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Chambers ES, Byrne CS, Rugyendo A, Morrison DJ, Preston T, Tedford C, Bell JD, Thomas L, Akbar AN, Riddell NE, Sharma R, Thursz MR, Manousou P, Frost G. The effects of dietary supplementation with inulin and inulin-propionate ester on hepatic steatosis in adults with non-alcoholic fatty liver disease. Diabetes Obes Metab. 2019 Feb;21(2):372-376.

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

Despite the propagation of research into the area broadly known as 'the microbiome', in most respects we are still in the space of 'starting to know'. At the broadest level, we definitely know that dietary fibre is a critical component of healthy dietary patterns, associated with lower incidence of cardio-metabolic disease and colorectal cancer [1-3] through diverse mechanisms, from gastrointestinal effects, and effects on cholesterol, glucose tolerance and insulin sensitivity [4,5].

The trajectory of research has, however, become more focused on specific fibre types and their effect on bacterial species associated with health benefits, and the production of short-chain fatty acids [6]. These fibre types are broadly known as 'prebiotics', which to refresh your memory are defined as:

"a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microbiota that confers benefits." [7]

The 'selectively fermented' criteria means that specific species of bacteria are responsible for the degradation of these fibres in the colon, evident in the increasing populations of these bacteria following prebiotic intakes [7]. To date, only three fibre types have been classified as prebiotics: inulin, oligofructose [FOS] and galactooligosaccharides [GOS], for each of which there is evidence of benefits to human gastrointestinal health [8].

But what of other health effects, i.e., 'extra-intestinal' effects? This is where interest has been directed at short-chain fatty acids* [SCFA], which are produced through fermentation but may be absorbed into by the liver and into systemic circulation [6]. Mechanistic research suggests that SCFA may increase insulin sensitivity, satiety, decrease inflammation [6]. As a prebiotic, inulin has a well-established effect on increase populations of beneficial *Bifidobacteria* in the gut and SCFA concentrations [6,7]. The investigators behind the present study previously showed that a supplemental of inulin combined with the SCFA, propionate, led to reductions in liver fat over 24-weeks [8]. The present study aimed to provide further proof in concept of the effects of this supplement in participants with diagnosed non-alcoholic fatty liver [NAFLD].

*Geek Box: Short-Chain Fatty Acids

SCFA are fatty acids of between 1 - 6 carbons in length, that are produced through fermentation of fibres, proteins and peptides, in the colon. There are three primary SCFAs: butyrate, propionate, and acetate. Butyrate is the primary fuel source for intestinal cells in the colon ['colonocytes'], and is used preferentially over other colonocyte fuel sources like the amino acid, glutamine. Butyrate is associated the suppression of pathogenic bacteria, anti-inflammatory effects, and improve peristalsis [movement of food through the GI tract]. Propionate is absorbed directly to the liver via the hepatic portal vein, while acetate is also absorbed to the liver but enters circulation and is metabolised by peripheral tissues, e.g., skeletal muscle. SCFA's are very efficiently absorbed, and only 5-10% of these fatty acids are excreted in faeces. As you can see from the different metabolic fates of these three SCFA, they each influence different processes. Acetate is used for the creation of new fat [lipogenesis], and is also used for cholesterol synthesis. Conversely, propionate may inhibit cholesterol synthesis and decrease free fatty acid concentrations. The majority of butyrate, however, between 70-90% is taken up by colonocytes. Thus, for the so-called 'extra-intestinal' effects on cardio-metabolic processes, it may be that the ratio of propionate to acetate is an important determinant. It is important to stress that at this stage, much of the research is in animal models and in vitro studies, and well-conducted human studies are scarce, albeit continuing to accumulate. Nevertheless, the increase in SCFA levels, and the populations of bacteria which produce SCFA, are a consistent observation in populations with low prevalence of gastrointestinal diseases. The mechanistic and human data is suggestive of a number of explanations for these associations, but there is still a way to go in establishing effects of different fibre types on SCFAs, the precise metabolic fate and activity of SCFAs in humans, and the potential therapeutic application of targeting the production of SCFAs for specific conditions.

The Study

The study recruited male and female participants with a diagnosis of NAFLD confirmed by liver biopsy within the previous 5yrs, but with stable and controlled blood glucose levels. The study was designed as a randomised, placebo-controlled, and double-blinded intervention. The study intervention and control consisted of:

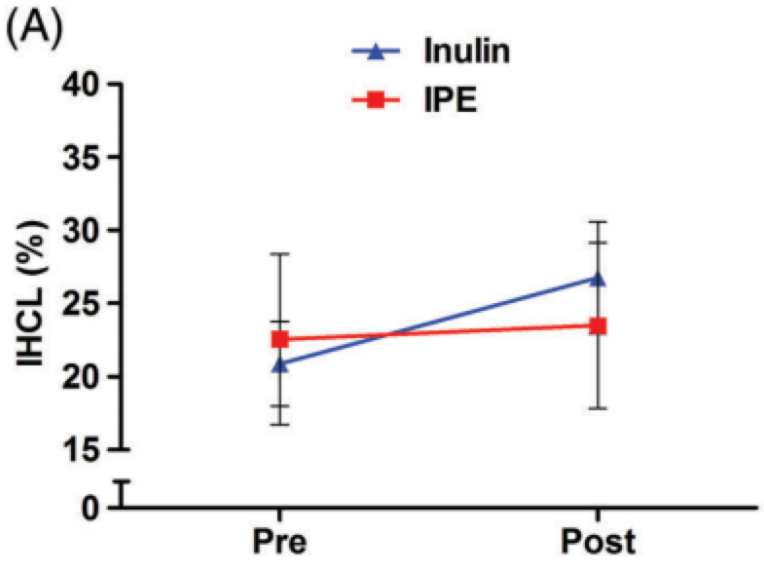
- Intervention: 20g/d of inulin-propionate ester [IPE; providing 14.6g/d inulin and 5.4g propionate]. IPE is a specific supplement with the SCFA propionate bound to the prebiotic inulin. When the inulin is metabolised by bacteria in the colon, it releases the propionate, which can be absorbed.
- Control: 20g/d of inulin alone.

The intervention lasted 42-days. Sachets containing 10g of either the IPE or inulin were provided to participants, with instructions to consumed 2 per day mixed into their habitual diet. Participants attended at the investigators' research centre to undergo testing for the outcome measures at baseline and on day 42.

The primary outcome measure was change in intra-hepatocellular lipids [IHCL, i.e., fat within liver cells]. The authors hypothesised that the IPE intervention would significantly reduce IHCL compared to the control.

Results: 9 participants completed the study in each group [*n* = 18 total]. Average age of the participants was 49 and 51 in the control and intervention groups, respectively. The male/female allocations were 5/4 and 4/5 in the control and intervention groups, respectively.

- ***IHCL*:** IHCL did not change significantly in the IPE group [22.6% at baseline to 23.5% post-intervention]. However, in the control inulin group, IHCL increased from 20.9% at baseline to 26.8% post-intervention.



- ***Metabolic Outcomes*:** There was a significant difference in insulin resistance [measured by HOMA-IR] between groups, driven primarily by an increase in the inulin control group.

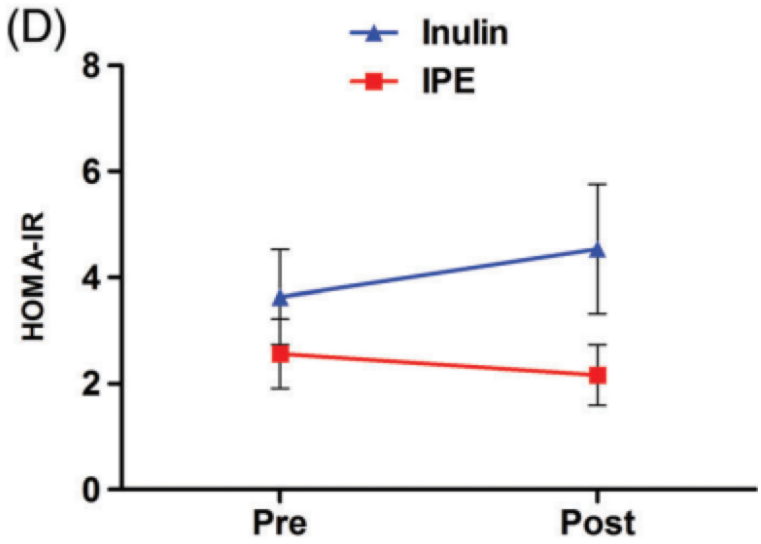


Figure *from paper illustrating (**top**) the group changes in IHCL from before and after the intervention. As can be clearly seen from the blue line representing the inulin control group, there was a significant increase in liver fat in that group over the course of the intervention. On **bottom** is the difference in insulin resistance between groups, which also increased in the inulin control group. It is plausible that the increase in liver fat provides an explanation for the deterioration in insulin resistance, given the strong relationship between these two factors.*

There were no significant differences in any other cardio-metabolic markers between groups.

The Critical Breakdown

Pros: Randomisation method was appropriate [internet-based randomisation] and stratified for sex, which was balanced between study groups. Both participants and investigators were blinded to the allocation of participants. The use of a 'positive control' was a thoughtful way of navigating the challenge of a placebo in a nutrition trial [more under ***Key Characteristic***, below]. The study was part of a 'first-in-human' trial of the effects of IPE supplementation. Participants had biopsy-confirmed NAFLD and baseline levels of IHCL of ~20%, thus the trial was conducted in a targeted at-risk population subgroup. Compliance was good at 90% and 95% in the control and intervention groups, respectively. The trial was pre-registered and there do not appear to have been any material changes with the protocol or outcomes.

Cons: The study was very small, with only n = 18 completing the trial. However, the power calculation provided did state that a minimum of 16 [8 per group] participants would be required to detect an 8% change in IHCL from baseline, thus while small it may have been adequately powered to detect effects and avoid error. For some reason, the bulk of additional methodological information, e.g., methods of randomisation, power calculations, etc., is provided only in the supplementary data and not the main paper; one shouldn' t have to go digging for such rudimentary information pertaining to the study quality.

