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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 rate[1]; 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 NAFLD[9]; 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/m$^2$) are also diagnosed with NAFLD[27]; 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 TMS6F2 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 fat[48] 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 palmitate[58]; 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 $^{13}C$ tracer and using $^{13}C$-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 $^{13}C$-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 $^{13}C$-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 (PPARα) activity,[79] while insulin and glucose downregulate β-oxidation by suppressing adipose tissue TG lipolysis, reducing the delivery of NEFA to the liver[80] 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-Järvinen[102] 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 NALFD.[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 females[105]; 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/m$^2$ 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 potently 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 1990[121]; 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 allele[131]; 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<br>24.7±1.3 years<br>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)<br>19–25 kg/m$^{2a}$ | StdD versus StdD+Fru (3.5 g/kg/FFM) | 35% | 7 days | T2DM offspring: ↑ 1.0 kg$^{b}$*<br>Con: ↑ 0.6 kg$^{b*}$<br>NSD between groups | T2DM off: ↑ 79%*<br>Controls: ↑ 76%*<br>NSD between groups |
| 114 | R/P | 32 M<br>18-50 years$^{a}$<br>25.9–32.2 kg/m$^{2c}$ | StdD+Glu (25%TE) versus Fru (25%TE) | 25% | 2 weeks | Fru: ↑ 1.0±1.4 kg*<br>Glu: ↑ 0.6±1.0 kg*<br>NSD between groups | Fru: ↑ 24%$^{b}$*<br>Glu: ↑ 26%$^{b}$*<br>NSD between groups |
| 116 | R/C | 11 M<br>24.6±0.6 years<br>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$^{b}$*<br>Glu: ↑ 1 kg$^{b}$* | Fru: ↑ 52%±13%*<br>Glu: ↑ 58%±23%<br>NSD between groups |
| 125 | R/P | 20 M+F<br>30.5±2.0 years<br>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*<br>Fru: NSD | NSD in either group |
| 128 | R/P | 47 M+F<br>20–50 years$^{a}$<br>26–40 kg/m$^{2a}$ | StdD+1 L/day of cola, semi-skimmed milk, diet cola, or water | Cola: 430 kcal/day<br>Milk: 450 kcal/day<br>Diet-cola: N/A<br>Water: N/A | 6 months | NSD between groups<br>No comparisons made relative to baseline | Cola: ↑132%–143% compared with other gps.*<br>NSD between milk, diet-cola or water |
| 129 | SG | 16 M+F<br>54 years$^{d}$ (40–59 years)$^{e}$<br>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<br>20-38 years$^{a}$<br>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<br>PUFA: ↑ 1.6±0.85 kg.<br>NSD between groups | SFA: ↑ 58%.$^{b}$<br>PUFA: ↑53%$^{b\#}$ |
| 132 | SG | 15 M<br>25±6.6 years$^{f}$<br>23.4±2.5 kg/m$^{2f}$ | Habitual diet+280 g/day fat (800 mL cream) | 2632 kcal/day | 3 days | NSD in BMI<br>Body mass not presented | ↑ 112%*$^{b}$ |
| 134 | R/C | 10 M<br>24±1 years<br>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%$^{b}$<br>HF+high-pro: 49%$^{b}$ | 4 days | HF: NSD<br>HF+high-pro: ↑ 1.1 kg$^{a}$* | HF: ↑ 90%*<br>HF+high-pro: ↑ 68%$^{b*\#}$ |
| 139 | R/P | 36 M<br>22 years (19–27)$^{c}$<br>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$^{g}$*<br>NSD between groups | NSD in gps consuming beverages with meals<br>HFHS ↑ 45%* and HS, ↑ 110%*when consumed between meals<br>NSD in 'between meals' groups |
| 117 | P | 30 M<br>23.9±0.4 years<br>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%<br>SFA: 30%<br>Fru+SFA: 65% | 7 days | ↑ 0.3±0.1 kg$^{g}$<br>NSD between groups | Fru: ↑ 16%*<br>SFA: ↑ 86%*<br>Fru+SFA: ↑ 133%$^{*\#}$ |
| 135 | R/C | 9 M<br>23.3±0.9 years<br>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%$^{b}$<br>High-Fru+EAA: 38%$^{b}$ | 6 days | NSD | High-Fru+placebo: ↑ 115%*$^{b}$<br>High-Fru+EAA: ↑ 81%$^{b*\#}$ |

Subject data presented as mean±SEM unless otherwise stated.
