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Liver deposits of fat metabolic processes: The impact of dietary habits and phenotypes on the risk of intrahepatic triglycerides formation

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A B S T R A C T

The liver is a metabolically adaptable organ that is essential for controlling blood glucose and cholesterol levels. Fatty acids (FAs) are continuously flowing into the liver from a variety of sources, such as dietary sources, adipose tissue, endogenous synthesis from non-lipid precursors, intrahepatic lipid droplets, and recycling of triglyceride-rich leftovers. Triglycerides, which can be oxidised, stored, or released into the bloodstream as very low-density lipoproteins, are produced in the liver using FAs. Many factors can influence intrahepatic FA partitioning, and while there is good evidence that both phenotype (e.g., sex, ethnicity, and adiposity) and dietary macronutrient composition can play a role in intrahepatic triglyceride accumulation, their interaction is still poorly understood. The processes of FA uptake, FA synthesis, and the intracellular partitioning of FAs into storage, oxidation, or secretory pathways are tightly regulated; an imbalance in these processes leads to intrahepatic triglyceride accumulation and is linked to the development of metabolic dysfunction-associated steatotic liver disease. This review's objectives are to investigate how phenotypes affect the delivery, synthesis, and disposal of FAs and to comprehend how the makeup of dietary macronutrients may affect how FAs are partitioned in the human liver in vivo.

Keywords: Liver, Fat Metabolism, Triglycerides, Intrahepatic, oxidation, phenotypes

INTRODUCTION

The largest and most important internal organ for metabolism in the human body is the liver. In response to changes in energy demands, it may quickly switch between the metabolic responsibilities of energy storage and supply since it is a homeostatic regulator of systemic lipid and glucose metabolism and has a high degree of metabolic flexibility (Hodson & Gunn, 2019).

Overview of the human liver's anatomy

The liver acts as a mediator between the extra-hepatic organs that need energy and external and endogenous energy sources by occupying the metabolic crossroads. The portal vein, which supplies approximately 75% of the total blood flow, connects the liver, which is mainly made up of two lobes, to the gut. The hepatic artery provides the remaining blood supply, which is oxygenated blood from the systemic circulation (Trefts et al., 2017). The portal triad—the hepatic artery, the bile duct, and the portal vein—is essential to the transport of hepatic substrates. For instance, chylomicron remnants, long-chain FAs, lactate, pyruvate, and AAs reach the liver by the hepatic artery, while medium chain fatty acids (FAs), amino acids (AAs), and glucose enter through the portal vein (Trefts et al., 2017). Following metabolic conversions, the corresponding products—such as CO2, VLDL, ketones, and urea—are released into the systemic circulation and the hepatic venous drainage (from the left, right, and middle hepatic veins) (Trefts et al., 2017).

Hepatocytes make up around 80% of the liver's parenchyma, with minor contributions from Kupffer cells, stellate cells, sinusoidal endothelial cells, and cholangiocytes (Ben-Moshe & Itzkovitz, 2019). According to Porat-Shliom (2024), each lobe is composed of lobules, which are characterized as either periportal or pericentral based on how close they are to the portal vein or the hepatic artery, respectively. These lobules are composed of plates of hepatocytes and sinusoids. According to Porat-Shliom (2024), hepatocytes in the periportal zone receive the most nutrients, whereas nutrient

concentrations gradually decrease as one moves toward the pericentral zone. The preservation of metabolic health depends on the cellular integrity and function of hepatocytes, which are the primary drivers of hepatic metabolic activity (Green et al., 2015) (Hodson & Gunn, 2019).

Overview of the metabolism of FA in the human liver

The liver has metabolic flexibility in health, meaning it may quickly switch between anabolic and catabolic processes in response to changes in nutrient flux (such as when a person is fasting and then fed) and in response to hormonal and nutritional demands (Samuel & Shulman, 2016). Insulin plays a key role in this by changing how substrates (such as FAs, glycerol, glucose, and lactate) are delivered to the liver and by changing post-translational events (such as phosphorylation and dephosphorylation) that shift hepatocellular metabolism from energy utilization to energy storage [i.e., the conversion of glucose to glycogen and FAs to triglyceride (TG) and the attenuation of hepatic glucose production] (Hodson & Gunn, 2019; Samuel & Shulman, 2016). Large amounts of FA (approximately 100 g/day; Frayn et al., 2006) are processed daily by the liver from a variety of sources, such as TG-rich remnants (i.e., chylomicron remnants (in the fed state) and the ongoing recycling of VLDL-TG) and non-esterified fatty acids (NEFAs) released from adipose TG lipolysis. By means of de novo lipogenesis (DNL), FAs can also be produced inside the hepatocyte from non-lipid precursors, such as monosaccharides (glucose and fructose) or AAs. The channeling into these respective pathways depends on both nutritional state and metabolic health. The majority of FAs in the liver are primarily divided between two pathways: esterification to form primarily glycerolipids (TG and phospholipids) or mitochondrial oxidation (Hodson & Frayn, 2011).

The accumulation of intrahepatic TG (IHTG) is thought to be caused by an imbalance between the transport of FAs to the liver, the synthesis of FAs inside the liver, and their removal from the liver (Diraison & Beylot, 1998; Sidossis et al., 1998). Type 2 diabetes (T2D), cardiovascular disease, and the metabolic syndrome are all strongly linked to the net retention of IHTG, which has pathological repercussions (Targher et al., 2024). Few studies have looked into the underlying mechanisms causing changes in IHTG content, despite strong evidence that an individual's phenotype (age, ethnicity, adiposity, and sex), genetics, and dietary macronutrient composition may all contribute to IHTG accumulation (Marjot et al., 2020). Part of the reason for the paucity of the literature is the difficulty of studying human liver metabolism in vivo. In order to comprehend how dietary macronutrient composition can affect the partitioning of FAs in the liver in vivo in humans, this review will examine how phenotypes impact the corresponding pathways of FA delivery, synthesis, and disposal. Preclinical murine models and in vitro cell-line models have been demonstrated to replicate certain elements of human hepatic metabolism that cannot be performed in vivo in humans, despite the fact that this review concentrates on findings in humans (Gunes & Estall, 2024). Therefore, when available, we also present information from preclinical murine models to give mechanistic support for the findings observed in people.

