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Dietary carbohydrates and fats in nonalcoholic fatty liver disease

Hannele Yki-Järvinen^{1,2}✉, Panu K. Luukkonen^{1,2,3}, Leanne Hodson^{4,5} and J. Bernadette Moore⁶

Abstract | The global prevalence of nonalcoholic fatty liver disease (NAFLD) has dramatically increased in parallel with the epidemic of obesity. Controversy has emerged around dietary guidelines recommending low-fat–high-carbohydrate diets and the roles of dietary macronutrients in the pathogenesis of metabolic disease. In this Review, the topical questions of whether and how dietary fats and carbohydrates, including free sugars, differentially influence the accumulation of liver fat (specifically, intrahepatic triglyceride (IHTG) content) are addressed. Focusing on evidence from humans, we examine data from stable isotope studies elucidating how macronutrients regulate IHTG synthesis and disposal, alter pools of bioactive lipids and influence insulin sensitivity. In addition, we review cross-sectional studies on dietary habits of patients with NAFLD and randomized controlled trials on the effects of altering dietary macronutrients on IHTG. Perhaps surprisingly, evidence to date shows no differential effects between free sugars, with both glucose and fructose increasing IHTG in the context of excess energy. Moreover, saturated fat raises IHTG more than polyunsaturated or monounsaturated fats, with adverse effects on insulin sensitivity, which are probably mediated in part by increased ceramide synthesis. Taken together, the data support the use of diets that have a reduced content of free sugars, refined carbohydrates and saturated fat in the treatment of NAFLD.

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It is widely accepted that nonalcoholic fatty liver disease (NAFLD), an umbrella term encompassing a range of liver pathologies including steatosis (nonalcoholic fatty liver (NAFL)), nonalcoholic steatohepatitis (NASH), advanced fibrosis (fibrosis stages 3–4) and cirrhosis (fibrosis stage 4), is a complex phenotype¹. The prevalence of NAFL, defined as steatosis in which at least 5–10% of hepatocytes exhibit macrovesicular steatosis, or the intrahepatic triglyceride (IHTG) content exceeds 5.5%, averages 25% worldwide². The prevalence of NASH, which requires a liver biopsy for diagnosis, has been estimated to be 1.5–6.5%². On the basis of a meta-analysis of small, paired-biopsy studies, fibrosis progresses by one stage in approximately 14 years in patients with NAFLD and in 7 years in those with NASH³. Although all stages of fibrosis increase both overall and liver-specific mortality, in general, liver-specific mortality in patients with NAFLD is much lower (0.77 incidence rate per 1,000 person-years) than in patients with cardiovascular disease (CVD) (4.79) or overall mortality (15.44) according to a systematic review based on global data². NASH increases the risk of liver-specific mortality in absolute terms (11.77 incidence rate per 100 person-years) and in relation to overall mortality (25.56 deaths per 1,000 patient-years)².

NAFLD is frequently associated with features of the metabolic syndrome and predicts, independent of obesity, CVD and type 2 diabetes mellitus (T2DM)^{4,5}. These data imply that it is important to consider the effects of any given NAFLD intervention both on liver pathology and on cardiovascular risk factors.

The objective of this Review is to address the question, predominantly using human data, of whether IHTG accumulates primarily due to the consumption of excess energy (that is, calories) regardless of origin, or whether specific sugars or fats matter. To assess this question, we first review cross-sectional data on dietary habits and patterns in NAFLD. Then, we discuss how pathways of IHTG synthesis and disposal are altered in NAFLD and regulated by dietary macronutrients. This discussion includes an examination of mechanisms by which macronutrients regulate IHTG synthesis and disposal, alter pools of bioactive lipids and influence insulin sensitivity. Finally, we review randomized controlled studies that have examined the effects of altering total carbohydrate and fat content, or the effects of changing fat quality (that is, saturated, monounsaturated and polyunsaturated) or sugar type (specifically the monosaccharides glucose and fructose), on IHTG. Studies addressing effects of other dietary constituents,

Key points

- Nonalcoholic fatty liver disease (NAFLD), total energy intake and intake of free sugars and refined carbohydrates have increased in parallel; *de novo* lipogenesis, which produces saturated fat from sugars, contributes to NAFLD.
- Saturated fat intakes have remained well above the recommended maximum of 10% total energy in many developed countries/regions worldwide, which is of concern in NAFLD as well as cardiovascular disease.
- The American Association for the Study of Liver Diseases, in contrast to the European Association for the Study of the Liver, did not make any recommendation regarding macronutrient intake in NAFLD and instead called for rigorous, prospective, longer-term trials with histopathological end points.
- Analysis of existing trials shows that high-fat–low-carbohydrate diets containing high saturated fat increase intrahepatic triglyceride (IHTG) content more than low-fat–high-carbohydrate diets.
- Saturated fat-enriched diets increase IHTG more than polyunsaturated or monounsaturated diets; ceramides probably contribute to saturated fat-induced adverse metabolic and cardiovascular consequences.
- The limited data available support the use of a Mediterranean diet that is low in saturated fat with high amounts of monounsaturated fat and dietary fibre in the treatment of NAFLD.

such as red meat⁶, vitamin D⁷, vitamin E⁸, probiotics, prebiotics and synbiotics^{9,10}, omega-3 fatty acids¹¹, coffee^{12,13} and alcohol¹⁴ on the risk of NAFLD, are beyond the scope of this Review.

NAFLD and obesity

NAFLD progression is determined by dynamic interactions between diet, lifestyle and genetic factors, and involves crosstalk between multiple organs and the intestinal microbiome¹. The heterogeneity of NAFLD presentation and progression, as well as its close relationship with metabolic dysfunction, led in 2020 to a consensus-driven proposal for a name change to metabolic-associated fatty liver disease (MAFLD)^{15,16}. Considerations behind the proposed name change included a recognition that although genetic risk influences NAFLD pathogenesis, the phenotypic threshold is strongly influenced by environmental factors such as adiposity, insulin resistance and diet^{15,16}. Multiple endorsements and significant effort will be required to ultimately change the diagnostic and symptom codes of the International Classification of Diseases. Given the close association between NAFLD and obesity, weight loss through dietary and lifestyle intervention is the mainstay of current clinical management in the absence of licensed pharmaceutical agents^{17–19}.

In the context of an obesogenic world, maintaining individual energy balance and a healthy weight throughout the lifespan is challenging²⁰. Population level survey data and long-term prospective cohort studies have illustrated that, in addition to lifestyle factors (such as physical activity, alcohol use, television watching and smoking habits), changes in the consumption of specific foods and beverages are associated with long-term weight gain²¹. During 20 years of follow-up of the 120,877 women and men involved in the Nurses' Health Study and the Health Professionals Follow-up Study in the USA, increased intakes of potato chips, potatoes and sugar-sweetened beverages (SSBs) were, independent of confounders, the top three predictors of weight

gain²². Dietary sugars, occasionally referred to as 'simple sugars', include monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, lactose and maltose). The term 'total sugars' found on food labels includes both sugars that occur naturally in food and beverages and those added during processing and preparation. The term 'free sugars' excludes sugars present in intact fruits and vegetables and lactose naturally present in milk and milk products. It is these free sugars, in particular fructose and those consumed as beverages (in both soft drinks and fruit or vegetable juices), that are implicated in the development and progression of NAFLD and other chronic metabolic diseases²³.

Collectively, these studies raise the possibility that the obesity and NAFLD epidemics are a consequence of weight gain due to excessive intake of carbohydrates including free sugars. In addition, they have led to a debate over historical dietary guidelines in the USA, which recommend low-fat (considered <35% of daily energy from fat with an 'acceptable distribution' of 20–35%) and low saturated fat diets (7–10% of daily energy) for the prevention of CVD^{24,25}. However, the often polarized debates on sugar versus fat in the aetiology of obesity and metabolic disease^{26,27} (or low-fat versus low-carbohydrate diets in the treatment and prevention) omit the crucial point that, at a population level, identifying individual culpable nutrients in diverse diets for complex diseases is problematic.

With the exception of one large study ($n = 293$)²⁸ and one small study ($n = 31$)²⁹ addressing the effects of weight loss on liver histology in patients with NASH, intervention data on the effects of altering diets (energy or macronutrient composition) on histopathological end points in NASH are virtually non-existent. Therefore, the American Association for the Study of Liver Diseases could not provide any recommendations for macronutrient intake in NAFLD, and instead called for rigorous, large, prospective, longer-term trials with histopathological end points before recommendations could be made¹⁸. However, repeat biopsies before and after weight loss might not be clinically indicated and are therefore difficult to justify for clinical trials. The European associations for the study of diabetes, obesity and the liver (EASD, EASO and EASL, respectively), based on only one randomized trial that measured changes in IHTG content in 12 patients with NAFLD³⁰, has suggested that a Mediterranean diet might be the diet of choice for NAFLD¹⁷. Data are equally sparse regarding the effects of different diets on progression of liver fibrosis, although this can be determined non-invasively using techniques such as transient or magnetic resonance elastography³¹. Thus, although there is consensus that weight loss through diet and lifestyle³² or clinical³³ interventions are beneficial for NAFLD, the data available to date are fairly uninformative regarding the effect of different diets on NASH and fibrosis. However, as steatosis predicts progression to NASH and fibrosis^{34,35}, diets that reduce IHTG are likely to be helpful in the prevention of NAFLD progression. Furthermore, as CVD is the main cause of death in patients with NASH³⁶, diets that reduce cardiometabolic risk factors are likely to be of benefit to patients and should be considered.

