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Influence of dietary macronutrients on liver fat accumulation and metabolism

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ABSTRACT

The liver is a principal metabolic organ within the human body and has a major role in regulating carbohydrate, fat, and protein metabolism. With increasing rates of obesity, the prevalence of non-alcoholic fatty liver disease (NAFLD) is growing. It remains unclear why NAFLD, which is now defined as the hepatic manifestation of the metabolic syndrome, develops but lifestyle factors such as diet (ie, total calorie and specific nutrient intakes), appear to play a key role. Here we review the available observational and intervention studies that have investigated the influence of dietary macronutrients on liver fat content. Findings from observational studies are conflicting with some reporting that relative to healthy controls, patients with NAFLD consume diets higher in total fat/saturated fatty acids, whilst others find they consume diets higher in carbohydrates/sugars. From the limited number of intervention studies that have been undertaken, a consistent finding is a hypercaloric diet, regardless of whether the excess calories have been provided either as fat, sugar, or both, increases liver fat content. In contrast, a hypocaloric diet decreases liver fat content. Findings from both hyper- and hypo-caloric feeding studies provide some suggestion that macronutrient composition may also play a role in regulating liver fat content and this is supported by data from isocaloric feeding studies; fatty acid composition and/or carbohydrate content/type appear to influence whether there is accrual of liver fat or not. The mechanisms by which specific macronutrients, when consumed as part of an isocaloric diet, cause a change in liver fat remain to be fully elucidated.

INTRODUCTION

The prevalence of obesity is increasing at a rapid rate¹; obesity is strongly associated with an increased risk of metabolic disease including: type 2 diabetes mellitus (T2DM),² cardiovascular disease (CVD),³ and non-alcoholic fatty liver disease (NAFLD).⁴ NAFLD represents a spectrum of disease encompassing simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis and hepatocellular carcinoma.⁵ It is estimated that between 6% and 51% of the adult population have NAFLD,^{6–8} with ~70% of individuals with T2DM having NAFLD⁹; however, estimates of prevalence are dependent on the assessment method used and

ethnicity of cohort studied. The accumulation of intrahepatic fat (steatosis) is considered by some to be benign^{10,11}; however, it is now recognised as the hepatic manifestation of the metabolic syndrome and a contributor to the metabolic complications associated with obesity.¹²

Diagnosing NAFLD

For NAFLD to be diagnosed, there has to be evidence that liver fat content exceeds 5% of liver tissue,^{4,13} which has been reported to correspond with the 95th percentile of liver fat in the general population.¹⁴ By definition, NAFLD is only present in individuals who do not consume large amounts of alcohol (>30 and 20 g of alcohol daily for men and women, respectively).^{6,8} However, alcohol intake is often self-reported and there exists a large degree of heterogeneity regarding alcohol consumption by NAFLD patients in the literature; it is challenging to delineate the contribution of alcohol intake in the progression of disease.

NAFLD can be diagnosed in a number of ways. Liver biopsy is considered the ‘gold standard’; however, this is an invasive procedure which is prone to sampling error as only a small quantity of tissue is taken (~1/50 000th relative to total liver size),¹⁵ from one part of the liver which may not be representative of the whole organ.¹⁶ The analysis of liver enzymes in blood (eg, alanine transaminase (ALT) and aspartate aminotransferase (AST)), are often used to determine abnormalities in liver function, although changes in these markers do not always correlate with the diagnosis of NAFLD obtained from liver biopsies.^{9,17} Non-invasive liver imaging techniques are being increasingly used and these include ultrasound, fibroscan (elastography), CT, and MRI with or without spectroscopy (MRS),¹⁸ with the latter being reported to accurately quantify liver fat.^{14,19}

Risk factors for NAFLD: an overview

Although total body fatness is an independent risk factor for NAFLD,^{4,20,21} it is suggested that the distribution of body fat is also important. Increased visceral (intra-abdominal) adiposity is strongly associated with NAFLD pathogenesis.^{22,23} An example of a strong association between visceral fat and NAFLD is from patients with genetic defects that cause partial whole-body or depot-specific lipodystrophy;



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these individuals exhibit an increased visceral fat depot with little or no change in subcutaneous adiposity.^{24–26} A number of healthy-weight individuals (body mass index (BMI) 19–25 kg/m²) are also diagnosed with NAFLD²⁷; the factors regulating liver fat content in these individuals remain to be elucidated. Thus, it is plausible that adipose tissue function rather than mass, along with dietary or lifestyle factors, may be critical in the development of NAFLD.²⁸

Susceptibility to NAFLD is known to have a strong heritable component,²⁹ with genetic variants in patatin-like phospholipase domain containing three protein (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2) and membrane bound O-acyltransferase domain-containing seven gene (MBOAT7, also called LPIAT1)^{30–31} being identified. The prevalence of the PNPLA3 and TM6SF2 alleles has been estimated to be between 30%–50% and ~15%, respectively^{32–33}; the prevalence of the MBOAT7 alleles remains unclear. It has recently been suggested that polymorphisms of MBOAT7 are linked to fibrosis; the TM6SF2 variant appears to modulate hepatic fat accumulation, with hepatic lipid synthesis from n-6 polyunsaturated fatty acids (PUFAs) being impaired,³⁴ while the PNPLA3 polymorphism confers risk of both increased steatosis and fibrosis.³¹ Carriers of the PNPLA3 and TM6SF2 variants exhibit a higher liver fat content than non-carriers but do not exhibit features of the metabolic syndrome/insulin resistance and therefore have been suggested to not be at increased risk of T2DM or CVD compared with non-variant carriers.^{32–33–35–36} It has been suggested that PNPLA3, a lipase, is involved in hepatocellular lipid remodeling and retinol metabolism, TM6SF2 is involved in hepatic very low-density lipoproteins (VLDL) secretion and MBOAT7 is involved in phosphatidylinositol acyl-chain remodeling.³⁰ Environmental factors such as diet have been suggested to interact with PNPLA3 variants; high intakes of PUFA are associated with increased liver fat in carriers of the PNPLA3 rs738409 variant when compared with non-carriers.³⁷ This finding is in line with the ‘multiple hit’ hypothesis of NAFLD,³⁸ whereby genetic variants may predispose individuals to NAFLD and lifestyle factors may be an additional catalyst. To date, there is limited work investigating the interaction between genes, environment, and nutrients on NAFLD development. Furthermore, as each of these genetic variants is related to an increased risk of NAFLD, it is plausible to speculate that carriers of multiple variants are at an even greater risk of NAFLD development, and progression, than carriers of single variants.

Sexual dimorphism in NAFLD prevalence was first suggested over two decades ago where relatively small studies reported an increased prevalence of NAFLD in females.^{39–40} However, more recent population-based studies have suggested the prevalence of NAFLD is greater in males.^{41–45} It has recently been suggested that there is sexual dimorphism in intrahepatic fatty acid synthesis, and partitioning of dietary fat, which may in part explain some of the reported difference in liver fat content between men and women.⁴⁶ Population-based studies have also suggested ethnic differences in NAFLD prevalence, with a higher prevalence reported in Hispanic and Asian populations compared with Caucasian and Black populations.^{45–47} The mechanisms underpinning these ethnic differences are

unclear but are likely related to a combination of genetic, environmental, and lifestyle factors.

Lifestyle factors such as increased calorie consumption and lack of physical activity/exercise have also been reported to be key mediators in the accumulation of liver fat⁴⁸ and appear to be predominantly mediated by changes in body fatness (eg, weight gain or loss). As there are currently no licensed pharmaceutical agents for treatment of NAFLD, dietary changes and increased physical activity leading to decreased body fatness is the recommended management/treatment strategy^{17–49–50}; however, these are difficult to achieve and maintain for many individuals.⁵¹ Whether specific dietary components influence NAFLD risk independently of total calorie intake and changes in body weight remains unclear.

Intrahepatic fatty acid metabolism: an overview

The liver rapidly adapts to altered nutrient fluxes that occur during the transition from the postabsorptive to postprandial state. In the postabsorptive state, there is an increase in circulating non-esterified fatty acids (NEFAs) which are derived from the intracellular lipolysis of adipose (subcutaneous and visceral) tissue triglyceride (TG). Circulating NEFAs are taken up by the liver via fatty acid transporter proteins (FATP) including, FATP2, FATP5, and fatty acid translocase/cluster of differentiation 36 (FAT/CD36).⁴ Once within the cytosol, fatty acids are rapidly converted to fatty acyl-coenzyme As by fatty acyl-coenzyme A synthetases, or the FATPs directly which have been proposed to exhibit synthetase-like properties^{52–53}; acyl-coenzyme As are then partitioned toward esterification or oxidation pathways.⁵⁴ Within the liver, fatty acids may also be synthesised from non-lipid precursors such as dietary carbohydrates via the process of de novo lipogenesis (DNL). During this process, pyruvate arising from glycolysis enters the mitochondria where it is converted to acetyl-coenzyme A for metabolism within the tricarboxylic acid (TCA) cycle.⁵⁵ When energy stores are in excess, TCA cycle intermediates begin to accumulate resulting in the transportation of citrate into the cytoplasm where it is broken down by ATP citrate lyase to acetyl-coenzyme A and oxaloacetate.⁵⁶ Citrate is an allosteric activator of acetyl-coenzyme A carboxylase (ACC) and catalyses the conversion of acetyl-coenzyme A to malonyl-coenzyme A, which is the primary carbon source for fatty acid synthesis and is considered the rate-limiting step in the DNL pathway.⁵⁷ Synthesis of fatty acids are then catalysed by fatty acid synthase (FAS), which sequentially uses malonyl-coenzyme A to elongate the fatty acid chain eventually forming the 16 carbon fatty acid palmitate⁵⁸; this can then be further metabolised through elongation and/or desaturation pathways. The transcriptional regulation of the DNL pathway involves sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP) and these increase glycolytic and lipogenic gene expression when activated by increased circulating insulin and glucose concentrations.^{53–59}

In the postprandial period, following consumption of a mixed meal, TG-rich chylomicrons are synthesised in enterocytes before being transferred to the intestinal lymph and secreted into the systemic circulation via the thoracic duct.⁶⁰ The majority of chylomicron-TG (~70%)

is hydrolysed by lipoprotein lipase (LPL) in the capillaries of extrahepatic tissues, such as adipose tissue, and chylomicron remnants within the circulation^{61 62} can be taken up by the liver via the low-density lipoprotein (LDL) receptor-related protein-1.^{63 64} It has been demonstrated that a proportion of fatty acids liberated by the action of LPL on chylomicron-TG are not taken up by subcutaneous adipose tissue and 'spillover' into the systemic NEFA pool.^{65 66} Thus, dietary fatty acids can enter the liver as either chylomicron remnants or spillover-derived NEFA and once taken up they are further metabolised.⁶⁷ Work has been undertaken demonstrating that after consumption of a high fat (62% total energy from fat) meal, liver fat content is increased within 3 hours and remains elevated 5 hours after meal consumption in lean healthy subjects.⁶⁸ By feeding a mixed meal, where the dietary fat was labelled with ¹³C tracer and using ¹³C-MRS to assess dietary fat trafficking through the liver, Ravikumar *et al*⁶⁹ observed that in a control group, with low liver fat, the increment in intrahepatic ¹³C-TG reached peak enrichment by 6 hours, after which there was a sharp decrease back to baseline levels. In contrast, in individuals with T2DM who had notably higher liver fat content, peak enrichment in intrahepatic ¹³C-TG was achieved by 4 hours after which it decreased back to baseline levels.⁶⁹ Taken together, these observations highlight the dynamic nature of the intrahepatocellular TG pool.