Triglyceride Intrahepatic Accumulation

IHTG accumulation is characterized by changes in hepatic FA partitioning (Fabbrini et al., 2010), and pathological IHTG accumulation, when unrelated to excessive alcohol consumption, is the first stage of steatotic liver disease associated with metabolic dysfunction [(MASLD); formerly known as non-alcoholic fatty liver disease (NAFLD)]. In 2023, Rinella et al. Because steatosis and systemic metabolic dysfunction are closely related, the term MASLD was coined (Rinella et al., 2023). Approximately 95% to 99% of individuals with NAFLD fit the criteria for MASLD, despite the fact that the two conditions have different classifications (Hagstrom et al., 2024). For the sake of simplicity, we shall refer to this review as MASLD. Metabolic dysfunction-associated steatotic liver disease includes a range of liver pathologies, from metabolic-associated steatohepatitis, advanced fibrosis, and more severe liver disease (i.e., cirrhosis) to steatosis, which is histologically defined as the presence of intracellular TG in >5% of hepatocytes, or >5.6% when evaluated by proton MRI or spectroscopy (Fabbrini et al., 2009).

The clinical burden of MASLD encompasses more than just liver-related issues; it also includes cardiovascular disease, chronic renal disease, and other extra hepatic symptoms of some malignancies (Targher et al., 2024). Steatotic liver disease has been directly linked to the development of cardiovascular disease (Ren et al., 2023), the primary cause of death for individuals with MASLD (Younossi et al., 2023, 2024), according to Mendelian randomization studies. The most common type of liver disease globally is metabolic dysfunction-associated steatotic liver disease (Kalligeros et al., 2023), and it is more common in those with type 2 diabetes (T2D) and those who are overweight or obese (Riazi et al., 2022). According to a number of studies, MASLD and T2D commonly coexist and work in concert to raise the risk of unfavorable extra hepatic and hepatic outcomes (Mantovani et al., 2020). T2D and MASLD have a complicated and reciprocal relationship in which having one increases the risk of having the other. According to Hazlehurst et al. (2016), the likelihood of acquiring T2D is correlated with the severity of MASLD, and MASLD may either precede or encourage the development of T2D.

Several anti-hyperglycaemic agents used to treat type 2 diabetes (T2D) have been shown in phase 2 and 3 clinical trials to have positive effects on MASLD and cardioprotective effects, despite the lack of an approved pharmacotherapy for the condition. These agents include sodium-glucose cotransporter-2 inhibitor (SGLT2i), glucagon-like peptide-1 (GLP-1) receptor agonists, and incretin receptor co-agonists (Mantovani et al., 2022).

Triglyceride Intrahepatic evaluation

In vivo metabolism in humans Because the portal vein is inaccessible, direct evaluations of the human liver (by measurements of arteriovenous difference) are rarely carried out; as a result, the mechanisms behind the aetiology and course of disease have not yet been well clarified. Liver biopsies provided insight into molecular variations that may be important in the aetiology of illness. Because these techniques are intrusive and only capture one moment in time, they are unable to shed light on the causes and development of MASLD. A different strategy is to use surrogate indicators of liver fat metabolism, such as 3-hydroxybutyrate and VLDL-TG plasma concentrations, which provide some information about the oxidation and esterification pathways of hepatic FA, respectively (Donnelly et al., 2005; Havel et al., 1970; Kotronen et al., 2009). Using radioactive or stable-isotope tracers (Figure 1) is a more sophisticated method that offers the chance to track the fate of particular FA sources via hepatic esterification and oxidation pathways (Donnelly et al., 2005; Havel et al., 1970; Hodson et al., 2007; Luukkonen et al., 2018; Miles et al., 2004; Parry et al., 2020; Sunny et al., 2011), as reviewed by Umpleby (2015).

VLDL-TG has been labeled ex vivo using radio-isotopes, which also enable tracking of the particles' in vivo destiny (Gormsen et al., 2006). When radio-tracers and positron emission tomography (PET) are used together, it is possible to investigate the metabolism of FA in the splanchnic bed and calculate flux rates by simultaneously measuring blood flow (Iozzo et al., 2010; Risikesan et al., 2024). Given that the liver receives an influx of nutrients from multiple FA sources and that humans spend a large proportion of the day in the postprandial state (McQuaid et al., 2011), the use of stable-isotope tracer methodology has provided the opportunity to trace the fate of these through different hepatic pathways (Figure 1).