Dietary intakes and patterns in NAFLD

Population level survey data from the National Health and Nutrition Examination Survey in the USA show that between 1971–1974 and 2001–2004, the average energy intake increased by 22% among women and by 10% among men²¹ (Supplementary Fig. 1a). During this time period, the percentage of energy consumed from fat declined (from 36.9% to 33.4% in men and from 36.1% to 33.8% in women), whereas that from carbohydrates, both as foods (starches and grains) and as SSBs, increased (from 42.4% to 48.2% in men and from 45.4% to 50.6% in women)²¹ (Supplementary Fig. 1a). However, although the percentage of energy consumed from fat in the USA reduced slightly in the 1990s, it has been increasing since 2010, with an average of 35.2% (95% CI 34.8–35.6%) of daily energy intake from fat in the most recent estimate (2015 to 2016) for both sexes³⁷, which is not particularly low-fat (Supplementary Fig. 1b). A concern in NAFLD as well as CVD is that saturated fat intakes have remained at ~11.7% for the past two decades, well above the recommended maximum of 10%³⁷ (Supplementary Fig. 1b).

Memory-based, self-reported dietary survey data have been both highly criticized as unreliable and defended as useful in yielding insights in relation to dietary factors and health outcomes^{38,39}. In this context, food supply data, although they do not translate precisely to what a single individual might eat, are useful for giving additional perspective on the food environment. Data from the Food and Agricultural Organization of the United Nations support the concept that for most developed countries/regions, the per capita supply of energy has increased dramatically in the past 50 years⁴⁰. In the USA, although increased energy supply has indeed come in part from carbohydrates, there has also been a marked increase in the per capita supply of energy from fat^{40,41} (Supplementary Fig. 1c).

The relationship between NAFLD (NAFL and NASH, diagnosed by a variety of means) and dietary intakes or patterns (typically assessed via food frequency questionnaires) has been investigated in multiple prospective longitudinal or cross-sectional cohort studies including cohorts of varying sizes, ethnicities and age groups. Early studies in Italian⁴², Japanese⁴³, Israeli⁴⁴ and US⁴⁵ populations found that increased consumption of meat and SSBs, as well as low consumption of fish, were associated with NAFLD. In 2018 and 2019, larger and multi-ethnic population studies confirmed that high consumption of red meat and processed meat is associated with NAFLD^{46,47}. For example, in a nested case–control study within the Multi-Ethnic Cohort study of US adults aged 45–75 years, higher intakes of red meat (OR 1.15; *P* trend 0.010), poultry (OR 1.16; *P* trend 0.005), processed red meat (OR 1.18; *P* trend 0.004) and cholesterol (OR 1.16; *P* trend 0.005) were significantly associated with NAFLD (2,974 patients, 518 with cirrhosis) compared with 29,474 matched controls⁴⁷. On the other hand, dietary fibre was inversely associated (OR 0.84; *P* trend 0.003) with risk. Associations were stronger in patients with NAFLD and with cirrhosis for red meat (OR 1.43 versus 1.10) and cholesterol (OR 1.52 versus 1.09) than in patients with NAFLD without cirrhosis⁴⁷. By contrast,

in a larger cohort study in Chinese adults (*n* = 4,365), patients with NAFLD (diagnosed by ultrasonography) consumed a diet higher in carbohydrates and free sugars than participants without NAFLD⁴⁸.

Multiple studies suggest a relationship between Western dietary patterns, which are typically characterized by high intakes of red meat and processed meat, refined grains, fat and added sugars as well as high SSB consumption, and NAFLD in both children and adults. For example, in Australian adolescents assessed at 14 and 17 years of age (*n* = 995), a Western dietary pattern characterized by high intakes of SSBs, confectionary, takeaway foods, sauces and dressings at 14 years of age was associated with an increased incidence of NAFLD (assessed by ultrasonography) at 17 years of age⁴⁹. The relationship was independent of sex, physical activity and sedentary behaviour, but not of BMI⁴⁹. In adult Iranian patients with NAFLD (*n* = 170), a Western dietary pattern characterized by high intakes of red meat, hydrogenated fats and SSBs was associated with fibrosis as diagnosed by transient elastography (OR 4.21)⁵⁰. Conversely, improving diet quality (as assessed by either the Alternative Healthy Eating Index or the Mediterranean Diet Score) over time has been shown in a longitudinal study of middle-aged to older US adults (51 ± 10 years at baseline; *n* = 1,521) to be significantly associated (*P* trend <0.001) with a lower incidence and risk of severe fatty liver⁵¹. Similarly, in a cross-sectional analysis of two large adult cohorts (England, UK, *n* = 9,645; Switzerland, *n* = 3,957), greater adherence to a Mediterranean diet was significantly associated with a reduced risk of hepatic steatosis as assessed by either ultrasonography or fatty liver index (prevalence ratios 0.86 (95% CI 0.81–0.90) for the English cohort and 0.82 (95% CI 0.78–0.86) for the Swiss cohort). However, associations were greatly reduced (to 0.94 and 0.98), and in the case of the Swiss cohort no longer significant, when adjusted for BMI, which suggests that lower adiposity associated with greater adherence to a Mediterranean diet explained the reduced risk⁵².

The majority of studies have found that excess SSB consumption is linked to NAFLD, with multiple meta-analyses finding a positive statistically significant association between SSB consumption and the risk of NAFLD^{53,54}. In 2019, Chen and colleagues⁵⁴ reviewed 12 studies with >35,000 participants and suggested that consumption of low doses (<1 cup per week), middle doses (1–6 cups per week) and high doses (≥7 cups per week) of SSBs increased the relative risk of NAFLD by 14%, 26% and 53%, respectively (*P* = 0.01, *P* < 0.00001 and *P* = 0.03, respectively). Thus, in the majority of studies investigating dietary intake and NAFLD, the evidence linking sugar intake and NAFLD risk seems to be a consistent finding.

Pathways of IHTG synthesis and disposal

Within the liver, fatty acids used for the synthesis of IHTG can originate from non-lipid dietary sources when consumed in excess, including the monosaccharides glucose and fructose, or amino acids in the context of a high-protein diet (32% of total energy intake)⁵⁵. When consumed in excess, these substrates are converted into

Fig. 1 | Metabolic fates of free sugars and SFAs. Fatty acids released by intestinal lipolysis are packaged into chylomicron (CM) particles, which are transported via chyle to the systemic circulation. Free fatty acids are released from CMs (and VLDL produced by the liver, not shown) via intravascular lipolysis and are taken up by peripheral tissues such as adipose tissue or spillover to the systemic circulation²¹⁴. Fatty acids stored in adipose tissue triglycerides (Tgs) undergo lipolysis, which releases free fatty acids, especially under fasting conditions. Non-esterified fatty acids (NEFAs) are transported to the liver bound to albumin and constitute the major source of liver Tgs both after an overnight fast and after a meal⁵⁸. Saturated fatty acid (SFA)-enriched diets increase CMs²¹⁵, peripheral lipolysis and liver SFAs such as palmitate (16:0), which is needed for synthesis of ceramides¹⁰⁵, compared with polyunsaturated fatty acid (PUFA)-containing diets. SFAs but not PUFAs stimulate ceramide synthesis in both animals¹⁴⁴ and humans^{105,117}. Ceramides induce hepatic insulin resistance, inflammation and mitochondrial dysfunction¹⁴⁶. Compared with high-carbohydrate diets, SFAs increase serum LDL and HDL cholesterol and decrease Tgs²¹⁶. Added sugars such as saccharose and high-fructose corn syrup contain glucose and fructose. If consumed in excess, these sugars might be converted to SFAs such as 16:0 palmitate and 18:0 oleate in the liver via stimulation of de novo lipogenesis (DNL). Fructose but not glucose also increases CMs²¹⁷. Nonalcoholic fatty liver disease (NAFLD) is characterized by increased free sugar intake, CMs, lipolysis, hepatic and circulating ceramides, insulin resistance of hepatic glucose production (HGP), increased VLDL synthesis and serum (S) Tgs, which lead to lowering of HDL cholesterol. DAG, diacylglycerol; fS, fasting serum; IHTG, intrahepatic triglyceride.

