Lipid metabolism in MASLD and MASH: From mechanism to the clinic

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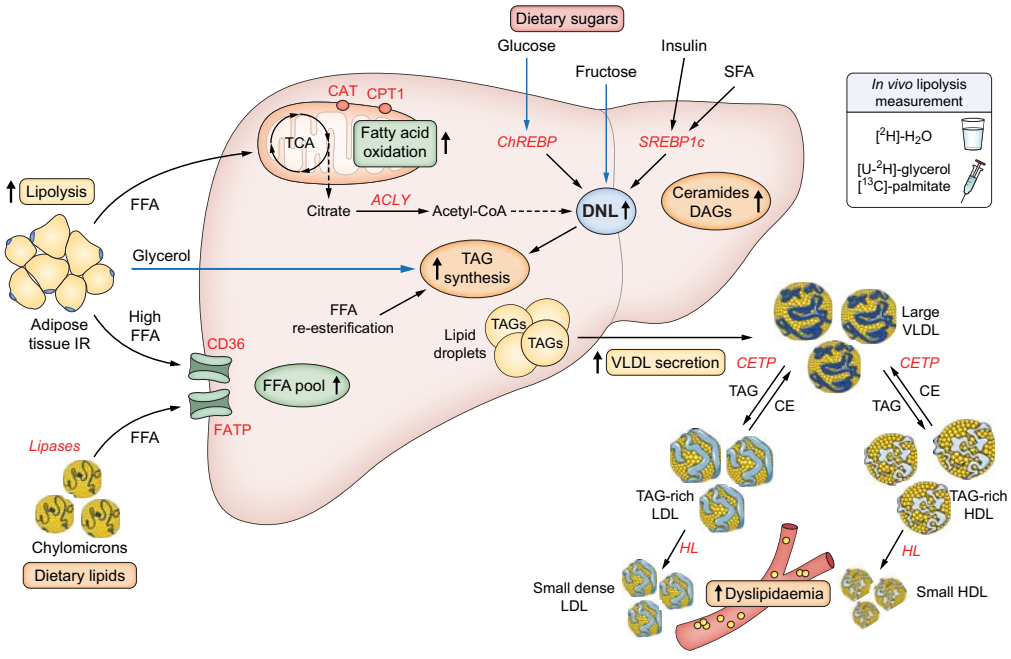
Graphical abstract



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Lipid metabolism in MASLD and MASH: From mechanism to the clinic

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Summary

Metabolic dysfunction-associated steatotic liver disease/steatohepatitis (MASLD/MASH) is recognised as a metabolic disease characterised by excess intrahepatic lipid accumulation due to lipid overflow and synthesis, alongside impaired oxidation and/or export of these lipids. But where do these lipids come from? The main pathways related to hepatic lipid accumulation are *de novo* lipogenesis and excess fatty acid transport to the liver (due to increased lipolysis, adipose tissue insulin resistance, as well as excess dietary fatty acid intake, in particular of saturated fatty acids). Not only triglycerides but also other lipids are secreted by the liver and are associated with a worse histological profile in MASH, as shown by lipidomics. Herein, we review the role of lipid metabolism in MASLD/MASH and discuss the impact of weight loss (diet, bariatric surgery, GLP-1RAs) or other pharmacological treatments (PPAR or THRβ agonists) on hepatic lipid metabolism, lipidomics, and the resolution of MASH.

Mechanisms of hepatic lipid synthesis and accumulation

The liver synthesises several lipids, including triacylglycerols (TAGs), diacylglycerols (DAGs) and sterols, which are synthesised at the level of the endoplasmic reticulum (ER) by re-esterification of free fatty acids (FFAs) derived from a) adipose tissue lipolysis, b) dietary chylomicrons taken up from the circulation or c) synthesised through hepatic *de novo* lipogenesis (DNL) and then stored within lipid droplets (LDs).

