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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<sup>1</sup>. 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<sup>2</sup>. The prevalence of NASH, which requires a liver biopsy for diagnosis, has been estimated to be 1.5–6.5%<sup>2</sup>. 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<sup>3</sup>. 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<sup>2</sup>. 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)<sup>2</sup>. NAFLD is frequently associated with features of the metabolic syndrome and predicts, independent of obesity, CVD and T2DM<sup>4,5</sup>. 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<sup>6</sup>, vitamin D<sup>7</sup>, vitamin E<sup>8</sup>, probiotics, prebiotics and synbiotics<sup>9,10</sup>, omega–3 fatty acids<sup>11</sup>, coffee<sup>12,13</sup> and alcohol<sup>14</sup> 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<sup>1</sup>. 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)<sup>15,16</sup>. 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<sup>15,16</sup>. 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<sup>17–19</sup>.

In the context of an obesogenic world, maintaining individual energy balance and a healthy weight throughout the lifespan is challenging<sup>20</sup>. 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<sup>21</sup>. 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<sup>22</sup>. 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<sup>23</sup>.

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<sup>24,25</sup>. However, the often polarized debates on sugar versus fat in the aetiology of obesity and metabolic disease<sup>26,27</sup> (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)<sup>28</sup> and one small (n=31) study<sup>29</sup> 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<sup>18</sup>. 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<sup>30</sup>, has suggested that a Mediterranean diet might be the diet of choice for NAFLD<sup>17</sup>. 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<sup>31</sup>. Thus, although there is consensus that weight loss through diet and lifestyle<sup>32</sup> or clinical<sup>33</sup> 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<sup>34,35</sup>, 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<sup>36</sup>, 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)<sup>21</sup>. 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)<sup>21</sup>. 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<sup>37</sup>, 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)<sup>37</sup>.

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<sup>38,39</sup>. 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<sup>40</sup>. 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)<sup>40,41</sup>.

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<sup>42</sup>, Japanese<sup>43</sup>, Israeli<sup>44</sup> and US<sup>45</sup> 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<sup>46,47</sup>. 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<sup>47</sup>. 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<sup>47</sup>. 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<sup>48</sup>.

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<sup>49</sup>. The relationship was independent of sex, physical activity and sedentary behavior, but not of body mass index (BMI)<sup>49</sup>. 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)<sup>50</sup>. 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<sup>51</sup>. 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<sup>52</sup>.

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<sup>12,53,54</sup>. In 2019, Chen and colleagues<sup>54</sup> 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 ( $\geq 7$  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)<sup>55</sup>. When consumed in excess, these substrates are converted into saturated fatty acids via hepatic de novo lipogenesis (DNL)<sup>56</sup>. In addition, fatty acids are released by intravascular hydrolysis and adipose tissue lipolysis, or they can be derived from dietary fat (Fig. 2)<sup>57</sup>. Fatty acids derived from adipose tissue lipolysis are the source of the majority of fatty acids used for triglyceride synthesis in the liver<sup>58</sup>. This observation is true both in the fasting and postprandial state, despite postprandial insulin-mediated suppression of adipose tissue lipolysis<sup>57</sup>. 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<sup>57</sup>. 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<sup>59</sup>. 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<sup>57</sup>. 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<sup>60</sup>.

***De novo lipogenesis.*** In humans, DNL occurs predominantly in the liver, and palmitate (a 16–carbon saturated fatty acid) is the end product<sup>61,62</sup>. However, DNL is not the pathway of first resort for disposal of dietary carbohydrates in humans<sup>56</sup>. 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<sup>62</sup>. However, during carbohydrate overfeeding, fractional DNL, which is the contribution of DNL to very–low–density lipoprotein (VLDL)–triglyceride, will be stimulated<sup>63</sup>. 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<sup>63</sup>. 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<sup>64</sup>, 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<sup>61</sup>, with DNL accounting for 15% to 38% of IHTG palmitate production<sup>58,59,65</sup> (Fig. 1). This proportion is notably higher than the 1% to 10% reported by different studies for individuals without NAFLD<sup>59,66–68</sup>. 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<sup>69,70</sup>. 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<sup>71–74</sup>. 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 NAFLD (n=26, DNL 19%) and lean individuals (n=14, DNL 11%), deuterated water was administered for 3–5 weeks<sup>61</sup>.

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<sup>59,61</sup> 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<sup>61</sup>. 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<sup>64</sup>. 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<sup>75</sup>. Several pharmacological inhibitors of DNL, including MK-4074<sup>76</sup>, GS-0976<sup>77</sup> and NDI-010976<sup>78</sup>, which all inhibit acetyl-coenzyme A carboxylase (ACC), are currently being tested in humans and have been reviewed previously<sup>79</sup>.

**Lipolysis.** Multiple studies have documented increased adipose tissue lipolysis in individuals with and without diabetes but who have NAFLD<sup>80–82</sup>. 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<sup>83</sup>. Interestingly, a study of adolescent girls with equivalent BMI and with either lower IHTG and visceral fat ( $n=7$ , BMI 36.6kg/m<sup>2</sup>) or higher IHTG and visceral fat ( $n=8$ , BMI 36.0kg/m<sup>2</sup>) 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<sup>84</sup>. For both groups, the turnover rate (lipolysis and synthesis) of triglyceride in adipose tissue was correlated with IHTG content<sup>84</sup>. 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<sup>84</sup>.

**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<sup>85</sup>. 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<sup>86</sup>. 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<sup>87–89</sup>. Although VLDL-triglyceride secretion correlates positively with IHTG<sup>87</sup>, there might be a plateau beyond 10% IHTG<sup>88</sup>. Very few studies have investigated the effect of dietary macronutrients or fat composition on VLDL-triglyceride secretion or production rates<sup>89,90</sup>. 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<sup>89</sup>. 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<sup>90</sup>.

***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<sup>85</sup>. Blood levels of the ketone body 3-hydroxybutyrate (3OHB) are often used as a surrogate marker of hepatic fatty acid oxidation<sup>91–93</sup>. Data on plasma 3OHB concentrations in individuals with and without NAFLD are inconsistent. Levels have been reported as being decreased<sup>94</sup>, similar<sup>95,96</sup> or increased<sup>97</sup>. A limited number of studies have assessed mitochondrial oxidation using stable isotope tracers. Again, results have varied. For example, Sunny and colleagues<sup>96</sup> 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<sup>98</sup>.

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)<sup>99</sup>. This randomized crossover study of 8 healthy individuals measured postprandial blood 3OHB concentrations and expired <sup>13</sup>CO<sub>2</sub> 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<sup>100,101</sup>, data in humans are sparse. Two small studies (n=6<sup>102</sup> and n=4<sup>103</sup>) that used metabolic tracers and measured expired <sup>13</sup>CO<sub>2</sub> 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 <sup>13</sup>CO<sub>2</sub>, 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<sup>104</sup>.

## **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<sup>105</sup>) 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<sup>70,106</sup>. Although a ketogenic low-carbohydrate diet decreased IHTG more than a standard hypoenergetic diet when measured after 2 days<sup>106</sup> or 2 weeks in 22 individuals with obesity, this difference was no longer observed after 11 weeks<sup>107</sup>. Similarly, in a longer (6 month) hypoenergetic intervention in 102 participants with overweight or obesity<sup>108</sup>, 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.<sup>109–111</sup> 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<sup>112</sup>. Notably, all three high-fat arms provided intakes of much higher saturated

and polyunsaturated fats than World Health Organization recommendations<sup>113</sup> (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<sup>114</sup>. 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<sup>105,115</sup>. 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<sup>17</sup>. 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<sup>107–111</sup> (Table 1).

## **Fat quality and IHTG**

In four studies, saturated fat consistently increased IHTG more than polyunsaturated fat when total energetic intake was similar<sup>105,116–118</sup> (Table 2, Fig. 2B). Rosqvist and colleagues<sup>118</sup> 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<sup>118</sup> and later in 60 individuals with overweight<sup>117</sup>. 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<sup>118</sup> and by 50% in individuals with obesity<sup>117</sup>, which is in line with earlier work showing this in an isoenergetic intervention in individuals with obesity<sup>116</sup>. These data were extended by Luukkonen and colleagues<sup>105</sup> 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<sup>105</sup>. PUFAs might be preferentially partitioned into oxidation pathways<sup>103</sup>, 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<sup>105,119</sup> or plasma beta-hydroxybutyrate concentrations<sup>120</sup> 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)<sup>5</sup>. 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)<sup>4</sup>. Given that SFAs increase concentrations of LDL cholesterol<sup>121</sup>, and that substitution of polyunsaturated fat with saturated fat reduces cardiovascular and all-cause mortality<sup>122,123</sup>, 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<sup>121</sup>.

Insulin resistance is perhaps the most important risk factor for CVD in patients with NAFLD, with effects that are mediated independent of LDL cholesterol<sup>124–127</sup>. 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<sup>106</sup> and 6 months<sup>108</sup>) 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<sup>128</sup>.

Although IHTG positively correlates closely with insulin resistance<sup>80,82,129–132</sup>, NAFL can exist without features of insulin resistance, such as in individuals with familial hypobetalipoproteinemia<sup>133</sup>, or in those carrying the common patatin-like phospholipase domain-containing 3 (PNPLA3) I148M variant<sup>134</sup>. These findings in humans and in numerous animal models<sup>135</sup> suggest that high IHTG levels are not sufficient to cause insulin resistance<sup>136</sup>. Rather than IHTG per se, accumulation of bioactive fatty acid metabolites, such as ceramides and diacylglycerols (DAGs), has been suggested to mediate insulin resistance<sup>137,138</sup>. 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<sup>105,117</sup>. Furthermore, multiple prospective studies have shown that circulating concentrations of ceramides predict CVD, independent of classic risk factors such as LDL cholesterol level<sup>139,140,141</sup>. High circulating ceramide concentrations are also predictors of prediabetes<sup>142</sup>, T2DM<sup>140</sup> and NASH in humans<sup>143</sup>.

**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<sup>138</sup>. 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<sup>144,145</sup>.

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<sup>146</sup>. In addition, ceramides increase hepatic mitochondrial acetyl-CoA concentrations, which can

allosterically activate pyruvate carboxylase and thereby stimulate gluconeogenesis<sup>147</sup>. Through direct interaction with protein kinase C zeta (PKC $\zeta$ ), ceramides induce translocation of the lipid transport protein CD36 to the cell membrane, stimulating fatty acid uptake<sup>148,149</sup>. 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)<sup>146</sup>. Simultaneously, ceramides impair fatty acid oxidation by inhibiting mitochondrial citrate synthase and electron transport chain activities<sup>147,148,150</sup> (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<sup>151</sup>. By impairing mitochondrial electron transport activities, ceramides can stimulate generation of reactive oxygen species in cultured hepatocytes<sup>152</sup>, which characterizes progression from steatosis to NASH<sup>153</sup>. 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<sup>154</sup>, which triggers caspase-induced apoptosis<sup>155</sup>.