saturated fatty acids (SFAs) via hepatic de novo lipogenesis (DNL)⁵⁶. In addition, fatty acids are released by intravascular hydrolysis and adipose tissue lipolysis, or they can be derived from dietary fat⁵⁷ (FIG. 1). Fatty acids derived from adipose tissue lipolysis are the source of the majority of fatty acids used for triglyceride synthesis in the liver⁵⁸. This observation is true both in the fasting and postprandial state, despite postprandial insulin-mediated suppression of adipose tissue lipolysis⁵⁷. After consumption of a meal containing a mixture of fats, carbohydrate and protein, chylomicron particles carry dietary triglycerides in the systemic circulation to peripheral tissues such as adipose tissue and skeletal muscle for intravascular hydrolysis mediated by lipoprotein lipase (FIG. 1). Fatty acids that are not taken up by these tissues spill over into the systemic circulation and can be taken up by the liver and used for triglyceride synthesis⁵⁷. Chylomicron remnants also deliver fatty acids to the liver, but the contribution of this pathway to triglyceride synthesis is believed to be small compared with that of other fatty acid sources⁵⁹. The overall contribution of dietary fat to triglyceride synthesis has been reported to increase by up to 30–40% in healthy men studied for 10 hours after ingesting two meals containing

~30% fat⁵⁷. The contribution of de novo fatty acids to triglyceride synthesis is low in the fasting state but increases postprandially, as any excess carbohydrate can be synthesized to triglyceride via the DNL pathway⁶⁰.

De novo lipogenesis. In humans, DNL occurs predominantly in the liver, and palmitate (a 16-carbon SFA) is the end product^{61,62}. However, DNL is not the pathway of first resort for disposal of dietary carbohydrates in humans⁵⁶. In healthy individuals, DNL produces 1–2 g of fat per day, which is minor compared with 50–100 g of dietary fat consumed by UK adults⁶². However, during carbohydrate overfeeding, fractional DNL, which is the contribution of DNL to VLDL triglyceride, will be stimulated⁶³. Nonetheless, even after 5 days of consuming 50% more energy from carbohydrates than baseline intakes it was found in six healthy adults that DNL contributed <5 g of fat to VLDL triglyceride secretion⁶³. Although the quantitative contribution of DNL to triglyceride synthesis might be low, the process of DNL is of physiological importance in the regulation of fatty acid oxidation. As demonstrated by McGarry and colleagues⁶⁴, when DNL is upregulated, malonyl-CoA, an intermediate in the DNL pathway, potentially inhibits

carnitine palmitoyltransferase 1, which leads to suppression of fatty acid oxidation. Thus, upregulation of DNL shifts cellular metabolism towards esterification (anabolic) and away from oxidation (catabolic) pathways.

It is thought that hepatic DNL is an important contributor to IHTG in people with NAFLD⁶¹, with DNL accounting for 15% to 38% of IHTG palmitate production^{58,59,65} (FIG. 1). This proportion is notably higher than the 1% to 10% found in different studies in individuals without NAFLD^{59,66–68}. This proportion might be specific to NAFLD associated with the metabolic syndrome and does not seem to characterize individuals with NAFLD due to the *PNPLA3* I148M gene variant^{69,70}. In addition to inter-individual variation, the estimated contribution of DNL to IHTG might depend on the methodology used to assess DNL. Hepatic DNL can be directly measured using metabolic tracers, for example deuterated water, which is typically consumed between 12–48 h prior to the assessment^{71–74}. In a study by Smith and colleagues where deuterated water was administered for 3–5 weeks, in individuals with obesity and NAFLD ($n=27$) the contribution of DNL-derived fatty acids (specifically, the percentage contribution of DNL to palmitate in triglyceride-rich lipoproteins) was the highest at 38% compared with 19% and 11% in individuals with obesity but without NAFLD ($n=26$) and in lean individuals ($n=14$), respectively⁶¹.

Therefore, if increased sugar intakes are contributing to the NAFLD epidemic, these could at least in part explain the higher rates of DNL observed in patients with NAFLD^{59,61} and be an important treatment target. Notably, 10% diet-induced weight loss in individuals with NAFLD significantly ($n=6$; $P<0.05$) decreased DNL and IHTG, as well as 24-h plasma glucose and insulin concentrations⁶¹. With the decrease in DNL, it is likely that there was an increase in fatty acid oxidation due to a lower production of malonyl-CoA, an intermediate in the DNL pathway, and a potent inhibitor of carnitine palmitoyltransferase I⁶⁴. This was demonstrated in a study in 19 healthy men who received an ω -3 fatty acid supplement (4 g per day) for 8 weeks. Compared with baseline, fasting and postprandial hepatic DNL decreased significantly ($P<0.05$) and there was a significant increase in fatty acid oxidation⁷⁵. Several pharmacological inhibitors of DNL, including MK-4074 (REF.⁷⁶), GS-0976 (REF.⁷⁷) and NDI-010976 (REF.⁷⁸), which all inhibit acetyl-coenzyme A carboxylase, are currently being tested in humans and have been reviewed previously⁷⁹.

Lipolysis. Multiple studies have shown increased adipose tissue lipolysis in individuals with and without diabetes but who have NAFLD^{80–82}. Whether this finding is because patients with NAFLD have a higher BMI than age-matched and gender-matched individuals without NAFLD is less certain. Lipolysis, which is the rate of non-esterified free fatty acid (NEFA) appearance into the systemic circulation, increases in direct proportion to fat mass⁸³. Interestingly, a study of adolescent girls with equivalent BMI and with either lower IHTG and visceral fat ($n=7$, BMI 36.6 kg/m²) or higher IHTG

and visceral fat ($n=8$, BMI 36.0 kg/m²) found that those with higher IHTG and visceral fat had higher rates of adipose tissue triglyceride turnover (representing both lipolysis and synthesis at steady state), as measured using a novel stable isotope method, than those with lower IHTG and visceral fat⁸⁴. In both groups, the turnover rate (lipolysis and synthesis) of triglyceride in adipose tissue was correlated with IHTG content⁸⁴. Therefore, contrary to the belief that ectopic fat deposition is a result of an inability to store triglycerides in adipose tissue, this study in humans suggests that the problem is reduced retention of NEFAs in adipose tissue⁸⁴.

Fates of fatty acids. Once in the liver, fatty acids are partitioned into oxidation and ketogenesis or esterification to form predominantly triglycerides, which might be secreted in VLDL particles⁸⁵. Many factors are involved in the regulation of fatty acid partitioning, perhaps most importantly insulin, which regulates the supply of fatty acids to the liver from adipose tissue and suppresses VLDL production⁸⁶. Individuals with NAFLD have been reported to have an overproduction of VLDL particles that contain >40% more triglycerides compared with age-matched and BMI-matched individuals without NAFLD^{87–89}. Although VLDL triglyceride secretion correlates positively with IHTG⁸⁷, there might be a plateau beyond 10% IHTG⁸⁸. Very few studies have investigated the effect of dietary macronutrients or fat composition on VLDL triglyceride secretion or production rates^{89,90}. In men with NAFLD ($n=11$), consumption of a diet enriched with sugars for 12 weeks did not notably alter VLDL1 triglyceride production rates compared with a diet lower in sugars. In contrast, consumption of a sugar-enriched diet in men without NAFLD ($n=14$) significantly ($P<0.05$) increased the VLDL1 triglyceride production rate compared with a diet containing less sugar⁸⁹. Consumption of a diet high in monounsaturated fat (13.7% total energy) did not alter VLDL1 production rates compared with consumption of a diet low in monounsaturated fat (7.8% total energy) for 6 weeks in 17 middle-aged adults (mean age 55 years) with moderate hypercholesterolaemia⁹⁰.

Oxidation and ketogenesis. A further branch point in intrahepatic fatty acid metabolism is within the oxidation pathway. Here, intra-mitochondrial acetyl-CoA is partitioned between either complete oxidation via the tricarboxylic acid cycle and electron transport chain for ATP production, or ketogenesis⁸⁵. Blood levels of the ketone body 3-hydroxybutyrate (3OHB) are often used as a surrogate marker of hepatic fatty acid oxidation, although 3OHB is influenced both by rates of ketogenesis, β -oxidation and terminal mitochondrial oxidation of fatty acids^{91–93}. Data on plasma 3OHB concentrations in individuals with and without NAFLD are inconsistent. Levels have been reported to be decreased⁹⁴, similar^{95,96} or increased⁹⁷. A limited number of studies have assessed mitochondrial oxidation using stable isotope tracers. Again, results have varied. For example, Sunny and colleagues⁹⁶ found fasting mitochondrial oxidation to be twice as high in individuals with NAFLD ($n=8$, 17% IHTG) than in those without NAFLD ($n=8$,

3% IHTG), whereas others have found similar rates in individuals with high IHTG ($n=4$, ~9%) and low IHTG ($n=4$, ~2%)⁹⁸.