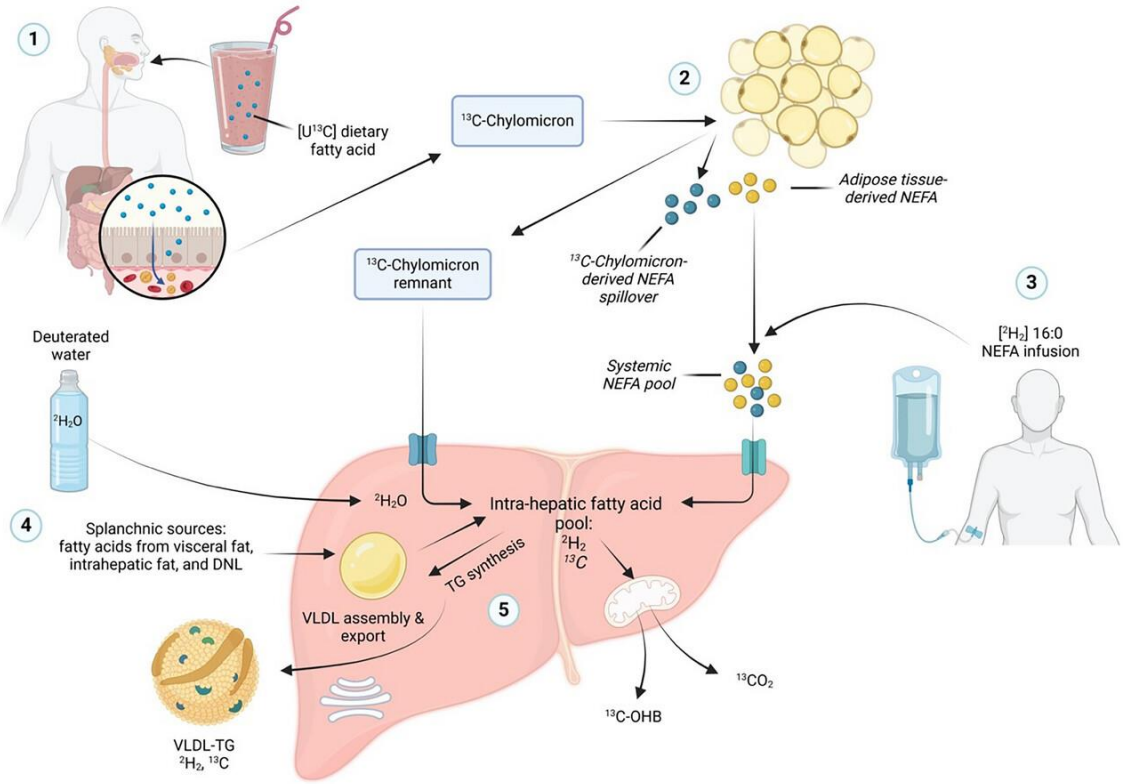

Figure 1: *The ins and outs of hepatic FA metabolism using stable-isotope tracer methodology. (1) The ingestion of a meal labelled with a [U-^{13}C]FA (e.g., [U-^{13}C]palmitic acid) allows for the tracing of exogenous fats. The [U-^{13}C]FA is incorporated into chylomicrons in the enterocyte and appears in systemic circulation as TG-rich ^{13}C-chylomicrons, (2) where it is quickly hydrolysed by adipose tissue lipoprotein lipase, creating ^{13}C-chylomicron remnants that are taken up by the liver. Not all FAs from the hydrolysis of TG-rich ^{13}C-chylomicrons are taken up across adipose tissue, causing a spillover of ^{13}C-NEFAs that appear in the systemic plasma NEFA pool. (3) Endogenous pathways are labelled using an intravenous infusion of [$^{2}H_2$]palmitate complexed with human albumin, which equilibrates with the systemic plasma NEFA pool to allow for the tracing of the whole-body rate of appearance of NEFAs. Plasma NEFAs are taken up by the liver and introduced to the intrahepatic NEFA pool. (4) Given that water is used in the synthesis of FAs, the consumption of deuterated water ($^{2}H_2O$) is used to calculate the fractional synthesis of FA de novo. The $^{2}H_2O$ equilibrates with the total body pool, and ^{2}H labels NADPH that is subsequently used for the formation of fatty acyl-chains during the process of DNL. (5) Depending on nutritional state and metabolic health (i.e., insulin resistance, liver fat content), intrahepatic FAs are partitioned towards oxidation or are esterified to form TGs and are incorporated into VLDL and secreted into*

circulation, or stored within the hepatocyte. Abbreviations: CO_2, carbon dioxide, DNL, de novo lipogenesis; FA, fatty acid; NEFA, non-esterified fatty acid; TG, triglyceride; VLDL, very-low density lipoprotein; OHB, 3-hydroxybutyrate.

Delivery of Fatty Acid to Liver

The intracellular lipolysis of subcutaneous and visceral adipose tissue TG provides the largest contribution to the continuous delivery of NEFAs to the liver, whereas the FAs released by the hydrolysis of dietary TG carried in chylomicrons (chylomicron-derived spillover NEFAs) constitute a smaller contribution (Piche et al., 2018). Insulin controls the lipolysis of adipose tissue TG by blocking the actions of hormone-sensitive lipase and adipose TG lipase. As a result, after consuming a mixed test meal, plasma concentrations of NEFAs reach their lowest level and are highest in the post-absorptive state when insulin levels are low (McQuaid et al., 2011). On the other hand, intestinally produced chylomicrons transport dietary TG into the bloodstream and rise until they reach their maximum 2–4 hours after a mixed meal (Barrows et al., 2005; Hodson et al., 2007; McQuaid et al., 2011; Piche et al., 2018). Lipoprotein lipase (LPL), which is anchored on the endothelium surface of adipose tissue, hydrolyzes the TG content of chylomicrons in about five minutes, leaving behind residual particles. While most released FAs are absorbed in adipose tissue, some can be absorbed by the liver and spill into the systemic NEFA pool (Miles et al., 2004) (Figure 1).

We and others have shown that chylomicron-derived spillover NEFAs can make up around 50% of the total plasma NEFA pool during the lowest postprandial NEFA concentrations (Barrows et al., 2005; McQuaid et al., 2011; Nelson et al., 2013; Piche et al., 2018). Some, but not all, people with obesity and insulin resistance have elevated plasma NEFAs (Karpe et al., 2011), which are frequently attributed to an increase in adipose tissue mass. Though the release of NEFAs from subcutaneous adipose tissue (per 100 g of tissue) decreases with increasing adiposity (Frayn & Humphreys, 2012; Karpe et al., 2011), the rate of appearance of NEFAs across the fasting and postprandial states has been demonstrated to be lower in obese individuals compared to lean individuals (McQuaid et al., 2011). Plotting the rate of NEFA appearance versus fasting insulin concentrations revealed similar results (Karpe et al., 2011), indicating that obese people may suppress adipocyte lipolysis to help get plasma NEFA concentrations back to normal (McQuaid et al., 2011).