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<sup>156</sup>. 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<sup>156</sup>. 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<sup>156</sup>. 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<sup>157</sup>. 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<sup>105,117</sup> or free sugars<sup>105</sup> in the face of similar energy excess<sup>105</sup>. The saturated fat diet was also the only diet to induce insulin resistance<sup>105</sup>. 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<sup>143</sup>. 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 (PKC $\epsilon$ ), which is associated with decreased activation of insulin receptor tyrosine kinase<sup>158,159</sup>, resulting in reduced hepatic glycogen synthesis. This mechanism mainly impairs the direct insulin action of stimulating hepatic glycogen synthesis<sup>160</sup>. The specific molecular mechanism underlying the PKC $\epsilon$ –mediated inhibition of insulin receptor tyrosine kinase is through phosphorylation of the receptor at Thr160 as demonstrated in mice<sup>161</sup>. Deletion of *Prkce* and inactivation of this phosphorylation site both protect mice from high-fat diet-induced insulin resistance<sup>161,162</sup>.

In humans, hepatic DAG concentrations have been repeatedly shown to correlate with steatosis and insulin resistance<sup>95,156,160,163–166</sup>, 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<sup>167,168</sup>. One potential cause of this inflammation is the gut microbiome, which is a rich source of inflammatory mediators, such as endotoxin<sup>169</sup>. Acute administration of small intravenous bolus of endotoxin increases systemic and adipose tissue inflammation, lipolysis and induces insulin resistance in healthy humans (n=20)<sup>170</sup>. In large cross-sectional human studies, endotoxaemia has been found to be positively correlated with both insulin resistance (n=1,347)<sup>171</sup> and histological severity of NAFLD (n=237)<sup>172</sup>. A potential mechanism by which endotoxin could mediate liver injury is Kupffer cell activation, as is well-recognized in experimental alcohol-related liver injury<sup>173</sup>.

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



fat meal enriched with butter<sup>177</sup>, or cream compared with an isocaloric amount of orange juice<sup>178</sup>, 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<sup>179</sup>. 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<sup>105</sup>. In addition to saturated fat, fructose intake has been shown to increase bacterial endotoxin concentrations and markers of liver injury in rodents<sup>180</sup>, non-human primates<sup>181</sup> and humans<sup>182,183</sup>. 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<sup>183</sup>. Endotoxin is found in chylomicrons and increases in response to a meal<sup>184</sup>. Chylomicrons contain more endotoxin in insulin-resistant individuals with obesity than insulin-sensitive lean individuals<sup>184</sup>. 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  $23 \pm 1$  kg/m<sup>2</sup>, age  $22 \pm 1$  years) without altering intestinal permeability, as measured via a four-sugar probe test<sup>185</sup>.

***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<sup>186</sup>. 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<sup>187</sup>. The fibre content is twice the current average US fibre intake<sup>188</sup>.

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<sup>189</sup>, 28<sup>190</sup> and 12<sup>30</sup> individuals, the MUFA or Mediterranean diets decreased IHTG more than a standard diet, despite containing more fat than the control diets<sup>30,120,190</sup>. 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<sup>191,192</sup>. 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  $-4.2 \pm 7.1\%$  versus low–fat  $-3.8 \pm 6.7\%$ ;  $P = 0.036$ )<sup>192</sup>. 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<sup>191</sup>. In a study by Properzi and colleagues<sup>193</sup>, 51 individuals with NAFLD on a Mediterranean diet had statistically significant improvements in several cardiometabolic risk factors, including blood lipids and glycated haemoglobin (HbA<sub>1c</sub>), 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<sup>23</sup>, 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<sup>194</sup>.

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<sup>23,195,196</sup>. 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<sup>79,197</sup>), thereby potentially providing more substrate to the DNL pathway and IHTG than glucose does<sup>197</sup>. 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<sup>198</sup>. Work in mice showed that the small intestine converted low doses of fructose almost entirely into glucose, lactate and glycerate<sup>199</sup>. 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<sup>199</sup>. 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<sup>200</sup>. Thus, it is possible that fructose has roles in other metabolic pathways that influence IHTG content<sup>198</sup>.

### ***Fructose versus glucose or other carbohydrate intervention studies.***

Although the lipogenic effect of glucose is acutely exacerbated by fructose<sup>60</sup>, 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<sup>201–206</sup>. 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<sup>201,202</sup>, exchanging fructose for other carbohydrates, whereas the other trials were hyperenergetic<sup>203–206</sup>, 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<sup>206</sup>.

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<sup>207</sup>. 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<sup>208</sup>. 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<sup>209</sup>. 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<sup>210</sup>. 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<sup>210</sup>. 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<sup>23,195</sup>. 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)<sup>211</sup>. 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<sup>212</sup>. 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 ( $\text{BMI } 22.2\text{kg/m}^2$ ) healthy adults and found that IHTG content remained unchanged, along with other metabolic parameters including hepatic glycogen and markers of insulin sensitivity<sup>213</sup>. 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<sup>208</sup>. 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<sup>214</sup>. 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.



## REFERENCES

- 1 Moore, J. B. From sugar to liver fat and public health: systems biology driven studies in understanding non-alcoholic fatty liver disease pathogenesis. *Proceedings of the Nutrition Society* **78**, 290–304, doi:10.1017/S0029665119000570 (2019).
- 2 Younossi, Z. M. *et al.* Global epidemiology of nonalcoholic fatty liver disease–Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64**, 73–84, doi:10.1002/hep.28431 (2016).
- 3 Singh, S. *et al.* Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **13**, 643–654 e641–649; quiz e639–640, doi:10.1016/j.cgh.2014.04.014 (2015).
- 4 Yki-Jarvinen, H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *The lancet. Diabetes & endocrinology* **2**, 901–910, doi:10.1016/S2213-8587(14)70032-4 (2014).
- 5 Anstee, Q. M., Targher, G. & Day, C. P. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature reviews. Gastroenterology & hepatology* **10**, 330–344, doi:10.1038/nrgastro.2013.41 (2013).
- 6 Johnston, B. C. *et al.* Unprocessed Red Meat and Processed Meat Consumption: Dietary Guideline Recommendations From the Nutritional Recommendations (NutriRECS) Consortium. *Annals of internal medicine*, doi:10.7326/M19-1621 (2019).
- 7 Zhang, Z., Thorne, J. L. & Moore, J. B. Vitamin D and nonalcoholic fatty liver disease. *Current opinion in clinical nutrition and metabolic care* **22**, 449–458, doi:10.1097/mco.0000000000000605 (2019).
- 8 Sato, K. *et al.* Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Nutrition (Burbank, Los Angeles County, Calif.)* **31**, 923–930, doi:10.1016/j.nut.2014.11.018 (2015).
- 9 Safari, Z. & Gerard, P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci* **76**, 1541–1558, doi:10.1007/s00018-019-03011-w (2019).
- 10 Liu, L., Li, P., Liu, Y. & Zhang, Y. Efficacy of Probiotics and Synbiotics in Patients with Nonalcoholic Fatty Liver Disease: A Meta-Analysis. *Digestive diseases and sciences* **64**, 3402–3412, doi:10.1007/s10620-019-05699-z (2019).
- 11 Jump, D. B., Lytle, K. A., Depner, C. M. & Tripathy, S. Omega-3 polyunsaturated fatty acids as a treatment strategy for nonalcoholic fatty liver disease. *Pharmacol Ther* **181**, 108–125, doi:10.1016/j.pharmthera.2017.07.007 (2018).
- 12 Wijarnpreecha, K., Thongprayoon, C. & Ungprasert, P. Coffee consumption and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *European journal of gastroenterology & hepatology* **29**, e8–e12, doi:10.1097/meg.0000000000000776 (2017).
- 13 Marventano, S. *et al.* Coffee and tea consumption in relation with non-alcoholic fatty liver and metabolic syndrome: A systematic review and meta-analysis of observational studies. *Clinical nutrition* **35**, 1269–1281, doi:10.1016/j.clnu.2016.03.012 (2016).
- 14 Rehm, J. & Patra, J. Different guidelines for different countries? On the scientific basis of low-risk drinking guidelines and their implications. *Drug Alcohol Rev* **31**, 156–161, doi:10.1111/j.1465-3362.2011.00395.x (2012).
- 15 Eslam, M., Sanyal, A. J., George, J. & International Consensus, P. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* **158**, 1999–2014 e1991, doi:10.1053/j.gastro.2019.11.312 (2020).
- 16 Eslam, M. *et al.* A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *Journal of hepatology*, doi:10.1016/j.jhep.2020.03.039 (2020).

- 17 EASO, E. E. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Journal of hepatology* **64**, 1388–1402, doi:10.1016/j.jhep.2015.11.004 (2016).
- 18 Chalasani, N. *et al.* The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* **67**, 328–357, doi:10.1002/hep.29367 (2018).
- 19 Glen, J., Floros, L., Day, C., Pryke, R. & Guideline Development, G. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. *BMJ* **354**, i4428, doi:10.1136/bmj.i4428 (2016).
- 20 Moore, J. B. & Boesch, C. Getting energy balance right in an obesogenic world. *Proc Nutr Soc* **78**, 259–261, doi:10.1017/S0029665118002720 (2019).
- 21 Writing Group, M. *et al.* Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation* **121**, e46–e215, doi:10.1161/CIRCULATIONAHA.109.192667 (2010).
- 22 Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C. & Hu, F. B. Changes in diet and lifestyle and long-term weight gain in women and men. *The New England journal of medicine* **364**, 2392–2404, doi:10.1056/NEJMoa1014296 (2011).
- 23 Moore, J. B. & Fielding, B. A. Sugar and metabolic health: is there still a debate? *Current opinion in clinical nutrition and metabolic care* **19**, 303–309, doi:10.1097/mco.0000000000000289 (2016).
- 24 Wise, J. Major report backs overhaul of US dietary guideline process. *BMJ (Clinical research ed.)* **358**, j4340, doi:10.1136/bmj.j4340 (2017).
- 25 Teicholz, N. The scientific report guiding the US dietary guidelines: is it scientific? *BMJ (Clinical research ed.)* **351**, h4962, doi:10.1136/bmj.h4962 (2015).
- 26 Clifton, P. We need more data before rejecting the saturated fat hypothesis. *BMJ (Clinical research ed.)* **347**, f6847, doi:10.1136/bmj.f6847 (2013).
- 27 Lim, D. C. Sugar, not fat, is the culprit. *BMJ (Clinical research ed.)* **347**, f6846, doi:10.1136/bmj.f6846 (2013).
- 28 Vilar-Gomez, E. *et al.* Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* **149**, 367–378.e365; quiz e314–365, doi:10.1053/j.gastro.2015.04.005 (2015).
- 29 Promrat, K. *et al.* Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* **51**, 121–129, doi:10.1002/hep.23276 (2010).
- 30 Ryan, M. C. *et al.* The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *Journal of hepatology* **59**, 138–143, doi:10.1016/j.jhep.2013.02.012 (2013).
- 31 Castera, L., Friedrich-Rust, M. & Loomba, R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* **156**, 1264–1281.e1264, doi:10.1053/j.gastro.2018.12.036 (2019).
- 32 Kenneally, S., Sier, J. H. & Moore, J. B. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. *BMJ Open Gastroenterol* **4**, e000139, doi:10.1136/bmjgast-2017-000139 (2017).
- 33 Koutoukidis, D. A. *et al.* Association of Weight Loss Interventions With Changes in Biomarkers of Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. *JAMA internal medicine*, doi:10.1001/jamainternmed.2019.2248 (2019).
- 34 McPherson, S. *et al.* Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *Journal of hepatology* **62**, 1148–1155, doi:10.1016/j.jhep.2014.11.034 (2015).
- 35 Pais, R. *et al.* A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *Journal of hepatology* **59**, 550–556, doi:10.1016/j.jhep.2013.04.027 (2013).
- 36 Hagstrom, H. *et al.* Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *Journal of hepatology* **67**, 1265–1273, doi:10.1016/j.jhep.2017.07.027 (2017).