Dietary macronutrients might also have an effect on hepatic fatty acid oxidation. However, studies using stable isotope tracers to examine metabolism in humans are technically difficult and somewhat invasive. Therefore, few studies have investigated the effects, and sample size is typically limited. For example, it was demonstrated that in the short term (3 days), consumption of a low-fat-high-carbohydrate diet (10% fat and 75% carbohydrate) did not change fasting or postprandial fatty acid oxidation (measured by indirect calorimetry) but significantly decreased postprandial fatty acid oxidation (measured using stable-isotope methodologies) when compared with a high-fat-lower-carbohydrate diet ($n=8$; 40% fat and 45% carbohydrate)⁹⁹. This randomized crossover study in eight healthy individuals measured postprandial blood 3OHB concentrations and expired ¹³CO₂ levels as a marker of whole-body fatty acid oxidation. Although it is often suggested, based on evidence from rodent studies, that dietary polyunsaturated fatty acids (PUFAs) preferentially enter oxidation pathways compared with SFAs^{100,101}, data in humans are sparse. Two small studies in healthy men ($n=6$ (REF.¹⁰²) and $n=4$ (REF.¹⁰³)) that used metabolic tracers and measured expired ¹³CO₂ suggested

a greater oxidation of monounsaturated fatty acids (MUFAs) and PUFAs compared with SFAs. In a study in healthy adults (12 men and 12 women) using metabolic tracers and measuring expired ¹³CO₂, oxidation of dietary linoleate was found to be significantly ($P<0.05$) greater than oxidation of dietary palmitate¹⁰⁴.

Macronutrient composition and IHTG

Several trials have examined IHTG responses to dietary macronutrient manipulation. Evaluation of the dietary energy changes elicited by such interventions is important. The trials were heterogeneous in type and length of intervention and are summarized in TABLE 1. In three trials the dietary interventions were hypoenergetic (reduced calorie content), in three trials the interventions were isoenergetic and, in two trials (one trial with two interventions)¹⁰⁵, the interventions were hyperenergetic; these are discussed in detail in this section. In FIG. 2 (left), the percentage changes in IHTG relative to baseline are illustrated to enable comparison across the trials.

Hypoenergetic comparisons. Weight loss is remarkably effective at decreasing IHTG. A hypoenergetic (>500 calorie deficit per day), low-carbohydrate (<60 g of carbohydrate per day) diet has been shown to decrease IHTG by 30–45% in less than a week in individuals with

Table 1 | Studies comparing the effects of low-fat-high-carbohydrate and high-fat-low-carbohydrate diets on IHTG and insulin sensitivity

Study	Participant characteristics (n; BMI (kg/m ²); age (years); NAFLD (%))	Duration	Design	Energy content (relative to baseline diet)	Diet	Fat and carbohydrate (% of total energy)	Fat quality (SFA; PUFA; MUFA) (% of total energy)	IHTG (%): before, after	Insulin sensitivity
Kirk et al. (2009) ¹⁰⁶	22; 37; 44; 54	11 weeks	P	Hypo	LF-HC	20, 65	No data	11.2, 6.2↓	↑
					HF-LC	75, 10		12.4, 8.1↓	↑
Browning et al. (2011) ¹⁰⁷	18; 35; 45; 100	2 weeks	P	Hypo	LF-HC	34, 50	14.7; 13; 6.3	19, 14↓	No data
					HF-LC	59, 8	24; 25; 10	22, 10↓ ^a	No data
Haufe et al. (2011) ¹⁰⁸	102; 32; 45; 54	6 months	P	Hypo	LF-HC	'Reduced fat'	Less saturated ^b	9.6, 5.6↓	↑
					HF-LC	'Reduced carb'		7.6, 4.0↓	↑
Westerbacka et al. (2005) ¹⁰⁹	10; 33; 43; 50	2 weeks	C	Iso	LF-HC	16, 61	7.2; 6.6; 2.2	10, 8 ^a	↑ ^a
					HF-LC	56, 31	25.2; 23.0; 7.8	10, 13	↓
van Herpen et al. (2011) ¹¹⁰	20; 29; 55; no data	3 weeks	P	Iso	LF-HC	22, 57	9.0; 8.1; 4.8	4.0, 3.5↓	NS
					HF-LC	49, 34	20.1; 18.6; 10.3	2.2, 2.6	NS
Utzschneider et al. (2013) ¹¹¹	35; 27; 69; 15	4 weeks	P	Iso	LF-HC	23, 57	8.5; 9.7; 4.8	2.2, 1.7↓	↑
					SFA-LC	43, 38	25.8; 13.8; 3.4	1.2, 1.4	NS
Sobrecases et al. (2010) ¹¹⁵	39; 23; 25; no data	7 days	P	Hyper	LF-HC	High fructose ^d	No data	12 ^c , 14 ^c	NS
					HF-LC	High fat ^e		11 ^c , 21 ^c	NS
Luukkonen et al. (2018) ¹⁰⁵	26; 31; 46; 27	3 weeks	P	Hyper	LF-HC	24, 64	9.8; 9.8; 4.3	4.3, 5.7↑	NS
					SFA-LC	60, 26	37.8; 16.2; 6.0	4.9, 7.6↑ ^a	↓
	24; 32; 48; 25	3 weeks	P	Hyper	LF-HC	24, 64	9.8; 9.8; 4.3	4.3, 5.7↑	NS
					PUFA-LC	59, 23	17.7; 28.9; 12.4	4.8, 5.5↑	NS

The fat quality column shows the percentages of total energy consumed as saturated, polyunsaturated and monounsaturated fatty acids or measured from change in fatty acid composition of phospholipids¹⁰⁹. ↓, significant decrease from baseline; ↑, significant increase from baseline; C, crossover design; HC, high carbohydrate; HF, high fat; Hyper, hyperenergetic; Hypo, hypoenergetic; IHTG, intrahepatocellular triglycerides (as measured by proton magnetic resonance spectroscopy); Iso, isoenergetic; LC, low carbohydrate; LF, low fat; MUFA, high-fat diet enriched with monounsaturated fatty acids; n, number of completers; NAFLD, nonalcoholic fatty liver disease; NS, no significant change; P, parallel design; PUFA, high-fat diet enriched with polyunsaturated fatty acids; SFA, high-fat diet enriched with saturated fatty acids. ^aSignificant difference in change between the diets. ^bThe LF-HF group consumed, in absolute terms, less saturated and n-6 fatty acids and similar n-3 fatty acids compared with the HF-LC group. ^cIHTG units are millimoles per kilogram wet weight (not percentage). ^dAddition of 3.5 g per day of fructose per kilogram of fat free mass. ^eAddition of 30% of total energy as fat. For corresponding figure, see FIG. 2 (left). Adapted from REF.²¹³, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

Fig. 2 | Effects of fats and carbohydrates on liver fat content. Effects of low-fat-high-carbohydrate diets as compared to high-fat-low-carbohydrate diets on liver fat content (left). Expressed as relative (percentage) change from baseline measured by ^1H -magnetic resonance spectroscopy (^1H -MRS). Interventions comparing hypoenergetic, isoenergetic and hyperenergetic low-fat-high-carbohydrate diets to high-fat-low-carbohydrate diets are shown. Upper-left panel: 1 (REF.¹⁰⁶), 2 (REF.¹⁰⁷), 3 (REF.¹⁰⁸); middle-left panel: 4 (REF.¹⁰⁹), 5 (REF.¹¹⁰), 6 (REF.¹¹¹); bottom-left panel: 7 (REF.¹¹⁵), 8 (REF.¹⁰⁵). Effects of fat quality on liver fat content, expressed as relative (percentage) change from baseline measured by ^1H -MRS (right). Interventions comparing isocaloric or hypocaloric diets (upper-right panel) or hypercaloric diets (bottom-right panel) low in saturated fat and high in polyunsaturated fat or high in saturated fat and low in polyunsaturated fat are shown. Upper-right panel: 1 (REF.¹¹⁶), 2 (REF.¹⁸⁸), 3 (REF.¹⁸⁹), 4 (REF.³⁰), 5 (REF.¹⁹¹); lower-right panel: 6 (REF.¹¹⁸), 7 (REF.¹⁰⁵), 8 (REF.¹¹⁷). carb, carbohydrate; CONT, control; LF, low-fat diet; MED/LC, Mediterranean low-carbohydrate diet; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. Adapted from REF.²¹³, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

overweight or obesity^{70,106}. Although a ketogenic low-carbohydrate diet decreased IHTG more than a standard hypoenergetic diet when measured after 2 days¹⁰⁶ or 2 weeks in 22 individuals with obesity, this difference was no longer observed after 11 weeks¹⁰⁷. Similarly, in a longer (6-month) hypoenergetic intervention in 102 participants with overweight or obesity¹⁰⁸, there was no statistically significant difference in the reduction in IHTG between those receiving the low-fat diet (42% decrease in IHTG) and those receiving the low-carbohydrate diet (47% decrease).