Esterification

Within the liver, acyl-coenzyme As in the cytosol are esterified by glycerol-3-phosphate acyltransferase (GPAT) to form lysophosphatidic acid and then by 1-acylglycerol-3-phosphate acyltransferase (AGPAT) to form phosphatidic acid (PA).⁷⁰ PA is then dephosphorylated by lipin-1 to form diacylglycerol,⁷¹ which is esterified to another acyl-coenzyme A to form TG.⁵³ In the endoplasmic reticulum (ER), TG are transferred to apolipoprotein B-100 by microsomal TG transfer protein to form VLDL-TG, which are then secreted into the systemic circulation.^{63 72 73} VLDL-TG assembly is regulated by TG availability within the liver and insulin; increased hepatic fatty acid concentrations have a stimulatory effect,⁷⁴ while increased circulating insulin concentrations suppress production.⁷⁵

Oxidation

If partitioned toward oxidation, acyl-coenzyme As are converted to acylcarnitine and transported across the mitochondrial membrane by carnitine palmitoyl transferase (CPT)-1 and CPT-2, and carnitine acylcarnitine translocase.⁷⁶ Once within the mitochondria, acylcarnitine is converted back into acyl-coenzyme A by CPT-2 before being sequentially metabolised by β -oxidation into acetyl-coenzyme A. This process requires the activation of four enzymes: acyl-coenzyme A dehydrogenase, 2-enoyl-coenzyme A hydratase, 3-hydroxyacyl-coenzyme A dehydrogenase, and 3-oxoacyl-coenzyme A thiolase.⁵³ Acetyl-coenzyme As produced via β -oxidation can then be further metabolised by the TCA cycle. When concentrations of acetyl-coenzyme A are high, ketone bodies (β -hydroxybutyrate, acetoacetate, and acetone) are formed through a series of reactions for which 3-hydroxy-3-methylglutaryl-coenzyme A synthase has been reported to be

the rate-limiting enzyme.⁷⁷ Ketone bodies are secreted into the circulation to be used as a fuel source in extrahepatic tissues.⁷⁸ The ketone body, β -hydroxybutyrate is often used as a surrogate marker of hepatic fatty acid oxidation.⁵⁴ β -Oxidation is upregulated in the postabsorptive state and reduced in the postprandial state, a response mediated by systemic substrate and hormone concentrations. Glucagon stimulates β -oxidation by upregulation of peroxisome proliferator-activated receptor α (PPAR α) activity,⁷⁹ while insulin and glucose downregulate β -oxidation by suppressing adipose tissue TG lipolysis, reducing the delivery of NEFA to the liver⁸⁰ along with increasing hepatic DNL.^{81 82} For the latter, an intermediate in the DNL pathway, malonyl-coenzyme A, is a potent inhibitor of CPT-1,⁸³ thus when hepatic DNL is increased, hepatic fatty acid oxidation may be attenuated.

Liver fat accumulation: potential metabolic pathways

Why the liver starts to accumulate fat is not fully understood but it has been proposed that when hepatic fatty acid availability exceeds the capacity for removal then they are stored within the liver as TG.^{63 84 85} The storage of TG leads to a net retention of fat which is a prerequisite for the development of NAFLD.⁸⁶ There are multiple pathways through which fat may start to accumulate within the liver, these include: (1) a greater delivery of fatty acids to the liver from the lipolysis of adipose tissue; (2) increased delivery of dietary fatty acids to the liver; (3) increased intrahepatic DNL; (4) a reduction in fatty acid oxidation; and (5) a reduction in VLDL-TG production and secretion.^{87 88}

Expansion of adipose tissue mass has previously been reported to be associated with increased whole-body lipolysis and increased delivery of NEFA to the liver.^{89 90} This would go some way in explaining the association between obesity and NAFLD; however, the observation that circulating NEFAs are elevated in obesity has recently been challenged by Karpe *et al*.⁹¹ Adipose tissue insulin resistance, resulting in increased lipolytic activity is commonly reported in patients with NAFLD.^{92 93} When investigating individuals with the metabolic syndrome, it has been observed that those with higher liver fat also have increased hepatic DNL.⁹⁴ This is likely due to the paradoxical observation that the stimulation of DNL remains insulin sensitive even in insulin-resistant individuals, such as those with NAFLD.⁹⁵ It has been reported that DNL is notably higher (up to threefold) in individuals with NAFLD when compared with healthy individuals without NAFLD.^{94 96} Taken together, it is plausible that increased hepatic DNL may be an important process that contributes to liver fat accumulation. Typically, when DNL is increased, hepatic fatty acid oxidation is low,⁹⁷ which may result in a greater secretion of TG in VLDL or storage within hepatocytes. It has been reported that both β -oxidation and the secretion of VLDL-TG are increased in patients with NAFLD^{98–100}; the inability of these compensatory responses to normalise liver fat content has been suggested to be a key mediating feature in the progression of NAFLD.^{98 99} Thus, it would appear that alterations in multiple facets of hepatic fatty acid metabolism converge and may contribute to the development of NAFLD.

Overview of review

As individuals spend the majority of a 24-hour period in the postprandial, rather than postabsorptive state,¹⁰¹ it is reasonable to suggest that diet in regard to calorie intake and nutrient composition plays a key role in mediating liver fat content. We have previously reviewed the literature regarding the effect of dietary fat on liver fat,¹⁸ while Yki-Järvinen¹⁰² has reviewed the literature on the impact of nutritional modulation of fat and carbohydrate intakes in the context of hypercaloric, hypocaloric, and isocaloric diets on liver fat content and insulin resistance. Taken together, it was found that hypercaloric diets increased, while hypocaloric diets decreased liver fat and saturated, when compared with mono-unsaturated and poly-unsaturated fatty acids increased liver fat and insulin resistance to a greater extent.^{12 18} Since the publication of these reviews more relevant studies have become available, although studies are still limited. Therefore, the purpose of this review is to assess the available studies that have investigated the influence of calories, along with macronutrient intake/composition (fat, carbohydrate, and protein) on liver fat accumulation and metabolism in humans. Although evidence is increasing that alterations in the gut-microbiome (dysbiosis) may influence NAFLD development (as reviewed),¹⁰³ data from human studies where manipulation of dietary macronutrients, changes in the gut-microbiome and changes in liver fat content have been measured, are lacking and therefore cannot be commented on. The studies presented in this review will focus on human observational and interventional studies investigating the influence of the macronutrient content of the diet on liver fat content and/or changes in liver metabolism (eg, hepatic DNL). Relevant studies were determined by searching PubMed, using the search terms 'Fatty liver, NAFLD, liver steatosis, dietary carbohydrates, dietary fats, dietary proteins, and human'. We have, for completeness, included all available interventional studies (n=32), where dietary macronutrient composition has been modified and liver fat content measured, which has meant that between studies, participants are heterogeneous as some have studied healthy, lean participants and other patients with NAFLD and/or T2DM.

OBSERVATIONAL EVIDENCE

The observational evidence comes from cohort studies of varying sizes, ethnicities, and age groups. Liver fat content (to stage NAFLD) has been determined by a variety of means, including liver ultrasound,^{104–106} biopsy,¹⁰⁷ and MRS.¹⁰⁸ Dietary intakes have been assessed with various food frequency questionnaires, which have typically been performed at baseline and then NAFLD incidence measured in follow-up or analysed and compared between patients with no NAFLD and NAFLD; with neither approach accounting for any changes in dietary intakes made over time. Surprisingly, given the prevalence of NAFLD and proposed effect of diet, there are a limited number of studies that have assessed the association of diet and NAFLD.^{104–109}

By assessing the dietary intakes of 995 Australian adolescent females aged 14 and 17 years, Oddy *et al*¹⁰⁶ observed that a high-fat Western style diet at baseline was associated with an increased incidence of NAFLD at follow-up (ie, after 3 years). This finding was supported by data from a

smaller Chinese cohort comparing the dietary intakes of patients with NAFLD and healthy controls, where it was reported that increased liver fat content was associated with high-energy, high-total fat, and high-saturated fatty acid (SFA) intakes.¹⁰⁸ In contrast, data from 4365 individuals enrolled in the Tianjin Chronic Low-grade Systemic Inflammation and Health Cohort Study indicated that NAFLD prevalence was associated with a high-carbohydrate/high-sugar diet in females¹⁰⁵; the authors were unable to make any association between dietary intake and NAFLD prevalence in males.¹⁰⁵ The discrepancies in these findings remain when reviewing the data from small European studies; Cortez-Pinto *et al*¹⁰⁷ reported that when compared with healthy controls, patients with NASH consumed a greater quantity of dietary fat and less carbohydrate than non-NASH control subjects. Others have reported that patients with NAFLD consume a high-carbohydrate, and in particular a high-added sugar (eg, sucrose and high-fructose corn syrup (HFCS)) diet than subjects with no NAFLD.¹⁰⁹ In line with this, Assy *et al*¹⁰⁴ observed when comparing Israeli patients with NAFLD with healthy controls that 80% of patients with NAFLD consumed an 'excessive' amount of soft drinks (defined as greater than two drinks per day) such as Coca-Cola and flavored fruit juices; soft drink consumption was the only independent predictor of NAFLD in this cohort,¹⁰⁴ suggesting a role for sugar-sweetened beverages in the development of NAFLD.

