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Title

Dietary carbohydrates and fats in nonalcoholic fatty liver disease

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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 poly–unsaturated or mono–unsaturated fats, with adverse effects on insulin sensitivity, which are likely mediated in parts 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.

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 (DNL), 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 worldwide, which is of concern to 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 endpoints.
- 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 likely 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.

Introduction

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 stage 3–4) and cirrhosis (fibrosis stage 4), is a complex phenotype[1]. The prevalence of NAFL, defined as steatosis in which at least 5–10% of hepatocytes exhibit macrovesicular steatosis, or the intrahepatic triglyceride content (IHTG) exceeds 5.5%, averages 25% worldwide[2]. 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 NASH[3]. 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 1000 person–years) than that of mortality from cardiovascular disease (CVD) (4.79) or overall mortality (15.44) according to a systematic review based on global data[2]. 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 1000 patient years)[2]. NAFLD is frequently associated with features of the metabolic syndrome and predicts, independent of obesity, CVD and 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, such as red meat[6], vitamin D[7], vitamin E[8], probiotics, prebiotics and synbiotics[9,10], omega–3 fatty acids[11], coffee[12,13] and alcohol[14] 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 microbiome[1]. The heterogeneity of NAFLD presentation and progression, as well as its close relationship with metabolic dysfunction, has 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 include 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 challenging[20]. 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 gain[21]. During 20 years of follow–up of the 120,877 women and men involved in the Nurses Health and the Health Professionals Follow–up studies in the USA, increased intakes of potato chips, potatoes and sugar–sweetened beverages (SSBs) were, independent of confounders, the top three predictors of weight gain[22]. 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), which are implicated in the development and progression of NAFLD and other chronic metabolic diseases[23].

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 US, 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 cardiovascular disease[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–carb 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 (n=293)[28] and one small (n=31) study[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 endpoints in NASH are virtually nonexistent. Therefore, the American Association for the Study of Liver Diseases (AASLD) could not provide any recommendations for macronutrient intake in NAFLD, and instead called for rigorous, large, prospective, longer–term trials with histopathological endpoints before recommendations could be made[18]. 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 randomised trial that measured changes in IHTG content in 12 patients with NAFLD[30], has suggested that a Mediterranean diet might be the diet of choice for NAFLD[17]. 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 elastography[31]. Thus, although there is consensus that weight loss through diet and lifestyle[32] or clinical[33] 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 helpful in the prevention of NAFLD progression.

Furthermore, as CVD is the main cause of death in patients with NASH[36], 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 men (Supplementary Fig. 1a)[21]. 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) (Supplementary Fig. 1a)[21]. However, although the percentage of energy consumed from fat in the USA reduced slightly in the 1990s, it has been increasing since 2010, and was 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 sexes[37], which is not particularly low–fat (Fig 1b). Of concern to 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% (Supplementary Fig. 1b)[37].

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

nations, the per capita supply of energy has increased dramatically in the past 50 years[40]. In the USA, although increased energy supply has indeed come in part from carbohydrates, there has also been a marked increase in per capita supply of energy from fat (Supplementary Fig. 1c)[40,41] .

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 of varying sizes, ethnicities and age groups. Early studies in Italian[42], Japanese[43], Israeli[44] and US[45] populations found increased consumption of meat and SSBs, as well as low consumption of fish, to be associated with NAFLD cases. In 2018 and 2019, larger and multi–ethnic population studies have confirmed the association between high consumption of red and processed meats with NAFLD[46,47]. For example, in a nested case–control study within the Multi–Ethnic Cohort study of older (45–75 years) US adults, 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 controls[47]. 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 cirrhosis[47]. By contrast, in a larger cohort study of Chinese adults (n=4,365), patients with NAFLD (diagnosed by ultrasonography) consumed a diet higher in carbohydrates and free sugars than non–NAFLD participants[48].

Multiple studies suggest a relationship between Western dietary patterns, which are typically characterized by high intakes of red 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 ages 14 and 17 (n=995), a Western dietary pattern characterized by high intakes of SSBs, confectionary, takeaway foods, sauces and dressings at age 14 was associated with an increased incidence of NAFLD (assessed by ultrasonography) at 17 years of age[49]. The relationship was independent of sex, physical activity and sedentary behavior, but not of body mass index (BMI)[49]. 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 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 risk of incidence and severity of fatty liver[51]. Similarly, in a cross–sectional analysis of two large adult cohorts (England, 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 and 95% CI were 0.86 (0.81, 0.90) and 0.82 (0.78, 0.86) for English and Swiss cohorts). 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 Mediterranean diet explained the reduced risk[52].

The majority of studies have found excess SSB consumption to be linked to NAFLD, with multiple meta–analyses finding a positive statistically significant association between SSB consumption and risk of NAFLD[12,53,54]. In 2019, Chen and colleagues[54] 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, P = 0.03, respectively). Thus, for 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 saturated fatty acids 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 fat (Fig. 2)[57]. Fatty acids derived from adipose tissue lipolysis are the source of the majority of fatty acids used for triglyceride synthesis in the liver[58]. This observation is true both in the fasting and postprandial state, despite postprandial insulin–mediated suppression of adipose tissue lipolysis[57]. 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. 2). Fatty acids that are not taken up by these tissues spillover into the

systemic circulation and can be taken up by the liver and used for triglyceride synthesis[57]. 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 sources[59]. 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% fat[57]. 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 pathway[60].

De novo lipogenesis. In humans, DNL occurs predominantly in the liver, and palmitate (a 16–carbon saturated fatty acid) is the end product[61,62]. However, DNL is not the pathway of first resort for disposal of dietary carbohydrates in humans[56]. In healthy individuals, DNL produces 1–2 grams of fat per day, which is minor compared with 50–100 grams of dietary fat consumed in UK adults[62]. However, during carbohydrate overfeeding, fractional DNL, which is the contribution of DNL to very–low–density lipoprotein (VLDL)–triglyceride, will be stimulated[63]. Nonetheless, even after 5 days of consuming 50% more energy from carbohydrates than baseline intakes it was found in 6 healthy adults that DNL contributed <5 grams of fat to VLDL–triglyceride secretion[63]. 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 colleagues[64], when DNL is upregulated, malonyl–CoA, an intermediate in the DNL pathway, potently inhibits carnitine palmitoyltransferase 1, which leads to a suppression of fatty acid oxidation. Thus, an 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 NAFLD[61], 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% reported by different studies for individuals without NALFD[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 hours prior to the assessment[71–74]. In a study by Smith and colleagues, which reported that individuals with obesity and NAFLD (n=27) had the greatest contribution of DNL–derived fatty acids (specifically, percentage contribution of DNL to palmitate in triglyceride–rich lipoproteins), at 38% compared with individuals with obesity but without NALFD (n=26, DNL 19%) and lean individuals (n=14, DNL 11%), deuterated water was administered for 3–5 weeks[61].