- 37 National Center for Health Statistics USA. in *NCHS Fact Sheet* (2017).
- 38 Davy, B. M. & Estabrooks, P. A. The Validity of Self-reported Dietary Intake Data: Focus on the "What We Eat In America" Component of the National Health and Nutrition Examination Survey Research Initiative. *Mayo Clinic proceedings* **90**, 845–847, doi:10.1016/j.mayocp.2015.05.009 (2015).
- 39 Archer, E., Pavea, G. & Lavie, C. J. The Inadmissibility of What We Eat in America and NHANES Dietary Data in Nutrition and Obesity Research and the Scientific Formulation of National Dietary Guidelines. *Mayo Clinic proceedings* **90**, 911–926, doi:10.1016/j.mayocp.2015.04.009 (2015).
- 40 UN Food and Agricultural Organization. FAOstat food balance sheets (<http://www.fao.org/faostat/en/#home>). (2017).
- 41 Ritchie H, R. M. Our World in Data: Diet Compositions (<https://ourworldindata.org/diet-compositions#diet-compositions-by-macronutrient>). (2017).
- 42 Musso, G. *et al.* Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology (Baltimore, Md)* **37**, 909–916, doi:10.1053/jhep.2003.50132 (2003).
- 43 Toshimitsu, K. *et al.* Dietary habits and nutrient intake in non-alcoholic steatohepatitis. *Nutrition (Burbank, Los Angeles County, Calif.)* **23**, 46–52, doi:10.1016/j.nut.2006.09.004 (2007).
- 44 Zelber-Sagi, S. *et al.* Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *Journal of hepatology* **47**, 711–717, doi:S0168-8278(07)00427-8 [pii]10.1016/j.jhep.2007.06.020 (2007).
- 45 Kim, C. H. *et al.* Nutritional assessments of patients with non-alcoholic fatty liver disease. *Obesity surgery* **20**, 154–160, doi:10.1007/s11695-008-9549-0 (2010).
- 46 Zelber-Sagi, S. *et al.* High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. *Journal of hepatology* **68**, 1239–1246, doi:10.1016/j.jhep.2018.01.015 (2018).
- 47 Nouredin, M. *et al.* Diet associations with nonalcoholic fatty liver disease in an ethnically diverse population: the Multiethnic Cohort. *Hepatology (Baltimore, Md)*, doi:10.1002/hep.30967 (2019).
- 48 Jia, Q. *et al.* Dietary patterns are associated with prevalence of fatty liver disease in adults. *European journal of clinical nutrition* **69**, 914–921, doi:10.1038/ejcn.2014.297 (2015).
- 49 Oddy, W. H. *et al.* The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. *The American journal of gastroenterology* **108**, 778–785, doi:10.1038/ajg.2013.95 (2013).
- 50 Soleimani, D. *et al.* Dietary patterns in relation to hepatic fibrosis among patients with nonalcoholic fatty liver disease. *Diabetes, metabolic syndrome and obesity : targets and therapy* **12**, 315–324, doi:10.2147/dmso.S198744 (2019).
- 51 Ma, J. *et al.* Improved Diet Quality Associates With Reduction in Liver Fat, Particularly in Individuals With High Genetic Risk Scores for Nonalcoholic Fatty Liver Disease. *Gastroenterology* **155**, 107–117, doi:10.1053/j.gastro.2018.03.038 (2018).
- 52 Khalatbari-Soltani, S. *et al.* The association between adherence to the Mediterranean diet and hepatic steatosis: cross-sectional analysis of two independent studies, the UK Fenland Study and the Swiss CoLaus Study. *BMC medicine* **17**, 19, doi:10.1186/s12916-019-1251-7 (2019).
- 53 Asgari-Taee, F. *et al.* Association of sugar sweetened beverages consumption with non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Eur J Nutr* **58**, 1759–1769, doi:10.1007/s00394-018-1711-4 (2019).
- 54 Chen, H. *et al.* Consumption of Sugar-Sweetened Beverages Has a Dose-Dependent Effect on the Risk of Non-Alcoholic Fatty Liver Disease: An Updated Systematic Review and Dose-Response Meta-Analysis. *International journal of environmental research and public health* **16**, doi:10.3390/ijerph16122192 (2019).

- 55 Charidemou, E. *et al.* A randomized 3–way crossover study indicates that high–protein feeding induces de novo lipogenesis in healthy humans. *JCI Insight* **4**, doi:10.1172/jci.insight.124819 (2019).
- 56 Aarsland, A. & Wolfe, R. R. Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. *Journal of lipid research* **39**, 1280–1286 (1998).
- 57 Jacome–Sosa, M. M. & Parks, E. J. Fatty acid sources and their fluxes as they contribute to plasma triglyceride concentrations and fatty liver in humans. *Current opinion in lipidology* **25**, 213–220, doi:10.1097/MOL.0000000000000080 (2014).
- 58 Donnelly, K. L. *et al.* Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *The Journal of clinical investigation* **115**, 1343–1351, doi:10.1172/JCI23621 (2005).
- 59 Lambert, J. E., Ramos–Roman, M. A., Browning, J. D. & Parks, E. J. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* **146**, 726–735, doi:10.1053/j.gastro.2013.11.049 (2014).
- 60 Parks, E. J., Skokan, L. E., Timlin, M. T. & Dingfelder, C. S. Dietary sugars stimulate fatty acid synthesis in adults. *The Journal of nutrition* **138**, 1039–1046 (2008).
- 61 Smith, G. I. *et al.* Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. *The Journal of clinical investigation*, doi:10.1172/JCI134165 (2019).
- 62 Hellerstein, M. K. De novo lipogenesis in humans: metabolic and regulatory aspects. *Eur J Clin Nutr* **53 Suppl 1**, S53–65 (1999).
- 63 Schwarz, J. M., Neese, R. A., Turner, S., Dare, D. & Hellerstein, M. K. Short–term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole–body fuel selection. *The Journal of clinical investigation* **96**, 2735–2743, doi:10.1172/JCI118342 (1995).
- 64 McGarry, J. D., Mannaerts, G. P. & Foster, D. W. A possible role for malonyl–CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *The Journal of clinical investigation* **60**, 265–270, doi:10.1172/JCI108764 (1977).
- 65 Hodson, L. *et al.* Docosahexaenoic acid enrichment in NAFLD is associated with improvements in hepatic metabolism and hepatic insulin sensitivity: a pilot study. *European journal of clinical nutrition* **71**, 973–979, doi:10.1038/ejcn.2017.9 (2017).
- 66 Hellerstein, M. K. *et al.* Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *The Journal of clinical investigation* **87**, 1841–1852, doi:10.1172/JCI115206 (1991).
- 67 Marques–Lopes, I., Ansorena, D., Astiasaran, I., Forga, L. & Martinez, J. A. Postprandial de novo lipogenesis and metabolic changes induced by a high–carbohydrate, low–fat meal in lean and overweight men. *The American journal of clinical nutrition* **73**, 253–261, doi:10.1093/ajcn/73.2.253 (2001).
- 68 Diraison, F., Moulin, P. & Beylot, M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non–alcoholic fatty liver disease. *Diabetes & Metabolism* **29**, 478–485, doi:10.1016/s1262–3636(07)70061–7 (2003).
- 69 Mancina, R. M. *et al.* Paradoxical dissociation between hepatic fat content and de novo lipogenesis due to PNPLA3 sequence variant. *The Journal of clinical endocrinology and metabolism* **100**, E821–825, doi:10.1210/jc.2014–4464 (2015).
- 70 Sevastianova, K. *et al.* Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss–induced decrease in liver fat in humans. *The American journal of clinical nutrition* **94**, 104–111, doi:10.3945/ajcn.111.012369 (2011).
- 71 Wilke, M. S. *et al.* Synthesis of specific fatty acids contributes to VLDL–triacylglycerol composition in humans with and without type 2 diabetes. *Diabetologia* **52**, 1628–1637, doi:10.1007/s00125–009–1405–9 (2009).
- 72 Semple, R. K. *et al.* Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *The Journal of clinical investigation* **119**, 315–322, doi:10.1172/jci37432 (2009).