[a]Inclusion criteria or estimated range within which all participants are included.
[b]Estimated from table/graph.
[c]Range.
[d]Median.
[e]IQR.
[f]SD.
[g]Pooled 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/m$^2$, 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/m$^2$, 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 isolation[148] 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<br>45.5 years[a]<br>(34.5–51.5 years)[b]<br>31.1 kg/m$^{2a}$<br>(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<br>43.6±2.5 years<br>36.5±0.8 kg/m$^2$ | High-CHO (>180 g/day) versus low-CHO (<60 g/day) | ↓1000 kcal/day | ~6 weeks<br>Followed by 4 week weight maintenance | ↓ 7.5%±0.4%[c]*<br>NSD between groups | Low-CHO:<br>↓38.0%±4.5%*<br>High-CHO:<br>↓44.5%±13.5%*<br>NSD between groups |
| 149 | P | 18 M+F NAFLD<br>45±12 years[d]<br>35±7 kg/m$^{2d}$ | Low-CHO (<20 g/day) diet versus low kcal (1200–1500 kcal/day) | Low-CHO: 1553±517 kcal/day<br>Low kcal: 1325±180 kcal/day | 2 weeks | Low-CHO: ↓ 4.6±1.5 kg*<br>Low-kcal: ↓ 4.0±1.5 kg*<br>NSD between groups | Low-CHO: ↓ 55%±14%*<br>Low-kcal: ↓ 28%±23%*[#] |
| 143 | R/P | 102 M+F<br>30–60 years[e]<br>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*<br>Low-fat: ↓ 6.5±0.7 kg*<br>NSD between groups | Low-CHO: ↓ 47%*<br>Low-fat: ↓ 42%*<br>NSD between groups |
| 144 | R/P | 70 F<br>50-70 years[e]<br>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<br>2 years | 6 months:<br>Paleo: ↓ 9 kg[f]*<br>Low-fat: ↓ 4 kg[f]*[#]<br>2 years:<br>Paleo: ↓ 8 kg[f]*<br>Low-fat:↓ 5 kg[f]*<br>NSD between gps at 2 years | 6 months:<br>Paleo: ↓ 64%*<br>Low-fat: ↓43%*[#]<br>2 years:<br>Paleo: ↓50%*<br>Low-fat: ↓49%*<br>NSD between gps at 2 years |
| 146 | SG | 18 M+F<br>50 years[a] (34–57 years)[g]<br>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+FT2DM<br>25–80 years[e]<br>27–45 kg/m$^{2e}$ | VLCD<br>Participants separated based on FPG at follow-up<br>Responders: <7 mmol/L<br>Non-responders: >7 mmol/L | 624–700 kcal/day | 8 weeks<br>Followed by 6 months weight maintenance | ↓ 14%[f]* | Responders: ↓ 83%*<br>Non-responders: ↓ 73%*<br>NSD between groups |
| 129 | SG | 16 M+F<br>54 years[a] (40–59 years)[g]<br>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)<br>20–50 years[e]<br>28–35 kg/m$^{2e}$ | Targeted weight loss (balanced diet). Participants separated based on baseline liver fat;<br>High: >5%<br>Low: <5% | ↓ 600–800 kcal/day | 3–6 months (dependent on time to ↓ BM by 8%) | High: ↓ 7.4%±0.2%*<br>Low: ↓ 7.7%±0.3%*<br>NSD between groups. | High: ↓ 49%*<br>Low: ↓ 39%*[#] |
| 142 | R/P | 37 M+F<br>35–70 years[e]<br>28–35 kg/m$^{2e}$ | Hypocaloric+placebo versus hypocaloric+rimonabant (20 mg) | ↓ 600–800kcal/day | 11 months | Placebo: ↓ 1.7±1.0 kg<br>Rimonabant:↓ 8.5±1.4 kg* | Placebo: ↓ 28%<br>Rimonabant: ↓ 69%*[#] |

Subject data presented as mean±SEM unless otherwise stated.