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–hour plasma glucose and insulin concentrations[61]. 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 palmitoyl–transferase I[64]. This was demonstrated in 19 healthy male participants supplemented with omega–3 fatty acids (4 g per day) for 8 weeks. Compared with baseline, fasting and post–prandial hepatic DNL decreased

significantly (P<0.05) and there was a significant increase in fatty acid oxidation[75]. Several pharmacological inhibitors of DNL, including MK–4074[76], GS–0976[77] and NDI–010976[78], which all inhibit acetyl–coenzyme A carboxylase (ACC), are currently being tested in humans and have been reviewed previously[79].

Lipolysis. Multiple studies have documented 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 their age– and gender–matched controls is less certain. Lipolysis, which is the rate of free fatty acid appearance into the systemic circulation, increases in direct proportion to fat mass[83]. Interestingly, a study of adolescent girls with equivalent BMI and with either lower IHTG and visceral fat (n=7, BMI 36.6kg/m^2) or higher IHTG and visceral fat (n=8, BMI 36.0kg/m^2) found that the group with increased IHTG and visceral fat had higher rates of adipose tissue triglyceride turnover (representing both lipolysis and synthesis at steady state), measured using a novel stable isotope method, compared with the group with group with lower IHTG and visceral fat[84]. For both groups, the turnover rate (lipolysis and synthesis) of triglyceride in adipose tissue was correlated with IHTG content[84]. Therefore, contrary to the belief that ectopic fat deposition results from an inability to store triglycerides in adipose tissue, this human study suggests that the problem is reduced retention of free fatty acids in adipose tissue[84].

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 particles[85]. 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 production[86]. Individuals with NAFLD have been reported to have an overproduction of VLDL particles that contain

>40% more triglycerides compared with age– and BMI–matched individuals without NAFLD[87–89]. Although VLDL–triglyceride secretion correlates positively with IHTG[87], there might be a plateau beyond 10% IHTG[88]. 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 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 VLDL1–triglyceride production rate compared with a lower sugar diet[89]. Consumption of a high monounsaturated fat diet (13.7% total energy) did not alter VLDL1 production rates compared with consumption of a low monounsaturated fat diet (7.8% total energy) for 6 weeks in 17 moderately hypercholesterolemic, middle–aged (mean 55 years) adults[90].

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 ketogenesis[85]. Blood levels of the ketone body 3–hydroxybutyrate (3OHB) are often used as a surrogate marker of hepatic fatty acid oxidation[91–93]. Data on plasma 3OHB concentrations in individuals with and without NAFLD are inconsistent. Levels have been reported as being decreased[94], similar[95,96] or increased[97]. A limited number of studies have assessed mitochondrial oxidation using stable isotope tracers. Again, results have varied. For example, Sunny and colleagues[96] found fasting mitochondrial oxidation to be twice as high in individuals with NAFLD (n=8, 17% IHTG) compared with those without NAFLD

(3% IHTG, n=8), whereas others have reported similar rates between individuals classified as having high (n=4, ~9%) and low (n=4, ~2%) IHTG[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 (75% carbohydrate and 10% fat) 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 of 8 healthy individuals measured postprandial blood 3OHB concentrations and expired $^{13}CO_2$ levels as a marker of whole–body fatty acid oxidation. Although it is often suggested, based on evidence from rodent studies, that dietary polyunsaturated FAs (PUFAs) preferentially enter oxidation pathways compared to saturated fatty acids[100,101], data in humans are sparse. Two small studies (n=6[102] and n=4[103]) that used metabolic tracers and measured expired $^{13}CO_2$ suggested a greater oxidation of mono–unsaturated and poly–unsaturated fatty acids compared with saturated fatty acids in healthy male participants. By using metabolic tracers and measuring expired $^{13}CO_2$, it was reported in healthy adults (n=12 male and 12 female participants) that oxidation of dietary linoleate was significantly ($P<0.05$) greater than dietary palmitate[104].

Macronutrient composition and IHTG

Several trials have examined IHTG response to dietary macronutrient

manipulation. These are important to evaluate in relation to the dietary energy change elicited by the intervention. The trials have been heterogeneous in type and length of intervention and are summarized in Table 1. Three trials intervened with hypoenergetic (reduced calorie content) diets, three used isoenergetic diets, and three interventions (two in one trial[105]) were hyperenergetic; these are discussed in detail in this section. In Fig. 2A, 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 grams of carbohydrate per day) diet has been shown to decrease IHTG by 30–45% in less than a week in individuals with overweight or obesity[70,106]. Although a ketogenic low–carbohydrate diet decreased IHTG more than a standard hypoenergetic diet when measured after 2 days[106] or 2 weeks in 22 individuals with obesity, this difference was no longer observed after 11 weeks[107]. Similarly, in a longer (6 month) hypoenergetic intervention in 102 participants with overweight or obesity[108], there was no statistically significant difference in the reduction in IHTG between the low–fat (42% decrease in IHTG) and low–carbohydrate (47% decrease) groups.

Isoenergetic comparisons. Isoenergetic low–fat (16–23% of total energy), high–carbohydrate (57–65% of total energy) diets have been compared to 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 response to the low–fat/high–carbohydrate diet and increased during the high–fat/low–carbohydrate diet (Fig. 2A). 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 IHTG[112]. Notably, all three high–fat arms provided intakes of much higher saturated

and polyunsaturated fats than World Health Organization recommendations[113] (Table 1), which are <10% and 6–11% of total energy respectively, conditions that were met in the low–fat trial arms. 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 with 480 individuals that examined data from both magnetic resonance imaging and changes in liver enzymes as a marker of liver health in patients with or obesity with or without NAFLD reached the same conclusion[114]. 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 to low–fat–high–carbohydrate diets during overfeeding are listed in Table 1[105,115]. In these studies, overfeeding with saturated, 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 NAFLD[17]. This conclusion seems justified, although the fat quality (as measured by the percentage saturated, monounsaturated, and polyunsaturated fat of total energy) varied markedly between the treatment arms in the few studies that reported 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] (Table 2, Fig. 2B). Rosqvist and colleagues[118] compared hyperenergetic diets enriched with saturated

fatty acids (SFAs) at the expense of PUFAs with a diet rich in PUFAs but lower in SFAs in 39 lean individuals[118] and later in 60 individuals with overweight[117]. The excess energy was served in similar–looking muffins. In the studies, overfeeding SFAs, but not PUFAs, statistically significantly increased IHTG content 40% in lean individuals[118] and by 50% in individuals with obesity[117], which is in line with earlier work showing this in an isoenergetic intervention in individuals with obesity[116]. These data were extended by Luukkonen and colleagues[105] who, 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 diet[105]. PUFAs might be preferentially partitioned into oxidation pathways[103], 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 beta–hydroxybutyrate concentrations[120] have yielded inconsistent results.

Metabolic effects of saturated fat

In the majority of large observational longitudinal studies, NAFLD is associated with an approximately two–fold increased risk of death from CVD as well as predisposition to type 2 diabetes mellitus (T2DM)[5]. 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 triglycerides, low HDL cholesterol and increased small dense LDL cholesterol)[4]. Given that SFAs increase concentrations of LDL cholesterol[121], 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 for 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 levels[121].