- 73 Santoro, N. *et al.* Hepatic De Novo Lipogenesis in Obese Youth Is Modulated by a Common Variant in the GCKR Gene. *The Journal of clinical endocrinology and metabolism* **100**, E1125–1132, doi:10.1210/jc.2015–1587 (2015).
- 74 Pramfalk, C. *et al.* Fasting Plasma Insulin Concentrations Are Associated With Changes in Hepatic Fatty Acid Synthesis and Partitioning Prior to Changes in Liver Fat Content in Healthy Adults. *Diabetes* **65**, 1858–1867, doi:10.2337/db16–0236 (2016).
- 75 Green, C. J. *et al.* Hepatic de novo lipogenesis is suppressed and fat oxidation is increased by omega–3 fatty acids at the expense of glucose metabolism. *BMJ Open Diabetes Res Care* **8**, doi:10.1136/bmjdr-2019–000871 (2020).
- 76 Kim, C. W. *et al.* Acetyl CoA Carboxylase Inhibition Reduces Hepatic Steatosis but Elevates Plasma Triglycerides in Mice and Humans: A Bedside to Bench Investigation. *Cell metabolism* **26**, 576, doi:10.1016/j.cmet.2017.08.011 (2017).
- 77 Loomba, R. *et al.* GS–0976 Reduces Hepatic Steatosis and Fibrosis Markers in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* **155**, 1463–1473 e1466, doi:10.1053/j.gastro.2018.07.027 (2018).
- 78 Stiede, K. *et al.* Acetyl–coenzyme A carboxylase inhibition reduces de novo lipogenesis in overweight male subjects: A randomized, double–blind, crossover study. *Hepatology* **66**, 324–334, doi:10.1002/hep.29246 (2017).
- 79 Hodson, L. & Gunn, P. J. The regulation of hepatic fatty acid synthesis and partitioning: the effect of nutritional state. *Nature reviews. Endocrinology* **15**, 689–700, doi:10.1038/s41574–019–0256–9 (2019).
- 80 Kotronen, A., Juurinen, L., Tiikkainen, M., Vehkavaara, S. & Yki–Jarvinen, H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. *Gastroenterology* **135**, 122–130, doi:10.1053/j.gastro.2008.03.021 (2008).
- 81 Fabbrini, E. *et al.* Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 15430–15435, doi:10.1073/pnas.0904944106 (2009).
- 82 Gastaldelli, A. *et al.* Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* **133**, 496–506, doi:10.1053/j.gastro.2007.04.068 (2007).
- 83 Mittendorfer, B., Magkos, F., Fabbrini, E., Mohammed, B. S. & Klein, S. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity* **17**, 1872–1877, doi:10.1038/oby.2009.224 (2009).
- 84 Nouws, J. *et al.* Altered In Vivo Lipid Fluxes and Cell Dynamics in Subcutaneous Adipose Tissues Are Associated With the Unfavorable Pattern of Fat Distribution in Obese Adolescent Girls. *Diabetes* **68**, 1168–1177, doi:10.2337/db18–1162 (2019).
- 85 Hodson, L. & Frayn, K. N. Hepatic fatty acid partitioning. *Current opinion in lipidology* **22**, 216–224, doi:10.1097/MOL.0b013e3283462e16 (2011).
- 86 Malmstrom, R. *et al.* Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes* **47**, 779–787 (1998).
- 87 Adiels, M. *et al.* Acute suppression of VLDL1 secretion rate by insulin is associated with hepatic fat content and insulin resistance. *Diabetologia* **50**, 2356–2365, doi:10.1007/s00125–007–0790–1 (2007).
- 88 Fabbrini, E. *et al.* Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* **134**, 424–431, doi:10.1053/j.gastro.2007.11.038 (2008).
- 89 Umpleby, A. M. *et al.* Impact of liver fat on the differential partitioning of hepatic triacylglycerol into VLDL subclasses on high and low sugar diets. *Clin Sci (Lond)* **131**, 2561–2573, doi:10.1042/cs20171208 (2017).
- 90 Gill, J. M. *et al.* Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics: dose–dependent effects on LDL. *The American journal of clinical nutrition* **78**, 47–56, doi:10.1093/ajcn/78.1.47 (2003).

- 91 Havel, R. J., Kane, J. P., Balasse, E. O., Segel, N. & Basso, L. V. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *The Journal of clinical investigation* **49**, 2017–2035, doi:10.1172/jci106422 (1970).
- 92 Weiss, M., Keller, U. & Stauffacher, W. Effect of epinephrine and somatostatin-induced insulin deficiency on ketone body kinetics and lipolysis in man. *Diabetes* **33**, 738–744, doi:10.2337/diab.33.8.738 (1984).
- 93 Nosadini, R. *et al.* Acetoacetate and 3-hydroxybutyrate kinetics in obese and insulin-dependent diabetic humans. *The American journal of physiology* **248**, R611–620, doi:10.1152/ajpregu.1985.248.5.R611 (1985).
- 94 Croci, I. *et al.* Whole-body substrate metabolism is associated with disease severity in patients with non-alcoholic fatty liver disease. *Gut* **62**, 1625–1633, doi:10.1136/gutjnl-2012-302789 (2013).
- 95 Kotronen, A. *et al.* Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. *Diabetes* **58**, 203–208, doi:10.2337/db08-1074 (2009).
- 96 Sunny, N. E., Parks, E. J., Browning, J. D. & Burgess, S. C. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell metabolism* **14**, 804–810, doi:10.1016/j.cmet.2011.11.004 (2011).
- 97 Bugianesi, E. *et al.* Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* **48**, 634–642, doi:10.1007/s00125-005-1682-x (2005).
- 98 Petersen, K. F., Befroy, D. E., Dufour, S., Rothman, D. L. & Shulman, G. I. Assessment of Hepatic Mitochondrial Oxidation and Pyruvate Cycling in NAFLD by (13)C Magnetic Resonance Spectroscopy. *Cell metabolism* **24**, 167–171, doi:10.1016/j.cmet.2016.06.005 (2016).
- 99 Roberts, R. *et al.* Reduced oxidation of dietary fat after a short term high-carbohydrate diet. *The American journal of clinical nutrition* **87**, 824–831, doi:10.1093/ajcn/87.4.824 (2008).
- 100 Leyton, J., Drury, P. J. & Crawford, M. A. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *The British journal of nutrition* **57**, 383–393, doi:10.1079/bjn19870046 (1987).
- 101 Bessesen, D. H., Vensor, S. H. & Jackman, M. R. Trafficking of dietary oleic, linolenic, and stearic acids in fasted or fed lean rats. *American journal of physiology. Endocrinology and metabolism* **278**, E1124–1132, doi:10.1152/ajpendo.2000.278.6.E1124 (2000).
- 102 Jones, P. J., Pencharz, P. B. & Clandinin, M. T. Whole body oxidation of dietary fatty acids: implications for energy utilization. *The American journal of clinical nutrition* **42**, 769–777, doi:10.1093/ajcn/42.5.769 (1985).
- 103 DeLany, J. P., Windhauser, M. M., Champagne, C. M. & Bray, G. A. Differential oxidation of individual dietary fatty acids in humans. *The American journal of clinical nutrition* **72**, 905–911, doi:10.1093/ajcn/72.4.905 (2000).
- 104 Parry, S. A., Rosqvist, F., Cornfield, T., Barrett, A. & Hodson, L. Oxidation of dietary linoleate occurs to a greater extent than dietary palmitate in vivo in humans. *Clinical nutrition*, doi:10.1016/j.clnu.2020.07.013 (2020).
- 105 Luukkonen, P. K. *et al.* Saturated Fat Is More Metabolically Harmful for the Human Liver Than Unsaturated Fat or Simple Sugars. *Diabetes care* **41**, 1732–1739, doi:10.2337/dc18-0071 (2018).
- 106 Kirk, E. *et al.* Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology* **136**, 1552–1560, doi:10.1053/j.gastro.2009.01.048 (2009).
- 107 Browning, J. D. *et al.* Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. *The American journal of clinical nutrition* **93**, 1048–1052, doi:10.3945/ajcn.110.007674 (2011).

- 108 Haufe, S. *et al.* Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology* **53**, 1504–1514, doi:10.1002/hep.24242 (2011).
- 109 Westerbacka, J. *et al.* Dietary fat content modifies liver fat in overweight nondiabetic subjects. *The Journal of clinical endocrinology and metabolism* **90**, 2804–2809, doi:10.1210/jc.2004–1983 (2005).
- 110 van Herpen, N. A., Schrauwen–Hinderling, V. B., Schaart, G., Mensink, R. P. & Schrauwen, P. Three weeks on a high–fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. *The Journal of clinical endocrinology and metabolism* **96**, E691–695, doi:10.1210/jc.2010–2243 (2011).
- 111 Utzschneider, K. M. *et al.* Beneficial effect of a weight–stable, low–fat/low–saturated fat/low–glycaemic index diet to reduce liver fat in older subjects. *The British journal of nutrition* **109**, 1096–1104, doi:10.1017/S0007114512002966 (2013).
- 112 Mardinoglu, A. *et al.* An Integrated Understanding of the Rapid Metabolic Benefits of a Carbohydrate–Restricted Diet on Hepatic Steatosis in Humans. *Cell metabolism* **27**, 559–571.e555, doi:10.1016/j.cmet.2018.01.005 (2018).
- 113 Elmadfa, I. & Kornsteiner, M. Fats and fatty acid requirements for adults. *Ann Nutr Metab* **55**, 56–75, doi:10.1159/000228996 (2009).
- 114 Ahn, J., Jun, D. W., Lee, H. Y. & Moon, J. H. Critical appraisal for low–carbohydrate diet in nonalcoholic fatty liver disease: Review and meta–analyses. *Clinical nutrition* **38**, 2023–2030, doi:10.1016/j.clnu.2018.09.022 (2019).
- 115 Sobrecases, H. *et al.* Effects of short–term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. *Diabetes & metabolism* **36**, 244–246, doi:10.1016/j.diabet.2010.03.003 (2010).
- 116 Bjermo, H. *et al.* Effects of n–6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *The American journal of clinical nutrition* **95**, 1003–1012, doi:10.3945/ajcn.111.030114 (2012).
- 117 Rosqvist, F. *et al.* Overeating Saturated Fat Promotes Fatty Liver and Ceramides Compared With Polyunsaturated Fat: A Randomized Trial. *The Journal of clinical endocrinology and metabolism* **104**, 6207–6219, doi:10.1210/jc.2019–00160 (2019).
- 118 Rosqvist, F. *et al.* Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* **63**, 2356–2368, doi:10.2337/db13–1622 (2014).
- 119 Kien, C. L., Bunn, J. Y. & Ugrasbul, F. Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. *The American journal of clinical nutrition* **82**, 320–326, doi:10.1093/ajcn.82.2.320 (2005).
- 120 Bozzetto, L. *et al.* Reduction in liver fat by dietary MUFA in type 2 diabetes is helped by enhanced hepatic fat oxidation. *Diabetologia* **59**, 2697–2701, doi:10.1007/s00125–016–4110–5 (2016).
- 121 Eckel, R. H. *et al.* 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* **129**, S76–99, doi:10.1161/01.cir.0000437740.48606.d1 (2014).
- 122 Hooper, L., Martin, N., Abdelhamid, A. & Davey Smith, G. Reduction in saturated fat intake for cardiovascular disease. *The Cochrane database of systematic reviews*, CD011737, doi:10.1002/14651858.CD011737 (2015).
- 123 Farvid, M. S. *et al.* Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta–analysis of prospective cohort studies. *Circulation* **130**, 1568–1578, doi:10.1161/CIRCULATIONAHA.114.010236 (2014).
- 124 Yaghootkar, H. *et al.* Genetic evidence for a normal–weight "metabolically obese" phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. *Diabetes* **63**, 4369–4377, doi:10.2337/db14–0318 (2014).
- 125 Lotta, L. A. *et al.* Association of Genetic Variants Related to Gluteofemoral vs Abdominal Fat Distribution With Type 2 Diabetes, Coronary Disease, and