It is plausible that the discrepancies in findings between studies regarding the associations of specific nutrients and/or foods and individuals with and without NAFLD are partly attributable to a number of different factors including the different cultural and environmental characteristics of the participants and dietary assessment methodology used. Dietary assessment techniques are typically confounded by under-reporting, incorrect estimations of portion sizes, behavior modification of the participant when being examined, and error in analysing data using nutritional software either from incorrect coding and computation or from the software not being up to date,^{110 111} making it challenging to delineate the contribution of individual dietary components and to separate the influence of specific macronutrients from that of total calorie intake in regard to the accumulation of liver fat. The complementary use of biomarkers with dietary assessment methodology provides additional, objective information. Although there is currently no biomarker for total fat intakes, the use of the composition of blood fatty acids to determine the type of fats that had been consumed would be informative.¹¹¹ Biomarkers to assess sugar intakes that would allow discrimination between low and high consumers of sugar-sweetened beverages are currently being developed.^{112 113}

INTERVENTIONAL EVIDENCE

A variety of interventional approaches in heterogeneous cohorts have been undertaken to investigate the effect of macronutrients on liver fat content and metabolism. Notably, between studies there is a wide spectrum for the duration the experimental diet is consumed, how the experimental diet is implemented and the phenotype and metabolic characteristics of participants across studies.

Hypercaloric diets

The majority of studies (n=13) investigating the influence of diet on liver fat content have been undertaken using a hypercaloric feeding regime and the studies are presented in [table 1](#). Males, with a BMI <27 kg/m² have made up the majority of participants in these studies which range in duration from 3 days to 6 months, and vary in both degree of energy excess (from 17% to 65% excess energy) and macronutrient of interest; a large proportion of the studies have overfed fructose ([table 1](#)). Findings from this work, have consistently demonstrated that acute (7–14 days) consumption of a high-fructose diet (from 3 to 4 g/kg) leads to a significant increase in liver fat content.^{114–117} Although not directly demonstrated, it is plausible that hepatic DNL was upregulated in response to these dietary interventions, as increased fructose intakes have been demonstrated to potentially stimulate the DNL pathway.^{118 119} The majority of the high-fructose studies observed significant increases in body weight in response to the dietary interventions ([table 1](#)); thus, it is challenging to disentangle the effect of fructose from those of increased body fat on liver fat accumulation. Of note, the quantity of fructose consumed in the majority of these studies was 3–4 g/kg body mass (BM) or fat free mass,^{115–117 120} which would theoretically translate to the consumption of >180 g/d of fructose for an 85 kg male with 25% body fat. This far exceeds the reported average intakes of fructose of 30–50 g/day since the year 1990¹²¹; although fructose consumption has been reported to be significantly higher (up to 70 g/day) in specific populations, such as adolescents.^{122 123} Overfeeding excess calories, while reducing the quantity of fructose consumed (<3 g/kg/BM) does not appear to elicit an increase in liver fat content, even when the duration of overfeeding is extended to 28 days^{124 125}; the magnitude of overfeeding in these studies was not sufficient to increase BM ([table 1](#)).

When comparing hypercaloric diets enriched in either fructose or glucose it would appear that both influence liver fat accumulation in a similar manner ([table 1](#)), suggesting no difference between high-glucose and high-fructose diets in risk of NAFLD development. However, humans typically do not consume fructose in isolation, with the majority of dietary fructose coming from fruit or products containing ‘free’ (also known as added or non-milk extrinsic) sugars (ie, sucrose and HFCS), such as sugar-sweetened beverages and confectionary.¹²⁶ Both sucrose and HFCS are composed of approximately 50% fructose and 50% glucose. It has been demonstrated that compared with the ingestion of fructose or glucose in isolation, hepatic DNL is augmented when glucose and fructose are consumed in combination.¹¹⁹ Despite both glucose and fructose being similar molecules, the divergent effect on hepatic DNL can be explained by differences in hepatic metabolism. Although the metabolism of both sugars begins with phosphorylation, fructose is phosphorylated by fructokinase, to form fructose-1-phosphate, therefore bypassing the enzyme phosphofructokinase, a major rate-limiting step in glucose metabolism.¹²⁷ This leads to fructose having a greater flux through this pathway and being metabolised more rapidly, ultimately providing a larger precursor pool available for DNL than glucose.¹²⁷ Only two studies^{128 129} have investigated the effects of overfeeding dietary free sugars. Maersk *et al*¹²⁸

compared the effects of ingesting 1 L/day of a sugar-sweetened beverage, calorie-matched milk, non-nutritive sweetened beverage, or water for 6 months. They demonstrated that ingestion of the sugar-sweetened beverage significantly increased liver fat by 132%–149% when compared with the other beverage groups, despite calorie intake and weight gain being similar between the sugar-sweetened beverage and milk groups ([table 1](#)).¹²⁸ These findings should be interpreted with some degree of caution as the groups were not well matched for sex, and diet was not standardised between groups.¹²⁸ Sevastianova *et al*¹²⁹ examined the effects of a 3-week hypercaloric (+1000 kcal/day) diet, where 98% of the excess calories were provided as added sugars (eg, candy, fruit juice, and sugar-sweetened beverages). Liver fat was significantly increased by ~27% at the end of the high-sugar diet. The increase in liver fat observed by Sevastianova *et al*¹²⁹ was associated with an increase in the secretion of VLDL-TG and an increase in the lipogenic index, which is the ratio of palmitate (16:0) to lineoleate (18:2 n-6) in VLDL-TG. As hepatic DNL primarily results in the production of SFA in humans,¹³⁰ alterations in the lipogenic index can be used as an indirect measure of DNL.¹¹⁹ Thus, the findings of Sevastianova *et al*¹²⁹ suggest that increased liver fat following a high-energy, high-sugar diet may be attributable to increased hepatic DNL.¹²⁹ Moreover, when the data were analysed according to PNPLA3 genotype it was found that DNL increased in proportion to liver fat, in subjects with the PNPLA3-148II but not the PNPLA3-148MM genotype.¹²⁹ It has been reported that individuals with the genetic variant PNPLA3 148M have significantly lower fasting hepatic DNL and expression of the lipogenic transcription factor SREBP1c, despite having greater liver fat than individuals without the PNPLA3 148M allele¹³¹; it is unknown if hepatic DNL is altered in individuals with the TM6SF2 or MBOAT7 variant, when compared with non-variant carriers, or how alterations in dietary macronutrients alter liver fat content and metabolism in these individuals. In line with the findings of studies overfeeding fructose, the significant increase in liver fat observed by Sevastianova *et al*¹²⁹ also occurred alongside a significant increase (~1%–2%) in BM ([table 1](#)) making it difficult to determine the specific effect of dietary sugars on liver fat accumulation.

When comparing a 7-day hypercaloric diet enriched with fructose with a hypercaloric diet enriched with saturated fatty acids (SFA), Sobrecases *et al*¹¹⁷ demonstrated that the SFA-enriched diet increased liver fat to a greater extent than fructose (~86% vs ~16% change in liver fat content, respectively, [table 1](#)), suggesting excess SFA is more deleterious to liver fat accumulation than excess added sugars. The authors also observed that liver fat increased to a greater extent when overfeeding a diet enriched with excess quantities of both fructose and SFA, compared with the high-fructose or high-SFA diets alone, however the high-fructose and SFA diet was considerably higher in calories ([table 1](#)). van der Meer *et al*¹³² observed a significant increase in liver fat (from 2.0±1.8% to 4.3±2.8%), after just 3 days of overfeeding a high-fat high-energy diet which provided a substantial fat (+280 g/day) and calorie (+2600 kcal/day) excess. It remains unclear if notable increases in liver fat are seen after more modest increases in fat intake. Furthermore, as liver fat was measured (using MRI) 4 hours after

Table 1 Overview of intervention studies which have investigated the effect of hypercaloric feeding on liver fat content

Ref	Design	Subjects	Intervention	Energy excess	Duration	Change in body mass	Change in liver fat
124	SG	7 M 24.7±1.3 years 19–25 kg/m ^{2a}	StdD+Fru (1.5 g/kg)	18%	4 weeks	NSD	NSD
115	R/P	16 M T2DM offspring (24.7±1.3 years) 19–25 kg/ m ^{2a} versus 8M Con (24±1 years) 19–25 kg/m ^{2a}	StdD versus StdD+Fru (3.5 g/kg/FFM)	35%	7 days	T2DM offspring: ↑ 1.0 kg ^{b*} Con: ↑ 0.6 kg ^{b*} NSD between groups	T2DM off: ↑ 79%* Controls: ↑ 76%* NSD between groups
114	R/P	32 M 18–50 years ^a 25.9–32.2 kg/m ^{2c}	StdD+Glu (25%TE) versus Fru (25%TE)	25%	2 weeks	Fru: ↑ 1.0±1.4 kg* Glu: ↑ 0.6±1.0 kg* NSD between groups	Fru: ↑ 24% ^{b*} Glu: ↑ 26% ^{b*} NSD between groups
116	R/C	11 M 24.6±0.6 years 19–25 kg/m ^{2a}	StdD+Fru (3.5 g/kg/FFM) versus Glu (3.5 g/kg/FFM)	35%	7 days	Fru: ↑ 0.6 kg ^{b*} Glu: ↑ 1 kg ^{b*}	Fru: ↑ 52%±13%* Glu: ↑ 58%±23% NSD between groups
125	R/P	20 M+F 30.5±2.0 years 25.9±0.5 kg/m ²	StdD+Fru (150 g/day) versus Glu (150 g/day)	600 kcal/day	4 weeks	Glu: ↑ 1.7±0.4 kg* Fru: NSD	NSD in either group
128	R/P	47 M+F 20–50 years ^d 26–40 kg/m ^{2a}	StdD+1 L/day of cola, semi-skimmed milk, diet cola, or water	Cola: 430 kcal/day Milk: 450 kcal/day Diet-cola: N/A Water: N/A	6 months	NSD between groups No comparisons made relative to baseline	Cola: ↑132%– 143% compared with other gps.* NSD between milk, diet- cola or water
129	SG	16 M+F 54 years ^d (40–59 years) ^e 30.6±1.2 kg/m ²	StdD+added sugars (‘candy diet’)	1000 kcal/day	3 weeks	↑ 1.8±0.3 kg*	↑ 27%*
133	R/P	37 M+F 20–38 years ^a 18–27 kg/m ^{2a}	Habitual diet+high SFA or high-n-6 PUFA muffins	Adjusted to achieve 3% wt gain at follow-up	7 weeks	SFA: ↑ 1.6±0.96 kg PUFA: ↑ 1.6±0.85 kg. NSD between groups	SFA: ↑ 58%. ^b PUFA: ↑ 53%. ^{b#}
132	SG	15 M 25±6.6 years ^f 23.4±2.5 kg/m ^{2f}	Habitual diet+280 g/day fat (800 mL cream)	2632 kcal/day	3 days	NSD in BMI Body mass not presented	↑ 112% ^{*b}
134	R/C	10 M 24±1 years 22.4±0.6 kg/m ²	StdD+HF (+100% fat intake ^b) versus HF+high- pro (+100% fat +>100% pro intake ^b)	HF: 30% ^b HF+high-pro: 49% ^b	4 days	HF: NSD HF+high-pro: ↑ 1.1 kg ^{a*}	HF: ↑ 90%* HF+high-pro: ↑ 68% ^{b**}
139	R/P	36 M 22 years (19–27) ^c 22.5 kg/m ² (19.5–24.5) ^c	Habitual diet+3 L/day HFHS or HS beverage, consumed 3x daily with meals or 2–3 hours after meal ingestion	40%	6 weeks	↑ 2.5±1.7 kg ^{g*} NSD between groups	NSD in gps consuming beverages with meals HFHS ↑ 45%* and HS, ↑ 110%* when consumed between meals NSD in ‘between meals’ groups
117	P	30 M 23.9±0.4 years 22.6±0.2 kg/m ²	StdD+Fru (3.5 g/kg/FFM), SFA (+30% TE), versus StdD+Fru+SFA (3.5 g/kg/ FFM and +30% TE)	Fru: 35% SFA: 30% Fru+SFA: 65%	7 days	↑ 0.3±0.1 kg ^g NSD between groups	Fru: ↑ 16%* SFA: ↑ 86%* Fru+SFA: ↑ 133% ^{**}
135	R/C	9 M 23.3±0.9 years 22.6±0.5 kg/m ²	StdD+high Fru (3 g/ kg)+placebo (6.6 g/day maltodextrin) versus high-Fru (3 g/kg)+EAA (20.3 g/day)	High-Fru+placebo: 36% ^b High-Fru+EAA: 38% ^b	6 days	NSD	High-Fru+placebo: ↑ 115% ^{*b} High-Fru+EAA: ↑ 81% ^{b**}

