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Luukkonen PK, Dufour S, Lyu K, Zhang XM, Hakkarainen A, Lehtimäki TE, Cline GW, Petersen KF, Shulman GI, Yki-Järvinen H. Effect of a ketogenic diet on hepatic steatosis and hepatic mitochondrial metabolism in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A. 2020 Mar 31;117(13):7347-7354.

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

In previous Deepdive' s where we have looked at non-alcoholic fatty liver disease [NAFLD], we have seen that both total energy intake and dietary composition modify the accumulation of fat in the liver [1,2]. Fatty liver is diagnosed when the net retention of triglycerides in hepatic [liver] cells has accumulated in >5% of liver cells [and is not attributable to excessive alcohol intake].

It is important to understand the main pathways* through which fatty acids are delivered to the liver, and the metabolic fate of fatty acids once they arrive in the liver. When fatty acids are delivered to the liver, there are two main fates: esterification or beta-oxidation [3]. Beta-oxidation is the production of energy from fatty acids. Esterification is the production of new triglycerides from free fatty acids [known as 'non-esterified fatty acids' , or NEFA]. Esterification can result in new triglycerides either being packaged into very-low-density-lipoproteins [VLDL] and exported from the liver into circulation, or these triglycerides may be stored in liver cells.

There is a third potential pathway through which circulating NEFA may be processed: ketogenesis, i.e., the production of ketone bodies as an alternate fuel source when entry of fatty acids into the Krebs Cycle to produce energy is limited by the unavailability of dietary carbohydrate. Hypocaloric ketogenic diets have been shown to reduce intra-hepatic triglycerides [IHTG] by up to 30% in as little as 6-days [4]. However, the mechanisms by which the ketogenic diet may reduce liver fat [IHTG] is not well understood. One of the hypotheses regarding the ketogenic diet is that despite elevations in circulating NEFA, these fatty acids may be shuttled into production of ketones and away from esterification into triglycerides.

The present study investigated the short-term effects of a ketogenic diet on liver fat, and investigated pathways of fatty acid utilisation to determine the underlying mechanisms of effect.

*Geek Box: Origins of Hepatic Fat

In the fasted state, adipose tissue lipolysis (i.e., the breakdown of stored TGs and release of NEFA) constitutes the primary endogenous pathway delivering NEFA to the liver. As humans spend a majority 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, and 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

10 participants with hepatic steatosis [i.e., fatty liver] and with low daily alcohol intake were recruited to undertake a 6-day ketogenic diet. The diet was designed to provide ~1,440kcal/d total energy, with <6% carbohydrate [<25g/d], 64% fat, and 28% protein. All meals were prepared by the study team and provided to participants for the full 6-days of the intervention. Participants had intra-hepatic triglyceride [IHTG] content determined by imaging, and underwent metabolic testing using stable isotope analysis [more under ***Key Characteristic,*** below] at baseline and after the 6-day dietary intervention.

Results: The diet resulted in a significant reduction in carbohydrate from an average of 183g/d before the intervention to 22g/d during the intervention: this indicated high compliance, which was verified by measuring circulating ketones.Fat and protein intake did not change significantly, increasing by 14g and 17g, respectively. Beta-hydroxybutyrate and acetoacetate, the main circulating ketones, increased by 10-fold and 6-fold, respectively, over the study. Bodyweight decreased by 3%, from an average of 93.5kg to 90.7kg.

- ***IHTG:*** Liver fat decreased from an average baseline level of 10.3% to 7.1%, a 31% relative reduction from baseline to post-intervention. The AST/ALT liver enzyme ratio, a marker of liver function [normal is <1.0] increased significantly by 34% from 0.84 to 1.13.

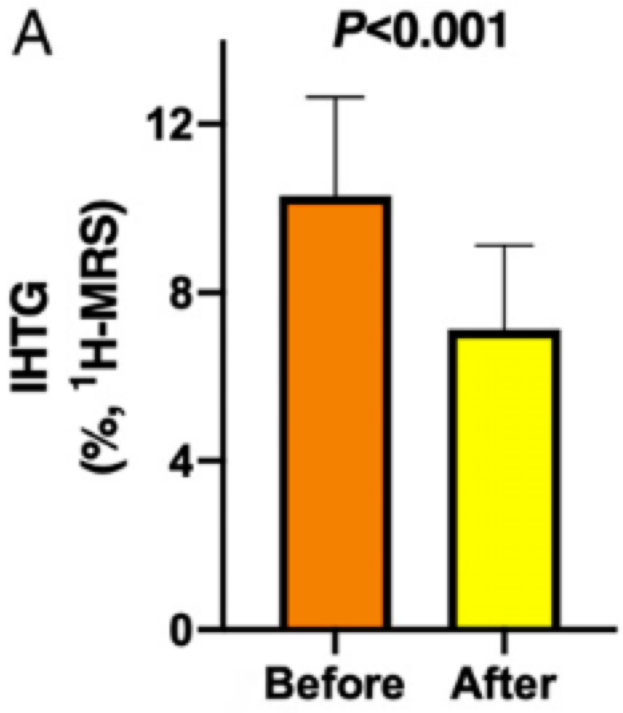

Figure *from paper indicating the change in liver fat (as a percentage) from baseline to the end of the 6-day intervention.*