Although there is a noticeable decrease in systemic NEFA concentrations in the postprandial state, adipose-derived NEFAs remain the main source of FAs utilized for TG synthesis within the liver in both the fasting and postprandial states (Donnelly et al., 2005; Hodson et al., 2007, 2010; Jacome-Sosa & Parks, 2014; Lambert et al., 2014; McQuaid et al., 2011; Parry et al., 2020; Pramfalk et al., 2016). Lean and obese people, as well as those with and without MASLD, have different relative contributions of FAs from adipose tissue to VLDL-TG (Fabbrini et al., 2008; Hodson et al., 2007, 2010; Umpleby et al., 2017). This could be because splanchnic-derived FA sources, such as IHTG, visceral adipose tissues, and DNL, contribute more to VLDL-TG in these individuals (Hodson et al., 2007, 2010; Nelson, Basu, Johnson, et al., 2007). Approximately 10–30% of hepatic FA delivery during the fasting state is thought to be due to visceral adipose tissue lipolysis, with the amount being proportionate to total visceral fat mass (Nielsen et al., 2004; Nielsen & Jensen, 2024; Nielsen et al., 2004; Risikesan et al., 2024).

Dietary Fat

The thoracic duct allows newly released chylomicrons containing dietary TG to reach the systemic circulation after being released into lymphatic channels. Only half of the chylomicron TG content is hydrolysed by LPL and absorbed by adipose tissue during the remnant generation process, according to evidence from a rat model (Hultin et al., 1996). Given that a normal chylomicron particle contains approximately 106 TG molecules, this is noteworthy (Frayn et al., 2006). FAs are released when chylomicron remnants in the bloodstream are hydrolysed by lysosomes after being absorbed by the liver through the LDL receptor or LDLR-related protein 1 (Hodson & Gunn, 2019). It is unknown how much chylomicron-derived TG contributes overall to IHTG synthesis; this will depend, in part, on the individual's phenotype and the frequency and quantity of dietary fat ingested.

The relative contribution of FAs from chylomicron remnants against FAs obtained from chylomicron spillover was higher (about 15% versus 10%, respectively) in VLDL-TG, according to studies tracking the destiny of dietary fat over a period of 12 to 24 hours (Barrows & Parks, 2006; Donnelly et al., 2005). Furthermore, a decrease in chylomicron TG absorption across adipose tissue may result in a higher flux of chylomicron-derived FAs to the liver in people with insulin resistance and/or obesity (McQuaid et al., 2011).

In line with the finding that the expression of adipose tissue LPL (and other FA trafficking genes) is down-regulated in obese compared to lean individuals (McQuaid et al., 2011), we have previously observed an inverse association between insulin resistance and chylomicron-derived spillover NEFAs (Piche et al., 2018). Reduced dietary fat extraction throughout adipose tissues may leave behind TG-rich chylomicron remnants, which the liver may absorb and use as a substrate for the synthesis of VLDL-TG or for storage in cytosolic lipid droplets.

Hepatic uptake of Systemic FAs

Fatty acid transport proteins (FATPs), fatty acid binding proteins, fatty acid translocase (CD36), and caveolins are among the transport proteins that absorb systemic FAs. The human liver's primary FATPs in health are FATP2 and FATP5, while CD36 is expressed at significantly lower levels (Miquilena-Colina et al., 2011). In contrast, patients with MASLD have

higher liver CD36 protein expression than control subjects (Greco et al., 2008; Miquilena-Colina et al., 2011). This is partially corroborated by human investigations that have shown that hepatic FA uptake is higher in obese people using PET combined with computed tomography (PET/CT) in conjunction with labeled palmitate (Immonen et al., 2018; Iozzo et al., 2010). Although FA transporters might exert a role in controlling hepatic FA flux, their role in the development of MASLD is unclear.

Partitioning Inteahepatic Fatty Acid

Intrahepatic FA production

Acetyl-CoA serves as a carbon source in de novo lipogenesis, which creates de novo FAs from non-lipid precursors. The 16-carbon FA, palmitate, is created when fatty acid synthase elongates malonyl-CoA, which is created when acetyl-CoA carboxylase carboxylates acetyl-CoA. Despite being frequently regarded as the main product of DNL, palmitate (palmitoyl-CoA) can be extended to generate stearate (18:0). Both palmitate and stearate can then undergo additional desaturation to form palmitoleate (16:1) and oleate (18:1), respectively (Hodson & Fielding, 2013). The glycerophosphate backbone provided by carbohydrate metabolism can esterify FAs after they have been produced, lengthened, and desaturated to create TG. Sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP) are the main transcriptional regulators of de novo lipogenesis. Due to the activation of ChREBP and SREBP1c brought on by rising glucose and insulin levels, DNL-derived FAs often contribute more to VLDL-TG in the postprandial state (Barrows & Parks, 2006; Timlin & Parks, 2005) (Hodson & Gunn, 2019). Fatty acid synthase, acetyl-CoA carboxylase, and other lipogenic genes are expressed when insulin increases the over-expression of SREBP1c (Hodson & Gunn, 2019).

Similarly, ChREBP promotes the activation of genes involved in glycolytic flux and gluconeogenesis as well as lipogenic genes in response to elevated blood glucose levels (Hodson & Gunn, 2019). Since insulin plays a role in the transcriptional control of DNL, hyperinsulinemia has been found to be closely linked to elevated DNL (Pramfalk et al., 2016), regardless of body BMI index (Schwarz et al., 2003). The quantitative contribution of DNL to whole-body TG synthesis is modest when compared to the systemic transport of FAs to the liver (Donnelly et al., 2005; Lambert et al., 2014). DNL is thought to generate only 1-2 g of freshly synthesised lipids per day in healthy persons (Hellerstein, 1999). Even following carbohydrate overfeeding, which significantly increases DNL, the contribution of de novo FAs to VLDL-TG is less than 5 g per day (Schwarz et al., 1995). However, malonyl-CoA inhibits carnitine palmitoyltransferase 1 when DNL is increased, which results in less fatty acyl-CoA being transported into the mitochondria. Therefore, lower FA oxidation may result from constitutive up-regulation of DNL, as shown in individuals with MASLD (Lambert et al., 2014; Smith et al., 2020) and insulin resistance (Pramfalk et al., 2016). It is unclear whether an increase in hepatic DNL is a cause or an effect of IHTG accumulation, despite the fact that DNL-derived FAs are thought to contribute 15% to 38% of VLDL-TG in people with MASLD and 1% to 10% in those without MASLD (Lambert et al., 2014). Although this has not yet been proven in humans, DNL is in fact higher in those who are classified as insulin resistant (Pramfalk et al., 2016; Smith et al., 2020) and is thought to be a factor in the buildup of IHTG (Lambert et al., 2014).