Key Characteristic

The 'positive control' was a thoughtful aspect to approaching control groups in nutrition intervention studies. Because there is no true placebo for food or nutrients, most studies tend to default to a control group of either an inactive placebo [where the control still have levels of what the placebo is supposed to be *no exposure* to], or a habitual diet [which still may not provide much contrast to an active treatment group].

Because this study was using a defined supplement that would not be habitually consumed in that form [i.e., a combination of inulin and propionate] in the diet, this did make life somewhat easier for the design. Adding the inulin-only control group meant that findings could be considered having 'controlled' for the the inulin content of the IPE, i.e., isolating propionate. However, if they had just had a control group continue with habitual diet, we would assume because it was a weight-maintaining that their liver fat would not have changed; because the intervention IPE supplement also showed no change, we would just be left with a null finding in relation to the treatment.

However, the positive control provided much more meaningful insight than just a null finding for the treatment, due to the outcome in the control group, i.e., the increase in IHCL. This difference in effect yielded comparisons in the potential metabolic effect of acetate vs. propionate. Without the positive control, the conclusion would simply be 'treatment does not work' , rather than the further insights into the potential moderating effects of dose of inulin, added propionate, related acetate and propionate uptake, and actions on liver fat accumulation. While these findings are in turn hypothesis-generating, it means that more can be taken out of this study than if the control had been an inactive control or habitual diet.

Interesting Finding

The interesting finding in this study is clearly the primary outcome, specifically the effects of the control group showing an increase in IHCL vs. no change in the IPE group. The main speculation as to why the control group lead to increased IHCL is that inulin fermentation produces high levels of acetate, and acetate is used for lipogenesis in circulation [10]. Interestingly, evidence for differences between acetate and propionate in effects on blood lipids goes back to the early 1990' s, where it was shown that acetate increases blood cholesterol levels, while propionate had the opposite effects [10]. Thus, it was suggested that the ratio of acetate to propionate may influence blood lipid levels.

To what extent this would influence the accumulation of fat in liver cells is currently speculative. The investigators note that inulin produces an SCFA ratio of 74:16:10 acetate, propionate, butyrate, respectively; IPE shifts this ratio to 25:69:6. The mechanistic evidence indicates that hepatic uptake of acetate my be used for fat synthesis. It may be that IPE, in shifting this ratio of acetate to propionate, protected the liver against excess fat synthesis from acetate. It would be interesting to see the effects of a pure propionate supplement, or one bound to a different fibrous substrate, like psyllium, which may not increase acetate to the same extent.

Relevance

It may be said that "mechanism does not = effect" until such purported effects are shown in human studies. The present study provides some example of this truism, albeit with the caveat that previous work had identified metabolic differences between acetate and propionate that provide at least some biological plausibility to the observed effects. Nonetheless, it is a first-in-human study and these mechanisms require further research in humans to examine the potential factors that explain both the lack of effect of IPE, and the potential for acetate uptake to induce accumulation of fat in the liver.

It is important to note that the previous study by this group using just 10g/d IPE did find a reduction in IHCL over 24-weeks; thus, whether dose and duration of exposure modify the effects of IPE remains to be further teased out. For example, it could be that a lower level of propionate delivered via inulin provides sufficient levels of this SCFA to induce a reduction in liver fat. Further studies could utilise stable isotope tracers to trace the metabolic fate of propionate from IPE, and indeed of acetate. However, for present purposes we are left to infer that acetate-induced fat synthesis may explain the increase in liver fat, with the increase in liver fat in turn explaining the deterioration in insulin resistance.

It could also be that the metabolic health of the host - in this study individuals with high levels of hepatic fat - and energy balance [participants consumed a weight-maintaining diet] also both influence the effects of SCFA on liver fat accumulation. The present study also brings to mind a previous NAFLD intervention covered in a Deepdive. That study also utilised a weight-maintenance protocol and compared a monounsaturated fat-rich diet to a fibre-rich diet, finding no effect of the fibre-rich diet [11]. Now, the fibre intake wasn' t *that* high, but it could it be that SCFA production from fibre is a moderating factor for the effects of fibre, or it could be that fibre simply has no effect. Either way, the potential effects of SCFA in extra-intestinal metabolic health remain to have more convincing human evidence.

Application to Practice

Whether specific supplementation with an inulin-propionate combination is warranted has not been demonstrated, and indeed this study provides some pause for caution for supplementing isolated inulin in individuals with impaired metabolic health. However, advice to consume fibre in the diet, and indeed prebiotic fibres found in garlic, onions, wheat, oats, soy, leeks, chicory, asparagus, Jerusalem artichokes and leeks, remains solid advice. What we are talking about is the difference between a food-based exposure, and an isolated specific component of food; it is common in nutrition that the latter may not show any effect in doses beyond that found in the former context. Further, it is also important to note that this is early doors for this research area, and the potential factors influencing extra-intestinal effects of SCFA remain to be determined. However, this does not negate the evidence for the intestinal benefits from SCFA, and a diverse array of fibre types in the diet facilitates this end. Just hold the prebiotic supplements for now.

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