- Cardiovascular Risk Factors. *JAMA : the journal of the American Medical Association* **320**, 2553–2563, doi:10.1001/jama.2018.19329 (2018).
- 126 Yaghootkar, H. *et al.* Genetic Evidence for a Link Between Favorable Adiposity and Lower Risk of Type 2 Diabetes, Hypertension, and Heart Disease. *Diabetes* **65**, 2448–2460, doi:10.2337/db15–1671 (2016).
- 127 Ji, Y. *et al.* Genome-Wide and Abdominal MRI Data Provide Evidence That a Genetically Determined Favorable Adiposity Phenotype Is Characterized by Lower Ectopic Liver Fat and Lower Risk of Type 2 Diabetes, Heart Disease, and Hypertension. *Diabetes* **68**, 207–219, doi:10.2337/db18–0708 (2019).
- 128 Vessby, B. *et al.* Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* **44**, 312–319 (2001).
- 129 Ryysy, L. *et al.* Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. *Diabetes* **49**, 749–758 (2000).
- 130 Sanyal, A. J. *et al.* Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* **120**, 1183–1192, doi:10.1053/gast.2001.23256 (2001).
- 131 Seppala-Lindroos, A. *et al.* Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *The Journal of clinical endocrinology and metabolism* **87**, 3023–3028, doi:10.1210/jcem.87.7.8638 (2002).
- 132 Korenblat, K. M., Fabbrini, E., Mohammed, B. S. & Klein, S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology* **134**, 1369–1375, doi:10.1053/j.gastro.2008.01.075 (2008).
- 133 Amaro, A. *et al.* Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. *Gastroenterology* **139**, 149–153, doi:10.1053/j.gastro.2010.03.039 (2010).
- 134 Romeo, S. *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nature genetics* **40**, 1461–1465, doi:10.1038/ng.257 (2008).
- 135 Sun, Z. & Lazar, M. A. Dissociating fatty liver and diabetes. *Trends in endocrinology and metabolism: TEM* **24**, 4–12, doi:10.1016/j.tem.2012.09.005 (2013).
- 136 Cohen, J. C., Horton, J. D. & Hobbs, H. H. Human fatty liver disease: old questions and new insights. *Science* **332**, 1519–1523, doi:10.1126/science.1204265 (2011).
- 137 Samuel, V. T. & Shulman, G. I. Nonalcoholic Fatty Liver Disease as a Nexus of Metabolic and Hepatic Diseases. *Cell metabolism* **27**, 22–41, doi:10.1016/j.cmet.2017.08.002 (2018).
- 138 Chaurasia, B. & Summers, S. A. Ceramides – Lipotoxic Inducers of Metabolic Disorders: (Trends in Endocrinology and Metabolism 26, 538–550; 2015). *Trends in endocrinology and metabolism: TEM* **29**, 66–67, doi:10.1016/j.tem.2017.09.005 (2018).
- 139 Havulinna, A. S. *et al.* Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. *Arteriosclerosis, thrombosis, and vascular biology* **36**, 2424–2430, doi:10.1161/ATVBAHA.116.307497 (2016).
- 140 Hilvo, M. *et al.* Ceramide stearic to palmitic acid ratio predicts incident diabetes. *Diabetologia* **61**, 1424–1434, doi:10.1007/s00125–018–4590–6 (2018).
- 141 Peterson, L. R. *et al.* Ceramide Remodeling and Risk of Cardiovascular Events and Mortality. *J Am Heart Assoc* **7**, doi:10.1161/JAHA.117.007931 (2018).
- 142 Lemaitre, R. N. *et al.* Circulating Sphingolipids, Insulin, HOMA–IR, and HOMA–B: The Strong Heart Family Study. *Diabetes* **67**, 1663–1672, doi:10.2337/db17–1449 (2018).
- 143 Gorden, D. L. *et al.* Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *Journal of lipid research* **56**, 722–736, doi:10.1194/jlr.P056002 (2015).
- 144 Holland, W. L. *et al.* Inhibition of ceramide synthesis ameliorates glucocorticoid–, saturated–fat–, and obesity–induced insulin resistance. *Cell metabolism* **5**, 167–179, doi:10.1016/j.cmet.2007.01.002 (2007).



- 145 Hu, W., Ross, J., Geng, T., Brice, S. E. & Cowart, L. A. Differential regulation of dihydroceramide desaturase by palmitate versus monounsaturated fatty acids: implications for insulin resistance. *The Journal of biological chemistry* **286**, 16596–16605, doi:10.1074/jbc.M110.186916 (2011).
- 146 Chaurasia, B. *et al.* Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science* **365**, 386–392, doi:10.1126/science.aav3722 (2019).
- 147 Xie, C. *et al.* An Intestinal Farnesoid X Receptor–Ceramide Signaling Axis Modulates Hepatic Gluconeogenesis in Mice. *Diabetes* **66**, 613–626, doi:10.2337/db16–0663 (2017).
- 148 Turpin, S. M. *et al.* Obesity–induced CerS6–dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell metabolism* **20**, 678–686, doi:10.1016/j.cmet.2014.08.002 (2014).
- 149 Xia, J. Y. *et al.* Targeted Induction of Ceramide Degradation Leads to Improved Systemic Metabolism and Reduced Hepatic Steatosis. *Cell metabolism* **22**, 266–278, doi:10.1016/j.cmet.2015.06.007 (2015).
- 150 Raichur, S. *et al.* CerS2 Haploinsufficiency Inhibits beta–Oxidation and Confers Susceptibility to Diet–Induced Steatohepatitis and Insulin Resistance. *Cell metabolism* **20**, 919, doi:10.1016/j.cmet.2014.10.007 (2014).
- 151 Hammerschmidt, P. *et al.* CerS6–Derived Sphingolipids Interact with Mff and Promote Mitochondrial Fragmentation in Obesity. *Cell* **177**, 1536–1552 e1523, doi:10.1016/j.cell.2019.05.008 (2019).
- 152 Garcia–Ruiz, C., Colell, A., Mari, M., Morales, A. & Fernandez–Checa, J. C. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *The Journal of biological chemistry* **272**, 11369–11377, doi:10.1074/jbc.272.17.11369 (1997).
- 153 Day, C. P. & James, O. F. Steatohepatitis: a tale of two "hits"? *Gastroenterology* **114**, 842–845, doi:10.1016/s0016–5085(98)70599–2 (1998).
- 154 Colombini, M. Ceramide channels and their role in mitochondria–mediated apoptosis. *Biochimica et biophysica acta* **1797**, 1239–1244, doi:10.1016/j.bbabi.2010.01.021 (2010).
- 155 Martinez, L. *et al.* Myristic acid potentiates palmitic acid–induced lipotoxicity and steatohepatitis associated with lipodystrophy by sustaining de novo ceramide synthesis. *Oncotarget* **6**, 41479–41496, doi:10.18632/oncotarget.6286 (2015).
- 156 Luukkonen, P. K. *et al.* Hepatic ceramides dissociate steatosis and insulin resistance in patients with non–alcoholic fatty liver disease. *Journal of hepatology* **64**, 1167–1175, doi:10.1016/j.jhep.2016.01.002 (2016).
- 157 Apostolopoulou, M. *et al.* Specific Hepatic Sphingolipids Relate to Insulin Resistance, Oxidative Stress, and Inflammation in Nonalcoholic Steatohepatitis. *Diabetes care* **41**, 1235–1243, doi:10.2337/dc17–1318 (2018).
- 158 Samuel, V. T. *et al.* Mechanism of hepatic insulin resistance in non–alcoholic fatty liver disease. *The Journal of biological chemistry* **279**, 32345–32353, doi:10.1074/jbc.M313478200 (2004).
- 159 Samuel, V. T. *et al.* Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *The Journal of clinical investigation* **117**, 739–745, doi:10.1172/JCI30400 (2007).
- 160 Ter Horst, K. W. *et al.* Hepatic Diacylglycerol–Associated Protein Kinase Cepsilon Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans. *Cell Rep* **19**, 1997–2004, doi:10.1016/j.celrep.2017.05.035 (2017).
- 161 Petersen, M. C. *et al.* Insulin receptor Thr1160 phosphorylation mediates lipid–induced hepatic insulin resistance. *The Journal of clinical investigation* **126**, 4361–4371, doi:10.1172/JCI86013 (2016).
- 162 Raddatz, K. *et al.* Time–dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia* **54**, 1447–1456, doi:10.1007/s00125–011–2073–0 (2011).

- 163 Puri, P. *et al.* A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* **46**, 1081–1090, doi:10.1002/hep.21763 (2007).
- 164 Kumashiro, N. *et al.* Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 16381–16385, doi:10.1073/pnas.1113359108 (2011).
- 165 Gordon, D. L. *et al.* Increased diacylglycerols characterize hepatic lipid changes in progression of human nonalcoholic fatty liver disease; comparison to a murine model. *PloS one* **6**, e22775, doi:10.1371/journal.pone.0022775 (2011).
- 166 Magkos, F. *et al.* Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology* **142**, 1444–1446 e1442, doi:10.1053/j.gastro.2012.03.003 (2012).
- 167 Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* **444**, 860–867, doi:10.1038/nature05485 (2006).
- 168 Weisberg, S. P. *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of clinical investigation* **112**, 1796–1808, doi:10.1172/JCI19246 (2003).
- 169 Sonnenburg, J. L. & Backhed, F. Diet–microbiota interactions as moderators of human metabolism. *Nature* **535**, 56–64, doi:10.1038/nature18846 (2016).
- 170 Mehta, N. N. *et al.* Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes* **59**, 172–181, doi:10.2337/db09–0367 (2010).
- 171 Lassenius, M. I. *et al.* Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes care* **34**, 1809–1815, doi:10.2337/dc10–2197 (2011).
- 172 Pang, J. *et al.* Significant positive association of endotoxemia with histological severity in 237 patients with non–alcoholic fatty liver disease. *Alimentary pharmacology & therapeutics* **46**, 175–182, doi:10.1111/apt.14119 (2017).
- 173 Thurman, R. G. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *The American journal of physiology* **275**, G605–G611, doi:10.1152/ajpgi.1998.275.4.G605 (1998).
- 174 Cani, P. D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772, doi:10.2337/db06–1491 (2007).
- 175 Caesar, R., Tremaroli, V., Kovatcheva–Datchary, P., Cani, P. D. & Backhed, F. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. *Cell metabolism* **22**, 658–668, doi:10.1016/j.cmet.2015.07.026 (2015).
- 176 Cani, P. D. *et al.* Changes in gut microbiota control metabolic endotoxemia–induced inflammation in high–fat diet–induced obesity and diabetes in mice. *Diabetes* **57**, 1470–1481, doi:10.2337/db07–1403 (2008).
- 177 Erridge, C., Attina, T., Spickett, C. M. & Webb, D. J. A high–fat meal induces low–grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *The American journal of clinical nutrition* **86**, 1286–1292, doi:10.1093/ajcn/86.5.1286 (2007).
- 178 Deopurkar, R. *et al.* Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll–like receptor–4 and suppressor of cytokine signaling–3. *Diabetes care* **33**, 991–997, doi:10.2337/dc09–1630 (2010).
- 179 Hernandez, E. A. *et al.* Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *The Journal of clinical investigation* **127**, 695–708, doi:10.1172/JCI89444 (2017).
- 180 Cho, Y. E. *et al.* Fructose Promotes Leaky Gut, Endotoxemia, and Liver Fibrosis Through Ethanol–Inducible Cytochrome P450–2E1–Mediated Oxidative and Nitrate Stress. *Hepatology*, doi:10.1002/hep.30652 (2019).
- 181 Kavanagh, K. *et al.* Dietary fructose induces endotoxemia and hepatic injury in calorically controlled primates. *The American journal of clinical nutrition* **98**, 349–357, doi:10.3945/ajcn.112.057331 (2013).