Subject data presented as mean±SEM unless otherwise stated.

^aInclusion criteria or estimated range within which all participants are included.

^bEstimated from table/graph.

^cRange.

^dMedian.

^eIQR.

^fSD.

^gPooled data.

*Significant effect of intervention ($p < 0.05$) according to the manuscript.

#Significant difference between groups ($p < 0.05$) according to the manuscript.

BMI, body mass index; C, crossover; EAA, essential amino acids; F, Female, T2DM, type 2 diabetes mellitus; FFM, fat-free mass; Fru, fructose; Glu, glucose; HF, high fat; HFHS, high fat and high sugar; HS, high sugar; M, male; N/A, not available; NSD, no significant difference; P, parallel groups; Pro, protein; PUFA, polyunsaturated fatty acids; R, randomised; Ref, reference; SFA, saturated fatty acids; SG, single group; StdD, standardised diet; TE, total energy.

the consumption of the last meal,¹³² it is plausible that the reported increase in liver fat content may be attributable to the greater fat content of the pretest meal. The findings of Rosqvist *et al*¹³³ suggest that alongside total fat intake the fatty acid composition of a high-energy diet might also be an important regulator of liver fat accumulation. They observed that when controlling for weight gain by adjusting the calorie excess for each participant on a daily basis to achieve a 3% increase in BM, 7 weeks of a hypercaloric diet enriched in SFA increased liver fat content to a greater extent than a hypercaloric diet enriched with PUFA.¹³³ Fasting concentrations of β -hydroxybutyrate (a surrogate for hepatic fatty acid oxidation) were reduced following both diets and the reduction was greater following the PUFA rather than SFA-enriched diet (−70% and −45% for the PUFA and SFA diets, respectively),¹³³ suggesting both diets reduced hepatic fatty acid oxidation. However, this cannot explain the divergence in liver fat accumulation implicating changes in other pathways such as VLDL-TG secretion and hepatic DNL.

The influence of a hypercaloric diet, with a high protein content, on liver fat accumulation has been investigated. Bortolotti *et al*¹³⁴ demonstrated that when overfeeding fat alone, liver fat increased by approximately 90%; supplementing the high-fat diet with protein led to a statistically significant reduction in liver fat accumulation compared with the high-fat diet (~22% reduction), despite providing a greater energy intake (table 1). This finding is supported by the work of Theytaz *et al*,¹³⁵ who observed that supplementing a high-fructose diet with a small amount (6.6 g/day) of essential amino acids led to a statistically significant blunting (~16% reduction) in liver fat accumulation when compared with a high-fructose diet alone (table 1). There are a number of potential mechanisms through which protein or amino acid supplementation may attenuate liver fat accumulation; these include increased postprandial hepatic fatty acid oxidation due to the substantial energy demand elicited by the metabolism of amino acids (ie, gluconeogenesis and the synthesis of urea),^{136–138} and increased secretion of newly synthesised fatty acids in VLDL-TG.¹³⁵ However, further mechanistic studies are required to fully elucidate these processes in humans.

The timing of food intake in the context of a hypercaloric diet may influence liver fat accumulation. Koopman *et al*¹³⁹ demonstrated that consuming a high-sugar, or high-fat and high-sugar beverage between meals led to a statistically significant increase in liver fat content, whereas consuming the same beverages alongside meals did not significantly affect liver fat (table 1). It has previously been reported that increasing meal frequency extends the duration for which glucose and insulin are elevated in healthy individuals.¹⁴⁰ Increased insulin and glucose concentrations throughout the day would have the dual effect of stimulating the DNL pathway and downregulating β -oxidation, providing a plausible explanation for the noted increase in liver fat when increasing meal frequency. However, alterations in substrate metabolism following increased meal frequency have not been found by others.¹⁴¹

Taken together, it is clear that hypercaloric feeding whether acute or chronic is associated with an accumulation in liver fat. Based on the findings from the limited studies available it would appear that excess consumption

of fat and/or sugars, in the context of a hypercaloric diet, results in an increased accumulation of liver fat. Excess calorie consumption typically results in increased BM, therefore disentangling the influence of calorie intake, increased BM and effects of specific dietary components on liver fat content is challenging. Moreover, the majority of these studies were undertaken in individuals with a BMI <25 kg/m², and therefore presumably relatively low liver fat content, it would be reasonable to assume that if these interventions were undertaken in cohorts with greater amounts of liver fat, and who were perhaps less metabolically flexible, the changes would be more marked. Finally, the mechanisms underpinning the observed changes in liver fat following these dietary interventions are likely to differ depending on the macronutrient being overfed.

Hypocaloric diets

To date, only a limited number of studies (n=10) have investigated the effect of a hypocaloric diet on liver fat accumulation and metabolism (table 2). Of note and in contrast to the participants involved in hypercaloric feeding studies, the majority of the participants in these studies had a BMI >25 kg/m², and one study focused on individuals who had T2DM for varying periods of time (0.5–23 years). Notably, even in studies including both males and females, a disproportionate amount of females participated in these studies (>75% of total subjects were female). In addition to heterogeneity between participants, there is marked variation between dietary interventions in regard to the calorie deficit achieved, and period of time undertaken, making direct comparisons between studies challenging. For example, a number of studies aimed for a relatively minor reduction in energy intake of approximately 600–800 kcal/day for an extended duration (6 months to 1 year),^{129 142–145} whereas others have undertaken a more extreme approach providing very low calorie diets where energy intake is as little as 450–800 kcal/day for periods between 7 and 9 weeks (table 2).^{146 147} Regardless of the participants studied or approach used to achieve a calorie deficit, a consistent finding is that a significant reduction in body weight is associated with a significant reduction in liver fat content, highlighting the effectiveness of weight loss as a prevention or treatment strategy for NAFLD. When looking at the changes in macronutrient composition, four of the studies have investigated the effects of a reduced carbohydrate intake, either in isolation¹⁴⁸ or compared with other hypocaloric diets.^{143 149 150} In these studies, although participants were advised to decrease a specific nutrient (eg, a specific sugar or total carbohydrate), given the nature of the diet it is likely that there will also be decreased intakes of other nutrients, such as fat. For instance, despite encouraging a decrease in fructose intake, Volynets *et al*,¹⁴⁸ found reductions in fructose, glucose, sucrose, total, and SFA intakes at follow-up. This may contribute to the mixed findings reported regarding the macronutrient composition of hypocaloric diets; Browning *et al*¹⁴⁹ demonstrated that reduced carbohydrate intake may be more effective at reducing liver fat content than reducing total calories without changing the macronutrient composition of the diet, while others^{143 150} have suggested carbohydrate intake has little influence on change in liver fat content in the context of

Table 2 Overview of intervention studies which have investigated the effect of hypocaloric feeding on liver fat content

