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Koopman KE, Caan MWA, Nederveen AJ, Pels A, Ackermans MT, Fliers E, et al. Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: A randomized controlled trial. Hepatology. 2014;60(2):545–53.

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

As an exposure in nutrition, meal frequency is largely considered to be "dead", largely because it doesn't help Bro's lose fat. Which, obviously, is the sole reason nutrition research exists. Saltiness aside, the very concept of meal frequency is nuanced, and whether the exposure is relevant or not depends on a number of factors.

First, what is meant by "meal"? This operational definition has been a bane of nutrition research, because 'meals' are primarily defined not by energy content or other relevant nutrition characteristics, but by cultural and social labels, such as 'breakfast' or 'dinner', which differ from one culture and context to another [(1)]. The lack of operational definitions for 'meals' or 'snacks' means that much of the data on frequency is not consistent [(2)]. Certain research groups interested in food timing have started to apply a minimum energy content to define snacks, usually 50kcal [i.e., a snack is only counted as a meal if it is 50kcal or over], and also applying a minimum period of 15-minutes to distinguish eating occasions [(2)]. This has provided some additional nuance: in an analysis of Australian adults, meal frequency was associated with higher diet quality - but snack frequency was not [(3)].

Second, what is the relationship between frequency and energy content of meals/snacks? In analysis of the US National Health and Examination Survey III data [1994-2004], the cohort with the lowest diet quality had the highest overall frequency of eating occasions, and extended duration of eating into the biological evening [(4)]. Conversely, in the INTERMAP study, higher meal frequency was associated with lower dietary energy density, greater fruit and vegetable intake, and lower Body Mass Index [(5)]. In addition, timing of food intake relative to frequency may be an important influence on energy balance: a high proportion of energy in the evening reflects shorter duration between meals and increasing meal size over the course of the day [(6)].

What emerges from this slightly confusing body of evidence is that eating frequency may be a secondary factor to energy balance, i.e., whether eating frequency is associated with positive or negative health outcomes relates to the influence of frequency on energy balance. But is it this simple? Could frequency itself as an exposure have a different effect to meal size? The present study investigated this question in relation to liver fat accumulation in healthy adults.

The Study

37 healthy young [mean age 22, range 19-27] men with a BMI of 22.4 [range 19.5-24.5] were enrolled in a randomised and controlled intervention trial investigating the effects of overfeeding high-fat/high-sugar, or high-sugar alone, consumed as either greater meal size or increased meal frequency.

Participants underwent a 1-week run-in phase where dietary intake was recorded: if body weight was similar at the start and end of the week, the calculated calorie intake from the diet record was deemed to be maintenance energy for that participant. Participants were then randomised to one of the four following overfeeding groups or a control group:

- High-fat/high-sugar + increased meal size [HFHS-S]
- High-fat/high-sugar + increased meal frequency [HFHS-F]
- High-sugar + increased meal size [HS-S]
- High-sugar + increased meal frequency [HS-F]
- Control group

Each diet was followed for 6-weeks. All diets contained a 40% energy surplus additional to the participant' s weight-maintenance diet. In the HFHS groups, to increase meal size, participants consumed the additional energy as a liquid meal replacement alongside 3 main meals. To increase meal frequency, participants consumed the liquid meal replacement as a snack between the three main meals [2-3hrs after the meal]. In the HS only groups, participants consumed 1L sucrose-sweetened soft drinks per day, either divided up alongside each meal [to increase size] or between meals [to increase frequency]. The control diet was a continuance of the weight-maintaining diet.

Resting energy expenditure, intra-hepatic triglycerides [IHTG]*, insulin sensitivity, and insulin resistance, in addition to glucose and free fatty acids, were all analysed.

*Geek Box: Pathways of Liver Fat

How does fat get into the liver? There are a number of origins of hepatic fat, and the respective contributions of different sources can differ whether we are looking at the fed vs. fasted state, or healthy individual's vs. individuals with obesity or non-alcoholic fatty liver disease [NAFLD] In the fasted state, the primary source of fatty acids delivered to the liver is from breakdown of stored fats [adipose tissue lipolysis]. In the fed state, dietary fats [triglycerides] and carbohydrate are absorbed through the small intestine. Dietary fats are packaged into chylomicrons and transported to body tissues. As the triglycerides are broken down, chylomicron remnants are formed, which are taken up by the liver: these account for about 24% of dietary triglycerides. In addition, fatty acids which "spillover" from chylomicrons are also taken up by the liver. Thus, dietary fat absorbed has two pathways to the liver: as chylomicron remnants or spillover fatty acids. In the fed state, the majority of liver fatty acids [45-75%] remain from systemic circulation taken up by the liver. The carbohydrate taken in through diet may be converted to fat through a process known as 'de novo lipogenesis'. However, the contribution of DNL is not as high as previously thought; in healthy individuals DNL may only contribute <5% of fatty acids delivered to the liver, while in individuals with NAFLD this can be up to 22-24%. There is also the splanchnic pathway, which if you haven't heard the term before, 'splanchnic' refers to visceral abdominal depots. 'Splanchnic lipolysis' thus describes the breakdown and release of fatty acids from visceral fat depots, and this is highly relevant for liver fat as the hepatic portal vein provides a rapid means of uptake for splanchnic fat. In healthy individuals in a fasted state, splanchnic lipolysis may account for between 5% and 10% of liver fatty acids, however, in individuals with high levels of visceral fat this may be up to 30%. Thus, there are multiple pathways through which the liver may accumulate fat, and the primary sources relate to fat metabolism and dietary intake, rather than the conversion of carbohydrate.