- 182 Jin, R. *et al.* Fructose induced endotoxemia in pediatric nonalcoholic Fatty liver disease. *Int J Hepatol* **2014**, 560620, doi:10.1155/2014/560620 (2014).
- 183 Nier, A., Brandt, A., Rajcic, D., Bruns, T. & Bergheim, I. Short-Term Isocaloric Intake of a Fructose– but not Glucose–Rich Diet Affects Bacterial Endotoxin Concentrations and Markers of Metabolic Health in Normal Weight Healthy Subjects. *Mol Nutr Food Res* **63**, e1800868, doi:10.1002/mnfr.201800868 (2019).
- 184 Vors, C. *et al.* Postprandial Endotoxemia Linked With Chylomicrons and Lipopolysaccharides Handling in Obese Versus Lean Men: A Lipid Dose–Effect Trial. *The Journal of clinical endocrinology and metabolism* **100**, 3427–3435, doi:10.1210/JC.2015–2518 (2015).
- 185 Bowser, S. M. *et al.* Serum endotoxin, gut permeability and skeletal muscle metabolic adaptations following a short term high fat diet in humans. *Metabolism: clinical and experimental* **103**, 154041, doi:10.1016/j.metabol.2019.154041 (2020).
- 186 EASL–EASD–EASO. EASL–EASD–EASO Clinical Practice Guidelines for the management of non–alcoholic fatty liver disease. *Journal of hepatology* **64**, 1388–1402, doi:10.1016/j.jhep.2015.11.004 (2016).
- 187 Zelber–Sagi, S., Salomone, F. & Mlynarsky, L. The Mediterranean dietary pattern as the diet of choice for non–alcoholic fatty liver disease: Evidence and plausible mechanisms. *Liver Int* **37**, 936–949, doi:10.1111/liv.13435 (2017).
- 188 Van Horn, L. *et al.* Recommended Dietary Pattern to Achieve Adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A Scientific Statement From the American Heart Association. *Circulation* **134**, e505–e529, doi:10.1161/CIR.0000000000000462 (2016).
- 189 Bozzetto, L. *et al.* Liver fat is reduced by an isoenergetic MUFA diet in a controlled randomized study in type 2 diabetic patients. *Diabetes care* **35**, 1429–1435, doi:10.2337/dc12–0033 (2012).
- 190 Errazuriz, I. *et al.* Randomized Controlled Trial of a MUFA or Fiber–Rich Diet on Hepatic Fat in Prediabetes. *The Journal of clinical endocrinology and metabolism* **102**, 1765–1774, doi:10.1210/jc.2016–3722 (2017).
- 191 Gepner, Y. *et al.* Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. *Circulation* **137**, 1143–1157, doi:10.1161/CIRCULATIONAHA.117.030501 (2018).
- 192 Gepner, Y. *et al.* The beneficial effects of Mediterranean diet over low–fat diet may be mediated by decreasing hepatic fat content. *Journal of hepatology* **71**, 379–388, doi:10.1016/j.jhep.2019.04.013 (2019).
- 193 Properzi, C. *et al.* Ad Libitum Mediterranean and Low–Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial. *Hepatology* **68**, 1741–1754, doi:10.1002/hep.30076 (2018).
- 194 Pepin, A., Stanhope, K. L. & Imbeault, P. Are Fruit Juices Healthier Than Sugar–Sweetened Beverages? A Review. *Nutrients* **11**, doi:10.3390/nu11051006 (2019).
- 195 Moore, J. B., Gunn, P. J. & Fielding, B. A. The role of dietary sugars and de novo lipogenesis in non–alcoholic fatty liver disease. *Nutrients* **6**, 5679–5703, doi:10.3390/nu6125679 (2014).
- 196 Maldonado, E. M. *et al.* Multi–scale, whole–system models of liver metabolic adaptation to fat and sugar in non–alcoholic fatty liver disease. *NPJ Syst Biol Appl* **4**, 33, doi:10.1038/s41540–018–0070–3 (2018).
- 197 Tappy, L. & Le, K. A. Metabolic effects of fructose and the worldwide increase in obesity. *Physiological reviews* **90**, 23–46, doi:10.1152/physrev.00019.2009 (2010).
- 198 Pinnick, K. E. & Hodson, L. Challenging metabolic tissues with fructose: tissue–specific and sex–specific responses. *The Journal of physiology* **597**, 3527–3537, doi:10.1113/jp277115 (2019).
- 199 Jang, C. *et al.* The Small Intestine Converts Dietary Fructose into Glucose and Organic Acids. *Cell metabolism* **27**, 351–361.e353, doi:10.1016/j.cmet.2017.12.016 (2018).
- 200 Francey, C. *et al.* The extra–splanchnic fructose escape after ingestion of a fructose–glucose drink: An exploratory study in healthy humans using a dual fructose isotope

- method. *Clinical nutrition ESPEN* **29**, 125–132, doi:10.1016/j.clnesp.2018.11.008 (2019).
- 201 Johnston, R. D. *et al.* No difference between high–fructose and high–glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. *Gastroenterology* **145**, 1016–1025 e1012, doi:10.1053/j.gastro.2013.07.012 (2013).
- 202 Schwarz, J. M. *et al.* Effect of a High–Fructose Weight–Maintaining Diet on Lipogenesis and Liver Fat. *The Journal of clinical endocrinology and metabolism* **100**, 2434–2442, doi:10.1210/jc.2014–3678 (2015).
- 203 Ngo Sock, E. T. *et al.* Effects of a short–term overfeeding with fructose or glucose in healthy young males. *The British journal of nutrition* **103**, 939–943, doi:10.1017/S0007114509992819 (2010).
- 204 Silbernagel, G. *et al.* Effects of 4–week very–high–fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *The British journal of nutrition* **106**, 79–86, doi:10.1017/S000711451000574X (2011).
- 205 Bravo, S., Lowndes, J., Sinnett, S., Yu, Z. & Rippe, J. Consumption of sucrose and high–fructose corn syrup does not increase liver fat or ectopic fat deposition in muscles. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **38**, 681–688, doi:10.1139/apnm–2012–0322 (2013).
- 206 Lecoultre, V. *et al.* Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* **21**, 782–785, doi:10.1002/oby.20377 (2013).
- 207 Stanhope, K. L. *et al.* Consuming fructose–sweetened, not glucose–sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *The Journal of clinical investigation* **119**, 1322–1334, doi:10.1172/JCI37385 (2009).
- 208 Taskinen, M. R. *et al.* Adverse effects of fructose on cardiometabolic risk factors and hepatic lipid metabolism in subjects with abdominal obesity. *Journal of internal medicine* **282**, 187–201, doi:10.1111/joim.12632 (2017).
- 209 Sun, S. Z., Anderson, G. H., Flickinger, B. D., Williamson–Hughes, P. S. & Empie, M. W. Fructose and non–fructose sugar intakes in the US population and their associations with indicators of metabolic syndrome. *Food Chem Toxicol* **49**, 2875–2882, doi:10.1016/j.fct.2011.07.068 (2011).
- 210 Marriott, B. P., Hunt, K. J., Malek, A. M. & Newman, J. C. Trends in Intake of Energy and Total Sugar from Sugar–Sweetened Beverages in the United States among Children and Adults, NHANES 2003–2016. *Nutrients* **11**, doi:10.3390/nu11092004 (2019).
- 211 Vos, M. B. *et al.* Added Sugars and Cardiovascular Disease Risk in Children: A Scientific Statement From the American Heart Association. *Circulation* **135**, e1017–e1034, doi:10.1161/CIR.0000000000000439 (2017).
- 212 Schwimmer, J. B. *et al.* Effect of a Low Free Sugar Diet vs Usual Diet on Nonalcoholic Fatty Liver Disease in Adolescent Boys: A Randomized Clinical Trial. *JAMA : the journal of the American Medical Association* **321**, 256–265, doi:10.1001/jama.2018.20579 (2019).
- 213 Smajis, S. *et al.* Metabolic effects of a prolonged, very–high–dose dietary fructose challenge in healthy subjects. *The American journal of clinical nutrition* **111**, 369–377, doi:10.1093/ajcn/nqz271 (2020).
- 214 Vilar–Gomez, E. *et al.* Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* **149**, 367–378 e365; quiz e314–365, doi:10.1053/j.gastro.2015.04.005 (2015).
- 215 Yki–Jarvinen, H. Nutritional Modulation of Non–Alcoholic Fatty Liver Disease and Insulin Resistance. *Nutrients* **7**, 9127–9138, doi:10.3390/nu7115454 (2015).
- 216 Frayn, K. N., Arner, P. & Yki–Jarvinen, H. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. *Essays in biochemistry* **42**, 89–103, doi:10.1042/bse0420089 (2006).

- 217 Weintraub, M. S., Zechner, R., Brown, A., Eisenberg, S. & Breslow, J. L. Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. *The Journal of clinical investigation* **82**, 1884–1893, doi:10.1172/JCI113806 (1988).
- 218 Mensink, R. P., Zock, P. L., Kester, A. D. & Katan, M. B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *The American journal of clinical nutrition* **77**, 1146–1155 (2003).
- 219 Desmarchelier, C., Borel, P., Lairon, D., Maraninchi, M. & Valero, R. Effect of Nutrient and Micronutrient Intake on Chylomicron Production and Postprandial Lipemia. *Nutrients* **11**, doi:10.3390/nu11061299 (2019).
- 220 Chaurasia, B. & Summers, S. A. Ceramides – Lipotoxic Inducers of Metabolic Disorders. *Trends in endocrinology and metabolism: TEM* **26**, 538–550, doi:10.1016/j.tem.2015.07.006 (2015).

## Acknowledgements

L.H. is a British Heart Foundation Senior Research Fellow (FS/15/56/31645). P.K.L. is supported by grants from the Sigrid Jusélius, Instrumentarium Science and Novo Nordisk Foundations.