Ref	Design	Subjects	Intervention	Energy	Duration	Change in body mass	Change in liver fat
148	SG	10 M+F NAFLD 45.5 years ^a (34.5–51.5 years) ^b 31.1 kg/m ^{2a} (25.6–40.6 kg/m ²) ^b	↓ 50% Fru	Ad libitum energy intake with repeat dietary consultations (every 2 weeks)	6 months	↓ 3%*	↓ 36%±26%*
150	R/P	22 M+F 43.6±2.5 years 36.5±0.8 kg/m ²	High-CHO (>180 g/day) versus low-CHO (<60 g/day)	↓1000 kcal/day	~6 weeks Followed by 4 week weight maintenance	↓ 7.5%±0.4% ^{c*} NSD between groups	Low-CHO: ↓38.0%±4.5%* High-CHO: ↓44.5%±13.5%* NSD between groups
149	P	18 M+F NAFLD 45±12 years ^d 35±7 kg/m ^{2d}	Low-CHO (<20 g/day) diet versus low kcal (1200–1500 kcal/day)	Low-CHO: 1553±517 kcal/day Low kcal: 1325±180 kcal/day	2 weeks	Low-CHO: ↓ 4.6±1.5 kg* Low-kcal: ↓ 4.0±1.5 kg* NSD between groups	Low-CHO: ↓ 55%±14%* Low-kcal: ↓ 28%±23%* [#]
143	R/P	102 M+F 30–60 years ^e 25–45 kg/m ^{2e}	Low-CHO (<90 g/day) versus low-fat (<20% TE)	↓ 30% TE	6 months	Low-CHO: ↓ 7.6±0.6 kg* Low-fat: ↓ 6.5±0.7 kg* NSD between groups	Low-CHO: ↓ 47%* Low-fat: ↓ 42%* NSD between groups
144	R/P	70 F 50-70 years ^e 25–40 kg/m ^{2e}	Paleo diet (40% TE fat) versus low-fat diet (25%– 30% TE fat)	Ad libitum energy intake, repeat dietary consultations	6 months 2 years	6 months: Paleo: ↓ 9 kg ^{f*} Low-fat: ↓ 4 kg ^{f*#} 2 years: Paleo: ↓ 8 kg ^{f*} Low-fat: ↓ 5 kg ^{f*} NSD between gps at 2 years	6 months: Paleo: ↓ 64%* Low-fat: ↓43%* [#] 2 years: Paleo: ↓50%* Low-fat: ↓49%* NSD between gps at 2 years
146	SG	18 M+F 50 years ^a (34– 57 years) ^g 44 kg/m ^{2a} (40–51 kg/ m ²) ^g	VLCD	450-800 kcal/day	6 weeks	↓ 15.1% ^a (9.6– 21.1%) ^{g*}	↓ 43%*
147	SG	30 M+F T2DM 25–80 years ^e 27–45 kg/m ^{2e}	VLCD Participants separated based on FPG at follow-up Responders: <7 mmol/L Non-responders: >7 mmol/L	624–700 kcal/day	8 weeks Followed by 6 months weight maintenance	↓ 14% ^{f*}	Responders: ↓ 83%* Non-responders: ↓ 73%* NSD between groups
129	SG	16 M+F 54 years ^a (40– 59 years) ^g 30.6±1.2 kg/m ²	Dietary consultation (repeated every 3 weeks)	Ad libitum energy	6 months	↓ 4%*	↓ 25%*
145	P	27 F (previous history of gestational diabetes) 20–50 years ^e 28–35 kg/m ^{2e}	Targeted weight loss (balanced diet). Participants separated based on baseline liver fat; High: >5% Low: <5%	↓ 600–800 kcal/day	3–6 months (dependent on time to ↓ BM by 8%)	High: ↓ 7.4%±0.2%* Low: ↓ 7.7%±0.3%* NSD between groups.	High: ↓ 49%* Low: ↓ 39%* [#]
142	R/P	37 M+F 35–70 years ^e 28–35 kg/m ^{2e}	Hypocaloric+placebo versus hypocaloric+rimonabant (20 mg)	↓ 600–800 kcal/day	11 months	Placebo: ↓ 1.7±1.0 kg Rimonabant: ↓ 8.5±1.4 kg*	Placebo: ↓ 28% Rimonabant: ↓ 69%* [#]

Subject data presented as mean±SEM unless otherwise stated.

^aMedian.

^bRange.

^cPooled data.

^dSD.

^eInclusion criteria or estimated range within which all participants are included.

^fEstimated from table/graph.

^gIQR.

*Significant effect of intervention ($p < 0.05$) according to the manuscript.

[#]Significant difference between groups ($p < 0.05$) according to the manuscript.

BM, body mass; CHO, carbohydrate; FPG, fasting plasma glucose; F, Female; T2DM, type 2 diabetes mellitus; Fru, fructose; M, male; NSD, no significant difference; P, parallel groups; R, randomised; Ref, reference; SG, single group; total energy; VLCD, very low calorie diet.

a hypocaloric diet. For example, a similar reduction in liver fat content (~44.5%) was observed by Haufe *et al*¹⁴³ when comparing diets which achieved a calorie deficit by reducing either carbohydrate or fat. This suggests that total calorie deficit is the mediating factor for decreasing liver fat and the macronutrient composition of the hypocaloric diet has little influence.

Taken together, the data from hypocaloric studies demonstrate that a negative energy balance leading to weight loss is associated with a reduction in liver fat content. This remains true irrespective of whether a calorie deficit is achieved by acute, very-low calorie interventions, or more modest reductions in calorie intake over an extended period. In contrast to the studies investigating hypercaloric feeding, the majority of hypocaloric feeding studies were undertaken in individuals with a BMI >25 kg/m² and presumably a relatively higher liver fat content. Thus, the effect of a hypocaloric diet on liver fat content was perhaps more notable in individuals starting with higher, rather than lower liver fat content.

The mechanisms underpinning the reductions in liver fat on a hypocaloric diet are difficult to determine, due to limited data. Of the available literature, Steven *et al*¹⁴⁷ demonstrate that the reduction in liver fat observed following a very low calorie diet intervention (~624–700 kcal/day) were associated with reduced fasting concentrations of VLDL-TG, which would suggest a change in the availability of TG for VLDL-TG production. A decrease in the TG within the liver maybe due to a number of factors, including a reduction in fatty acids entering the liver, from either the diet or from decreased lipolysis of adipose tissue TG; a decrease in hepatic DNL, due to decreased substrate availability or a repartitioning of fatty acids within the liver, away from esterification and into oxidation pathways.^{54 151}

Isocaloric diets

Eleven studies have investigated the effects of isocaloric diets on liver fat content (table 3). The effects of short-term (14–28 days) high-fat and low-fat diets on liver fat content have been compared by four studies,^{152–155} while the remaining seven studies compared differences in dietary fatty acids, or diets enriched with different sugars (table 3).^{114 156–160} Of note, studies have been undertaken in a wide variety of participants (ranging from young, healthy, lean individuals, to elderly, overweight patients with NAFLD) and using a variety of dietary approaches, for varying periods of time (from 9 days to 10 weeks). For example, some studies have modified dietary intakes using whole foods while others have supplemented the diet with specific nutrients. There is also variation in what defines a ‘low-fat diet’, with total fat intakes ranging from 16% to 30% total energy (TE) across studies^{152–155}; the amount of sugar provided also varies greatly from 8% to 30% TE (table 3).^{114 157 159} Moreover, some studies have provided all foods to participants while others have modified habitual dietary intakes using dietary counseling (table 3). Taking the data from short-term, high-fat versus low-fat studies suggests that isocaloric low-fat diets reduce liver fat content, whereas isocaloric high-fat diets increase liver fat content (table 3); a plausible explanation for this observation is that consumption of a high-fat diet would provide a

greater delivery of dietary fat to the liver compared with a low-fat diet.

The fatty acid composition of the fat consumed appears to influence liver fat content; isocaloric diets with a reduced SFA content, or increased MUFA or PUFA content, appear to reduce liver fat content (table 3).^{153 160 161} In the study by Ryan *et al*,¹⁵³ it is plausible that the greater reduction in liver fat on a Mediterranean diet (which consisted of 40% TE fat) compared with low-fat diet (30% TE fat) may in part be explained by the fiber content of the diet, rather than the fatty acid composition of the diet. The findings of Utzschneider *et al*¹⁶⁰ highlight the challenge of deciphering changes in liver fat when a notable change in total and SFA fat occur simultaneously in the context of an isocaloric diet; if fat intake is notably decreased another macronutrient must be increased.

It has previously been hypothesised that PUFA are oxidised more rapidly than SFA.¹⁶² Hypothetically, if a high PUFA diet was consumed, then this would partition fatty acids toward oxidation, rather than esterification pathways leading to a reduction in the amount of fatty acids available for esterification to TG, providing a plausible explanation for the divergence in liver fat accumulation between PUFA and SFA-enriched diets. However, this commonly accepted dogma is largely unsubstantiated and a direct demonstration in humans has yet to be undertaken. Alternatively, the lower accumulation of liver fat on the n-6 PUFA, compared with SFA diet could in part be attributable to a decrease in hepatic DNL; n-6 PUFA is a potent suppressor of lipogenic gene expression.¹⁶³ Indeed, Bjermo *et al*¹⁶¹ observed that the reduction in liver fat, which occurred to a greater extent on the n-6 PUFA diet, was strongly associated with changes in the stearoyl-coenzyme A desaturase 1 (SCD1) index in plasma cholesterol esters. Moreover, in the context of a hypercaloric diet, Rosqvist *et al*¹³³ also noted that the change in liver fat, was positively associated with a change in SCD1 index in plasma cholesterol esters. Given the reciprocal relationship between hepatic DNL and markers of hepatic fatty acid oxidation, it is likely that a decrease in DNL would lead to greater fatty acid oxidation.¹⁶⁴

Findings from *in vitro* cellular studies provide some evidence that may, in part explain the divergent responses between high-SFA and high-PUFA diets. Findings from cellular models of hepatic fatty acid metabolism have demonstrated the potent ability of SFA (eg, palmitic acid) to induce ER stress.^{165 166} It has been suggested that markers of hepatocellular ER stress are increased in patients with NAFLD.¹⁶⁷ Although, the mechanisms through which SFA may induce ER stress are yet to be fully elucidated, there is some evidence to suggest ER stress initiates an unfolded protein response that may lead to dysregulated phospholipid metabolism and the upregulation of multiple lipogenic genes (eg, SREBP-1c, FAS, and ACC).^{167 168}

Four studies have investigated the effect of isocaloric diets which differed in carbohydrate composition, on liver fat content.^{114 157–159} Two of these studies observed no difference in liver fat content following high-fructose or high-glucose diets in overweight adolescents¹⁵⁸ or adult males,¹¹⁴ respectively (table 3). Additionally, Bravo *et al*¹⁵⁷ reported no significant difference in liver fat content when comparing the effects of an isocaloric diet in which HFCS or sucrose was provided at 8, 18, or 30% total energy.