Ketogenesis and Oxidation

FAs can be directed toward mitochondrial oxidation pathways in the liver, and the levels of 3-hydroxybutyrate in the blood are utilized as a stand-in indicator for hepatic FA oxidation. Delivery of FAs from adipose tissue to the liver is a major regulator of β-oxidation and ketogenesis in metabolically healthy persons, and postprandial reduction in adipose tissue TG lipolysis significantly suppresses ketogenesis. The main regulator of β-oxidation is the transcriptional factor peroxisome proliferator-activated receptor alpha (PPAR-α). Insulin has a suppressive effect on β-oxidation, while FAs and glucagon increase PPAR-α activity and β-oxidation. It is difficult and complex to directly measure human hepatic mitochondrial oxidation in vivo under dynamic conditions (i.e., after eating). A measure of whole-body FA oxidation is provided by studies that combine the collection of expired breath samples for 13CO2 levels with the administration of oral or injected 13C-labelled tracers (Figure 1). It is possible to determine whether exogenous FAs (13C-FAs given orally) or adipose-derived FAs (intravenously administered 13C-FAs) have undergone the ketogenic route by measuring the incorporation of 13C in plasma 3-hydroxybutyrate.

For example, we previously demonstrated that the whole-body oxidation of dietary FAs is influenced by both FA composition and participant sex by adding uniformly labelled FAs (e.g., [U-13C]palmitate or [U-13C]linoleate) to a test meal (Parry et al., 2021). Others have adopted a different strategy, infusing many 13C-labelled tracers and using NMR to measure the 13C isotopomer analysis of plasma to ascertain the hepatic mitochondrial flow in people who have fasted overnight and those who have not (Sunny et al., 2011). In contrast to individuals with low IHTG, the scientists discovered that those with high IHTG had higher levels of mitochondrial fat metabolism pathways (such as the tricarboxylic acid cycle and anaplerosis), whereas there was no difference in 3-hydroxybutyrate turnover across the groups (Sunny et al., 2011). In contrast to individuals with low IHTG, the scientists discovered that those with high IHTG had higher levels of mitochondrial fat metabolism pathways (such as the tricarboxylic acid cycle and anaplerosis), whereas there was no difference in 3-hydroxybutyrate turnover across the groups (Sunny et al., 2011). Fu et al. (2023) used isotopomer analysis to show that fasting DNL in MASLD participants was linked to an increase in the tricarboxylic

acid cycle flow but a decrease in hepatic ketogenesis and β-oxidation. Hepatic DNL and ketogenesis have been reported to be strongly correlated in normoinsulinaemic persons, but not in hyperinsulinaemic individuals (Pramfalk et al., 2016). Since observations in a diet-induced mouse model of obesity and insulin resistance noted a progressive adaptation of hepatic ketogenesis during high-fat feeding, resulting in increased hepatic fat oxidation, our lack of association may be partially explained by the length of time an individual had been hyperinsulinaemic. This suggests that mitochondrial fat oxidation is stimulated rather than impaired during the onset of hepatic insulin resistance (Sunny et al., 2010). More recently, hepatic mitochondrial oxidation has been measured using the most advanced positional isotopomer NMR tracer analysis (PINTA) (Luukkonen et al., 2023; Petersen et al., 2024). Non-invasive understanding of hepatic mitochondrial function in both diseased and healthy individuals has been made possible by the use of PINTA (Luukkonen et al., 2023; Petersen et al., 2024). Despite having a similar FA delivery rate to the liver, Havel et al.'s early research (1970) showed that obese, hyperlipidemic, and normolipidemic people differed in how FAs were partitioned into their various pathways. By injecting 1-14C-palmitate in the post absorptive state, the authors discovered that while the percentage of FAs entering oxidation and esterification pathways was comparable in hyperlipidemic individuals, approximately two-thirds of FAs entering the liver in normolipidaemic individuals were partitioned towards oxidation rather than esterification pathways.

According to more recent research, hepatic FA oxidation decreases with increasing MASLD severity (Moore et al., 2022), indicating that FA oxidation deficits may play a role in the pathophysiology of metabolic dysfunction. According to Petersen et al. (2024), respondents in both groups responded similarly to glucagon, and hepatic mitochondrial oxidation, as measured by PINTA, was comparable between overweight people without hepatic steatosis and those with MASLD or metabolic-associated steatohepatitis. Methodological variations or variations in participant genotype may be the cause of the disparity in results (Luukkonen et al., 2023). Lipid hydrolysis within the liver It has been suggested that the breakdown of TG contained in lipid droplets is mediated by two key mechanisms in the liver: lipolysis and autophagy. Hepatic adipose TG lipase mediates lipolysis, and it has been observed that adipose TG lipase expression, along with that of its cofactor CG1-58, is down-regulated in liver biopsies of MASLD patients as compared to healthy participants (Kato et al., 2008). When lipid droplets are hydrolyzed in autolysosomes to release FAs into the cytosol, a highly selective type of lipid droplet-specific autophagy called lipophagy takes place; it has been proposed that lipophagy affects IHTG dynamics (Singh et al., 2009). Although the mechanisms governing lipophagy remain unclear, liver biopsies from MASLD patients have been found to have fewer protein markers of autophagy when compared to healthy control subjects (Rada et al., 2020). These findings are corroborated by preclinical rodent MASLD models that show impairments in autophagic flux. It is yet unknown how much each of these mechanisms contributes and if lipolysis and lipophagy happen concurrently or separately. Nevertheless, results from mouse models have demonstrated that IHTG accumulation is driven by a reduction in either pathway (Singh et al., 2009), pointing to a molecular connection between IHTG accumulation and either pathway malfunction.