## 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/m <sup>2</sup> ); 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) <sup>106</sup>	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) <sup>107</sup>	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↓ <sup>a</sup>	No data No data
Haufe et al. (2011) <sup>108</sup>	102; 32; 45; 54	6 months	P	Hypo	LF-HC HF-LC	'Reduced fat' 'Reduced carb'	Less saturated <sup>b</sup>	9.6, 5.6↓ 7.6, 4.0↓	↑ ↑
Westerbacka et al. (2005) <sup>109</sup>	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 <sup>a</sup> 10, 13	↑ <sup>a</sup> ↓
van Herpen et al. (2011) <sup>110</sup>	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) <sup>111</sup>	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) <sup>115</sup>	39; 23; 25; no data	7 days	P	Hyper	LF-HC HF-LC	High fructose <sup>d</sup> High fat <sup>e</sup>	No data	12 <sup>c</sup> , 14↑ <sup>c</sup> 11 <sup>c</sup> , 21↑ <sup>c</sup>	NS NS
Luukkonen et al. (2018) <sup>105</sup>	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↑ <sup>a</sup>	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<sup>109</sup>. ↓, 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. <sup>a</sup>Significant difference in change between the diets. <sup>b</sup>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. <sup>c</sup>IHTG units are millimoles per kilogram wet weight (not percentage). <sup>d</sup>Addition of 3.5 g per day of fructose per kilogram of fat free mass. <sup>e</sup>Addition of 30% of total energy as fat. For corresponding figure, see Fig. 2 (left). Adapted from<sup>213</sup>, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

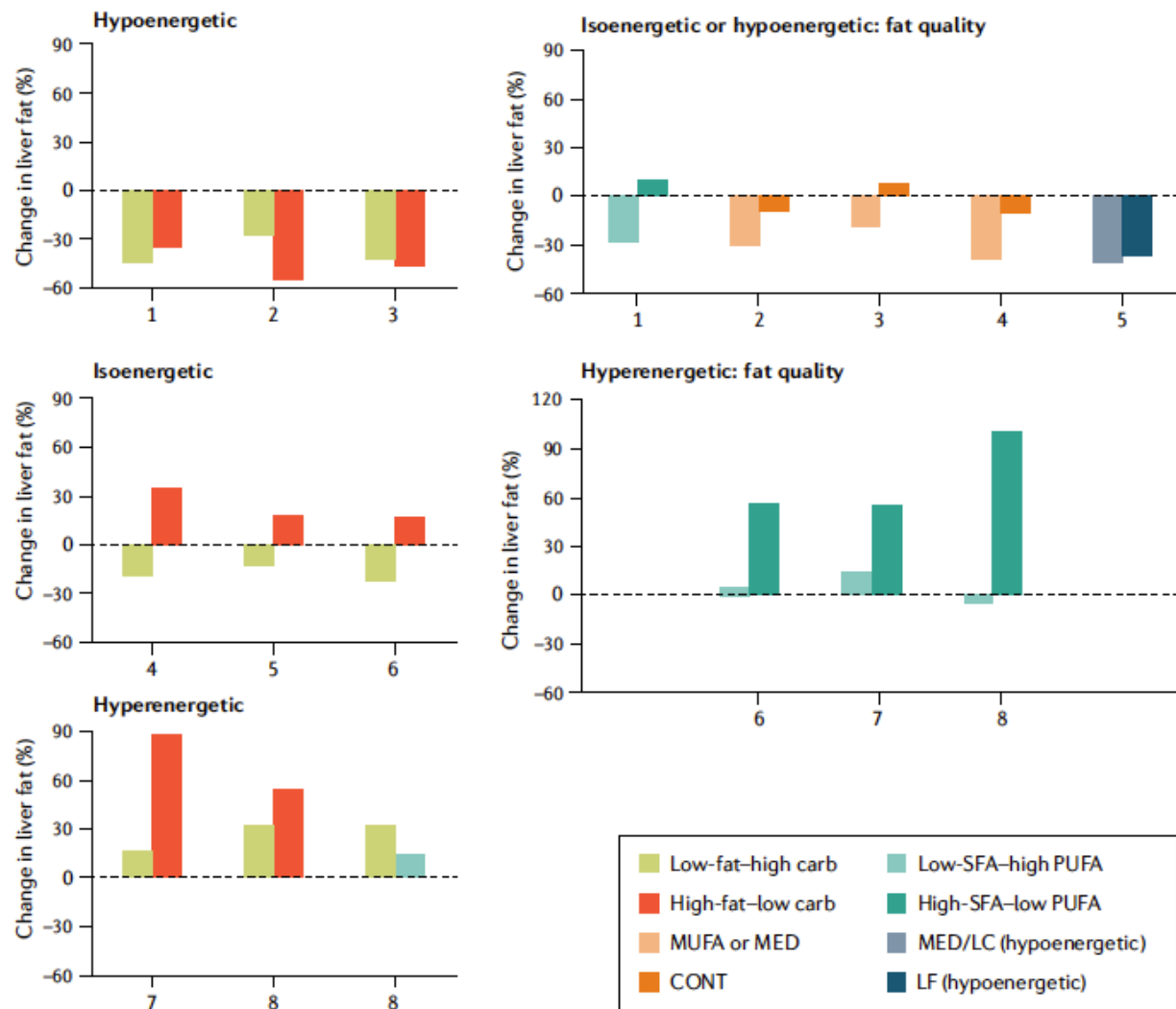
Table 2. studies comparing the effects of fat quality on liver fat

Study	Participant characteristics (n; BMI (kg/m <sup>2</sup> ); age (years); NAFLD (%))	Duration	Design	Energy content relative to baseline diet	Diet	Fat and carbohydrate (% of total energy)	IHTG (%): before, after
<b>SFA- enriched versus PUFA- enriched diets</b>							
Bjermo et al. (2012) <sup>116</sup>	61; 31; 30–65; no data	10 weeks	P	Iso	PUFA SFA	40 <sup>a</sup> , 39 43 <sup>b</sup> , 40	3.2, 2.3* 3.2, 3.5
Rosqvist et al. (2014) <sup>118</sup>	39; 20; 27; 0	7 weeks	P	Hyper	PUFA SFA	40 <sup>c</sup> , 43 36 <sup>d</sup> , 4	0.75, 0.79 0.96, 1.5*
Luukkonen et al. (2018) <sup>105</sup>	26; 30; 50; 27	3 weeks	P	Hyper	PUFA SFA	60 <sup>e</sup> , 23 59 <sup>f</sup> , 26	4.8, 5.5↑ 4.9, 7.6↑*
Rosqvist et al. (2019) <sup>117</sup>	61; 28; 42; 0	8 weeks	P	Hyper	PUFA SFA	51 <sup>g</sup> , 44 51 <sup>h</sup> , 44	2.0, 1.9 1.5, 3.0↑*
<b>MUFA and MED diets</b>							
Bozzetto et al. (2012) <sup>188</sup>	17; 30; 35–70; no data	8 weeks	P	Iso	CONT MUFA	28, 53 42 <sup>i</sup> , 40	17.7, 16.1 7.4, 5.2↓*
Errazuriz et al. (2017) <sup>189</sup>	28; 31; 61; 50	12 weeks	P	Iso	CONT MUFA	34 <sup>j</sup> , 49 46 <sup>k</sup> , 40	11.2, 11.9 9.7, 8.0↓*
Ryan et al. (2013) <sup>30</sup>	12; 32; 55; 100	6 weeks	C	Iso	CONT MUFA	21 <sup>l</sup> , 49 44 <sup>m</sup> , 34	11.2, 10.0 14.2, 8.6
Gepner et al. (2019) <sup>191</sup>	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) <sup>192</sup>	49; 31; 52; 100	12 weeks	P	Iso	CONT MUFA	31 <sup>n</sup> , 48 45 <sup>o</sup> , 37	21.5, 15.3↓ 34.2***, 24.0↓

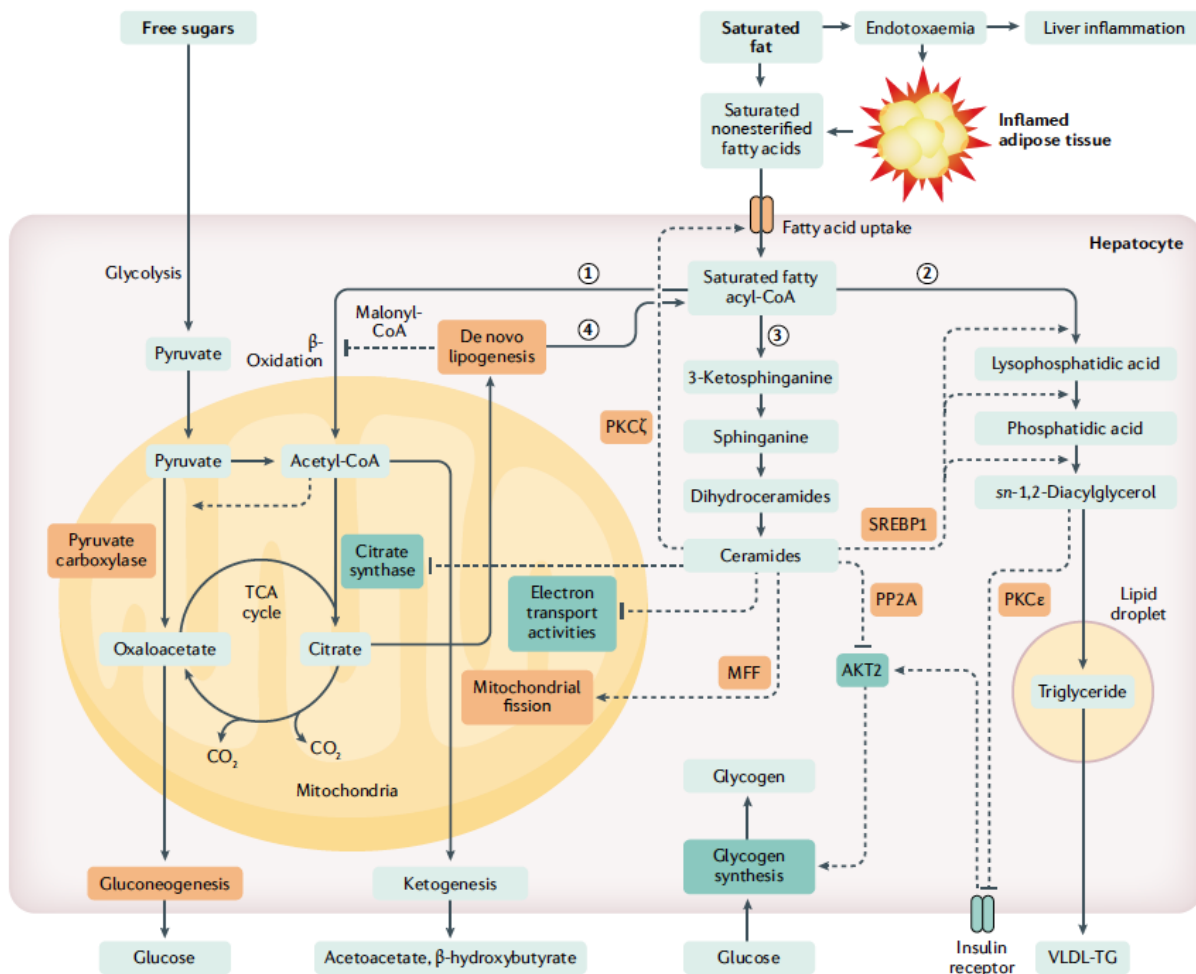
In all studies, there was no significant difference in change in body weight between groups. ↑, significant increase from baseline; ↓, significant decrease from baseline; C, crossover; CONT, standard control diet; Hyper, hyperenergetic; Hypo, hypoenergetic; Iso, isoenergetic; MED, Mediterranean diet; 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. <sup>a</sup>10% SFA, 17% MUFA, 13% PUFA. <sup>b</sup>20% SFA, 19% MUFA, 4% PUFA. <sup>c</sup>11% SFA, 12.4% MUFA, 13% PUFA. <sup>d</sup>16% SFA, 12.9% MUFA, 4% PUFA. <sup>e</sup>14% SFA, 28% MUFA, 11% PUFA. <sup>f</sup>33% SFA, 13% MUFA, 5% PUFA. <sup>g</sup>Sunflower oil. <sup>h</sup>Palm oil. <sup>i</sup>Enriched with MUFA, SFA similar in both arms. <sup>j</sup>12% SFA, 8% MUFA, 4% PUFA. <sup>k</sup>12% SFA, 22% MUFA, 5% PUFA. <sup>l</sup>14.4% SFA, 15.6% MUFA, 9.6% PUFA. <sup>m</sup>12.4% SFA, 20.4% MUFA, 7.2% PUFA. <sup>n</sup>9.3% SFA, 13.1% MUFA. <sup>o</sup>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.