Isoenergetic comparisons. Isoenergetic low-fat (16–23% of total energy), high-carbohydrate (57–65% of total energy) diets were compared with high-fat (43–56%), low-carbohydrate (31–38%) diets in three studies in

individuals with overweight or obesity^{109–111}. In all three studies, IHTG decreased in those receiving the low-fat-high-carbohydrate diet and increased in those receiving the high-fat-low-carbohydrate diet (FIG. 2, left). This difference can be attributed to the high-fat rather than the low-carbohydrate component as the latter should reduce rather than increase DNL and IHTG¹¹². Notably, all three high-fat diets provided intakes of saturated and polyunsaturated fats much higher than WHO recommendations¹¹³ (TABLE 1), which are <10% and 6–11% of total energy respectively, conditions that were met with the low-fat diets. Together, these few isoenergetic comparisons suggest that high-fat diets increase IHTG to a greater extent than high-carbohydrate diets. A meta-analysis including 11 studies in 480 individuals that examined data from both MRI and changes in liver

enzymes as a marker of liver health in individuals with obesity with or without NAFLD reached the same conclusion¹¹⁴. Namely, carbohydrate restriction is not helpful if it occurs at the expense of increased fat intake.

Hyperenergetic comparisons. Studies comparing high-fat–low-carbohydrate diets with low-fat–high-carbohydrate diets during overfeeding^{105,115} are listed in TABLE 1. In these studies, overfeeding with saturated fat, but not polyunsaturated fat, increased IHTG more than overfeeding with free sugars.

Overall, these data suggest that the energy content of a diet is an important factor influencing IHTG, which is consistent with current treatment recommendations, in which weight loss underpins the management of NAFLD¹⁷. This conclusion seems justified, although the fat quality (measured as the percentage saturated, monounsaturated, and polyunsaturated fat in relation to total energy) varied markedly between the treatment arms in the few studies that provided these data^{107–111} (TABLE 1).

Fat quality and IHTG

In four studies, saturated fat consistently increased IHTG more than polyunsaturated fat when total energetic intake was similar^{105,116–118} (FIG. 2, right; TABLE 2).

Rosqvist and colleagues¹¹⁸ compared hyperenergetic diets enriched with SFAs at the expense of PUFAs with a diet rich in PUFAs but lower in SFAs in 39 lean individuals¹¹⁸ and later in 60 individuals with overweight or obesity¹¹⁷. The excess energy was served in similar-looking muffins. In the studies, overfeeding SFAs, but not PUFAs, statistically significantly increased IHTG content by 40% in lean individuals¹¹⁸ and by 50% in individuals with overweight or obesity¹¹⁷, which is in line with earlier work showing the same result with an isoenergetic intervention in individuals with obesity¹¹⁶. Luukkonen and colleagues¹⁰⁵, in addition to comparing the effects of 3 weeks of overfeeding SFAs and PUFAs, also examined overfeeding free sugars (TABLE 1), demonstrating that SFAs increased IHTG statistically significantly more than either PUFAs or free sugars.

Data are limited regarding pathways that mediate the differential effects of SFAs and PUFAs and free sugars on IHTG synthesis and disposal. The overfeeding study by Luukkonen and colleagues examined these effects and found that overfeeding saturated fat increased adipose tissue lipolysis, whereas overfeeding free sugars increased DNL, thereby showing that the route of IHTG synthesis depends on the diet¹⁰⁵. PUFAs might be preferentially partitioned into oxidation

Table 2 | Studies comparing the effects of fat quality on liver fat

Study	Participant characteristics (n; BMI (kg/m ²); age (years); NAFLD (%))	Duration	Design	Energy content relative to baseline diet	Diet	Fat and carbohydrate (% of total energy)	IHTG (%): before, after
SFA-enriched versus PUFA-enriched diets							
Bjermo et al. (2012) ¹¹⁶	61; 31; 30–65; no data	10 weeks	P	Iso	PUFA	40 ^a , 39	3.2, 2.3*
					SFA	43 ^b , 40	3.2, 3.5
Rosqvist et al. (2014) ¹¹⁸	39; 20; 27; 0	7 weeks	P	Hyper	PUFA	40 ^c , 43	0.75, 0.79
					SFA	36 ^d , 48	0.96, 1.5*
Luukkonen et al. (2018) ¹⁰⁵	26; 30; 50; 27	3 weeks	P	Hyper	PUFA	60 ^e , 23	4.8, 5.5†
					SFA	59 ^f , 26	4.9, 7.6†*
Rosqvist et al. (2019) ¹¹⁷	61; 28; 42; 0	8 weeks	P	Hyper	PUFA	51 ^g , 44	2.0, 1.9
					SFA	51 ^h , 44	1.5, 3.0†*
MUFA and MED diets							
Bozzetto et al. (2012) ¹⁸⁸	17; 30; 35–70; no data	8 weeks	P	Iso	CONT	28, 53	17.7, 16.1
					MUFA	42 ⁱ , 40	7.4, 5.2↓*
Errazuriz et al. (2017) ¹⁸⁹	28; 31; 61; 50	12 weeks	P	Iso	CONT	34 ^j , 49	11.2, 11.9
					MUFA	46 ^k , 40	9.7, 8.0↓*
Ryan et al. (2013) ³⁰	12; 32; 55; 100	6 weeks	C	Iso	CONT	21 ^l , 49	11.2, 10.0
					MED	44 ^m , 34	14.2, 8.6
Gepner et al. (2019) ¹⁹¹	278; 31; 48; 53	18 months	P	Hypo	CONT	35, 44	10.1, 6.4↓*
					MED	38, 40	10.3, 6.1↓
Properzi et al. (2018) ¹⁹²	49; 31; 52; 100	12 weeks	P	Iso	CONT	31 ⁿ , 48	21.5, 15.3↓
					MED	45 ^o , 37	34.2**, 24.0↓

In all studies, there was no significant difference in change in body weight between groups. †, significant increase from baseline; ↓, significant decrease from baseline; C, crossover; CONT, standard control diet; Hyper, hyperenergetic; Hypo, hypoenergetic; Iso, isoenergetic; MED, Mediterranean diet; MUFA, diet enriched with monounsaturated fatty acids; n, number of completers; P, parallel; PUFA, diet enriched with polyunsaturated fatty acids; SFA, diet enriched with saturated fatty acids. ^a10% SFA, 17% MUFA, 13% PUFA. ^b20% SFA, 19% MUFA, 4% PUFA. ^c11% SFA, 12.4% MUFA, 13% PUFA. ^d16% SFA, 12.9% MUFA, 4% PUFA. ^e14% SFA, 28% MUFA, 11% PUFA. ^f33% SFA, 13% MUFA, 5% PUFA. ^gSunflower oil. ^hPalm oil. ⁱEnriched with MUFA, SFA similar in both arms. ^j12% SFA, 8% MUFA, 4% PUFA. ^k12% SFA, 22% MUFA, 5% PUFA. ^l14.4% SFA, 15.6% MUFA, 9.6% PUFA. ^m12.4% SFA, 20.4% MUFA, 7.2% PUFA. ⁿ9.3% SFA, 13.1% MUFA. ^o9.5% SFA, 24.7% MUFA. *Significant difference in change between the diets. **P=0.01 for difference in baseline liver fat. For corresponding figure, see Supplementary Fig. 1.

pathways¹⁰³, but this possibility has not been explored by direct measurements of hepatic fat oxidation during intervention studies. Data obtained using measurements of whole-body fat oxidation^{105,119} or plasma β -hydroxybutyrate concentrations¹²⁰ have yielded inconsistent results.

Metabolic effects of saturated fat

In the majority of large observational longitudinal studies, NAFLD was associated with an approximately twofold increased risk of death from CVD as well as a predisposition to T2DM⁵. NAFLD increases the risk of CVD and T2DM because in individuals with NAFLD and the metabolic syndrome, insulin is unable to normally suppress production of glucose and VLDL, leading to hyperglycaemia and atherogenic dyslipidaemia (characterized by high serum triglyceride levels, low HDL cholesterol levels and increased small dense LDL cholesterol levels)⁴. Given that SFAs increase concentrations of LDL cholesterol¹²¹, and that substitution of polyunsaturated fat with saturated fat reduces cardiovascular and all-cause mortality^{122,123}, it is likely that SFAs are particularly harmful in patients with NAFLD. Notably, the American Heart Association and American College of Cardiology guidelines recommend a dietary pattern that achieves 5–6% of energy from saturated fat for reduction of CVD risk in individuals with elevated LDL cholesterol levels¹²¹.