- ***Insulin, Glucose, & Triglycerides:*** Fasting insulin and insulin resistance significantly decreased by 53% and 57%, respectively. Fasting glucose levels decreased by 13%. While TGs decreased by 25%, circulating NEFA levels increased by 35%.

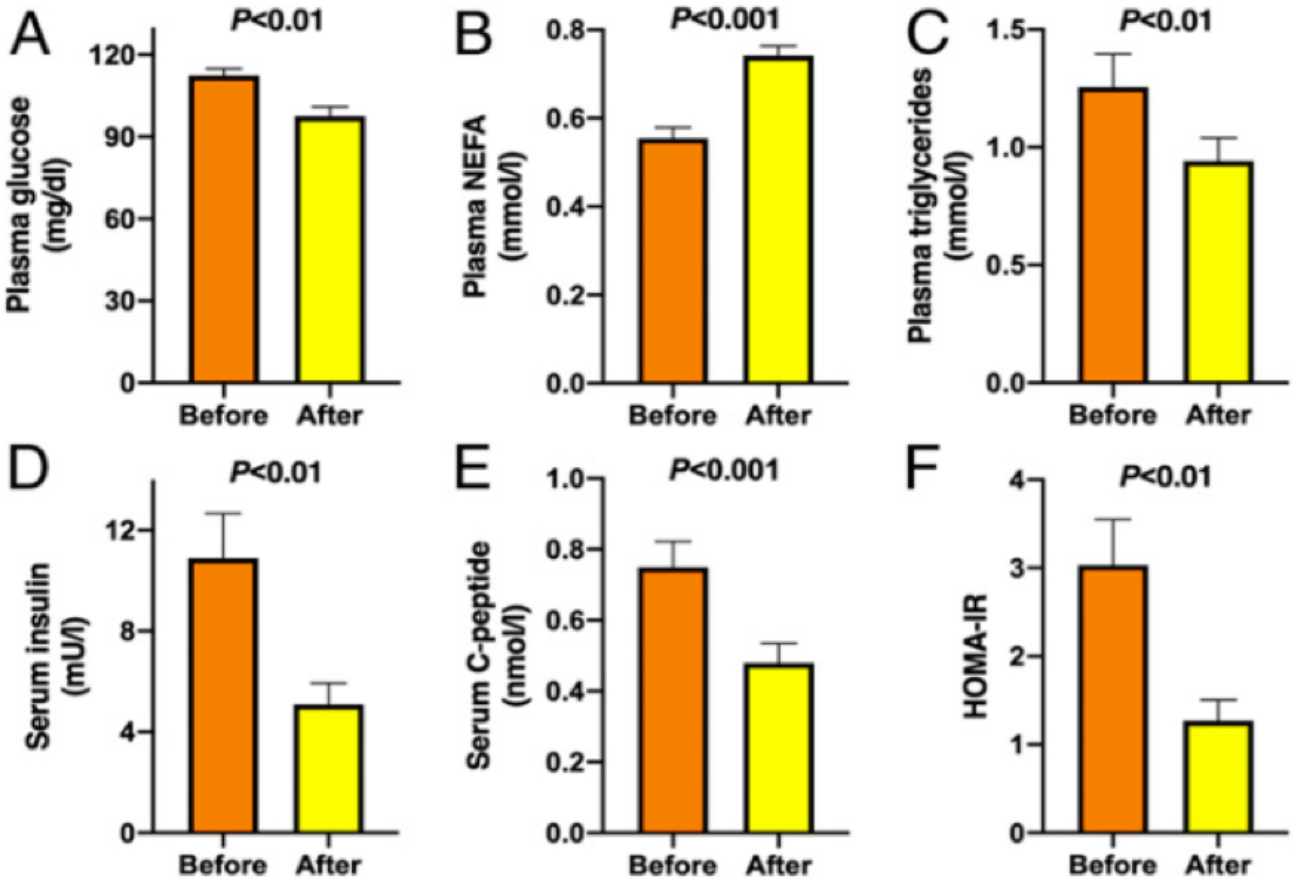

Figure *from paper illustrating (**A**) the reduction in plasma glucose, (**B**) the increase in plasma NEFA levels, (**C**) the reduction in triglycerides, (**D**) the decrease in insulin and (**E**) C-peptide [which precipitates insulin release from the pancreas], and (**F**) the decrease in insulin resistance.*