Intracellular lipid synthesis and storage in LDs

LDs are intracellular dynamic organelles that serve to both store neutral lipids for cellular metabolic needs and sequester lipotoxic lipids that would otherwise be toxic for the cell since they can act as membrane detergents or cause organelle dysfunction.[1]

LDs are present in many tissues, principally in the liver, adipose tissue and intestine, and enclose a core filled with neutral lipids, most commonly TAGs and sterol esters, surrounded by a phospholipid monolayer incorporating specific proteins from the perilipin family.[2–4] LD assembly is still poorly understood since it involves multiple steps. TAG and sterol ester synthesis, from the esterification of an activated fatty acid to a DAG or a sterol (such as cholesterol), respectively, involve different enzymes located primarily in the ER.[1]

TAG synthesis begins with the entry of fatty acids into the ER (Fig. 1) where they are converted to acyl-CoA and used for synthesis of DAGs by diacylglycerol acyltransferase 1 (DGAT1) and 2 (DGAT2). The DGAT1 enzyme is found exclusively on the membrane of the ER and re-esterifies the DAGs produced by the lipolysis of TAGs, while DGAT2 is found both in the ER and on the LD's surface and synthesises TAGs, which are incorporated into the LDs when the cytosolic concentration of fatty acids rises.[5] Both DGAT1 and DGAT2 prevent the intracellular accumulation of lipotoxic lipids, DAGs and fatty acids. DAGs are not only intermediates in TAG synthesis but they can also activate PKCε and, by impeding insulin signalling, are implicated in the development of hepatic insulin resistance (IR).[6,7]

LDs may expand through droplet–droplet fusion or transfer of TAG to LDs via ER membrane bridges or through TAG synthesis directly on the LD surface.[1] Thus, LDs act as a lipid buffering system by sequestering lipotoxic compounds while maintaining contact with many organelles, facilitating lipid transfer.

Adipose tissue lipolysis and DNL as endogenous sources of fatty acids

Individuals with metabolic dysfunction-associated steatotic liver/steatohepatitis (MASL/MASH) also exhibit adipose tissue IR (Adipo-IR), *i.e.* despite high insulin concentrations, lipolysis is not suppressed, especially during the fasting state[8–10]. Adipo-IR is associated with a lipidomic profile enriched with saturated lipids,[11] increased macrophage activity,[9] and severity of hepatic fibrosis.[10,12]

Subcutaneous adipose tissue (SAT) releases the majority of circulating FFAs, but visceral adipose tissue (VAT), although it is smaller than SAT, is highly lipolytic [13], and the FFAs from VAT are released directly into the portal vein and taken up mainly by the liver on their first pass.[14,15] VAT is increased with intrahepatic triglycerides (IHTGs) even in individuals without obesity,[11] and is associated with IR[11,16].

Another critical source of fatty acids is DNL from non-lipid nutrients,[17,18] *e.g.* carbohydrates, which occurs primarily in the liver and possibly the intestine[19] and adipose tissue.[20] The

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Keypoints

- Hepatic lipid accumulation results from high-fat diet, increased lipolysis caused by adipose tissue insulin resistance, high *de novo* lipogenesis and alterations in VLDL secretion.
- Dietary habits – both quantitative (caloric intake) and qualitative (saturated fatty acids, sucrose and fructose) – are also involved in the development and progression of MASLD.
- Resolution of MASH and improvement in serum lipids are associated with major weight loss (achieved with diet, bariatric surgery or drugs).
- Not only triglycerides, but also other hepatic and serum lipid species are associated with severe MASLD and cardiovascular disease.

saturated fatty acid (SFA) palmitic acid is the first FFA synthesised by DNL (Fig. 1). The non-essential FAs are produced through elongase and desaturase enzymes. Stearoyl-CoA-desaturase-1 inserts one double bond into palmitate and stearate and produces palmitoleic acid and oleic acid, respectively, the most abundant FAs present in TAGs, phospholipids, and cholesterol esters.[21]

To study DNL, *in vivo* and *in vitro* study protocols have been developed using labelled ^{13}C-acetate or deuterated water ($^{2}H_2O$) as metabolic tracers measuring their incorporation into fatty acids,[22] but $^{2}H_2O$ has the advantage of being administered per os (^{13}C-acetate needs to be infused) and rapidly equilibrates after ingestion. Donnelly *et al.*[23] infused ^{13}C-acetate for 5 days, showing high DNL variability (12.7%-37.0% range) but no association with IHTG, which has a long turnover rate (38 ± 16 days). IHTG was composed mainly of FFAs from SAT lipolysis (∼60%) and, to a lesser extent, FFAs from DNL (∼25%) or diet (∼15%). However, the study involved only nine participants (5M/4F, BMI 29-44 kg/m^2), and different diets might have a different effect on TAG composition and secretion.[23]