Insulin resistance is perhaps the most important risk factor for CVD in patients with NAFLD, with effects that are mediated independently of the concentration of LDL cholesterol^{124–127}. Changes in insulin sensitivity observed in studies comparing low-fat–high-carbohydrate and high-fat–low-carbohydrate diets are shown in TABLE 1, and those in studies comparing fat quality are highlighted in TABLE 2. However, it is important to note that these studies focused on measuring the effect of change in macronutrient composition on IHTG rather than insulin sensitivity, and the latter was mostly assessed by measuring fasting insulin concentrations. Therefore, perhaps unsurprisingly, no clear conclusions can be drawn from the studies manipulating carbohydrate and fat quantity (TABLE 1), and the majority of studies showed no changes in insulin sensitivity associated with the different diets. However, interestingly, the two longer (11 weeks¹⁰⁶ and 6 months¹⁰⁸) hypoenergetic interventions suggested a worsening of insulin sensitivities with both macronutrient interventions. The studies measuring the effect of fat quality support the view that saturated fat impairs insulin sensitivity whereas diets high in MUFAs enhance insulin sensitivity, a conclusion that is also supported by a fairly large study ($n = 162$) that measured insulin sensitivity, although not IHTG content¹²⁸.

Although IHTG positively correlates closely with insulin resistance^{80,82,129–132}, NAFL can exist without features of insulin resistance, for example in individuals with familial hypobetalipoproteinaemia¹³³, or in those carrying the common patatin-like phospholipase domain-containing protein 3 (PNPLA3) I148M variant¹³⁴. These findings in humans and in numerous animal models¹³⁵ suggest that high IHTG levels are not sufficient to cause insulin resistance¹³⁶. Rather than

IHTG per se, accumulation of bioactive fatty acid metabolites, such as ceramides and diacylglycerols (DAGs), has been suggested to mediate insulin resistance^{137,138}. In 2018 and 2019, as discussed below, two overfeeding studies in humans identified ceramides as potential mediators of insulin resistance induced by saturated fat^{105,117}. Furthermore, multiple prospective studies have shown that circulating concentrations of ceramides predict CVD, independent of classic risk factors such as LDL cholesterol level^{139–141}. High circulating ceramide concentrations are also predictors of prediabetes¹⁴², T2DM¹⁴⁰ and NASH in humans¹⁴³.

Ceramides. De novo synthesis of ceramides begins with condensation of palmitoyl-CoA and serine (FIG. 3). Later, a second fatty acyl chain is added by specific isoforms of ceramide synthase, and finally a double bond is added by dihydroceramide desaturase¹³⁸. Consistent with palmitoyl-CoA being the first precursor in the synthesis of ceramides, studies using lipid infusions in rats and cell cultures have found that ceramides increase in response to insulin resistance induced by saturated but not unsaturated fatty acids^{144,145}.

In experimental studies, ceramides have been shown to cause NAFL and insulin resistance via several mechanisms. In the mouse liver, ceramides impair insulin signalling by decreasing insulin-induced phosphorylation of AKT¹⁴⁶. In addition, ceramides increase hepatic mitochondrial acetyl-CoA concentrations, which can allosterically activate pyruvate carboxylase and thereby stimulate gluconeogenesis¹⁴⁷. Through direct interaction with protein kinase C ζ (PKC ζ), ceramides induce translocation of the lipid transport protein CD36 to the cell membrane, stimulating fatty acid uptake^{148,149}. Ceramides promote hepatic lipid synthesis by upregulating sterol regulatory element-binding protein 1 (SREBP1) and target genes such as those encoding diglyceride acyltransferase 1 (DGAT1) and DGAT2 (REF.¹⁴⁶). Simultaneously, ceramides impair fatty acid oxidation by inhibiting mitochondrial citrate synthase and electron transport chain activities^{147,148,150} (FIG. 3). In addition, a specific ceramide species with a saturated fatty acyl chain, C16:0 ceramide, synthesized by ceramide synthase 6 might induce mitochondrial fission and impair hepatic capacity to oxidize fatty acids in mice¹⁵¹. By impairing mitochondrial electron transport activities, ceramides can stimulate generation of reactive oxygen species in cultured hepatocytes¹⁵², which characterizes progression from steatosis to NASH¹⁵³. In addition, ceramides can increase the permeability of mitochondrial membranes and the release of cytochrome *c* from mitochondria into the cytosol in isolated rat mitochondria¹⁵⁴, which triggers caspase-induced apoptosis¹⁵⁵.

In a cross-sectional study investigating the liver lipid composition in 125 individuals with obesity and covering a spectrum from normal liver histology to various stages of NAFLD (20% with NASH), levels of almost all (14 out of 17) ceramide species were higher in insulin-resistant than insulin-sensitive individuals¹⁵⁶. Moreover, hepatic concentrations of SFAs and dihydroceramides (substrates and intermediates in the de novo ceramide synthetic pathway) were markedly increased in the

insulin-resistant compared with the insulin-sensitive human livers¹⁵⁶. Consistent with the findings in mouse studies, levels of the ceramide species with saturated fatty acyl chains, specifically C16:0 and C18:0 ceramides, correlated strongly with insulin resistance¹⁵⁶. In another study in 28 individuals with various degrees of NAFLD, hepatic ceramide levels were higher in insulin-resistant

individuals with NASH than in other groups, and ceramide concentrations positively correlated with hepatic oxidative stress and inflammation as determined by liver thiobarbituric acid reactive substances and liver phosphorylated JNK to total JNK ratios, respectively¹⁵⁷. In two overfeeding studies in humans comparing the effects of overfeeding saturated and polyunsaturated fat and free

Fig. 3 | Metabolic effects of excessive intakes of saturated fat and free sugars. Saturated fat intake increases saturated non-esterified (free) fatty acids in the circulation. Saturated fat intake can induce endotoxaemia, which promotes adipose tissue inflammation and lipolysis, thereby further increasing hepatic non-esterified fatty acid supply. In the liver, saturated fatty acyl-CoA can enter the mitochondria for β-oxidation (step 1) to acetyl-CoA, which can enter the TCA cycle. Alternatively, saturated fatty acyl-CoA can enter the Kennedy pathway (step 2), where it is metabolized into sn-1,2-diacylglycerols (sn-1,2-DAGs). sn-1,2-DAGs induce protein kinase Cε (PKCε) translocation from cytosol to the plasma membrane, which inhibits insulin signalling at the level of the insulin receptor¹³⁷. This process decreases activation of protein kinase AKT2 and subsequent glycogen synthesis. sn-1,2-DAGs are also precursors of triglycerides (Tgs), which can be stored in hepatic lipid droplets or hydrolysed and re-esterified in the endoplasmic reticulum as VLDL-Tg for secretion. Saturated fatty acyl-CoAs, particularly palmitoyl-CoA, can also enter the de novo ceramide synthetic pathway

(step 3). Ceramides can impair mitochondrial metabolism by promoting mitochondrial fission mediated by mitochondrial fission factor (MFF) as well as via inhibition of mitochondrial citrate synthase and electron transport activities¹⁴⁶. This decrease in mitochondrial metabolism may promote gluconeogenesis via accumulation of acetyl coenzyme A (acetyl-CoA) and its allosteric activation of pyruvate carboxylase. Ceramides can also stimulate the Kennedy pathway via upregulation of sterol regulatory element-binding protein 1 (SREBP1) and fatty acid uptake by PKCζ-mediated stimulation of the lipid transport protein CD36. In addition, ceramides can inhibit distal insulin signalling via protein phosphatase 2 (PP2A)-mediated inhibition of AKT2 (REF. 138). Excessive intake of free sugars can stimulate de novo lipogenesis (DNL) (step 4), which exclusively produces saturated fatty acids. An intermediate in the DNL pathway, malonyl-CoA, inhibits mitochondrial fatty acid uptake, thereby limiting β-oxidation. The orange boxes denote upregulation, and the dark green boxes denote downregulation. Dashed lines with a flat end denote inhibition, dashed lines with an arrow denote stimulation, and solid lines with an arrow denote substrate flux.

sugars (TABLE 2), saturated fat increased IHTGs and plasma ceramides more than polyunsaturated fat^{105,117} or free sugars¹⁰⁵ in the face of similar energy excess¹⁰⁵. The saturated fat diet was also the only diet to induce insulin resistance¹⁰⁵. In a study of 88 histologically-characterized adults with normal liver histology (35%), simple steatosis (19%), NASH (23%) and cirrhosis (23%), dihydroceramides and ceramides both in the liver and in plasma discriminated individuals with steatosis from those with NASH¹⁴³. Taken together, these data from experimental animal models and humans support the view that ceramides are important mediators of saturated fat-induced insulin resistance and NAFLD.

Diacylglycerols. DAGs, the immediate precursors of triglycerides, are a class of bioactive lipids that evidence suggests also mediate insulin resistance (FIG. 3). In the rat fatty liver, DAGs activate protein kinase C ϵ (PKC ϵ), which is associated with decreased activation of insulin receptor tyrosine kinase^{158,159}, resulting in reduced hepatic glycogen synthesis. This mechanism mainly impairs the direct insulin action of stimulating hepatic glycogen synthesis¹⁶⁰. The specific molecular mechanism underlying the PKC ϵ -mediated inhibition of insulin receptor tyrosine kinase is through phosphorylation of the receptor at Thr1160 as demonstrated in mice¹⁶¹. Deletion of *Prkce* and inactivation of this phosphorylation site both protect mice from high-fat diet-induced insulin resistance^{161,162}.