Table 3 Overview of intervention studies which have investigated the effect of isocaloric feeding on liver fat content

Ref	Design	Subjects	Intervention	Duration	Change in body mass	Change in liver fat
157	R/P	64 M+F 42.2±11.7 years 23–35 kg/m ^{2a}	StdD+HFCS or Suc at 8%, 18%, or 30% TE	10 weeks	↑ 1% ^{b*}	NSD at follow-up or between groups
114	R/P	32 M 18–50 years ^a 25.9–32.2 kg/m ^{2c}	Glu (25% TE) Fru (25% TE)	2 weeks	NSD	NSD at follow-up or between groups
158	R/P	21 M+F 11–18 years ^a BMI ≥85th percentile relative to age	Glu (99 g/day) Fru (99 g/day)	4 weeks	NSD	NSD at follow-up or between groups
159	C	8 M 42±3 years 24.4±1.6 kg/m ²	High-Fru (25% TE) Complex CHO (Fru, 5% TE)	9 days	NSD	High-Fru: 1.02% ^d (0.53%–3.07%) ^c Complex CHO: 0.70% ^d (0.39%–2.37%) ^{c#} Liver fat only assessed at the end of each dietary intervention
161	R/P	61 M+F 30–65 years ^a 25–40 kg/m ^{2a}	n-6 PUFA enriched (15% TE) SFA enriched (15% TE)	10 weeks	NSD	n-6 PUFA: ↓ 0.9% SFA: ↑ 0.3% [#]
152	R/P	13 M+F 35±2.9 years 33±1.3 kg/m ^{2a} 7 subjects completed both diet protocols	Con (fat 35% TE) Low-fat (20% TE) High-fat (55% TE)	4 weeks	NSD	Low-fat: ↓ 13.9±10.2% versus Con* High-fat: NSD versus Con NSD between low-fat and high-fat Data not listed separately for subjects who completed both diet protocols.
160	R/P	35 M+F 50–70 years ^a 25–30 kg/m ^{2a}	High-fat/high-SFA (43% and 25% TE) Low-fat/low-SFA (23% and 7% TE)	4 weeks	NSD	High-SFA: NSD Low-SFA: ↓ 19.8%±6%*
154	R/P	20 M 50–60 years ^a >27 kg/m ^{2a}	Low-fat (20% TE) Low-fat (20% TE)/high-fat (55% TE); participants switched from low-fat to high-fat after 3 weeks	6 weeks	NSD	Low-fat: ↓ 13% High-fat: ↑ 17% [#] Measures of liver fat made at 3 and 6 weeks
155	R/C	10 F 43±5 years ^e 33±4 kg/m ^{2e}	Low-fat (16% TE) High-fat (56% TE)	2 weeks	NSD	Low-fat: ↓ 20%±9%* High-fat: ↑ 35%±21%* [#]
153	R/C	12 M+F NAFLD 55±14 years 32±4.2 kg/m ²	Med diet (40% TE fat, MUFA, and n-3 PUFA enriched) Low-fat high-CHO (fat 30% TE, CHO 50% TE) diet	6 weeks	NSD	Med diet: ↓ 39%±4%* Low-fat high-CHO: ↓ 7%±2%* [#]
156	R/P	36 M+F T2DM 35–70 years ^a 25–35 kg/m ^{2a}	High-CHO (52% TE) High-CHO+Ex High-MUFA (28% TE) High-MUFA+Ex	8 weeks	NSD	High-CHO: NSD High-CHO+Ex: NSD MUFA: ↓ 29%* MUFA+Ex: ↓ 25%* MUFA gps significantly different to CHO groups. NSD between MUFA groups

Subject presented as mean±SEM unless otherwise stated.

^aInclusion criteria or estimated range within which all participants are included.

^bPooled data.

^cRange.

^dMedian.

^eSD.

*Significant effect of intervention ($p < 0.05$) according to the manuscript.

[#]Significant difference between groups ($p < 0.05$) according to the manuscript.

BMI, body mass index; C, crossover; CHO, carbohydrate; Con, control; Ex, exercise; F, Female, T2DM, type 2 diabetes mellitus; Fru, fructose; Glu, glucose; HFCS, high-fructose corn syrup; M, male; MUFA, monounsaturated fatty acids; NSD, no significant difference; P, parallel groups; PUFA, polyunsaturated fatty acids; R, randomised; Ref, reference; SFA, saturated fatty acids; StdD, standardised diet; TE, total energy.

These data suggest that in an isocaloric setting increased sugar consumption does not influence liver fat content; the accumulation of liver fat seen after hypercaloric high-fructose and high-glucose diets (table 1) are possibly related to increased substrate availability for DNL due to the ingestion of excess dietary carbohydrates.⁵⁹ In contrast to the findings

of Bravo *et al*,¹⁵⁷ Schwarz *et al*¹⁵⁹ reported that liver fat content was increased during a high-fructose diet when compared with a low-fructose diet where dietary fructose was substituted for 'complex carbohydrates' (ie, foods such as cereals, bread, pasta, rice, and potatoes) (table 3). The observed increase in liver fat content following the

high-fructose diet was associated with increased post-prandial hepatic DNL (measured by isotopic tracers), and reduced whole-body fatty acid oxidation.¹⁵⁹

There is limited data investigating the influence of specific nutrients (ie, high-carbohydrate as compared with high-fat) on liver fat accumulation. Bozetto *et al*¹⁵⁶ reported that when compared with a high-carbohydrate diet, an isoenergetic high-fat MUFA-enriched diet reduced liver fat content in patients with T2DM. A potential mechanism underpinning this MUFA-induced reduction in liver fat relates to the observation that MUFA-rich diets appear to increase the activity of LPL in adipose tissue,¹⁶⁹ which would lead to a decreased flux of dietary fatty acids toward the liver as more would be taken up into adipose tissue.

In summary, it would appear that even in the context of an isocaloric diet differences in nutrient intakes may modulate liver fat content. Based on the available evidence, it would seem that altering the macronutrient composition to have a higher fat (>50% TE) intake may lead to an increase in liver fat content. However, this observation appears to be attributable to increases in SFA, whereas increasing the quantity of PUFA or MUFA in the diet may reduce liver fat content. Findings from some, but not all studies assessing dietary carbohydrate suggest that diets which are high in free sugars have no influence on liver fat content. What remains unclear is if changes in macronutrient content alter intrahepatic fatty acid metabolism, even if liver fat content is not overtly altered.

CONCLUSIONS

The influence of specific dietary macronutrients on liver fat content and metabolism are not fully elucidated. Data from observational studies are conflicting, which are, at least in part, attributable to differences in cultural and environmental characteristics between and within the populations, methodological challenges inherent to assessing dietary intakes, and how and when liver fat content was assessed relative to dietary intakes. To determine the effect of nutritional intake on the development of NAFLD effectively, ideally, dietary intakes would be measured at baseline, and then repeated sequentially over time alongside measures of liver fat content in large cohorts of individuals; however, given the resources and time required this kind of study would be both financially and logistically challenging to undertake.

Comparing data from intervention studies is challenging, not least as there is marked heterogeneity in the phenotype of participants studied, and the duration and composition of the experimental diets varies across studies. It is challenging to determine what the most appropriate experimental design for a control group would be, as a change in any dietary component ultimately results in changing the composition of the diet in its entirety. A further challenge is blinding participants to their experimental diet, this can be in part achieved by the provision of some or all foods.

The evidence from the intervention studies highlight the importance of total calorie intake, independent of nutrient content, as a key regulator of liver fat content. Findings consistently show (to varying degrees) that regardless of the population or sex studied, consumption of a hypercaloric diet increased liver fat content and conversely consumption of a hypocaloric diet decreased liver fat content. It is likely

these changes are mediated primarily through a change in body weight and/or substrate availability. Findings from isocaloric feeding studies suggest that consumption of relatively high-fat diets will increase liver fat content although the fatty acid composition of the diet may influence this response. The effect of dietary sugars on liver fat accumulation and metabolism requires some clarity with only a few studies investigating the influence of dietary sugars in the form in which they are typically consumed (ie, sucrose and HFCS) rather than glucose and/or fructose in isolation. Future studies around this would ideally be undertaken as a randomised controlled trial.

Overall, there is a notable sparseness in the investigation of the mechanisms underpinning diet-induced changes in liver fat content. It is plausible that these mechanisms differ in a macronutrient-dependent manner. Addressing this knowledge gap in future research is vital in order to develop effective preventative and treatment strategies for NAFLD.

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REFERENCES

- Ng M, Fleming T, Robinson M, *et al*. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;384:766–81.
- Steppan CM, Bailey ST, Bhat S, *et al*. The hormone resistin links obesity to diabetes. *Nature* 2001;409:307–12.
- Poirier P, Eckel RH. Obesity and cardiovascular disease. *Curr Atheroscler Rep* 2002;4:448–53.
- Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010;51:679–89.
- Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ* 2011;343:d3897.
- Chalasani N, Younossi Z, Lavine JE, *et al*. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol* 2012;107:811–26.
- Moore JB. Non-alcoholic fatty liver disease: the hepatic consequence of obesity and the metabolic syndrome. *Proc Nutr Soc* 2010;69:211–20.
- Nascimbeni F, Pais R, Bellentani S, *et al*. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013;59:859–71.
- Byrne CD. Dorothy Hodgkin Lecture 2012: non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. *Diabet Med* 2012;29:1098–107.
- Ekstedt M, Franzén LE, Mathiesen UL, *et al*. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006;44:865–73.
- Musso G, Gambino R, Cassader M, *et al*. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011;43:617–49.
- Yki-Järvinen H. Liver fat in the pathogenesis of insulin resistance and type 2 diabetes. *Dig Dis* 2010;28:203–9.
- Brunt EM. Histopathology of non-alcoholic fatty liver disease. *Clin Liver Dis* 2009;13:533–44.
- Fabbrini E, Conte C, Magkos F. Methods for assessing intrahepatic fat content and steatosis. *Curr Opin Clin Nutr Metab Care* 2009;12:474–81.