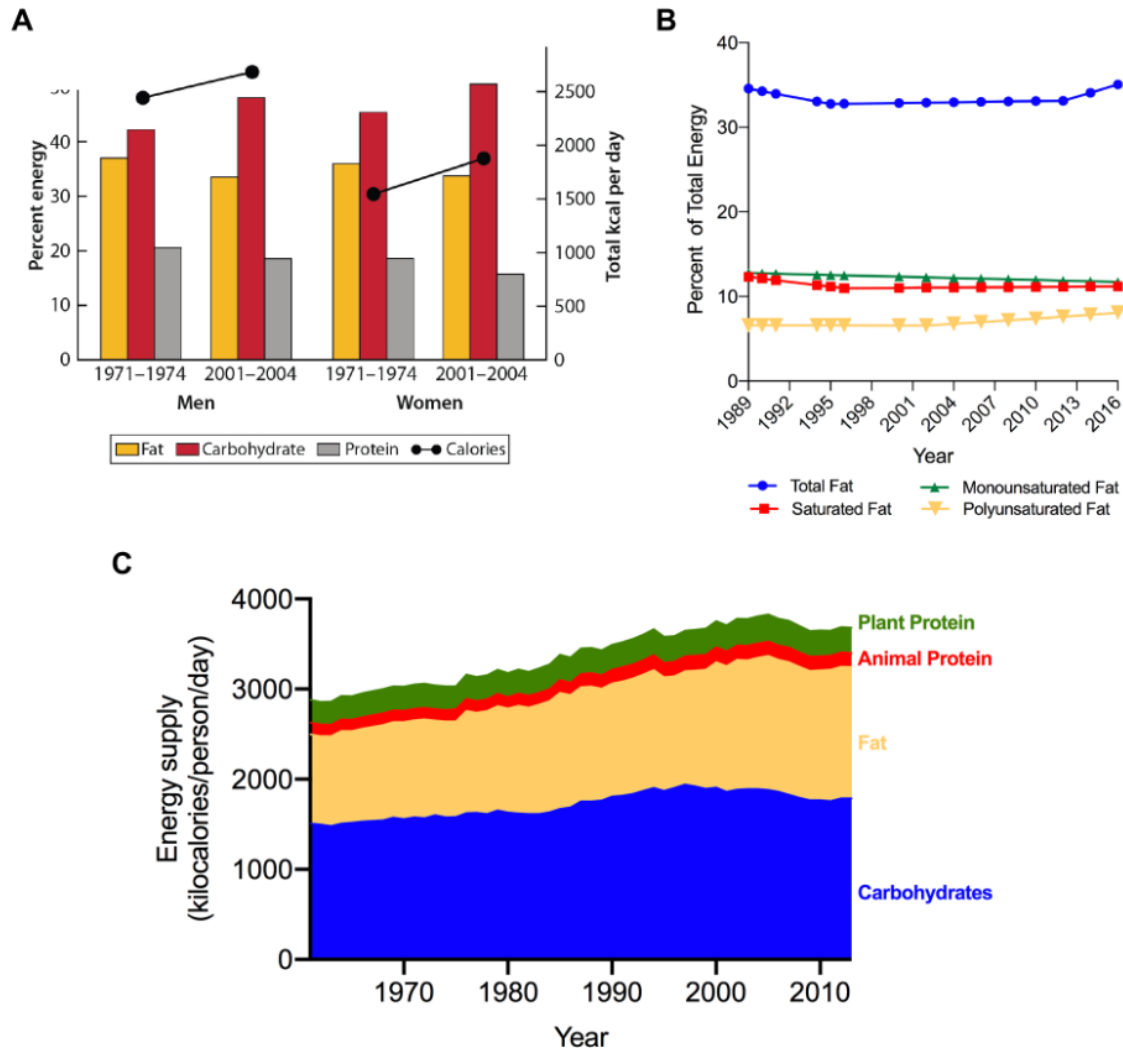
**Fig. 2. Effects of fats and carbohydrates on liver fat content.** Effects of low-fat-high-carbohydrate as compared to high-fat-low-carbohydrate diets on liver fat content (left side of figure). Expressed as relative (%) change from baseline measured by  $^1\text{H}$ -MRS. Interventions comparing hypoenergetic, isoenergetic and hyperenergetic low-fat-high-carbohydrate to high-fat-low-carbohydrate diets are shown. Upper-left panel: 1<sup>106</sup>, 2<sup>107</sup>, 3<sup>108</sup>; middle-left panel: 4<sup>109</sup>, 5<sup>110</sup>, 6<sup>111</sup>; bottom-left panel: 7<sup>115</sup>, 8<sup>105</sup>. Effect of fat quality on liver fat content, expressed as relative (%) change from baseline measured by  $^1\text{H}$ -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<sup>116</sup>, 2<sup>189</sup>, 3<sup>190</sup>, 4<sup>30</sup>, 5<sup>192</sup>; lower-right panel: 6<sup>118</sup>, 7<sup>105</sup>, 8<sup>117</sup>. Adapted from Ref.<sup>215</sup> 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 (PKC $\epsilon$ ) translocation from cytosol to the plasma membrane, which inhibits insulin signalling at the level of the insulin receptor<sup>137</sup>. 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<sup>146</sup>. 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 (Srebp1) and fatty acid uptake by PKC $\zeta$ -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<sup>220</sup>. 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 arrow denotes substrate flux.



## 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<sup>1,2</sup>. b | Fat intake as a percentage of total energy reported consumed by US adults from 1989–2016<sup>3</sup>. c | The average per capita supply of energy derived from carbohydrates, protein and fat<sup>4,5</sup>.

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

N	BMI (kg/m <sup>2</sup> )	Age (yrs)	Duration	Design	Cal	Diet	Fructose diet Comparator CARB diet	Liver fat (%) Before – After <sup>c</sup>	Insulin sensitivity Change	Year <sup>Ref</sup>
32	29	34	2 wks	P	ISO	FRU GLU	FRU 25% <sup>b</sup> GLU 25% <sup>b</sup>	7.2 - 7.5 8.0 - 7.9	↑ ↓	2013 <sup>6</sup>
8	24	42	9 days	C	ISO	FRU GLU	FRU 25% CCHO 25%	1.0* 0.7	↓ NS	2015 <sup>7</sup>
11	75 kg <sup>a</sup>	25	7 days	C	HYPER	FRU GLU	FRU 35% <sup>b</sup> GLU 35% <sup>b</sup>	2.1 - 3.2↑ 2.1 - 3.3↑	NS NS	2010 <sup>8</sup>
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 <sup>9</sup>
64	27	42	10 wks	P	HYPER	FRU GLU	HFCS 8-30% <sup>b</sup> SUCROSE 8-30% <sup>b</sup>	11.8 - 13.7 14.9 - 13.0	NS NS	2013 <sup>10</sup>
32	29	34	2 wks	P	HYPER	FRU GLU	FRU +25% <sup>b</sup> GLU +25% <sup>b</sup>	7.2 - 8.9↑ 8.0 - 10.1↑	↑ NS	2013 <sup>6</sup>
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 <sup>11</sup>

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, <sup>a</sup>=body weight, BMI not given, <sup>b</sup>=% of total energy intake, <sup>c</sup>The values denote liver fat before and after the dietary interventions, except for the study of Schwarz et al<sup>7</sup> 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 <sup>12</sup>, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

## References

1. National Center for Health Statistics US Department of Health and Human Services. Health, United States 2007, With Chartbook on Trends in the Health of Americans. (2007).
2. Writing Group, M., *et al.* Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation* **121**, e46-e215 (2010).
3. National Cancer Institute. Cancer Trends Progress Report 2018 Update (<http://progressreport.cancer.gov>). (2018).
4. Ritchie H, R.M. Our World in Data: Diet Compositions (<https://ourworldindata.org/diet-compositions#diet-compositions-by-macronutrient>). (2017).
5. UN Food and Agricultural Organization. FAOstat food balance sheets (<http://www.fao.org/faostat/en/#home>). (2017).
6. Johnston, R.D., *et al.* No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. *Gastroenterology* **145**, 1016-1025 e1012 (2013).
7. Schwarz, J.M., *et al.* Effect of a High-Fructose Weight-Maintaining Diet on Lipogenesis and Liver Fat. *The Journal of clinical endocrinology and metabolism* **100**, 2434-2442 (2015).
8. Ngo Sock, E.T., *et al.* Effects of a short-term overfeeding with fructose or glucose in healthy young males. *The British journal of nutrition* **103**, 939-943 (2010).
9. Silbernagel, G., *et al.* Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *The British journal of nutrition* **106**, 79-86 (2011).
10. Bravo, S., Lowndes, J., Sinnott, S., Yu, Z. & Rippe, J. Consumption of sucrose and high-fructose corn syrup does not increase liver fat or ectopic fat deposition in muscles. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **38**, 681-688 (2013).
11. Lecoultre, V., *et al.* Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* **21**, 782-785 (2013).
12. Yki-Jarvinen, H. Nutritional Modulation of Non-Alcoholic Fatty Liver Disease and Insulin Resistance. *Nutrients* **7**, 9127-9138 (2015).