In humans, hepatic DAG concentrations have been repeatedly shown to correlate with steatosis and insulin resistance^{95,156,160,163–166}, but there are no data on the effect of various diets on circulating or other DAGs in humans. To the best of our knowledge, there are no studies examining whether circulating DAGs predict the risk of CVD.

Although the causality between insulin resistance and both ceramides and DAGs has been well established in mice and rats, the currently available human data are mostly correlative. More studies are needed to establish causality between insulin resistance and these lipids in humans.

Endotoxaemia. Conditions associated with insulin resistance, such as NAFLD, are often characterized by a chronic low-grade inflammation^{167,168}. One potential cause of this inflammation is the gut microbiome, which is a rich source of inflammatory mediators, such as endotoxin¹⁶⁹. Acute administration of a small intravenous bolus of endotoxin increases systemic and adipose tissue inflammation and lipolysis, and induced insulin resistance in healthy humans ($n = 20$)¹⁷⁰. In large cross-sectional studies in humans, endotoxaemia has been found to be positively correlated with both insulin resistance ($n = 1,347$)¹⁷¹ and histological severity of NAFLD ($n = 237$)¹⁷². A potential mechanism by which endotoxin could mediate liver injury is Kupffer cell activation, as is well-recognized in experimental alcohol-related liver injury¹⁷³.

In mice, high-fat feeding increases the proportion of endotoxin-containing gut bacteria and induces endotoxaemia, inflammation, insulin resistance and hepatic

steatosis¹⁷⁴. These changes are induced by saturated but not polyunsaturated fat feeding¹⁷⁵, and are attenuated after antibiotic treatment¹⁷⁶, suggesting that saturated fat intake, gut microbiota and metabolic inflammation are causally linked. Both a study investigating the effects of a meal enriched with butter¹⁷⁷ and a study comparing the effects of saturated fat as cream with those of an isocaloric amount of orange juice¹⁷⁸ showed that high intakes of saturated fat induce endotoxaemia in humans. A single dose of palm oil (49% saturated fat) but not water impaired whole-body, hepatic and adipose tissue insulin sensitivity in humans and upregulated hepatic inflammation in mice¹⁷⁹. In agreement with these data, in a study in humans comparing the effects of over-consumption of either saturated or unsaturated fat or free sugars for 3 weeks in 38 healthy adults with overweight, only the saturated fat-enriched diet induced circulating endotoxaemia, adipose tissue inflammation and insulin resistance¹⁰⁵. In addition to saturated fat, fructose intake has been shown to increase bacterial endotoxin concentrations and markers of liver injury in rodents¹⁸⁰, non-human primates¹⁸¹ and humans^{182,183}. Interestingly, in a study in humans comparing isocaloric fructose-enriched and glucose-enriched diets, only the fructose-enriched diet was associated with increased endotoxaemia and alanine aminotransferase activities¹⁸³. Endotoxin is found in chylomicrons and levels increase in response to a meal¹⁸⁴. Chylomicrons contain more endotoxin in insulin-resistant individuals with obesity than in insulin-sensitive lean individuals¹⁸⁴. Endotoxaemia might be a consequence of increased fat absorption rather than altered gut permeability, which is another means for endotoxin to enter the circulation from the intestinal lumen. In support of this possibility, 5 days of an isocaloric high-fat (55% fat) diet, compared with a control diet containing 30% fat, increased fasting endotoxin in 13 young men (BMI 23 ± 1 kg/m², age 22 ± 1 years) without altering intestinal permeability, as measured via a four-sugar probe test¹⁸⁵.

Monounsaturated fatty acids, the Mediterranean diet and IHTG. The Mediterranean diet is currently recommended for patients with NAFLD by the EASD, EASO and EASL guidelines¹⁷. Primarily a plant-based diet, the Mediterranean diet contains high ratios of MUFAs and PUFAs, including ω -3 fatty acids, relative to SFAs. Typically high in MUFAs from the consumption of olive oil, nuts and seeds, the Mediterranean diet is also rich in vegetables and legumes, fruits, whole grains, fish and seafood but low in dairy and red and processed meat products¹⁸⁶. The fibre content is twice the current average US fibre intake¹⁸⁷.

Studies comparing a MUFA-enriched (namely, high intakes of olive oil and nuts) or the Mediterranean diet with diets that were lower in total fat and higher in carbohydrates are summarized in TABLE 2 and FIG. 2 (right). In three small isoenergetic intervention studies of 6–12 weeks, which included 45 (REF.¹⁸⁸), 28 (REF.¹⁸⁹) and 12 (REF.³⁰) individuals, the MUFA or Mediterranean diets decreased IHTG more than a standard diet, despite containing more fat than the control diets^{30,120,189}. In a longer and larger intervention (18 months, 278 individuals

with abdominal obesity or dyslipidaemia, 53% with NAFLD), a hypoenergetic Mediterranean–low-carbohydrate (MED–LC) diet was compared with a hypoenergetic low-fat control diet^{190,191}. Weight loss was of similar magnitude across the groups and averaged approximately 3 kg at 18 months compared with baseline. Individuals in both dietary groups had reduced IHTG levels compared with baseline, with those in the MED–LC group losing slightly more in absolute units (MED–LC diet $-4.2 \pm 7.1\%$, low-fat diet $-3.8 \pm 6.7\%$; $P = 0.036$)¹⁹¹. Notably, the MED–LC diet decreased waist circumference by approximately 2 cm more than the low-fat diet and improved cardiometabolic parameters to a greater extent¹⁹⁰. In a study by Properzi and colleagues¹⁹², 51 individuals with NAFLD on a Mediterranean diet had statistically significant improvements in several cardiometabolic risk factors, including blood lipids and glycated haemoglobin (HbA_{1c}), compared with those on a low-fat diet, resulting in similar decreases in body weight. Changes in IHTG are difficult to compare as baseline IHTG levels were far greater in the Mediterranean diet group than the low-fat diet group. Taken together, these studies suggest that saturated fat raises IHTG to a greater degree than polyunsaturated or monounsaturated fats or a Mediterranean diet.

Sugar quality and IHTG

Given that excess consumption of free sugars is strongly associated with NAFLD²³, a pertinent question is whether there are differential effects on IHTG elicited by different sugars, such as the disaccharides sucrose and lactose, and the monosaccharides fructose and glucose, found most commonly in the diet. Added sugars, including high-fructose corn syrup and sucrose, contain roughly equal proportions of glucose and fructose¹⁹³.

The role of fructose in metabolic health has been scrutinized, in part because in humans the pathways and tissues that metabolize glucose and fructose differ^{23,194,195}. Glucose is transported across the intestinal epithelium via the SGLT1 transporter, whereas fructose uses the GLUT5 transporter (FIG. 4). In hepatocytes, fructose bypasses the rate-limiting step of glycolysis catalysed by phosphofructokinase (reviewed previously^{79,196}), thereby potentially providing more substrate to the DNL pathway and IHTG than does glucose¹⁹⁶. Giving similar doses of dietary glucose and fructose to humans results in a tenfold lower increase in the concentration of circulating fructose than glucose, implying greater retention of fructose than glucose by the splanchnic bed¹⁹⁷. Work in mice has shown that the small intestine converts low doses of fructose almost entirely into glucose, lactate and glycerate¹⁹⁸. However, these data suggest that at higher fructose doses, metabolism within the mouse small intestine is not fully achieved, leading to delivery of non-metabolized fructose to both the liver and colon¹⁹⁸. Work in humans has suggested that fructose can induce DNL in the intestine and that about 15% of a 30-g fructose dose escapes extraction by the liver and gut¹⁹⁹. Thus, it is possible that fructose has roles in other metabolic pathways that influence IHTG content¹⁹⁷.

Fructose versus glucose or other carbohydrate intervention studies. Although the lipogenic effect of glucose is acutely exacerbated by fructose⁶⁰, human intervention studies comparing the effects of glucose and fructose on IHTGs have not documented differences. The trials performed in healthy individuals^{200–205} have been summarized in Supplementary Table 1. All were short, lasting a maximum of 10 weeks, thereby preventing conclusions on the differential effect of high fructose consumption in the long term. Two trials were isoenergetic^{200,201}, exchanging fructose for other carbohydrates, whereas the other trials were hyperenergetic^{202–205}, comparing the addition of fructose to other sugars. Notably, in only one study, in which fructose was overfed for 6–7 days, was fructose found to increase IHTG more than glucose²⁰⁵.