The increase in DNL, often observed in metabolic diseases, is also due to the overactivation of the transcription factors that stimulate DNL, *e.g*. sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate-responsive-element-binding-protein (ChREBP), which can promote the development of MASLD. Interestingly, DNL has been associated with hepatic and whole-body IR (glucose metabolism),[24,25] suggesting that IR may accelerate hepatic DNL.[25] Smith *et al.* hypothesised that there is a selective/partial IR pathway, *i.e*. it exists at the level of glucose metabolism but not of hepatic lipogenesis, where it would activate SREBP-1c and ChREBP, in line with studies conducted in rodent models of obesity and diabetes.[7] Sugars can activate ChREBP, while insulin, secreted in response to hyperglycaemia, activates SREBP1c (Fig. 2)[26]; SFAs can also activate SREBP1c and stimulate *de novo* synthesis of fatty acids while unsaturated fatty acids are capable of blocking SREBP1c.[27] These genes are overexpressed in the livers of individuals with MASLD.[24,28,29] Furthermore, the inhibition of acetyl-CoA carboxylase, the primary regulator of fatty acid synthesis, decreases DNL and IHTG content in mice fed a high-fat/high-sucrose diet and in individuals with MASLD.[30]

Reduced mitochondrial oxidation and MASLD

The liver contains a high number of mitochondria that provide the energy required to support its many metabolic functions. Hepatic mitochondria are also critical mediators of metabolic flexibility (the ability to adapt to fluctuations in energy demand and supply to maintain whole-body homeostasis) by dynamically modifying the oxidation of glucose or FFAs according to their availability, *i.e*. switching from FFA oxidation, during the fasting state, to enhanced glucose metabolism during the feeding state.[31]

In MASLD, excess lipid accumulation is due not only to excess FFAs but also to insufficient fatty acid oxidation. Whether this is due to mitochondrial dysfunction and/or reduced metabolic flexibility has been debated.

Several metabolic diseases, *e.g.* type 2 diabetes (T2D), obesity, and MASLD, are associated with reduced metabolic flexibility, with higher FFA oxidation even when glucose is the predominant energy supply, *e.g.* during the euglycaemic hyperinsulinaemic clamp.[31] In the early phases of MASLD, the impaired suppression of lipolysis by insulin is accompanied by increased FFA oxidation,[8,32] and mitochondrial activity and biogenesis are increased rather than decreased.[32,33] In the later stages of the disease, but not in isolated steatosis, mitochondrial respiration is reduced due to DNA and protein abnormalities, and hepatic IR is associated with reduced electron transport chain capacity; [32,33]however, it is not clear whether this is a cause or consequence of MASH(7). In humans and mice, high body weight, steatosis, and lipolysis from adipose tissue lead to an increase in fatty acids in the liver and their use in β-oxidation, the tricarboxylic acid cycle (probably due to excess production of acetyl-CoA) and ketogenesis.[34–36] In animal models, Einer *et al.* showed that as MASL progresses to MASH, megamitochondria are observed and accompanied by impaired mitophagy and reduced ATP production[33]; this was recently confirmed in the livers of individuals with obesity and MASH.[37] However, liver size is also increased in patients with MASH, and no study evaluated if the total number of mitochondria, not only their size, is changed since the lower number of mitochondria per g of liver could explain the reduced ATP production. It is also true that in the study by Einer *et al.*,[33] the megamitochondria had similar protein content, indicating that they probably include more lipids. Einer *et al.*[33] showed that megamitochondria with reduced mitochondrial oxidation did not increase the production of reactive oxygen species in animals fed a Western diet. In human livers, Sarabhai *et al.*[37] showed that individuals with obesity and MASH (not with MASL) had megamitochondria whose diameter was associated inversely with fusion/fission biomarkers and with oxidative capacity but positively with H_2O_2. Not only size but also structural changes were observed in individuals with MASH, like loss of mitochondrial cristae and paracrystalline inclusions.[38]

Fatty oxidation also occurs in mitochondria and produces ketone bodies. However, many studies have shown an increase in fatty acid oxidation and β-hydroxybutyrate in individuals