Results: There were 8 participants per diet group and 5 as controls. The control group weight remained stable, indicating compliance with weight-maintaining dietary intake, and there were no significant changes in any outcome measure in the control group. Dietary intake between the intervention groups was similar throughout the intervention, i.e., there was no difference in energy and macronutrient intake between the two HFHS groups, and between the two HS groups.

- ***Weight:*** Participants in each intervention group gained an average of 2.5kg over the 6-week intervention, with no significant differences between groups.
- ***IHTG***:
    - HFHS-Frequency: Increased significantly by 45% [from 0.98% to 1.38%]
    - HS-Frequency: Increased significantly by 110% [from 1.49% to 3.10%]
    - HFHS-Size: No significant change [0.85% to 1.05%]
    - HS-Size: No significant change [0.80% to 0.93%]

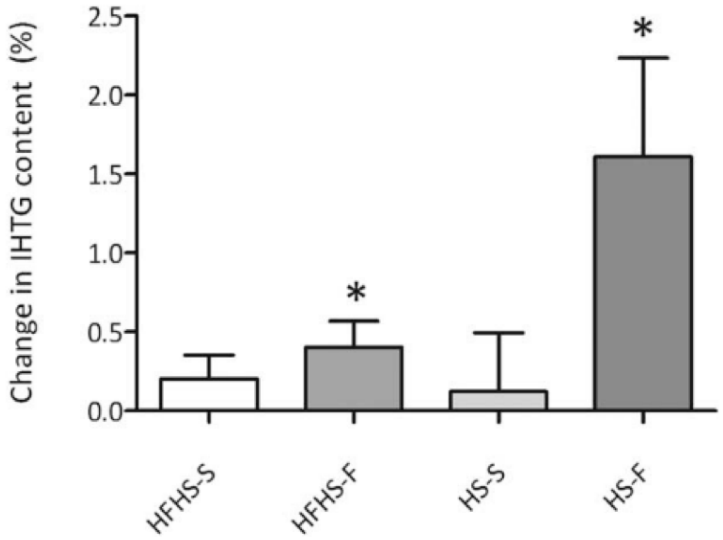

Figure *from paper illustrating the change in intra-hepatic triglyceride content, i.e, liver fat, after 6-weeks of overfeeding with high-fat/high-sugar [HFHS] or high-sugar only [HS], and either all energy consumed in 3 meals per day or consumed as 3 meals and three snacks [the high frequency intervention]. As is evident, the HFHS-F [**grey bar second from left**]] and HS-F [**grey bar far right**] exhibited the most significant increase in liver fat content following overfeeding.*

- ***Abdominal Adipose Tissue and Free Fatty Acids:*** There was a statistically significant increase in intra-abdominal adipose tissue and subcutaneous adipose tissue in the HFHS-Frequency group, and borderline significant effect in the HS-Frequency group. There was no significant change in either of the meal size groups. In the HFHS-Frequency group, fasting triglycerides significantly increased and the suppression of free-fatty acids by insulin significantly decreased.

Comparing the 4 diets, the overall effect of increasing IHTG and abdominal adipose tissue was driven by meal frequency, with no significant difference between HFHS vs. HS.

There were no other significant differences between diets.

The Critical Breakdown

Pros: The study was a first comparing the separate effects of frequency vs. meal size, at the same level of total energy intake. Participants were well-matched at baseline, were randomised and analysed against a control group. IHTG and abdominal fat were quantified using magnetic resonance spectrometry [MRS] and magnetic resonance imaging [MRI], both accurate techniques for these measures. Diet was monitored with weekly in-person visits, and reported daily online by participants.

Cons: The paper does not state what the primary outcome or secondary outcomes were. The power calculation for the sample size was based off data on insulin resistance, however, if liver fat, abdominal fat, or energy expenditure was the primary outcome there would have been sufficient wider literature to calculate the required number of participants for this outcome. Randomisation was conducted by drawing lots, and manual methods may introduce non-randomness and are generally not recommended. It would also have been useful to have presented individual data for the outcomes.

Key Characteristic

Comparing meal frequency to meal size was a novel design on a relevant research question for the modern food environment. In general, traditional 'three square meals a day' is a thing of the past in most populations in industrialised countries. Few studies examine the effects of overfeeding, and this was the first study to investigate the specific effect of eating frequency in the context of overfeeding. And this context is highly relevant to habitual population eating behaviours. If we consider that the effects were observed in both high-frequency diets - either HFHS or HS - this indicates that independent of the macronutrient composition of overfeeding, the increase in liver and abdominal fat resulted from higher frequency alone, and that the same level of carbohydrate and/or fat had little to no effect when consumed as 3 meals per day.