[a]Median.
[b]Range.
[c]Pooled data.
[d]SD.
[e]Inclusion criteria or estimated range within which all participants are included.
[f]Estimated from table/graph.
[g]IQR.
*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/m$^2$ 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 adolescents[158] 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<br>42.2±11.7 years<br>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<br>18-50 years[a]<br>25.9–32.2 kg/m$^{2c}$ | Glu (25% TE)<br>Fru (25% TE) | 2 weeks | NSD | NSD at follow-up or between groups |
| 158 | R/P | 21 M+F<br>11–18 years[a]<br>BMI ≥85th percentile relative to age | Glu (99 g/day)<br>Fru (99 g/day) | 4 weeks | NSD | NSD at follow-up or between groups |
| 159 | C | 8 M<br>42±3 years<br>24.4±1.6 kg/m$^2$ | High-Fru (25% TE)<br>Complex CHO (Fru, 5% TE) | 9 days | NSD | High-Fru: 1.02%[d] (0.53%–3.07%)[c]<br>Complex CHO: 0.70%[d](0.39%–2.37%)[c#]<br>Liver fat only assessed at the end of each dietary intervention |
| 161 | R/P | 61 M+F<br>30–65 years[a]<br>25–40 kg/m$^{2a}$ | n-6 PUFA enriched (15% TE) SFA enriched (15% TE) | 10 weeks | NSD | n-6 PUFA:↓ 0.9%<br>SFA:↑ 0.3%[#] |
| 152 | R/P | 13 M+F<br>35±2.9 years<br>33±1.3 kg/m$^{2a}$<br>7 subjects completed both diet protocols | Con (fat 35% TE)<br>Low-fat (20% TE)<br>High-fat (55% TE) | 4 weeks | NSD | Low-fat:↓ 13.9±10.2% versus Con*<br>High-fat: NSD versus Con<br>NSD between low-fat and high-fat<br>Data not listed separately for subjects who completed both diet protocols. |
| 160 | R/P | 35 M+F<br>50-70 years[a]<br>25–30 kg/m$^{2a}$ | High-fat/high-SFA (43% and 25% TE)<br>Low-fat/low-SFA (23% and 7% TE) | 4 weeks | NSD | High-SFA: NSD<br>Low-SFA: ↓ 19.8%±6%* |
| 154 | R/P | 20 M<br>50–60 years[a]<br>>27 kg/m$^{2a}$ | Low-fat (20%TE)<br>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%<br>High-fat: ↑ 17%[#]<br>Measures of liver fat made at 3 and 6 weeks |
| 155 | R/C | 10 F<br>43±5 years[e]<br>33±4 kg/m$^{2e}$ | Low-fat (16%TE)<br>High-fat (56% TE) | 2 weeks | NSD | Low-fat: ↓ 20%±9%*<br>High-fat: ↑ 35%±21%*[#] |
| 153 | R/C | 12 M+F NAFLD<br>55±14 years<br>32±4.2 kg/m$^2$ | Med diet (40% TE fat, MUFA, and n-3 PUFA enriched)<br>Low-fat high-CHO (fat 30% TE, CHO 50% TE) diet | 6 weeks | NSD | Med diet: ↓ 39%±4%*<br>Low-fat high-CHO: ↓ 7%±2%*[#] |
| 156 | R/P | 36 M+FT2DM<br>35-70 years[a]<br>25–35 kg/m$^{2a}$ | High-CHO (52% TE)<br>High-CHO+Ex<br>High-MUFA (28% TE)<br>High-MUFA+Ex | 8 weeks | NSD | High-CHO: NSD<br>High-CHO+Ex: NSD<br>MUFA:↓ 29%*<br>MUFA+Ex: ↓ 25%*<br>MUFA gps significantly different to CHO groups.<br>NSD between MUFA groups |

Subject data presented as mean±SEM unless otherwise stated.
[a]Inclusion criteria or estimated range within which all participants are included.
[b]Pooled data.
[c]Range.
[d]Median.
[e]SD.
*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 postprandial 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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