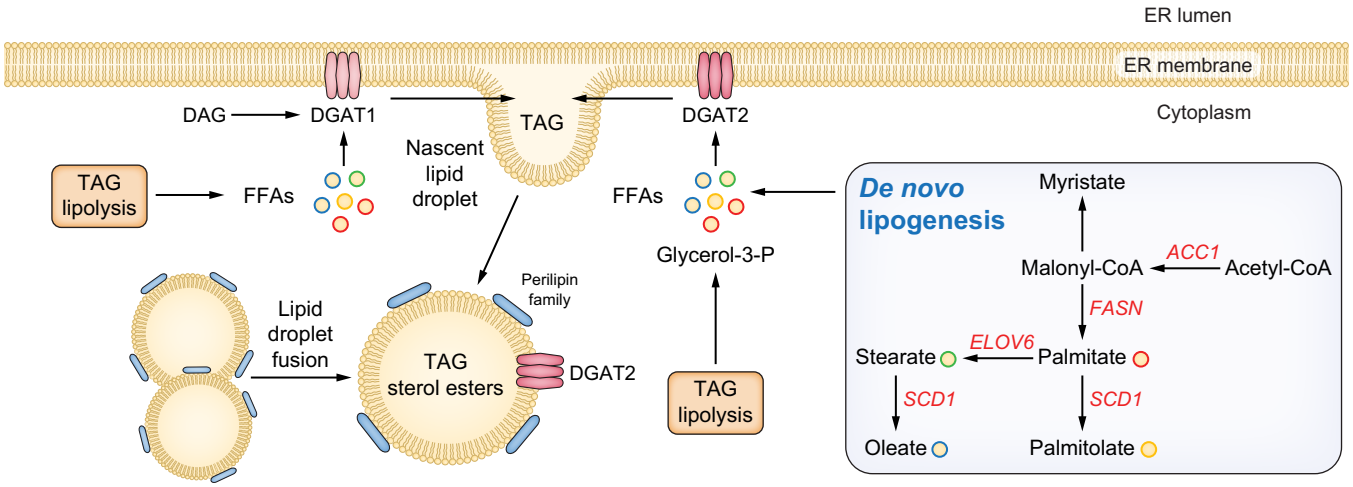

Fig. 1. Lipid droplet biogenesis. Non-esterified FFAs from lipolysis or *de novo* lipogenesis are re-esterified into TAGs by DGAT1 and DGAT2 in the ER. Palmitate is the first FFA synthesised in the DNL by the enzymatic complex FASN; synthesis begins by combining acetyl-CoA with malonyl-CoA. Palmitate is elongated to stearate by ELOV6 and desaturated by SCD1 to palmitolate and oleate. Lipid droplets contain TAGs and sterol esters and are surrounded by a phospholipid monolayer with proteins from PLIN family members. Lipid droplets expand through droplet-droplet fusion or TAG transfer to or synthesis on the lipid droplet surface. DGAT1/2, diacylglycerol acyltransferase 1/2; DNL, *de novo* lipogenesis; ELOV6, fatty acid elongase-6; ER, endoplasmic reticulum; FASN, fatty acid synthase; FFAs, free fatty acids; LDs, lipid droplets; PLIN, perilipin; SCD1, stearoyl-CoA desaturase-1; TAGs, triacylglycerols.

with MASH compared to those without liver steatosis.,[38,39] likely driven by the high fatty acid flux to the liver due to Adipo-IR. However, sexual dimorphism in lipid handling was observed, as women show high fatty acid oxidation and production of hydroxybutyrate but lower DNL compared to men matched for the same characteristics and liver fat content.[40] In part, this explains why men have a higher prevalence of MASLD.

Effects of feeding *vs.* fasting on hepatic lipid synthesis

Circulating lipid concentrations and composition (lipidome) change during the day and are influenced by the circadian state and hormones, diet composition, and type of lipids endogenously synthesised/released by organs like the liver, intestine, and adipose tissue.

Fasting state: adipose tissue FFAs are the primary substrate of hepatic TAG

Fasting is a catabolic state driven by the need for endogenous substrates to be used for energy supply, with low insulin and high glucagon concentrations. MASLD is characterised by high plasma concentrations of insulin and glucagon[41–44] that contribute to the pathophysiology of this disease.

During the fasting state, adipose tissue lipolysis and FFA release are maximal since insulin levels are relatively low. However, in the presence of Adipo-IR, FFA release is not suppressed despite high insulin concentrations.[45] The combination of high FFA flux and high insulin promotes the uptake and re-esterification of FFAs into TAGs by peripheral tissues, including the liver,[11,46] and has been associated with worse histology and increased fibrosis.[9,10,47]

During fasting, the contribution of DNL is related to high lipogenic substrate availability rather than altered molecular regulation of lipogenesis. Fasting DNL correlated with plasma TAG concentrations[48] and was higher in individuals with MASLD.[49] Fu *et al.*[48] found that DNL was still active after 24 h of fasting in individuals (with or without MASLD) with increased lipolysis but reduced ketone production, indicating a sort of metabolic inflexibility.

