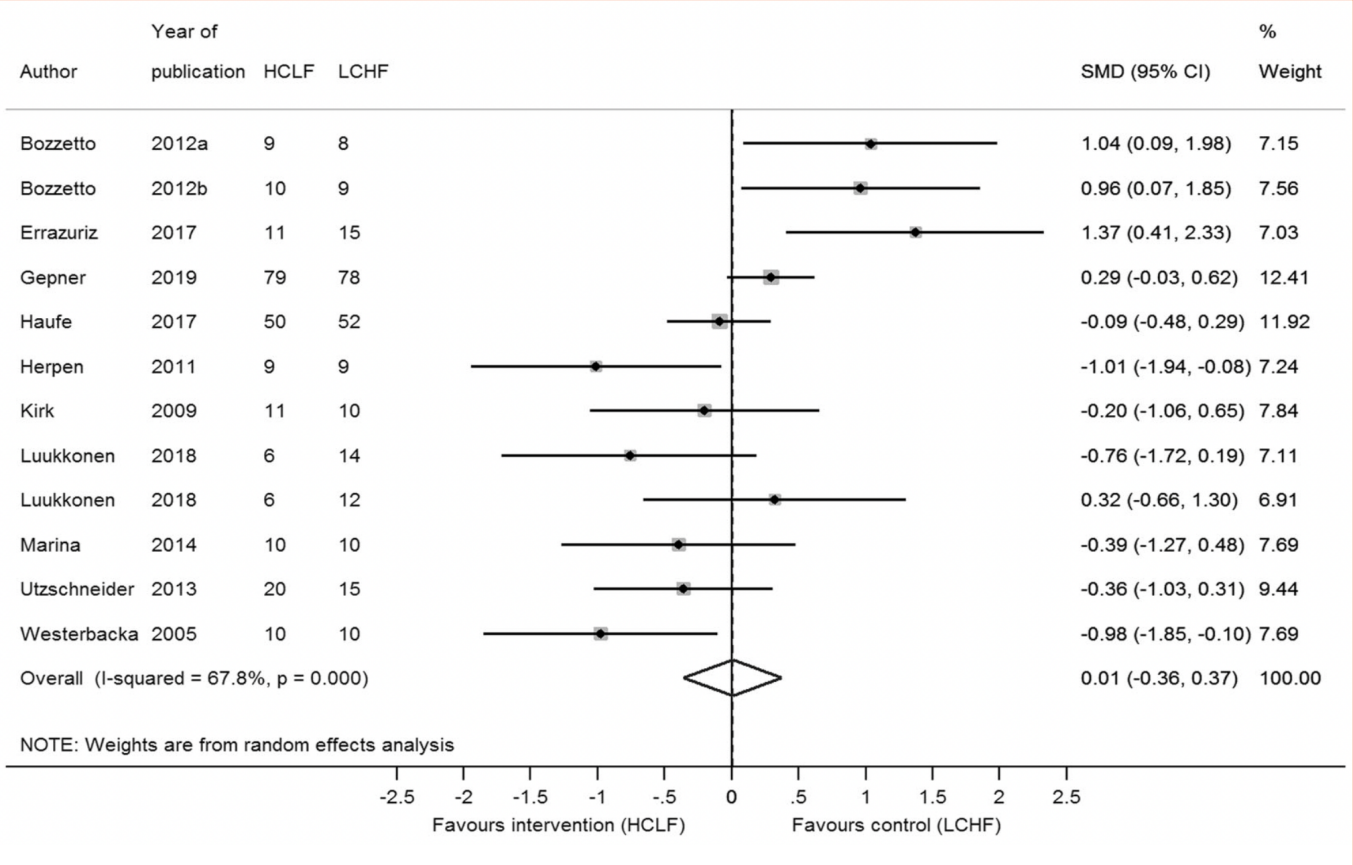
For the results, the study used standardised mean differences [SMD] between the intervention and control groups; an SMD of 0.2 was considered as small effect size, 0.5 was a moderate effect, and 0.8 was a high effect. If this was expressed as a negative and the studies were all energy deficit or energy balance trials, this reflects that the intervention had a greater effect compared to the control group. However, for overfeeding studies, a negative SMD reflected that the intervention had less increase in liver fat compared to the control group.