Insulin resistance is perhaps the most important risk factor for CVD in patients with NAFLD, with effects that are mediated independent of LDL cholesterol[124–127]. Changes in insulin sensitivity observed in studies comparing low–fat–high–carbohydrate to 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 change in insulin sensitivity on the different diets. However, interestingly, the two longer (11 weeks[106] and 6 months[108]) 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 content[128].

Although IHTG positively correlates closely with insulin resistance[80,82,129–132], NAFL can exist without features of insulin resistance, such as in individuals with familial hypobetalipoproteinemia[133], or in those carrying the common patatin–like phospholipase domain–containing 3 (PNPLA3) I148M variant[134]. These findings in humans and in numerous animal models[135] suggest that high IHTG levels are not sufficient to cause insulin resistance[136]. 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 later, two overfeeding studies in humans have 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[139,140,141]. High circulating ceramide concentrations are also predictors of prediabetes[142], T2DM[140] and NASH in humans[143].

Ceramides. De novo synthesis of ceramides begins with condensation of palmitoyl coenzyme A (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 desaturase[138]. Consistent with palmitoyl–CoA being the first precursor in the synthesis of ceramides, studies using lipid infusions into rats and cell cultures have found ceramides to 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 signaling by decreasing insulin–induced phosphorylation of Akt[146]. In addition, ceramides increase hepatic mitochondrial acetyl–CoA concentrations, which can

allosterically activate pyruvate carboxylase and thereby stimulate gluconeogenesis[147]. Through direct interaction with protein kinase C zeta (PKCζ), 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 transcription factor 1 (SREBF1) and target genes such as those encoding diglyceride acyltransferase 1 and 2 (DGAT1 and DGAT2)[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 mice[151]. By impairing mitochondrial electron transport activities, ceramides can stimulate generation of reactive oxygen species in cultured hepatocytes [152], which characterizes progression from steatosis to NASH[153]. In addition, ceramides can increase the permeability of mitochondrial membranes and the release of cytochrome c from mitochondria into the cytosol in isolated rat mitochondria[154], which triggers caspase–induced apoptosis[155].

In a cross–sectional study investigating the liver lipid composition of 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 livers[156]. 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 livers[156]. Consistent with mouse studies, levels of the ceramide species with saturated fatty acyl chains, specifically C16:0– and C18:0–ceramides, correlated strongly with insulin resistance[156]. In

another study of 28 individuals with various degrees of NAFLD, hepatic ceramides were higher in insulin–resistant individuals with NASH than in other groups, and their concentration positively correlated with hepatic oxidative stress and inflammation as determined by liver thiobarbituric acid reactive substances and liver phosphorylated JNK–total JNK ratios, respectively[157]. In two overfeeding studies in humans comparing the effects of overfeeding saturated and polyunsaturated fat and free sugars (Table 2), saturated fat increased IHTGs and plasma ceramides more than polyunsaturated fat[105,117] or free sugars[105] in the face of similar energy excess[105]. The saturated fat diet was also the only diet to induce insulin resistance[105]. In a study of 88 histologically–characterized adults with liver histology ranging from normal (35%) to 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 NASH[143]. 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 epsilon (PKCε), 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 synthesis[160]. The specific molecular mechanism underlying the PKCε–mediated inhibition of insulin receptor tyrosine kinase is through phosphorylation of the receptor at Thr1160 as demonstrated in mice [161]. 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 diacylglycerols has been well established in mice and rats, the currently available human data is 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 endotoxin[169]. Acute administration of small intravenous bolus of endotoxin increases systemic and adipose tissue inflammation, lipolysis and induces insulin resistance in healthy humans (n=20)[170]. In large cross–sectional human studies , 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 injury[173].

In mice, high–fat feeding increases the proportion of endotoxin–containing gut bacteria and induces endotoxaemia, inflammation, insulin resistance and hepatic steatosis[174]. These changes are induced by saturated but not polyunsaturated fat feeding[175], and are attenuated after antibiotic treatment[176], suggesting that saturated fat intake, gut microbiota and metabolic inflammation are causally linked. A single high–saturated–

fat meal enriched with butter[177], or cream compared with an isocaloric amount of orange juice[178], induces 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 mice[179]. In agreement with these data, in a human study comparing the effects of overconsumption 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 resistance[105]. In addition to saturated fat, fructose intake has been shown to increase bacterial endotoxin concentrations and markers of liver injury in rodents[180], non–human primates[181] and humans[182,183]. Interestingly, in a human study comparing isocaloric fructose–enriched and glucose–enriched diets, only the fructose–enriched diet was associated with increased endotoxaemia and ALT activities[183]. Endotoxin is found in chylomicrons and increases in response to a meal[184]. Chylomicrons contain more endotoxin in insulin–resistant individuals with obesity than insulin–sensitive lean individuals[184]. 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, five 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/m^2, age 22±1 years) without altering intestinal permeability, as measured via a four–sugar probe test[185].

Monounsaturated fatty acids, the Mediterranean diet and IHTG. The Mediterranean diet is currently recommended for patients with NAFLD by the EASD, EASO, and EASL guidelines[186]. Primarily a plant–based diet, the Mediterranean diet contains a high ratio of MUFAs and PUFAs, including omega–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 products[187]. The fibre content is twice the current average US fibre intake[188].

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. 2B. In three small isoenergetic intervention studies of 6–12 weeks, which included 45[189], 28[190] and 12[30] individuals, the MUFA or Mediterranean diets decreased IHTG more than a standard diet, despite containing more fat than the control diets[30,120,190]. In a longer and larger (18 months; 278 individuals with abdominal obesity or dyslipidemia; 53% with NAFLD) intervention, a hypoenergetic Mediterranean–low–carbohydrate (MED–LC) diet was compared with a hypoenergetic low–fat control diet[191,192]. Weight loss was of similar magnitude across the groups and averaged approximately 3 kg at 18 months compared with baseline. Both dietary groups had reduced IHTG levels compared with baseline, with the MED–LC group losing slightly more in absolute units (MED–LC –4.2 ± 7.1% versus low–fat –3.8 ± 6.7%; P = 0.036)[192]. 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 extent[191]. In a study by Properzi and colleagues[193], 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 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 poly–unsaturated or mono–unsaturated fats or a Mediterranean diet.

Sugar quality and IHTG

Given that excess consumption of free sugars is strongly associated with NAFLD[23], 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 fructose[194].

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[23,195,196]. 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 catalyzed by phosphofructokinase (reviewed previously[79,197]), thereby potentially providing more substrate to the DNL pathway and IHTG than glucose does[197]. Giving similar doses of dietary glucose and fructose to humans results in a 10–fold lower increase in the concentration of circulating fructose than glucose, implying greater retention of fructose than glucose by the splanchnic bed[198]. Work in mice showed that the small intestine converted low doses of fructose almost entirely into glucose, lactate and glycerate[199]. 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 colon[199]. Work in humans has suggested that fructose can induce DNL in the intestine and that about 15% of a 30g fructose

dose escapes extraction by the liver and gut[200]. Thus, it is possible that fructose has roles in other metabolic pathways that influence IHTG content[198].

Fructose versus glucose or other carbohydrate intervention studies.