- 15 Guido M, Ruge M. Liver biopsy sampling in chronic viral hepatitis. *Semin Liver Dis* 2004;24:89–97.
- 16 Bhatia LS, Curzen NP, Calder PC, et al. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J* 2012;33:1190–200.
- 17 Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. *Frontline Gastroenterol* 2014;5:277–86.
- 18 Green CJ, Hodson L. The influence of dietary fat on liver fat accumulation. *Nutrients* 2014;6:5018–33.
- 19 Reeder SB, Cruite I, Hamilton G, et al. Quantitative assessment of liver fat with magnetic resonance imaging and spectroscopy. *J Magn Reson Imaging* 2011;34:729–49.
- 20 Chang Y, Jung HS, Cho J, et al. Metabolically healthy obesity and the development of nonalcoholic fatty liver disease. *Am J Gastroenterol* 2016;111:1133–40.
- 21 Patell R, Dosi R, Joshi H, et al. Non-alcoholic fatty liver disease (NAFLD) in obesity. *J Clin Diagn Res* 2014;8:62–6.
- 22 Park BJ, Kim YJ, Kim DH, et al. Visceral adipose tissue area is an independent risk factor for hepatic steatosis. *J Gastroenterol Hepatol* 2008;23:900–7.
- 23 van der Poorten D, Milner KL, Hui J, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008;48:449–57.
- 24 Arioglu E, Rother KI, Reitman ML, et al. Lipotrophy syndromes: when 'too little fat' is a clinical problem. *Pediatr Diabetes* 2000;1:155–68.
- 25 Huang-Doran I, Sleigh A, Rochford JJ, et al. Lipodystrophy: metabolic insights from a rare disorder. *J Endocrinol* 2010;207:245–55.
- 26 Rietman A, Schwarz J, Tomé D, et al. High dietary protein intake, reducing or eliciting insulin resistance? *Eur J Clin Nutr* 2014;68:973–9.
- 27 Wattacheril J, Sanyal AJ. Lean NAFLD: an underrecognized outlier. *Curr Hepatol Rep* 2016;15:134–9.
- 28 Byrne CD. Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. *Proc Nutr Soc* 2013;72:412–9.
- 29 Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10:645–55.
- 30 Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TM6C4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology* 2016;150:1219–30.
- 31 Krawczyk M, Rau M, Schattenberg JM, et al. Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738 variants on NAFLD severity: a multicenter biopsy-based study. *J Lipid Res* 2017;58:247–55.
- 32 Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352–6.
- 33 Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461–5.
- 34 Luukkonen PK, Zhou Y, Nidhina Haridas PA, et al. Impaired hepatic lipid synthesis from polyunsaturated fatty acids in TM6SF2 E167K variant carriers with NAFLD. *J Hepatol* 2017;67:128–36.
- 35 He S, McPhaul C, Li JZ, et al. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010;285:6706–15.
- 36 Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7:e1001324.
- 37 Santoro N, Savoye M, Kim G, et al. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* 2012;7:e37827.
- 38 Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016;65:1038–48.
- 39 Ludwig J, Viggiano TR, McGill DB, et al. Nonalcoholic steatohepatitis: mayo clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980;55:434–8.
- 40 Powell EE, Cooksley WG, Hanson R, et al. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990;11:74–80.
- 41 Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387–95.
- 42 Clark MA, Rakowski W, Bonacore LB. Repeat mammography: prevalence estimates and considerations for assessment. *Ann Behav Med* 2003;26:201–11.
- 43 Ioannou GN, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999–2002. *Am J Gastroenterol* 2006;101:76–82.
- 44 Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2003;124:71–9.
- 45 Schneider AL, Lazo M, Selvin E, et al. Racial differences in nonalcoholic fatty liver disease in the U.S. population. *Obesity* 2014;22:292–9.
- 46 Pramfalk C, Pavlides M, Banerjee R, et al. Sex-specific differences in hepatic fat oxidation and synthesis may explain the higher propensity for NAFLD in men. *J Clin Endocrinol Metab* 2015;100:4425–33.
- 47 Pan JJ, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014;6:274–83.
- 48 Angulo P. Obesity and nonalcoholic fatty liver disease. *Nutr Rev* 2007;65:57–63.
- 49 Dyson J, Day C. Treatment of non-alcoholic fatty liver disease. *Dig Dis* 2014;32:597–604.
- 50 Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol* 2012;56:255–66.
- 51 Hallsworth K, Avery L, Trenell MI. Targeting lifestyle behavior change in adults with NAFLD during a 20-min consultation: summary of the dietary and exercise literature. *Curr Gastroenterol Rep* 2016;18:11.
- 52 Glatz JF, Luiken JJ. From fat to FAT (CD36/SR-B2): understanding the regulation of cellular fatty acid uptake. *Biochimie* 2017;136:21–6.
- 53 Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol* 2013;48:434–41.
- 54 Hodson L, Frayn KN. Hepatic fatty acid partitioning. *Curr Opin Lipidol* 2011;22:216–24.
- 55 Gray LR, Tompkins SC, Taylor EB. Regulation of pyruvate metabolism and human disease. *Cell Mol Life Sci* 2014;71:2577–604.
- 56 Strable MS, Ntambi JM. Genetic control of de novo lipogenesis: role in diet-induced obesity. *Crit Rev Biochem Mol Biol* 2010;45:199–214.
- 57 Leavens KF, Birnbaum MJ. Insulin signaling to hepatic lipid metabolism in health and disease. *Crit Rev Biochem Mol Biol* 2011;46:200–15.
- 58 Hellerstein MK, Schwarz JM, Neese RA. Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr* 1996;16:523–57.
- 59 Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. *Biol Rev Camb Philos Soc* 2016;91:452–68.
- 60 Xiao C, Lewis GF. Regulation of chylomicron production in humans. *Biochim Biophys Acta* 2012;1821:736–46.
- 61 Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005;115:1343–51.
- 62 Nestel PJ, Havel RJ, Bezman A. Sites of initial removal of chylomicron triglyceride fatty acids from the blood. *J Clin Invest* 1962;41:1915–21.
- 63 Babin PJ, Gibbons GF. The evolution of plasma cholesterol: direct utility or a 'spandrel' of hepatic lipid metabolism? *Prog Lipid Res* 2009;48:73–91.
- 64 Havel RJ, Hamilton RL. Hepatic catabolism of remnant lipoproteins: where the action is. *Arterioscler Thromb Vasc Biol* 2004;24:213–5.
- 65 Coppack SW, Fisher RM, Gibbons GF, et al. Postprandial substrate deposition in human forearm and adipose tissues in vivo. *Clin Sci* 1990;79:339–48.
- 66 Frayn KN, Coppack SW, Fielding BA, et al. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. *Adv Enzyme Regul* 1995;35:163–78.
- 67 Hodson L, Fielding BA. Trafficking and partitioning of fatty acids: the transition from fasted to fed state. *Clin Lipidol* 2010;5:131–44.
- 68 Lindeboom L, Nabuurs CI, Hesselink MK, et al. Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein. *Am J Clin Nutr* 2015;101:65–71.
- 69 Ravikumar B, Carey PE, Snaar JE, et al. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. *Am J Physiol Endocrinol Metab* 2005;288:E789–97.
- 70 Takeuchi K, Reue K. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *Am J Physiol Endocrinol Metab* 2009;296:E1195–E1209.
- 71 Csaki LS, Reue K. Lipins: multifunctional lipid metabolism proteins. *Annu Rev Nutr* 2010;30:257–72.
- 72 Blasiole DA, Davis RA, Attie AD. The physiological and molecular regulation of lipoprotein assembly and secretion. *Mol Biosyst* 2007;3:608–19.
- 73 Gibbons GF, Wiggins D, Brown AM, et al. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans* 2004;32:59–64.
- 74 Adiels M, Westerbacka J, Soro-Paavonen A, et al. Acute suppression of VLDL1 secretion rate by insulin is associated with hepatic fat content and insulin resistance. *Diabetologia* 2007;50:2356–65.
- 75 Malmström R, Packard CJ, Caslake M, et al. Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes* 1998;47:779–87.