- ***Hepatic Fat Oxidation and Glucose Production:*** Glucose production in the liver decreased by 22%, while the rate of ketogenesis increased by 232% during the diet. Concentrations of hepatic citrate synthase [VCS], which reflects oxidation in the mitochondria [i.e., energy production], decreased by 38%. Conversely, pyruvate carboxylase [VPC], which reflects glucose oxidation and anaerobic metabolism, remained unchanged. The ratio of VCS:VPC increased by 52% [largely reflecting the reduction in VCS (more under ***Interesting Finding***, below)].

The Critical Breakdown

Pros: Diet was fully controlled and all meals provided to participants. The study was well balanced for sex with 5/5 men and women. The study was highly controlled and employed a number of advanced methodology to quantify liver fat and to determine rates of fat oxidation and glucose production in the liver using stable isotope methods, in addition to extensive blood sampling for hormones and metabolites.

Cons: The study was a very small n=10 intervention, with no control group. While 6-days was sufficient to detect effects, it is not possible to extrapolate these effects beyond the period of the intervention. There were certain notable adverse effects of the intervention, in particular the the significant increase in liver enzymes which implies injury to the liver, and the increase in protein oxidation which indicates protein breakdown exceeded intake.

Key Characteristic

The use of stable isotope tracers to determine the metabolic fate of fat and glucose in the liver. A ' stable isotope' is a less abundant form of a common chemical element, for example a 13-carbon fatty acid instead of the most common 12-carbon form. 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 [(5)]. 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.

Interesting Finding

Despite increases in circulating NEFA levels, fatty acids were diverted into production of ketones [ketogenesis] rather than lipogenesis [for storage in fat] or re-esterification into triglycerides. The mechanistic findings support this preferential partitioning of energy substrates. First, the significant reduction in insulin levels led to greater mobilisation of fatty acids from triglycerides, evident in the increased circulating NEFA levels. Second, the profound increase in circulating ketone bodies indicates that fatty acid beta-oxidation diverted mitochondrial energy production toward ketogenesis. This shift in hepatic mitochondrial energy flux is reflected in the significant decrease in VCS, which implies inhibition of the Krebs Cycle. Finally, these shifts in mitochondrial energy production inhibited production of glucose in the liver. This mechanistic evidence supports a shift in liver metabolism on a hypocaloric ketogenic diet which favours mobilisation of fatty acids and suppressed liver glucose production, while diverting NEFA toward ketogensis rather than synthesis of new IHTG.

Relevance

While much of the earlier research focused on the effects of dietary sugars, in particular fructose, it appears this effect is mediated by energy intake, and the deleterious effects of sugars are primarily observed in conditions of energy surplus [6]. However, more recent research has focused on the effects of dietary fats, and demonstrated that in conditions of energy balance, high fat diets - in particular high saturated fat intake - increases liver fat independent of energy excess [1-3]. Conversely, unsaturated fats may protect against increased liver fat during energy excess, while during energy balance there may be reductions in liver fat with monounsaturated fat-enriched diets [7,8].

In energy balance studies using isocaloric diets, low-fat/high-carb diets consistently show greater reductions in liver fat compared to low-carb/high-fat diets: this appears to be attributable to the high-fat content of the diets [9]. Conversely, in energy deficit studies there appears to be a greater short-term effect of low-carb/high-fat diets, albeit this is not consistently evident over 12-24-months [9]. The present study therefore posits an important question: would the effect of a high-fat ketogenic diet on liver fat, and related mechanisms, be evident without an energy deficit? Does the ketogenic diet augment the effects of energy restriction, such that an additive effect is observed? And is the short-term duration sufficient for the desired effect, i.e., what happens to liver fat levels after the intervention?

These are questions to be teased out in further research. For now, one thing is clear: hypocaloric ketogenic diets do lead to profound reductions in liver fat content in as little as 1-week. Specific macronutrient manipulations may have some utility in the context of hypocaloric studies, however this remains to be fully elucidated; at this juncture it is evident that negative energy balance, whether short-term and more severe or moderate and longer-term, reduce liver fat content.

Application to Practice

Tightly controlled studies of dietary interventions for fatty liver remain small in number, but it is an expanding evidence-base. Although the present study provides evidence of specific metabolic effects of ketogenesis which may induce short-term reductions in liver fat, these effects require confirmation in larger comparative trials.

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