Esterification of fatty acids

Hepatic portal delivery, which primarily reflects the mobilization of FAs from the visceral compartment, and systemic circulation are the two ways that fatty acids are transported to the liver (Figure 1). Fatty acyl-CoAs are created in the hepatocyte when acyl-CoA synthetases activate freshly supplied FAs. As an alternative, the fatty acyl-CoA pool may be produced from DNL or may result from the absorption of lipoprotein remnants and their breakdown within lysosomes. Fatty acyl-CoA will be directed towards either esterification or mitochondrial oxidation for glycerolipid synthesis, depending on the nutritional and hormonal status. Diacylglycerol acyltransferase (DGAT) enzymes catalyse the last stage of TG synthesis (Hodson & Gunn, 2019). There are two types of DGAT in hepatocytes: DGAT1 utilizes DNL-derived diacylglycerols, while DGAT1 esterifies exogenous FAs. While DGAT1 TG may be more focused on storage in lipid droplets, DGAT2 TG has been proposed to be preferentially partitioned to endoplasmic reticulum pools for instantaneous secretion in VLDL particles (McFie et al., 2021). The hydrolysis and re-esterification of TG from the intrahepatic pool, which entails the addition of a fatty acyl-CoA to a preexisting monoacylglycerol, is another method of TG production (Hodson & Gunn, 2019).

Hepatic Fatty Acid Secretion

The liver can release fatty acids and cholesterol esters that are esterified to TG as VLDL (Boren et al., 2022). A second lipidation step is necessary to form a mature VLDL1 particle. VLDL formation starts in the endoplasmic reticulum, where TGs are transferred to a developing apolipoprotein B-100 particle by microsomal TG transfer protein, creating a primordial VLDL2 particle with a small TG core (Boren et al., 2022). The precise mechanisms that underlie and govern this process are yet unknown, but it most likely involves an intrahepatic lipolysis-re-esterification cycle that exploits VLDL2 fusion and luminal lipid droplets as substrate pools. The most well-characterized lipases linked to VLDL construction in humans are carboxyl-esterase enzymes 1 and 2, which are thought to hydrolyse luminal lipid droplets for the second stage of VLDL1 lipidation (Ruby et al., 2017). Specialised transport vesicles release mature VLDL1 particles into the circulation after they have undergone vesicle-mediated transfer to the Golgi apparatus (Boren et al., 2022). The creation of VLDL-TG, especially VLDL1 particles, seems to be significantly influenced by both substrate availability

and insulin; however, the production and secretion of VLDL2 do not seem to be reliant on either of these factors (Karpe & Hamsten, 1995) or insulin (Malmstrom et al., 1998).
Postprandially, an increase in insulin inhibits the release of VLDL1 by direct and indirect mechanisms, such as decreased NEFA flow to the liver and anti-lipolytic effects (Malmstrom et al., 1998). According to Mittendorfer et al. (2016), women release less moles of VLDL-TG than men do at a given insulin concentration. It is suggested that rather than possible variations in DNL, the observed differences in the secretion rate of VLDL-TG between men and women are due to variations in the integration of systemic FAs into VLDL-TG (Nielsen et al., 2003). Given that chylomicrons are more prevalent in the blood and compete with VLDL-TG for LPL activity, it is hypothesized that the direct inhibition of VLDL1 synthesis by insulin in the postprandial state may be a compensatory mechanism (Boren et al., 2022). The fractional extraction of TG from VLDL across subcutaneous abdominal adipose tissue is approximately four to five times lower than chylomicron TG extraction, as we have previously shown in healthy adults. This suggests that chylomicron TGs are the preferred substrate for lipoprotein lipase (Bickerton et al., 2007). An excess of TG in the endoplasmic reticulum promotes the production of VLDL1 and expands the amount of nascent VLDL particles that can enter the assembly and secretion route (Boren et al., 1990). VLDL, especially VLDL1 (Adiels et al., 2006), is overproduced in those with increased IHTG accumulation; this may be done to shield the liver from more TG overload. Fabbrini et al. (2008) found that the post-absorptive secretion rate of TG-rich VLDL1 particles was twice as high in people with elevated (~22.7%) IHTG content as in people with normal liver fat levels (~3.4% IHTG) after comparing individuals with body mass index and body fat matching.
According to Umpleby et al. (2017), VLDL particles released by individuals with MASLD are larger and more TG-rich than those secreted by those without MASLD. VLDL secretion seems to have a limit, as evidenced by the plateau in VLDL1 secretion rates seen at IHTG levels greater than 10% (Fabbrini et al., 2008). Transgenic mice that over-express SREBP-1a and develop steatosis provide evidence that very large VLDL particles can surpass the diameter of the sinusoidal endothelial pores, causing their accumulation as IHTG (Horton et al., 1999). However, it is unclear what factors limit the secretion of VLDL-TG in humans. Furthermore, the genes encoding apolipoprotein B and microsomal TG transfer protein are down-regulated when steatosis occupies more than 30% of the liver's volume (Higuchi et al., 2011), which may hinder the liver's ability to eliminate produced lipids. Lipids are partitioned toward their accumulation in intracellular lipid droplets rather than their export in carriers of a mutation in the Transmembrane 6 superfamily 2 gene, which also results in a decrease in VLDL secretion (Sliz et al., 2018). Kinetic investigations in humans have shown that those with higher IHTG content had less of the inhibitory effect of insulin on VLDL1 production (Adiels et al., 2007).
Although the exact mechanisms underlying insulin's direct action on VLDL1 are yet unknown, they may be connected to hepatic insulin resistance and the buildup of lipid species in cytosolic lipid droplets that prevent the last stage of lipidation (Boren et al., 2022). There are also obvious sex differences in VLDL-TG metabolism, with adult males having greater plasma VLDL-TG concentrations than females for any given level of adiposity. This is mostly due to an increase in VLDL-TG secretion (Mittendorfer et al., 2016). Furthermore, over-secretion of VLDL1 particles causes elevated plasma VLDL-TG concentrations in obese males relative to their lean counterparts, while decreased clearance of VLDL1 particles is the main cause of elevated systemic VLDL-TG concentrations in obese females (Mittendorfer et al., 2016). The development of hypertriglyceridemia and atherogenic dyslipidemia in individuals with elevated IHTG content is thought to be caused by the overproduction and poor clearance of VLDL1 (Taskinen et al., 2011).