Although the lipogenic effect of glucose is acutely exacerbated by fructose[60], human intervention studies comparing the effects of glucose and fructose on IHTGs have not documented differences. The trials performed in healthy individuals have been summarized in Supplementary Table 1[201–206]. 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 [201,202], exchanging fructose for other carbohydrates, whereas the other trials were hyperenergetic [203–206], comparing the addition of fructose to other sugars. Notably, only one study, in which fructose was overfed for 6–7 days, found fructose to increase IHTG more than glucose[206].

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 has previously been shown to impair insulin sensitivity in 32 individuals with overweight or obesity[207]. In addition, it was shown that 75g fructose per day for 12 weeks increased IHTG and homeostatic model assessment of insulin resistance (HOMA–IR) in a 71 men with abdominal obesity[208]. 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 are hypercaloric feeding studies in which participants consume excess amounts of a particular sugar over a short time period. Although the aim is 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 country to country on whether they report total, free or added sugars. However, fructose intakes from US adults (age 19–80 years, n=17,749 from the NHANES 1999–2006 databases) have previously been estimated by Sun and colleagues as 48 g per day[209]. This was approximately 37% of total sugar consumption, which was at the time ~129 g per day. From 2003–2016, total sugar intake decreased by 30% and total sugar intakes from SSBs (soft drinks, sports drinks, energy drinks, fruit drinks) by 46% in adults[210]. 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 day[210]. This would suggest an average fructose intake of ~40g per day, underscoring how high the amounts of single monosaccharides used in the studies examining 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[23,195]. This reduction is important not only for adults but also for children and adolescents, who consume, at least in the USA, three–fold more added

sugars than recommended by the American Heart Association (19 versus 6 teaspoons per day)[211]. Notably, in an open randomized trial in 40 adolescent boys (aged 11 to 16 years) with histologically–verified NAFLD, significant (P<0.001) improvement of steatosis was observed in response to strict restriction of free sugar intake (to <3% of total energy) for 8 weeks[212]. This effect was enabled by individualized menu planning and provision of study meals 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 very–high fructose diet (150 g per day for 8 weeks) in 10 lean (BMI 22.2kg/m^2) healthy adults and found that IHTG content remained unchanged, along with other metabolic parameters including hepatic glycogen and markers of insulin sensitivity[213]. 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 hypertriglyceridaemia[208]. Whether the latter changes were a consequence of fructose or weight gain remains, however, 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 loss[214]. Studies comparing 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–saturated fat intakes. Saturated fat–enriched diets increase IHTG more than PUFA 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 show 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).

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 and/or low refined carbohydrate diets on NAFLD and cardiovascular risk.

Research design

- Adequately powered multicentre studies using isoenergetic low fat–high carb versus high fat–low carb diets lasting months rather than weeks. Utilizing noninvasive 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.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Table 1. Studies comparing effects of low–fat–high–carbohydrate and high–fat–low–carbohydrate on IHTG and insulin sensitivity.

Study	Participant characteristics (n; BMI (kg/m2); 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	LC-HC HF-LC	20, 65 75, 10	No data	11.2, 6.2↓ 12.4, 8.1↓	↑ ↑
Browning et al. (2011)[107]	18; 35; 45; 100	2 weeks	P	Hypo	LF–HC HF–LC	34, 50 59, 8	14.7; 13; 6.3 24; 25; 10	19, 14↓ 22, 10↓[a]	No data No data
Haufe et al. (2011)[108]	102; 32; 45; 54	6 months	P	Hypo	LF–HC HF–LC	'Reduced fat' 'Reduced carb'	Less saturatedb	9.6, 5.6↓ 7.6, 4.0↓	↑ ↑
Westerbacka et al. (2005)[109]	10; 33; 43; 50	2 weeks	C	Iso	LF–HC HF–LC	16, 61 56, 31	7.2; 6.6; 2.2 25.2; 23.0; 7.8	10, 8[a] 10, 13	↑[a] ↓
van Herpen et al. (2011)[110]	20; 29; 55; no data	3 weeks	P	Iso	LF–HC HF–LC	22, 57 49, 34	9.0; 8.1; 4.8 20.1; 18.6; 10.3	4.0, 3.5↓ 2.2, 2.	NS NS
Utzschneider et al. (2013)[111]	35; 27; 69; 15	4 weeks	P	Iso	LF–HC SFA–LC	23, 57 43, 38	8.5; 9.7; 4.8 25.8; 13.8; 3.4	2.2, 1.7↓ 1.2, 1.4	↑ NS
Sobrecases et al. (2010)[115]	39; 23; 25; no data	7 days	P	Hyper	LF–HC HF–LC	High fructose[d] High fat[e]	No data	12[c], 14↑[c] 11[c], 21↑[c]	NS NS
Luukkonen et al. (2018)[105]	26; 31; 46; 27	3 weeks	p	Hyper	LF–HC SFA–LC	24, 64 60, 26	9.8; 9.8; 4.3 37.8; 16.2; 6.0	4.3, 5.7↑ 4.9, 7.6↑[a]	NS ↓
	24; 32; 48; 25	3 weeks	P	Hyper	LF–HC PUFA–LC	24, 64 59, 2	9.8; 9.8; 4.3 17.7; 28.9; 12.4	4.3, 5.7↑ 4.8, 5.5↑	NS 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 phospholipids[109]. ↓, 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. [a]Significant difference in change between the diets. [b]The 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. [cl]HTG units are millimoles per kilogram wet weight (not percentage). [d]Addition of 3.5 g per day of fructose per kilogram of fat free mass. [e]Addition of 30% of total energy as fat. For corresponding figure, see Fig. 2 (left). Adapted from[213], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

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 SFA	40[a], 39 43[b], 40	3.2, 2.3* 3.2, 3.5
Rosqvist et al. (2014)[118]	39; 20; 27; 0	7 weeks	P	Hyper	PUFA SFA	40[c], 43 36[d], 4	0.75, 0.79 0.96, 1.5*
Luukkonen et al. (2018)[105]	26; 30; 50; 27	3 weeks	P	Hyper	PUFA SFA	60[e], 23 59[f], 26	4.8, 5.5↑ 4.9, 7.6↑*
Rosqvist et al. (2019)[117]	61; 28; 42; 0	8 weeks	P	Hyper	PUFA SFA	51[g], 44 51[h], 44	2.0, 1.9 1.5, 3.0↑*
MUFA and MED diets							
Bozzetto et al. (2012)[188]	17; 30; 35–70; no data	8 weeks	P	Iso	CONT MUFA	28, 53 42[i], 40	17.7 , 16.1 7.4, 5.2↓*
Errazuriz et al. (2017)[189]	28; 31; 61; 50	12 weeks	P	Iso	CONT MUFA	34[j], 49 46[k], 40	11.2, 11.9 9.7 , 8.0↓*
Ryan et al. (2013)[30]	12; 32; 55; 100	6 weeks	C	Iso	CONT MUFA	21[l], 49 44[m], 34	11.2, 10.0 14.2, 8.6
Gepner et al. (2019)[191]	278; 31; 48; 53	18 months	P	Hyper	CONT MUFA	35, 44 38, 40	10.1, 6.4↓* 10.3, 6.1↓
Properzi et al. (2018)[192]	49; 31; 52; 100	12 weeks	P	Iso	CONT MUFA	31[n], 48 45[o], 37	21.5, 15.3↓ 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. [a]10% SFA, 17% MUFA, 13% PUFA. [b]20% SFA, 19% MUFA, 4% PUFA. [c]11% SFA, 12.4% MUFA, 13% PUFA. [d]16% SFA, 12.9% MUFA, 4% PUFA. [e]14% SFA, 28% MUFA, 11% PUFA. [f]33% SFA, 13% MUFA, 5% PUFA. [g]Sunflower oil. [h]Palm oil. [i]Enriched with MUFA, SFA similar in both arms. [j]12% SFA, 8% MUFA, 4% PUFA. [k]12% SFA, 22% MUFA, 5% PUFA. [l]14.4% SFA, 15.6% MUFA, 9.6% PUFA. [m]12.4% SFA, 20.4% MUFA, 7.2% PUFA. [n]9.3% SFA, 13.1% MUFA. [o]9.5% 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.