The meta-analysis, which was broken down into three distinct meta-analysis:

- High-carb/low-fat [HCLF] vs. Low-carb/high-fat [LCHF]
- Saturated fat [SFA] vs. Unsaturated fat [UFA]
- High-protein/low-carb [HPLC] vs. low-protein/high-carb [LPHC]

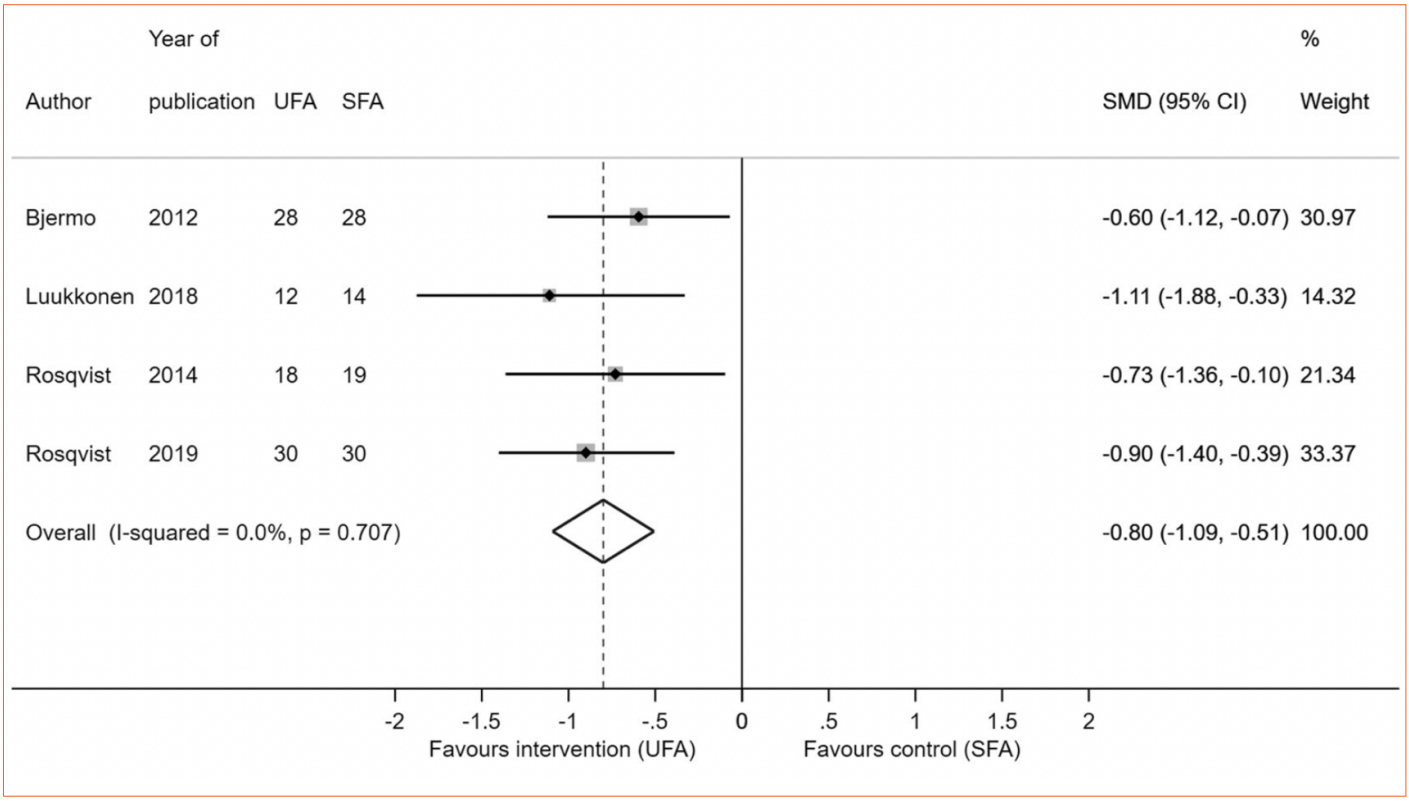
Results: The systematic review included 26 RCTs, of which all but two measured liver fat using MRI/MRS. 21 studies were included in the meta-analysis. The LCHF diets had a carbohydrate content of 10-40%, and fat content of 42-75%; the HCLF diets had a carb content of 53-65% and fat content of 16-34%. The LPHC diets ranged from 5-18.5% protein and 45-60% carbs; the HPLC diets ranged from 22.1-30.5% protein and 29.7-41% carbs.

- ***HCLF vs. LCHF*:** 12 trials compared HCLF vs. LCHF diets; 3 favoured LCHF diets, two favoured HCLF diets, and the remainder showed no difference. The SMD between these diets was 0.01 (95% CI. −0.36 to 0.37), a non-existent effect size.