- 76 Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid β -oxidation. *J Inherit Metab Dis* 2010;33:469–77.
- 77 McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* 1980;49:395–420.
- 78 Rui L. Energy metabolism in the liver. *Compr Physiol* 2014;4:177–97.
- 79 Longuet C, Sinclair EM, Maida A, et al. The glucagon receptor is required for the adaptive metabolic response to fasting. *Cell Metab* 2008;8:359–71.
- 80 Sidossis LS, Stuart CA, Shulman GI, et al. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. *J Clin Invest* 1996;98:2244–50.
- 81 Timlin MT, Barrows BR, Parks EJ. Increased dietary substrate delivery alters hepatic fatty acid recycling in healthy men. *Diabetes* 2005;54:2694–701.
- 82 Timlin MT, Parks EJ. Temporal pattern of de novo lipogenesis in the postprandial state in healthy men. *Am J Clin Nutr* 2005;81:35–42.
- 83 Saggerson D. Malonyl-CoA, a key signaling molecule in mammalian cells. *Annu Rev Nutr* 2008;28:253–72.
- 84 Diraison F, Beylot M. Role of human liver lipogenesis and reesterification in triglycerides secretion and in FFA reesterification. *Am J Physiol* 1998;274:E321–7.
- 85 Sidossis LS, Mittendorfer B, Walsler E, et al. Hyperglycemia-induced inhibition of splanchnic fatty acid oxidation increases hepatic triacylglycerol secretion. *Am J Physiol* 1998;275:E798–805.
- 86 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221–31.
- 87 Downman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010;103:71–83.
- 88 Lavoie JM, Gauthier MS. Regulation of fat metabolism in the liver: link to non-alcoholic hepatic steatosis and impact of physical exercise. *Cell Mol Life Sci* 2006;63:1393–409.
- 89 Mittendorfer B, Magkos F, Fabbrini E, et al. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity* 2009;17:1872–7.
- 90 Pardina E, Baena-Fustegueros JA, Llamas R, et al. Lipoprotein lipase expression in livers of morbidly obese patients could be responsible for liver steatosis. *Obes Surg* 2009;19:608–16.
- 91 Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes* 2011;60:2441–9.
- 92 Bugianesi E, Gastaldelli A, Vanni E, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005;48:634–42.
- 93 Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87:3023–8.
- 94 Lambert JE, Ramos-Roman MA, Browning JD, et al. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* 2014;146:726–35.
- 95 Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab* 2008;7:95–6.
- 96 Diraison F, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab* 2003;29:478–85.
- 97 McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002;51:7–18.
- 98 Fujita K, Nozaki Y, Wada K, et al. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology* 2009;50:772–80.
- 99 Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab* 2015;21:739–46.
- 100 Sunny NE, Parks EJ, Browning JD, et al. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab* 2011;14:804–10.
- 101 McQuaid SE, Hodson L, Neville MJ, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes* 2011;60:47–55.
- 102 Yki-Järvinen H. Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. *Nutrients* 2015;7:9127–38.
- 103 Aqel B, DiBaise JK. Role of the gut microbiome in nonalcoholic fatty liver disease. *Nutr Clin Pract* 2015;30:780–6.
- 104 Assy N, Nasser G, Kamayse I, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol* 2008;22:811–6.
- 105 Jia Q, Xia Y, Zhang Q, et al. Dietary patterns are associated with prevalence of fatty liver disease in adults. *Eur J Clin Nutr* 2015;69:914–21.
- 106 Oddy WH, Herbison CE, Jacoby P, et al. The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. *Am J Gastroenterol* 2013;108:778–85.
- 107 Cortez-Pinto H, Jesus L, Barros H, et al. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin Nutr* 2006;25:816–23.
- 108 Cheng Y, Zhang K, Chen Y, et al. Associations between dietary nutrient intakes and hepatic lipid contents in NAFLD patients quantified by ^1H -MRS and dual-echo MRI. *Nutrients* 2016;8:527.
- 109 Volynets V, Küper MA, Strahl S, et al. Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Dis Sci* 2012;57:1932–41.
- 110 Dhurandhar NV, Schoeller D, Brown AW, et al. Energy balance measurement: when something is not better than nothing. *Int J Obes* 2015;39:1109–13.
- 111 Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* 2008;47:348–80.
- 112 Cook CM, Alvig AL, Liu YQ, et al. The natural ^{13}C abundance of plasma glucose is a useful biomarker of recent dietary caloric sweetener intake. *J Nutr* 2010;140:333–7.
- 113 Hedrick VE, Davy BM, Wilburn GA, et al. Evaluation of a novel biomarker of added sugar intake ($\delta^{13}\text{C}$) compared with self-reported added sugar intake and the Healthy Eating Index-2010 in a community-based, rural U.S. sample. *Public Health Nutr* 2016;19:429–36.
- 114 Johnston RD, Stephenson MC, Crossland H, et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. *Gastroenterology* 2013;145:1016–25.
- 115 Lê KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. *Am J Clin Nutr* 2009;89:1760–5.
- 116 Ngo Sock ET, Lê KA, Ith M, et al. Effects of a short-term overfeeding with fructose or glucose in healthy young males. *Br J Nutr* 2010;103:939–43.
- 117 Sobrecases H, Lê KA, Bortolotti M, et al. Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. *Diabetes Metab* 2010;36:244–6.
- 118 Softic S, Cohen DE, Kahn CR. Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig Dis Sci* 2016;61:1282–93.
- 119 Hudgins LC, Parker TS, Levine DM, et al. A dual sugar challenge test for lipogenic sensitivity to dietary fructose. *J Clin Endocrinol Metab* 2011;96:861–8.
- 120 Lecoultré V, Egli L, Carrel G, et al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* 2013;21:782–5.
- 121 White JS. Challenging the fructose hypothesis: new perspectives on fructose consumption and metabolism. *Adv Nutr* 2013;4:246–56.
- 122 Pollock NK, Bundy V, Kanto W, et al. Greater fructose consumption is associated with cardiometabolic risk markers and visceral adiposity in adolescents. *J Nutr* 2012;142:251–7.
- 123 Vos MB, Kimmons JE, Gillespie C, et al. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *Medscape J Med* 2008;10:160.
- 124 Lê KA, Faeh D, Stettler R, et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. *Am J Clin Nutr* 2006;84:1374–9.
- 125 Silbernagel G, Machann J, Unmuth S, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *Br J Nutr* 2011;106:79–86.
- 126 Sun SZ, Anderson GH, Flickinger BD, et al. Fructose and non-fructose sugar intakes in the US population and their associations with indicators of metabolic syndrome. *Food Chem Toxicol* 2011;49:2875–82.
- 127 Frayn KN, Kingman SM. Dietary sugars and lipid metabolism in humans. *Am J Clin Nutr* 1995;62:250S–61.
- 128 Maersk M, Belza A, Stødkilde-Jørgensen H, et al. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomised intervention study. *Am J Clin Nutr* 2012;95:283–9.
- 129 Sevastianova K, Santos A, Kotroinen A, et al. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *Am J Clin Nutr* 2012;96:727–34.
- 130 Aarsland A, Wolfe RR. Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. *J Lipid Res* 1998;39:1280–6.
- 131 Mancina RM, Matikainen N, Maglio C, et al. Paradoxical dissociation between hepatic fat content and de novo lipogenesis due to PNPLA3 sequence variant. *J Clin Endocrinol Metab* 2015;100:E821–5.
- 132 van der Meer RW, Hammer S, Lamb HJ, et al. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab* 2008;93:2702–8.

- 133 Rosqvist F, Iggman D, Kullberg J, *et al.* Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* 2014;63:2356–68.
- 134 Bortolotti M, Kreis R, Debad C, *et al.* High protein intake reduces intrahepatocellular lipid deposition in humans. *Am J Clin Nutr* 2009;90:1002–10.
- 135 Theytaz F, Noguchi Y, Egli L, *et al.* Effects of supplementation with essential amino acids on intrahepatic lipid concentrations during fructose overfeeding in humans. *Am J Clin Nutr* 2012;96:1008–16.
- 136 Fukagawa NK, Bandini LG, Lim PH, *et al.* Protein-induced changes in energy expenditure in young and old individuals. *Am J Physiol* 1991;260:E345–52.
- 137 Hochstenbach-Waelen A, Veldhorst MA, Nieuwenhuizen AG, *et al.* Comparison of 2 diets with either 25% or 10% of energy as casein on energy expenditure, substrate balance, and appetite profile. *Am J Clin Nutr* 2009;89:831–8.
- 138 Veldhorst MA, Westerterp-Plantenga MS, Westerterp KR. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am J Clin Nutr* 2009;90:519–26.
- 139 Koopman KE, Caan MW, Nederveen AJ, *et al.* Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomised controlled trial. *Hepatology* 2014;60:545–53.
- 140 Munsters MJ, Saris WH. Effects of meal frequency on metabolic profiles and substrate partitioning in lean healthy males. *PLoS One* 2012;7:e38632.
- 141 Solomon TP, Chambers ES, Jeukendrup AE, *et al.* The effect of feeding frequency on insulin and ghrelin responses in human subjects. *Br J Nutr* 2008;100:810–9.
- 142 Bergholm R, Sevastianova K, Santos A, *et al.* CB(1) blockade-induced weight loss over 48 weeks decreases liver fat in proportion to weight loss in humans. *Int J Obes* 2013;37:699–703.
- 143 Haufe S, Engeli S, Kast P, *et al.* Randomised comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology* 2011;53:1504–14.
- 144 Otten J, Mellberg C, Ryberg M, *et al.* Strong and persistent effect on liver fat with a Paleolithic diet during a two-year intervention. *Int J Obes* 2016;40:747–53.
- 145 Tiikkainen M, Bergholm R, Vehkavaara S, *et al.* Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes* 2003;52:701–7.
- 146 Lewis MC, Phillips ML, Slavotinek JP, *et al.* Change in liver size and fat content after treatment with Optifast very low calorie diet. *Obes Surg* 2006;16:697–701.
- 147 Steven S, Hollingsworth KG, Al-Mrabeh A, *et al.* Very low-calorie diet and 6 months of weight stability in type 2 diabetes: pathophysiological changes in responders and nonresponders. *Diabetes Care* 2016;39:808–15.
- 148 Volynets V, Machann J, Küper MA, *et al.* A moderate weight reduction through dietary intervention decreases hepatic fat content in patients with non-alcoholic fatty liver disease (NAFLD): a pilot study. *Eur J Nutr* 2013;52:527–35.
- 149 Browning JD, Baker JA, Rogers T, *et al.* Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. *Am J Clin Nutr* 2011;93:1048–52.
- 150 Kirk E, Reeds DN, Finck BN, *et al.* Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology* 2009;136:1552–60.
- 151 Nielsen S, Karpe F. Determinants of VLDL-triglycerides production. *Curr Opin Lipidol* 2012;23:321–6.
- 152 Marina A, von Frankenberg AD, Suvag S, *et al.* Effects of dietary fat and saturated fat content on liver fat and markers of oxidative stress in overweight/obese men and women under weight-stable conditions. *Nutrients* 2014;6:4678–90.
- 153 Ryan MC, Itsiopoulos C, Thodis T, *et al.* The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol* 2013;59:138–43.
- 154 van Herpen NA, Schrauwen-Hinderling VB, Schaart G, *et al.* Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. *J Clin Endocrinol Metab* 2011;96:E691–5.
- 155 Westerbacka J, Lammi K, Häkkinen AM, *et al.* Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005;90:2804–9.
- 156 Bozzetto L, Prinster A, Annuzzi G, *et al.* Liver fat is reduced by an isoenergetic MUFA diet in a controlled randomised study in type 2 diabetic patients. *Diabetes Care* 2012;35:1429–35.
- 157 Bravo S, Lowndes J, Sinnett S, *et al.* Consumption of sucrose and high-fructose corn syrup does not increase liver fat or ectopic fat deposition in muscles. *Appl Physiol Nutr Metab* 2013;38:681–8.
- 158 Jin R, Welsh JA, Le NA, *et al.* Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. *Nutrients* 2014;6:3187–201.
- 159 Schwarz JM, Noworolski SM, Wen MJ, *et al.* Effect of a high-fructose weight-maintaining diet on lipogenesis and liver fat. *J Clin Endocrinol Metab* 2015;100:2434–42.
- 160 Utzschneider KM, Bayer-Carter JL, Arbuckle MD, *et al.* Beneficial effect of a weight-stable, low-fat/low-saturated fat/low-glycaemic index diet to reduce liver fat in older subjects. *Br J Nutr* 2013;109:1096–104.
- 161 Bjermo H, Iggman D, Kullberg J, *et al.* Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomised controlled trial. *Am J Clin Nutr* 2012;95:1003–12.
- 162 DeLany JP, Windhauser MM, Champagne CM, *et al.* Differential oxidation of individual dietary fatty acids in humans. *Am J Clin Nutr* 2000;72:905–11.
- 163 Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? *Prog Lipid Res* 2013;52:15–42.
- 164 Pramfalk C, Pavlides M, Banerjee R, *et al.* Fasting plasma insulin concentrations are associated with changes in hepatic fatty acid synthesis and partitioning prior to changes in liver fat content in healthy adults. *Diabetes* 2016;65:1858–67.
- 165 Cao J, Dai DL, Yao L, *et al.* Saturated fatty acid induction of endoplasmic reticulum stress and apoptosis in human liver cells via the PERK/ATF4/CHOP signaling pathway. *Mol Cell Biochem* 2012;364:115–29.
- 166 Park EJ, Lee AY, Park S, *et al.* Multiple pathways are involved in palmitic acid-induced toxicity. *Food Chem Toxicol* 2014;67:26–34.
- 167 Leamy AK, Egnatchik RA, Young JD. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. *Prog Lipid Res* 2013;52:165–74.
- 168 Han J, Kaufman RJ. The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* 2016;57:1329–38.
- 169 Rivellese AA, Giacco R, Annuzzi G, *et al.* Effects of monounsaturated vs. saturated fat on postprandial lipemia and adipose tissue lipases in type 2 diabetes. *Clin Nutr* 2008;27:133–41.