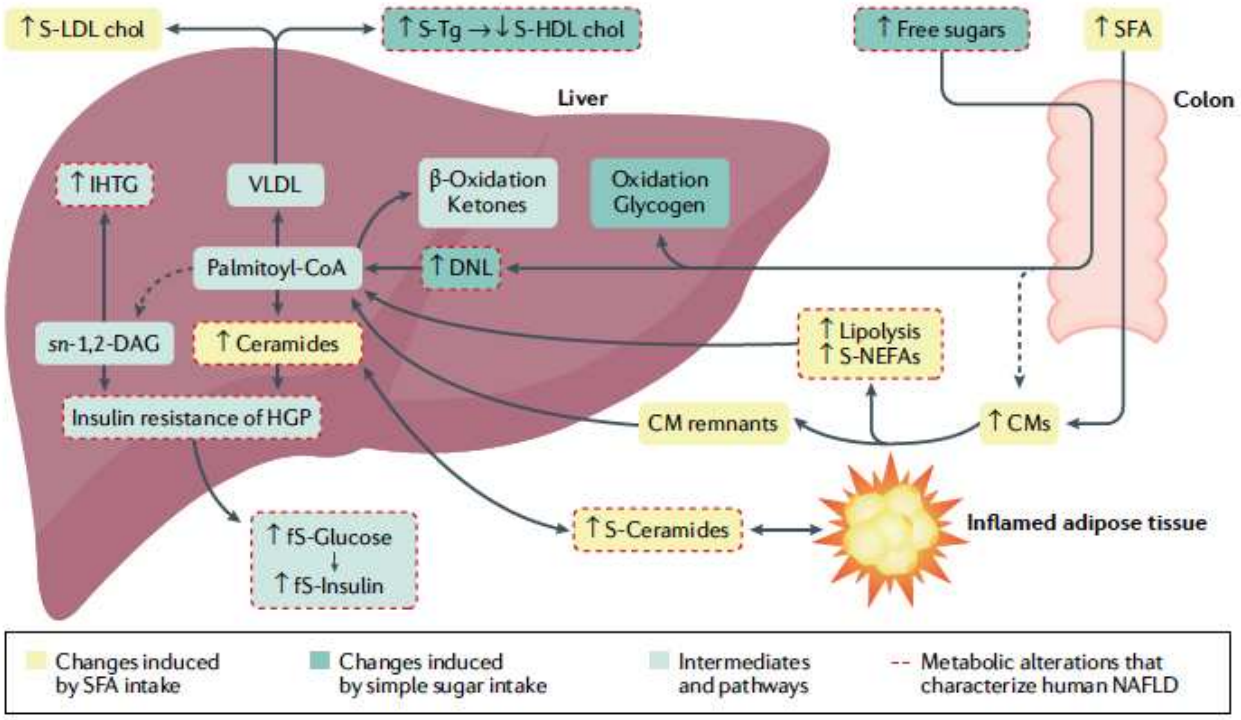


Fig. 1. Metabolic fates of free sugars and saturated fatty acids. Fatty acids released by intestinal lipolysis are packaged into chylomicron (CM) particles, which are transported via chyle to the systemic circulation. Free fatty acids are released from CMs (and very–low–density lipoprotein (VLDL) produced by the liver, not shown) via intravascular lipolysis and are taken up by peripheral tissues such as adipose tissue or spillover to the systemic circulation[216]. Fatty acids stored in adipose tissue triglycerides undergo lipolysis, which releases free fatty acids, especially under fasting conditions. Free fatty acids are transported to the liver bound to albumin and constitute the major source of liver triglycerides both after an overnight fast and after a meal[58]. Saturated fatty acid (SFA)–enriched diets (yellow) increase CMs[217], peripheral lipolysis and liver SFAs such as palmitate (16:0), which is needed for synthesis of ceramides[105], compared with polyunsaturated fatty acid (PUFA)–containing diets. SFAs but not PUFAs stimulate ceramide synthesis both in animals[144] and humans[105,117]. Ceramides induce hepatic insulin resistance, inflammation and mitochondrial dysfunction[146]. Compared with high carbohydrate diets, SFAs increase serum LDL and high–density lipoprotein (HDL) cholesterol and decrease triglycerides[218]. 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 CMs[219]. NAFLD is characterized by increased free sugar intake, CMs, lipolysis, hepatic and circulating ceramides, insulin resistance of hepatic glucose production, increased VLDL synthesis and circulating triglycerides, which lead to lowering of HDL cholesterol.