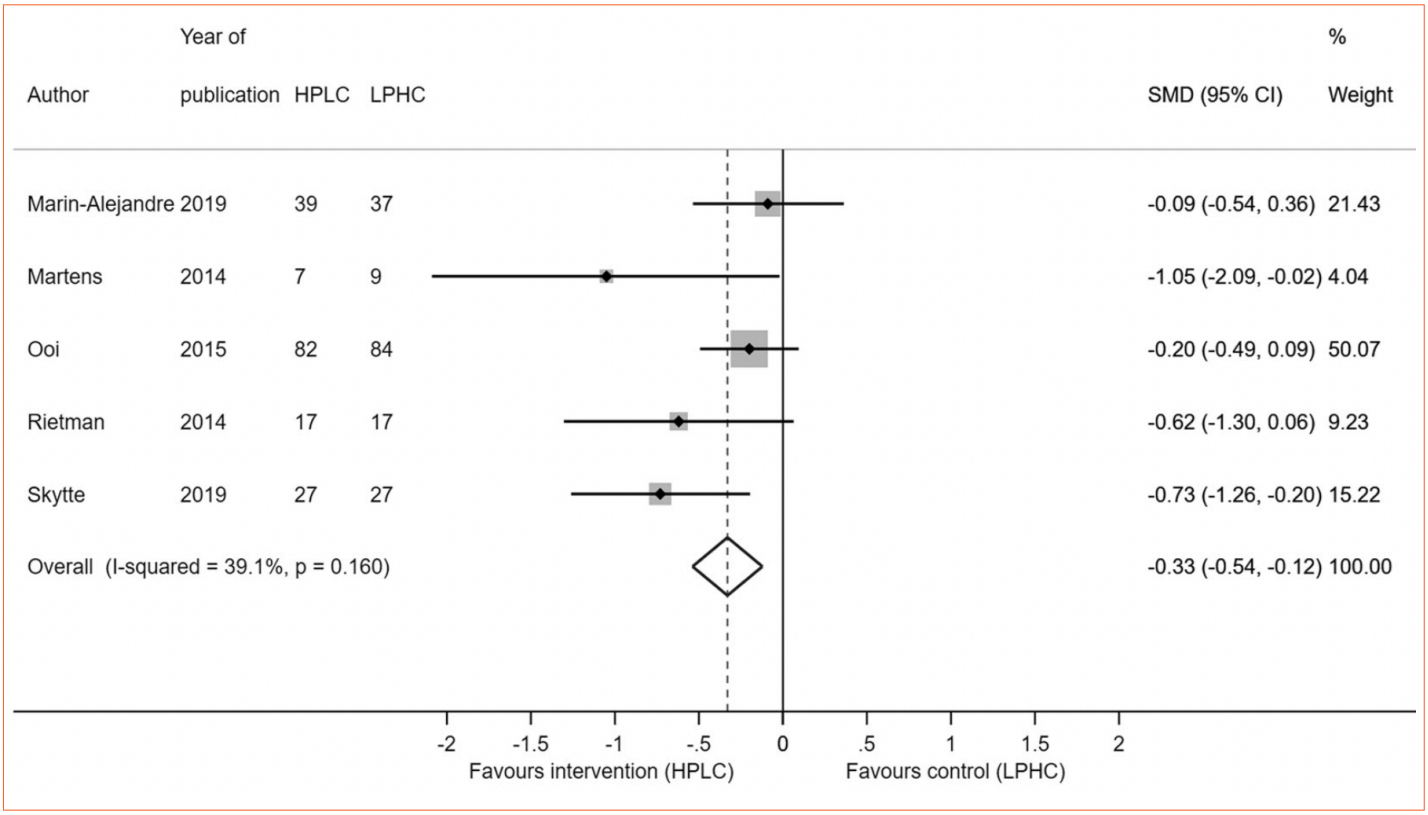
***Forrest plot** from the paper showing the 12 studies included in the HCLF vs. LCHF comparison. The heterogeneity was 67.8%, which is substantial heterogeneity and reflects the varying compositions of the diets in their range of fat and carbohydrate intakes.*

- ***SFA vs. UFA*:** 3 studies compared SFA vs. UFA, and all found that UFA reduced liver fat compared to SFA, with an SMD of –0.80 (95% CI, –0.51 to – 1.09), a large effect size in favour of UFA.



***Forrest plot** from the paper showing the studies which compared unsaturated to saturated fat. You can see that the SMD for each study was a large effect size, with only the Bjermo et al. 2012 study showing a moderate SMD of 0.60. There was no heterogeneity present, as you can see from the 0.0% result of the I2 test.*

- ***HPLC vs. LPHC*:** Of five studies included in the meta-analysis, two showed a significant effect of HPLC diets while the remainder showed no significant difference. The SMD was of –0.33 (95% CI, –0.12 to – 0.54), a small effect size in favour of HPLC.