Diet's impact on partitioning and intrahepatic fat acid accumulation

According to Yki-Jarvinen et al. (2021), a number of studies have looked at how dietary macronutrient manipulation affects IHTG responses, but very few have looked into the ways that diet or macronutrient composition can influence the mechanisms that underlie IHTG accumulation. In a 3-week overfeeding study, for instance, Luukkonen et al. (2018) showed that consuming a hyper-caloric diet (+1000 kcal/day) high in saturated fat led to a higher buildup of IHTG than consuming a hyper-caloric diet high in free sugars or unsaturated fats (+1000 kcal/day). According to the authors, compared to baseline responses, consumption of a diet enriched in free sugars or unsaturated fat had little effect on lipolysis, while saturated fat reduced the ability of insulin to suppress adipocyte lipolysis during a euglycaemic–hyperinsulinaemic clamp with the co-infusion of [2H5]glycerol. The high-sugar diet significantly raised DNL, but neither the saturated nor unsaturated fat diets did (Luukkonen et al., 2018). According to Rosqvist et al. (2014, 2019), in both lean and overweight people, a hyper caloric diet high in saturated fat causes a higher level of IHTG accumulation than a hyper caloric diet high in polyunsaturated fat.
The authors showed that dietary fat composition had no effect on the rate of hepatic FA uptake as determined by PET (Rosqvist et al., 2019). This suggests that factors other than hepatic FA uptake may be more important in regulating IHTG buildup. Polyunsaturated FAs may be partitioned preferentially to enter oxidation pathways in comparison to saturated FAs, according to limited data in humans (Parry et al., 2021; Sahini & Borlak, 2014; Schmidt et al., 1999; Srnic et al., 2024). We have recently shown that polyunsaturated FAs preferentially enter oxidation pathways over saturated FAs, even when hepatocellular metabolism is pushed towards esterification due to an over-expression of DNL (Srnic et al., 2024). According to our earlier research, persons without metabolic disorder who do not experience significant body weight gain also exhibit the negative effects of a diet high in saturated fat on IHTG accumulation (Parry et al., 2020).

While there were no discernible differences in whole-body FA oxidation, the rate at which NEFA and DNL appeared, or the contribution of FA sources in VLDL-TG between diets, we discovered that consuming a diet high in eucaloric saturated fats for four weeks increased IHTG content more than consuming a diet high in sugar (Parry et al., 2020).
Although the overfeeding of dietary saturated fats consistently increases an inflammatory response and stimulates the synthesis of ceramides (Luukkonen et al., 2018; Rosqvist et al., 2019), processes that may favor IHTG accumulation, it is unclear why a diet high in saturated fat had such a profound effect on IHTG. The role of ceramide synthesis in dietary saturated fat-mediated IHTG accumulation was supported by Rosqvist et al. (2019), who showed that the differential effects of a diet enriched in either saturated fat or polyunsaturated fat on IHTG content are eliminated after adjusting for changes in C16 serum ceramides (which are thought to reflect ceramides of hepatic origin).

Impact of food composition on the kinetics of VLDL-TG
IHTG content and VLDL-TG concentrations are strongly correlated (Adiels et al., 2006; Donnelly et al., 2005), and the FA composition of VLDL-TG is thought to represent the IHTG composition (Donnelly et al., 2005). Systemic VLDL-TG concentrations can be significantly impacted by changes in either VLDL secretion, clearance, or both (Mittendorfer et al., 2016). VLDL-TG concentrations are a combination of secretion and clearance. Nevertheless, little research has been done on the connection between food and VLDL dynamics. Umpleby et al. (2017) investigated the effects of a hyper-caloric diet with a low (6% total energy) or high (26% total energy) sugar content on post-absorptive lipoprotein kinetics, as determined by stable-isotope tracing, in a 12-week randomised crossover study involving men with (n = 11) and without (n = 14) MASLD. The authors discovered that when healthy participants consumed a diet rich in free sugars, their synthesis of TG-rich VLDL1 increased in comparison to those who consumed a low-sugar diet. This was accompanied by an increase in the contribution of DNL-derived FAs to VLDL1-TG. The high-sugar diet had no effect on DNL or the secretion or clearance rate of VLDL1 in MASLD participants, indicating that they were already functioning at their best.
Both the MASLD and control groups experienced a roughly twofold rise in IHTG content following the high-sugar diet, despite similar increases in body weight (about 2 kg) following either diet (Umpleby et al., 2017). However, the production and clearance rate of VLDL1 or VLDL2 particles did not change when adults with hypercholesterolemia were fed a standardised eucaloric diet for six weeks (52%/32%/16% total energy from carbohydrates, fats, and protein, respectively) that varied in FA composition of saturated fat/monounsaturated fat (14%/7% and 7%/14% of energy intake, respectively) (Gill et al., 2003). It's unknown if same results would be seen in diets that alter the kind and quantity of a macronutrient ingested (such as a high-fat diet made up of saturated fats).

Meal frequency's impact on IHTG accumulation
The frequency of nutrient consumption is a feature of human dietary patterns that is frequently disregarded in physiological feeding research. Koopman et al. (2014) showed in a short, thorough investigation that, regardless of total caloric intake and changes in body weight, meal frequency and macronutrient composition can both have significant effects on IHTG buildup. During the 6-week intervention period, the authors demonstrated that in lean males, consuming 40% excess energy from sugar-sweetened beverages 2-3 hours after each main meal led to a significant relative increase (+110%) in IHTG content. However, when the sugar-sweetened beverages were consumed with the main meal, they had no effect on IHTG. Additionally, snacking with a beverage high in fat similarly raised participants' IHTG level (relative increase +40%), but this impact was much less pronounced than that shown after snacking with beverages sweetened with sugar (Koopman et al., 2014).