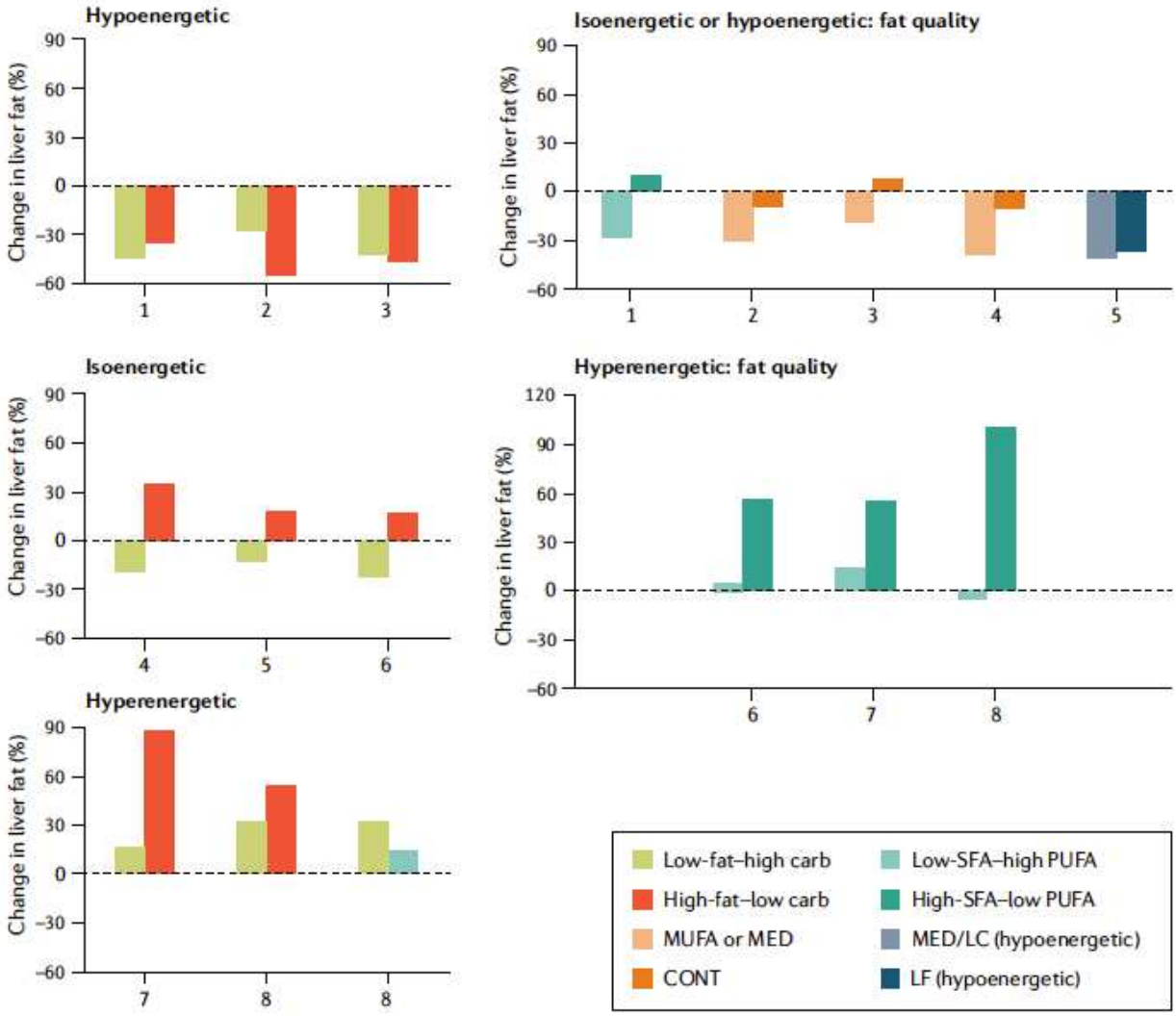


Fig. 2. **Effects of fats and carbohydrates on liver fat content**. Effects of low–fat–high–carbohydrate as compared to high–fat–low–carbohydrate diets on liver fat content (left side of figure). Expressed as relative (%) change from baseline measured by ^{1}H–MRS. Interventions comparing hypoenergetic, isoenergetic and hyperenergetic low–fat–high–carbohydrate to high–fat–low–carbohydrate diets are shown. Upper–left panel: 1[106], 2[107], 3[108]; middle–left panel: 4[109], 5[110], 6[111]; bottom–left panel: 7[115], 8[105]. Effect of fat quality on liver fat content, expressed as relative (%) change from baseline measured by ^{1}H–MRS (right side of figure). Interventions comparing isocaloric or hypocaloric (upper–right panel) or hypercaloric (bottom–right panel) diets low in saturated and high in polyunsaturated or high in saturated and low in polyunsaturated fat are shown. CONT, control; MED/LC, Mediterranean low carbohydrate diet; LF, low–fat diet; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. Upper–right panel: 1[116], 2[189], 3[190], 4[30], 5[192]; lower–right panel: 6[118], 7[105], 8[117]. Adapted from Ref.[215] Effects of fat, CC by 4.0 (https://creativecommons.org/licenses/by/4.0/).