Interesting Finding

The increase in IHTG was only observed with the increased meal frequency diet, not the increased meal size. When both HFHS and HS high frequency diets were analysed together, there was a 78% increase in liver fat [although separately, the magnitude of effect was greater on the HS diet compared to the HFHS diet]. What could explain these effects?

First, the suppression of free-fatty acids significantly decreased, i.e., fatty acids remained elevated following meals. This is a crucial aspect of metabolism with regard to liver fat, as circulating FFA contribute the greatest influx of fat into the liver in the post-prandial period [(7)]. Secondly, greater meal frequency means more carbohydrate and/or fat circulating in the post-prandial period, and the effects of one meal are not independent of the effects of a prior meal. For example, the fat that appears in circulation over the first 30-mins following a meal is actually the fat stored from a previous meal [the "early peak"], while the primary peak may come 4-6hrs later [(8)]. As a result, a metabolic process known as "the second meal" effect occurs, where consumption of a second meal causes a greater increase in circulating triglycerides, even if the meals are identical [(9)]. With excess triglycerides from diet to break down, there can also be a "spillover" of FFA which pass to the liver [(10)].

Thirdly, previous research has also shown that overfeeding simple sugars from 1L soda per day resulted in significant increases in visceral fat and liver fat [(11)]. Thus, greater frequency may exacerbate these underlying metabolic processes, delivering greater amounts of fat to the liver.

Finally, why could the effect of the HS diet been greater than the HFHS diet, despite overfeeding? It is likely that this reflects the fat composition of the liquid meal replacement used in the HFHS interventions, which contained 35% energy from fat primarily as unsaturated fat. In a number of overfeeding studies, unsaturated fats have been shown to have minor effects on increasing liver fat, while saturated fats have been shown to generate substantial increases in IHTG by >50%, despite similar changes in bodyweight [12,13].

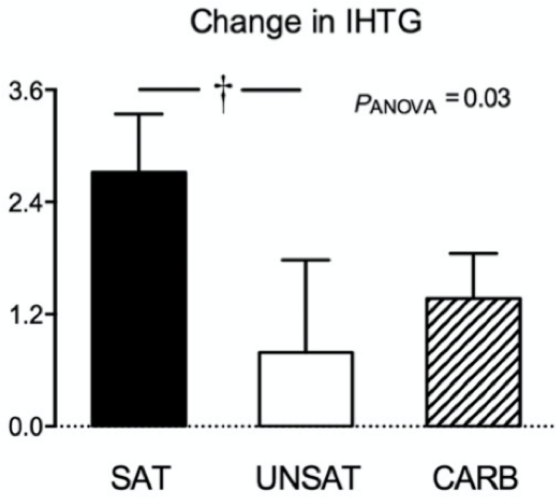

***Figure** from (**13**) indicating the increase in intra-hepatic triglycerides following overfeeding from 1,000kcal extra per day of saturated (**black bar, left**), unsaturated (**white bar, middle**), and simple sugars (**lined bar, right**): saturated fat increased liver by 58%, compared to 15% in the unsaturated fat diet.*

Relevance

The importance of post-prandial metabolism is not necessarily new: as early as 1979, Zilversmit described cardiovascular disease as a "post-prandial phenomenon" [14]. Non-alcoholic fatty liver disease is estimated to have a prevalence of 25% in the global population, and the relevance of dietary determinants of fatty liver cannot be overstated given the relationship between liver fat, cardiovascular disease and type-2 diabetes [15].

In this regard, numerous analysis in different populations have identified that 'grazing' or 'snacking' patterns of energy intake are highly prevalent [3-5]. However, there are ambiguous associations in epidemiology between meal frequency and health outcomes, which appears to relate to the relationship between meal frequency and energy balance [3-5]. The present study therefore provided important insight into this relationship in the context of energy surplus.

The fact that the increases in liver and abdominal fat occurred in otherwise healthy, lean participants also dispatches the fact that meal frequency has no relevance for health status. In particular, both subcutaneous abdominal adipose tissue [SAAT] and visceral adipose tissue are strongly associated with adverse cardiometabolic effects [16]. Intra-abdominal adipose tissue, which increased in the present study, is a fat depot particularly implicated in impaired glucose tolerance, insulin resistance and a stronger correlation to cardiometabolic risk than anthropometric measures [17].

It may be that frequency is relevant factor for underlying liver fat accumulation, effects which may not necessarily be evident from changes in body weight alone, but more research will need to corroborate these findings.

Application to Practice

Given the prevalence of 'grazing' or 'snacking' patterns of energy intake, in conditions of energy surplus it appears that meal frequency may be a modifiable factor in protecting against liver and abdominal fat increases, and associated long-term cardio-metabolic risk.

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