Forrest plot *from the paper showing the studies which compared HPLC to LPHCt. You can see that the SMD for the included studies ranged from small [Ooi et al. 2015, SMD 0.20] to very large [Martens et al., SMD 10.05]. The heterogeneity present in these studies was considered low, as it was <40.*

The Critical Breakdown

Pros: The inclusion criteria is strong in this one. Overfeeding and energy deficit trials could only be included if both the intervention and control groups were matched for energy intake. The sensitivity analysis excluded studies with physical activity as part of the intervention, to eliminate the potential for this factor to influence the outcome above diet alone. The included studies all used accurate measures of liver fat. The studies were all relatively recent, with 2002 the earliest but 22 from 2011 onwards.

Cons: Obviously researchers had to play the cards as their dealt, and in this case that means the definition of 'low' or 'high' for any of the macronutrients analysed. For example, can we say 40% carbohydrate is a truly 'low-carb diet'? And certainly would qualitatively differ to a 10% carbohydrate diet. As a result of these differences, there was substantial heterogeneity in the meta-analysis of carb/fat studies. Although most studies were at least 4-weeks long, a number of studies were less than this and it would have been useful to conduct sensitivity analysis to see whether study duration and energy balance influenced the findings.

Key Characteristic

Any meta-analysis is defined by the input data, but with NAFLD as the outcome of interest, there is one key determinant and moderating factor of the effects of different macronutrients: energy balance. This is the major limitation of this analysis, is not being more granular with the energetic content of the diets. Even their table for the systematic review which sets out the characteristics of the included studies does not state whether the trial was hyper-caloric, iso-caloric, or hypo-caloric.

Just looking at the primary studies included in the comparison of high/low protein diets indicates a mix of studies in which participants' weight was maintained (Skytte et al., 2019), participants were overfed for 2-weeks (Rietman et al., 2014), or put on an energy-restricted diet (Marin-Alejandre et al., 2019). Bear in mind *the analysis looked at the mean difference between diet groups*. So in the Rietman et al. 2019 overfeeding study, *both diets increased* liver fat; it is just that the high-protein diet resulted in much less of an increase.

These studies all had different effects on liver fat, likely mediated by the energy balance of the study diets. So we can't quite conclude that the effects are independent of energy balance; an important caveat.

Interesting Finding

Sticking with the protein theme here, but is the effect on liver fat mediated by the high protein component of the diet, or the low carb? The role of dietary protein has been somewhat overlooked in this literature, so the meta-analysis in this study adds some interesting evidence. In general, it is suggestive that high-protein/low-carb diets may be advantageous. The Rietman et al. paper suggests that 25% protein vs. 15% [with similar fat and carbohydrate intakes] leads to less liver fat accumulation.