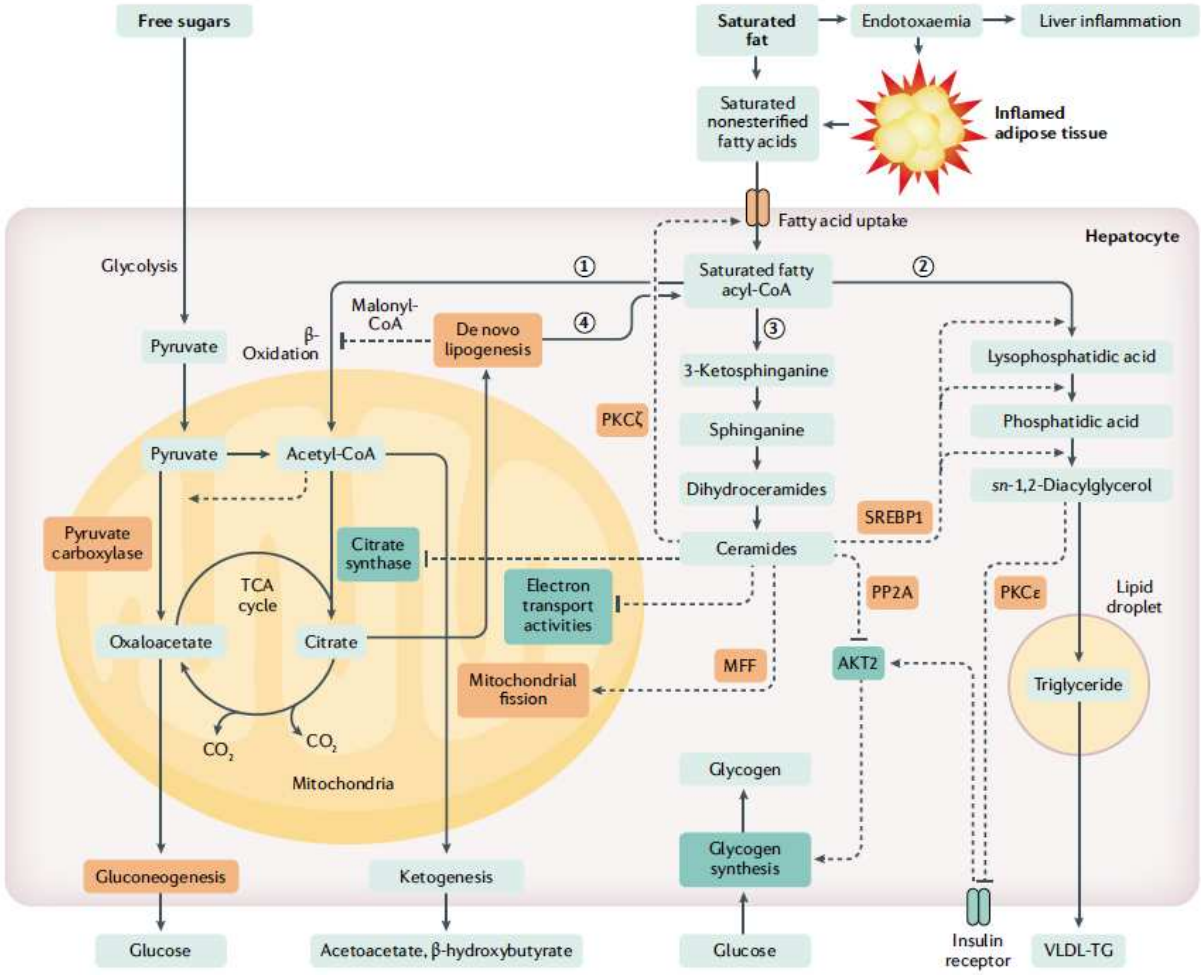


Fig. 3. Metabolic effects of excessive intakes of saturated fat and free sugars. Saturated fat intake increases saturated nonesterified (free) fatty acids in the circulation. Saturated fat intake can induce endotoxaemia, which promotes adipose tissue inflammation and lipolysis, thereby further increasing hepatic nonesterified fatty acid supply. In the liver, saturated fatty acyl–CoA can enter the mitochondria for beta oxidation (1) to acetyl–CoA, which can enter ketogenesis or be oxidized to carbon dioxide in the tricarboxylic acid (TCA) cycle. Alternatively, saturated fatty acyl–CoA can enter the Kennedy pathway (2), where it is metabolized into *sn*–1,2–diacylglycerols (*sn*–1,2–DAGs). *Sn*–1,2–DAGs induce protein kinase C epsilon (PKCε) translocation from cytosol to the plasma membrane, which inhibits insulin signalling at the level of the insulin receptor[137]. This process decreases activation of AKT serine–threonine kinase 2 (AKT2) and subsequent glycogen synthesis. *Sn*–1,2–DAGs are also precursors of triglycerides, which can be stored in hepatic lipid droplets or hydrolyzed and re–esterified in the endoplasmic reticulum as very–low–density lipoprotein–triglyceride (VLDL–TG) for secretion. Saturated fatty acyl–CoAs, particularly palmitoyl–CoA, can also enter the de novo ceramide synthetic pathway (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 activities[146]. 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 transcription factor 1 (Srebpf1) and fatty acid uptake by PKCζ–mediated stimulation of the lipid transport protein CD36. In addition, ceramides can inhibit distal insulin signaling via protein phosphatase 2 (PP2A)–mediated inhibition of AKT2[220]. Excessive intake of free sugars can stimulate DNL (4), which exclusively produces saturated fatty acids. An intermediate in the DNL pathway, malonyl–CoA, inhibits mitochondrial fatty acid uptake, thereby limiting beta oxidation. An orange box denotes upregulation, whereas a dark green box denotes downregulation. A dashed line with a flat end denotes inhibition, a dashed line with an arrow denotes stimulation, whereas a solid line with an arow denotes substrate flux.

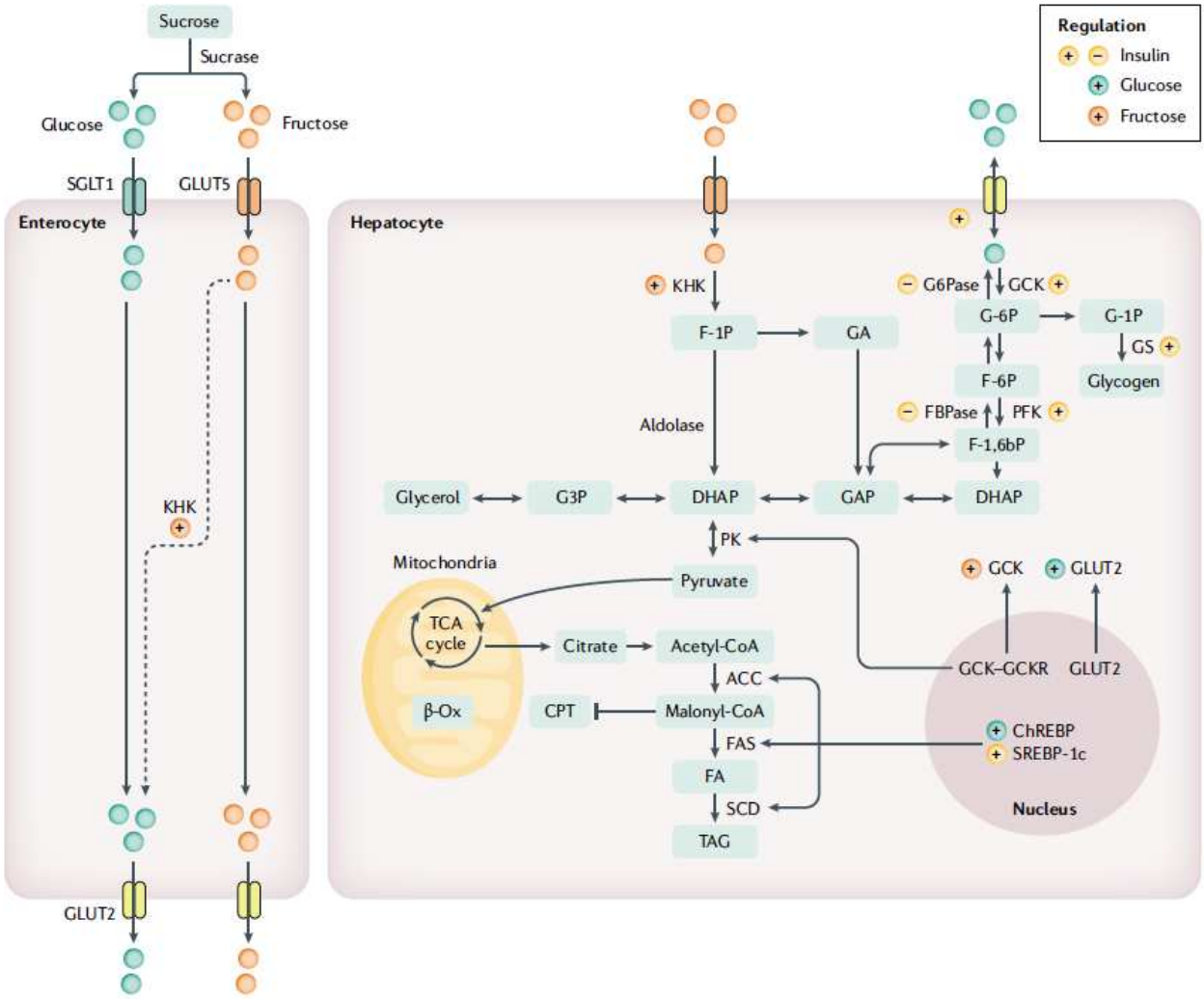
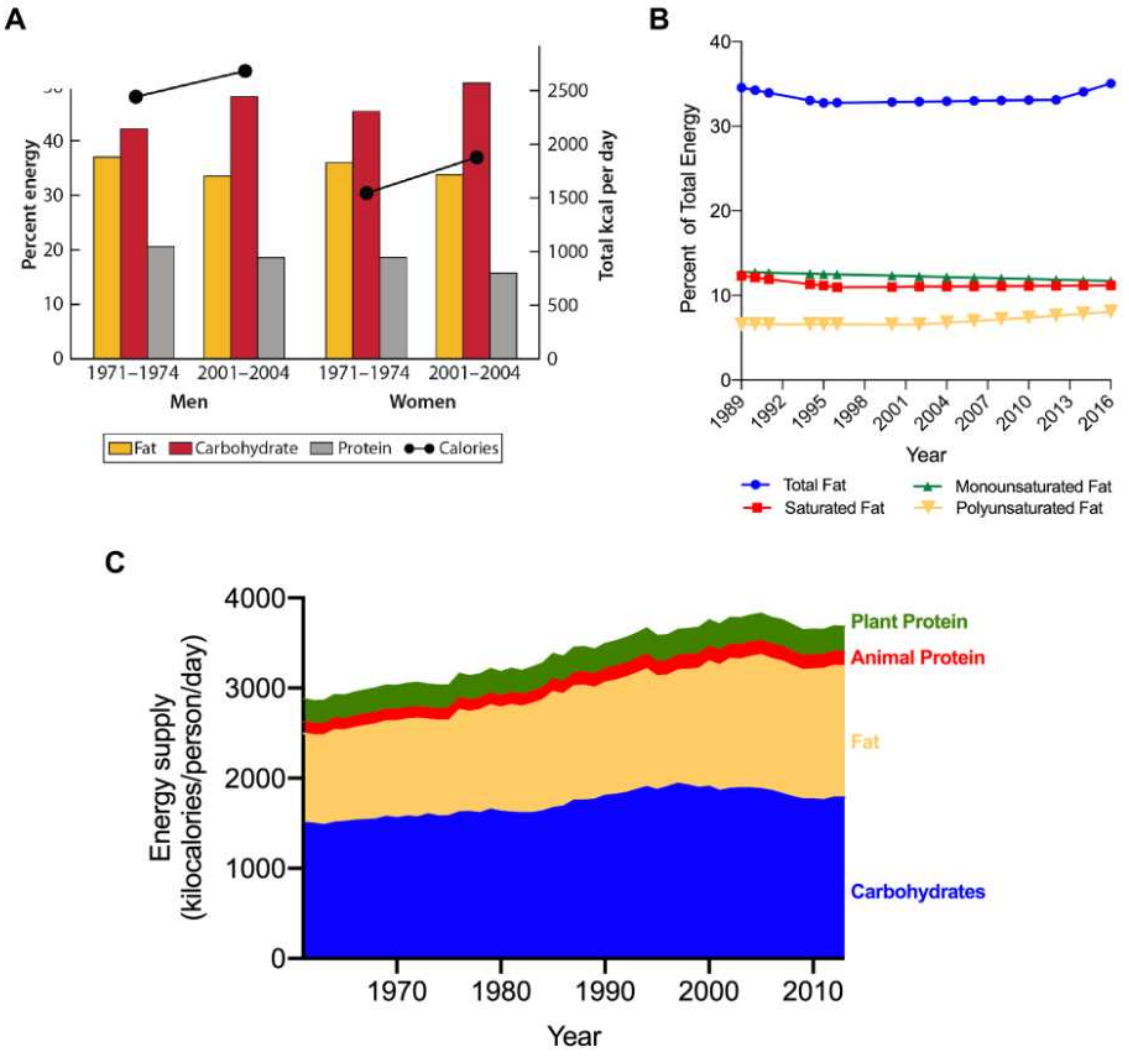