Although not observed in these studies that quantified IHTG as an outcome (Supplementary Table 1), isoenergetic fructose compared with glucose (25% of total daily energetic intake) consumption for 10 weeks was found to impair insulin sensitivity in 32 individuals with overweight or obesity²⁰⁶. In addition, it was shown that 75 g fructose per day for 12 weeks increased IHTG and homeostatic model assessment of insulin resistance (HOMA-IR) in 71 men with abdominal obesity²⁰⁷. Individuals also gained on average 1.1 kg, so it is not clear whether the increases in IHTG and HOMA-IR were due to the increase in fructose intake or weight gain. Also, there was no comparator arm. Taken together, these human data are insufficient to justify conclusions regarding the differential effects of fructose and high-fructose corn syrup compared with glucose or sucrose consumption on NAFLD.

Many of the studies comparing the effects of glucose and fructose on IHTG were hypercaloric feeding studies in which participants consumed excess amounts of a particular sugar over a short time period. Although the aim was to understand the effects of the specific monosaccharides, it is important to emphasize that fructose and glucose are not typically consumed in isolation. These monosaccharides are usually co-ingested in foods and beverages, making the studies challenging to translate to ‘real life’. Longer-term isocaloric feeding studies with amounts of monosaccharides that are typically consumed would be of interest. Monosaccharide intakes are not routinely reported in national dietary surveys, which vary from country to country as to whether they report total, free or added sugars. However, fructose intakes in US adults (aged 19–80 years, $n = 17,749$ from the NHANES 1999–2006 databases) were estimated by Sun and colleagues to be 48 g per day²⁰⁸. This was approximately 37% of total sugar consumption, which was at the time ~129 g per day. From 2003 to 2016, total sugar intake decreased by 30% and total sugar intakes from SSBs (soft drinks, sports drinks, energy drinks and fruit drinks) by 46% in adults²⁰⁹. The 2015–2016 NHANES data show that total sugar intake expressed as the percentage of daily energy intake in the US has declined by 17% to 107 g per day²⁰⁹. This would suggest an average fructose intake of ~40 g per day, underscoring how high the amounts of single monosaccharides used in the studies examining the effects of glucose and fructose on IHTG have been.

As has been previously observed, although differential effects of specific monosaccharides on body weight and health might not yet be clear, in the context of the obesity and NAFLD epidemics, reducing dietary sugar consumption is a prudent public health message^{23,194}. This reduction is important not only for adults but also for children and adolescents, who consume, at least in the USA, threefold more added sugars than recommended

by the American Heart Association (19 versus 6 teaspoons per day)²¹⁰. Notably, in an open randomized trial in 40 adolescent boys (aged 11 to 16 years) with histologically-verified NAFLD, significant ($P < 0.001$) improvement in steatosis was observed in response to strict restriction of intake of free sugar (to $< 3\%$ of total energy) for 8 weeks²¹¹. This effect was enabled by individualized menu planning and provision of study meals

Fig. 4 | Sugar metabolism and regulation. The digestive enzyme sucrase hydrolyses the disaccharide sucrose (table sugar) into its constitutive monosaccharide subunits, glucose and fructose, also found in high-fructose corn syrup, sugar-sweetened beverages and fruit juices. Glucose and fructose are transported at the apical enterocyte membrane by the sodium-dependent glucose cotransporter 1 (SGLT1) and the fructose transporter GLUT5, respectively. At low intakes fructose is almost completely metabolized by the enterocytes to glucose, lactate, glycerate and other amino and organic acids¹⁹⁸. On the other hand, high intakes of fructose saturate the intestinal clearance capacity, with fructose passing to the liver as well as the colonic microbiota and excreted in faeces. Glucose and fructose are transported into hepatocytes by the insulin-independent transporter GLUT2. In contrast to glucose, without inhibitory feedback fructose is first rapidly phosphorylated by fructokinase to fructose 1-phosphate (F-1P), then split into trioses by the activity of aldolase prior to converging with glucose

metabolism. Insulin and glucose, via sterol regulatory element-binding protein 1 (SREBP1) and carbohydrate-responsive element-binding protein (ChREBP), promote lipogenic gene expression of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD) to encourage de novo lipogenesis (DNL). DNL intermediates, such as malonyl-CoA, inhibit β -oxidation (β -ox), further promoting DNL and intrahepatic triglyceride accumulation from both fructose and glucose. CPT, carnitine palmitoyltransferase; DHAP, dihydroxyacetone phosphate; F-1,6bP, fructose 1,6-bisphosphate; F-6P, fructose 6-phosphate; FA, fatty acid; FBpase, fructose-1,6-bisphosphatase; G-1P, glucose 1-phosphate; G-6P, glucose 6-phosphate; G3P, glycerol 3-phosphate; G6Pase, glucose 6-phosphatase; GA, glyceraldehyde; GAP, glyceraldehyde 3-phosphate; GCK, glucokinase; GCKR, glucokinase regulatory protein; GS, glycogen synthase; KHK, ketohexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; TAG, triacylglycerol; TCA, tricarboxylic acid.

Box 1 | Unanswered questions and research needs

Unanswered questions

- Long-term effects of varying macronutrient composition on nonalcoholic fatty liver disease (NAFLD) and cardiovascular risk factors in the absence of changes in body weight
- Long-term effects of fat quality on NAFLD and cardiovascular risk factors
- Long-term effects of low sugar diets and/or low refined carbohydrate diets on NAFLD and cardiovascular risk

Research design

- Adequately powered multicentre studies using isoenergetic low-fat-high carbohydrate versus high-fat-low-carbohydrate diets lasting months rather than weeks, utilizing non-invasive imaging tools to quantify intrahepatic triglyceride (IHTG) levels and fibrosis, and also assessing markers of cardiometabolic risk
- Prospective long-term cohort studies addressing changes in IHTG and markers of cardiovascular disease risk varying fat quality in the face of unaltered macronutrient composition
- Prospective long-term cohort studies addressing changes in IHTG and markers of cardiovascular disease risk when reducing sugar and refined carbohydrates in the face of unaltered macronutrient composition

for the entire household in the intervention group (who lost an average of 1.4 kg body weight) but not the control group which was not instructed to restrict sugar intake (who gained an average of 0.6 kg). Although implementation of this study design in the real world might not be feasible, it is an important proof-of-concept study. Smajis et al. assessed the effects of a diet very high in fructose (150 g per day for 8 weeks) in ten lean (BMI 22.2 kg/m²) healthy adults, and found that IHTG content remained unchanged, along with other metabolic parameters including hepatic glycogen and markers of insulin sensitivity²¹². These data suggest that lean individuals can at least temporarily compensate for increased fructose intake. In men with abdominal obesity, overfeeding fructose 75 g per day for 12 weeks did increase IHTG by 10% and the individuals developed insulin resistance and mild hypertriglyceridaemia²⁰⁷. However, whether the latter changes were a consequence of fructose or weight gain remains unclear.

Conclusions

The epidemic of NAFLD has correlated with increased energy intakes, especially in the form of added sugars. Reducing energy intakes effectively reverses steatosis,

inflammation and fibrosis in direct proportion to weight loss²⁸. Studies comparing the effects of macronutrient composition as well as those comparing the effects of fat quality on NAFLD are restricted to measurement of changes in IHTG levels. Although studies to date have been small and mostly of short duration, they have been carefully controlled and performed in clinical research units. Despite their limitations, these data seem fairly consistent. Namely, hyperenergetic high-fat-low-carbohydrate diets increase IHTG more than equally hypercaloric low-fat-high-carbohydrate diets in individuals with and without NAFLD. From the available evidence, it would appear that the effect of monounsaturated fat on IHTG is minimal and the effects would most likely be attributable to high intake of saturated fat. Saturated fat-enriched diets increase IHTG more than PUFA-enriched or MUFA-enriched diets, and Mediterranean diets seem to be beneficial in NAFLD.

Ceramides are formed from saturated fat and might contribute to the deleterious effects of high SFA diets on IHTG and insulin resistance. Hyperenergetic high-carbohydrate diets also increase IHTG content, but perhaps less so than an equal amount of excess energy derived from saturated fat. Although the metabolism of fructose is predicted to have more harmful effects than glucose on the liver, the available intervention trials in humans have shown no differential effects, with both glucose and fructose increasing IHTG in the context of excess energy. Taken together, these data support the use of diets that have reduced amounts of sugars, refined carbohydrates and saturated fats. Although hypoenergetic diets will certainly reduce IHTG, isoenergetic Mediterranean diets with increased MUFAs and PUFAs derived from plant-based sources can also be beneficial for both reducing IHTG and improving cardiometabolic risk factors. Given the high prevalence of NAFLD and its metabolic complications in the form of CVD and T2DM, there is an urgent need for large multicentre studies with sufficient numbers of patients specifically with NAFLD and of sufficient duration to establish the composition of a diet that can prevent or reverse these problems (see BOX 1 for unanswered questions and research needs).

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