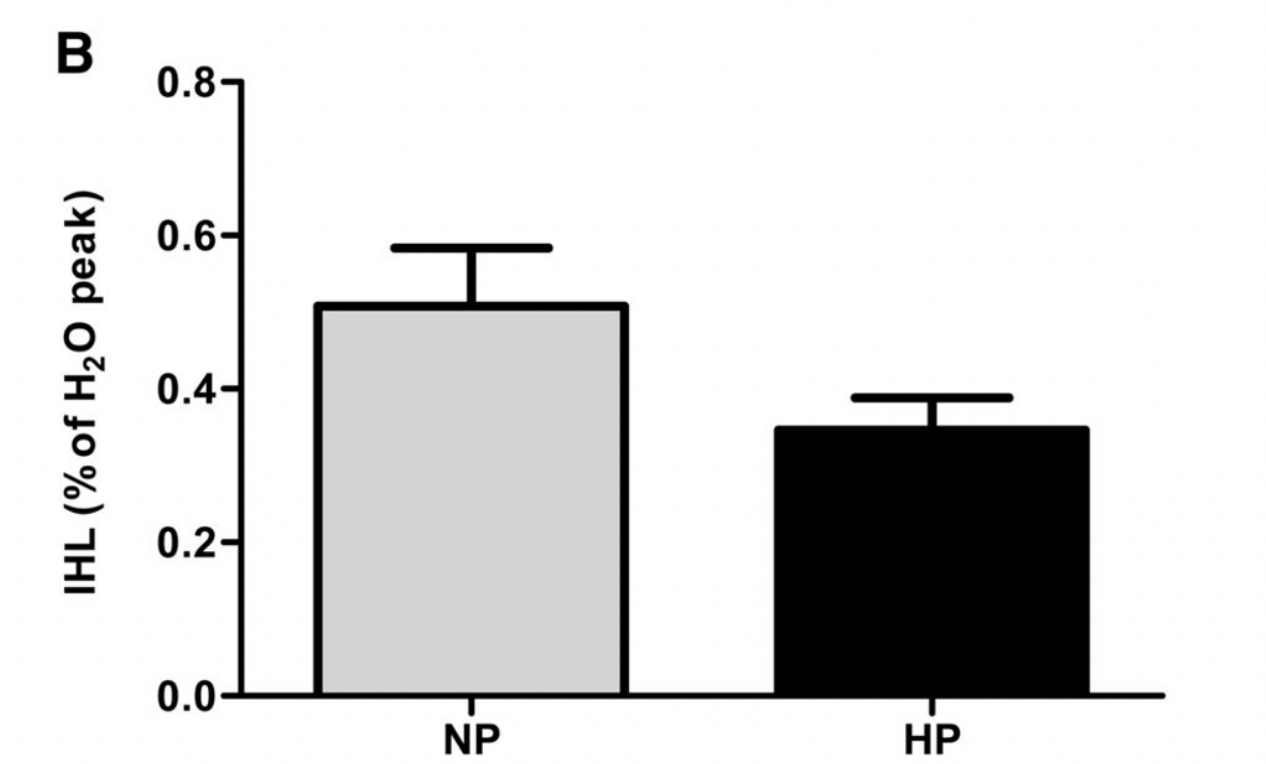


Figure *from the Rietman et al. 2019 paper included in the meta-analysis; you can see that both diets increased intra-hepatic lipids [IHL], i.e., liver fat, but the the high-protein [HP] diet attenuated this effect. The present meta-analysis used the difference between the HP and NP diets in the analysis, so caution is required if interpreting this effect as reduction in liver fat in all cases.*

Is this the full story for protein and overfeeding? Maybe not. The PROOF study from Bray et al. published in 2019 compared three different levels of protein while overfeeding participants: 5% vs. 14% vs. 25%. Carbohydrate was kept constant, so the different levels of protein diets varied content of dietary fat. In the analysis, the low-protein diet with the highest fat intake significantly increased liver fat; this effect was attributable to the dietary fat, not protein content [(11)].

Relevance

Per the ***Key Characteristic*** point above, there is a big caveat to this study insofar as the results are *not* inseparable from the energy balance in the primary study. However, this study has the strength of being the first meta-analysis of the available intervention studies investigating macronutrient manipulations for NAFLD.

From the wider literature, however, we can make the following observations breaking down the relationship with energy balance. First, overfeeding studies suggest a hierarchy to the effects of different nutrients on the liver: saturated fat having the largest effect on increasing liver fat, followed by simple sugars, followed by unsaturated fat [with PUFA showing the least increase/most protective effect] [5,8,9].

Secondly, in the context of energy balance, the effects of sugars is largely abolished, yet saturated fat has been shown to still increase liver fat levels [6,12]. Finally, studies using energy-restricted diets are largely equivocal as the energy deficit itself leads to liver fat reductions [13].

Under hypocaloric conditions, low-carb diets vs. low-fat diets [with protein intake matched] may result in faster short-term reductions in liver fat, but this also appears to be equivocal over time [14]. However, as we reviewed in this previous Deepdive, there may be utility for very low-carb diets to kickstart dramatic short-term reductions in liver fat in as little as 7-days.

Thus, it is important to consider the relationship with energy balance for the effects of any nutrient exposure in the diet. Of particular interest arising from the present study is the potential role of dietary protein, and as the PROOF study highlighted, the relationship of protein to dietary fat. The Rietman et al. study suggests that high protein intake may protect against liver fat accumulation on a high fat diet; the PROOF study suggests low-protein on a high fat diet may have the opposite effect. Interesting for future research to tease out.

Application to Practice

For those of you working clinically, there is some real application emerging from the NAFLD research, and a number of tools exist in the toolbox. For weight-stable interventions, modification of fat composition to enrich the diet with unsaturated fats appears to be the most effective approach, while energy-restricted diets very much depend on whether fast-and-furious or slow-and-steady would be best suited to the individual, in which case lower carb would favour the former while the latter may be equivocal between carbs and fats.

For those of you working with the general population, these principles still apply. However, perhaps the most underappreciated part of this evidence is still the effect of saturated fat; most people think fatty liver and think “fructose” or “sugar”. But sugars are only likely to increase liver fat where they contribute to energy excess. Being more mindful of saturated fats, even without overconsuming energy, remains important for hepatic health.

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