Fig. 4. Sugar metabolism and regulation. The digestive enzyme sucrase hydrolyzes the disaccharide sucrose (table sugar) into its constitutive monosaccharide subunits, glucose and fructose, also found in high–fructose corn syrup, sugar–sweetened beverages and fruit juices. Glucose and fructose are transported 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 acids[199]. 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, then split into trioses by the activity of aldolase prior to converging with glucose metabolism. Insulin and glucose, via sterol regulatory element–binding transcription factor 1 (SREBP1) and carbohydrate response 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, further promoting DNL and intrahepatic triglyceride (IHTG) accumulation from both fructose and glucose. TAG, triacylglycerol; G6Pase, glucose 6–phosphatase; GS, glycogen synthase; FBPase, fructose–1,6–bisphosphatase; PFK, phosphofructokinase; PK, pyruvate kinase; F–1P, fructose 1–phosphate; G–6P, glucose 6–phosphate; G–1P, glucose 1–phosphate; F–6P, fructose 6–phosphate; F–1,6bP, fructose 1,6–bisphosphate; GA, glyceraldehyde; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3–phosphate; G3P, glycerol 3–phosphate; CPT, carnitine palmitoyltransferase; GCKR, glucokinase regulatory protein.

SUPPLEMENTARY INFORMATION



Supplementary Fig. 1. Changes in energy and macronutrient intakes and supply over time. a | Age-adjusted trends in macronutrients and total energy reported consumed by US adults (20 to 74 years of age) from 1971–2004[1,2]. b | Fat intake as a percentage of total energy reported consumed by US adults from 1989-2016[3]. c | The average per capita supply of energy derived from carbohydrates, protein and fat[4,5].

Supplementary Table 1. Effects of fructose as compared to other carbohydrates on liver fat and insulin sensitivity.

N	BMI (kg/m^2)	Age (yrs)	Duration	Design	Cal	Diet	Fructose diet Comparator CARB diet	Liver fat (%) Before – After[c]	Insulin sensitivity Change	Year[Ref]
32	29	34	2 wks	P	ISO	FRU GLU	FRU 25%[b] GLU 25%[b]	7.2 - 7.5 8.0 - 7.9	↑ ↓	2013[6]
8	24	42	9 days	C	ISO	FRU GLU	FRU 25% CCHO 25%	1.0* 0.7	↓ NS	2015[7]
11	75 kg[a]	25	7 days	C	HYPER	FRU GLU	FRU 35%[b] GLU 35%[b]	2.1 - 3.2↑ 2.1 - 3.3↑	NS NS	2010[8]
20	25	30	10 wks	P	HYPER	FRU GLU	FRU +600 cal/day GLU +600 cal/day	1.3 - 1.8 1.6 - 2.1	NS NS	2011[9]
64	27	42	10 wks	P	HYPER	FRU GLU	HFCS 8-30%[b] SUCROSE 8-30%[b]	11.8 - 13.7 14.9 - 13.0	NS NS	2013[10]
32	29	34	2 wks	P	HYPER	FRU GLU	FRU +25%[b] GLU +25%[b]	7.2 - 8.9↑ 8.0 - 10.1↑	↑ NS	2013[6]
28	22	23	6-7 days	P	HYPER	FRU GLU	FRU 3 g/kg day GLU 3 g/kg day	9.0 - 18.5↑* 12.9 - 16.1	↓ NS	2013[11]

Abbreviations: N=number of completers, BMI=body mass index, yrs=years, wks=weeks, C=crossover, P=parallel, Cal=energetic content relative to baseline diet, ISO=isoenergetic, HYPER=hyperenergetic, CARB=carbohydrate, FRU=fructose, GLU=glucose, CCHO=complex carbohydrate, HFCS=high fructose corn syrup, [a]=body weight, BMI not given, [b]=% of total energy intake, [c]The values denote liver fat before and after the dietary interventions, except for the study of Schwarz et al[7] where the median values for the treatment periods are shown, *significant difference in change between the two diets, ↑ significant increase, ↓ significant decrease compared to baseline, Ref=reference. Table adapted from Ref [12], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

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