It is possible that repeated hepatic uptake of dietary sugars would lead to a sustained up-regulation in DNL and a corresponding down-regulation in hepatic FA oxidation, maintaining hepatocellular metabolism towards FA esterification, even though the mechanisms underlying these findings are still unclear. Apolipoprotein B-100 expression is decreased and microsomal TG transfer protein activity is impaired when SREBP1c is over-expressed in bovine hepatocytes. This results in decreased VLDL synthesis and VLDL-TG export, which in turn causes the deposition of IHTG (Li et al., 2014). It is necessary to do additional research to clarify the ways in which the frequency of nutrient intake influences the processes behind the accumulation of IHTG.

CONCLUSION

IHTG accumulation may be influenced alone or in combination by a variety of variables that control the synthesis, export, partitioning, and intrahepatic delivery of FAs (Figure 2). Hepatic FA metabolism disturbances have far-reaching effects and are strongly linked to a higher risk of cardiometabolic diseases, such as T2D and MASLD.

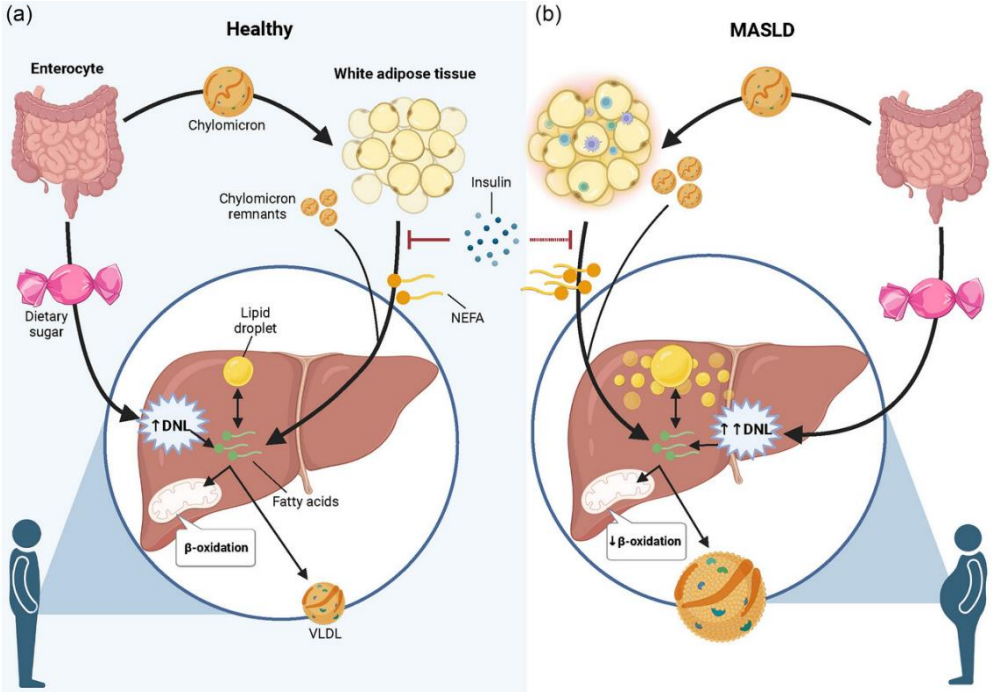

Figure 2: *Hepatic FA partitioning in healthy individuals (a) and in those with MASLD (b). (a) When insulin concentrations are low, as seen in the fasting state, the hydrolysis of adipose tissue TG releases NEFAs that are taken up by the liver and mix with FAs from the cytosolic TG pool. Within the liver, FAs can be used for energy production via β-oxidation or for TG synthesis. FAs esterified to TGs are either incorporated into VLDL and exported into the systemic circulation or partitioned to storage within cytosolic lipid droplets. Dietary fat is packaged into chylomicrons as TG in enterocytes and enters the circulation. Chylomicrons quickly undergo hydrolysis by adipose tissue lipolysis, liberating FAs to be taken up in adipose tissue, thereby creating chylomicron remnants, which are taken up by the liver. Some FAs will escape uptake across adipose tissue and appear in the systemic NEFA pool. Dietary sugars and other non-lipid precursors can be used to form FAs in the liver via DNL. The secretion of insulin suppresses adipose tissue lipolysis and stimulates FA uptake across adipose tissue and increases DNL, thereby shifting the cellular metabolism of FA from oxidative to esterification pathways. (b) The production and secretion of large, TG-rich VLDL particles is up-regulated in the presence of elevated intrahepatic TG content owing to the increased delivery and uptake of NEFAs and chylomicron remnants, increased DNL and excessive TG storage in cytosolic lipid droplets. Abbreviations: DNL, de novolipogenesis; FA, fatty acid; MASLD, metabolic dysfunction-associated steatotic liver disease; NEFA, non-esterified fatty acid; TG, triglyceride; VLDL, very-low density lipoprotein.*

Although it is generally known that food composition and an individual's phenotype (such as sex and obesity) have a significant impact on IHTG buildup, little is known about how these factors interact with liver metabolism in vivo. The intricacy of how food content and an individual's phenotype affect the control of intrahepatic FA metabolism has been partially outlined by studies employing stable-isotope tracers. According to available data, meals high in saturated fats or those characterized by a high consumption of free sugars cause the buildup of IHTG, likely through a number of different but poorly understood pathways, regardless of total body weight growth. Few studies have examined the processes behind the impact of variations in dietary fat composition on human IHTG content, despite the fact that it is widely known that intrahepatic metabolism of FA varies by saturation. Improvements in imaging methods, along with preclinical and in vitro models that mimic features of metabolic disease (Deja et al., 2024), may help clarify the additional underlying mechanisms that underlie the intricate series of events that can result in IHTG accumulation.

Conflict of Interests

None declared.

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