Postprandial state: impact of dietary lipids and sugars on hepatic TAG

The postprandial state is an anabolic state with high insulin secretion stimulated by ingested nutrients, which promotes glycogen, protein, and lipid synthesis in the liver, muscle, and adipose tissue. Dietary lipids are assembled as chylomicrons in enterocytes and then taken up by the liver and other peripheral tissues.[4] Once lipids enter the systemic circulation, TAGs in chylomicrons are hydrolysed by circulating lipases into FFAs and taken up by peripheral tissues (Fig. 2). Dietary lipids can also be temporarily stored in intestinal LDs that might be a buffer for excess lipids.[4] Tracer studies showed that dietary lipids remain in the circulation for up to 18 h after a high-fat meal but not after a low-fat meal,[50] raising the question of whether high-fat meals are retained in the intestine and continue to fuel the liver for a significant part of the day.

Different dietary lipids have a different effect on hepatic TAG accumulation. Costabile *et al.*[51] showed that isoenergetic substitution of SFAs with unsaturated fatty acids reduced IHTG. Luukkonen *et al.*[52] showed that 3 weeks of a hypercaloric diet rich in SFAs induced the highest increase in IHTG (+55%) compared to the diets high in unsaturated fatty acids

(+15%) or sugars (+33%, p <0.05). The high-SFA diet increased lipolysis, adipo-IR, and ceramide levels.

Studies on alterations in lipid metabolism over the years have focused on fasting, but postprandial lipid metabolism is also altered in MASLD.[53] Postprandial lipidomics highlight the different metabolic responses to food intake. Bonham *et al.*[54] showed that TAG species varied significantly in response to the type of food ingested, suggesting a different formation/clearance of chylomicrons, despite similar TAG concentrations in chylomicrons of patients with metabolic syndrome and controls.

The Mediterranean diet, food with low-glycaemic index and fibre-rich foods have a protective effect against steatosis, while high intake of refined sugars, in particular fructose, promotes MASLD.[17,18] Dietary sugars are critical energy substrates (Fig. 2). Luukkonen *et al.*[52] showed that DNL was high only in individuals consuming a high-sugar diet, rich in fructose, while a high-SFA diet was associated with higher IHTG, but lower DNL. It is well established that only certain sugars (*e.g.*, fructose and sucrose, but not glucose) are precursors of newly synthesised palmitate and stimulate DNL,[24,55] while glucose is used to produce energy through oxidation or stored as glycogen. Ter Horst *et al.* showed that fructose, but not glucose, ingestion stimulated DNL in patients with MASLD.[24] Geidl-Flueck *et al.* also showed that consumption of beverages containing fructose but not glucose for 7 weeks resulted in a 2-fold increase in basal hepatic fractional secretion rates of newly synthesised fatty acids compared to controls but no change in basal synthesis and secretion of very low-density lipoprotein (VLDL)-TAG.[55]

DNL occurs throughout the day but mainly in the postprandial state since its main precursors are exogenous sugars (sucrose and fructose, not glucose).[24,55] Other substrates may be used to produce the acyl-CoA used for DNL. Postprandial DNL contributes to 15-26% of liver TAGs in individuals with MASLD[49,56] *vs.* 1-6% in the fasting state in healthy individuals.[48] However, the combination of high levels of insulin and substrates could also explain the stimulation of DNL, as also proposed by Ter Horst *et al.*[24]

Lipid fluxes out of the liver: VLDL

The liver is not only a site for lipid accumulation, but synthesised lipids are usually secreted by lipoproteins (Fig. 2). Lipoproteins are micelles formed by a membrane lipid monolayer on the surface, consisting mainly of phosphatidylcholines and cholesterol and with an internal core primarily containing TAGs, cholesterol esters, and lipophilic vitamins. Lipoproteins differ in size, tissue of origin, and lipid composition, as well as in the type of associated apolipoprotein that confers specific functions. VLDLs serve to transport fatty acids to peripheral organs and are assembled in hepatocytes. The liver synthesises VLDL using apolipoprotein-B100 and both endogenously synthesised lipids, like TAGs and cholesterol, as well as lipids derived from chylomicron remnants. VLDLs are secreted by the liver to export TAGs as well as other lipids, such as phospholipids and ceramides, and are converted by lipolysis to intermediate density lipoprotein (not shown) and then to cholesterol-rich low-density lipoprotein (LDL) (Fig. 2). The monolayer membrane of VLDL is composed of phospholipids and mainly phosphatidylcholines (PCs), which are thus fundamental to their assembly. A recent study conducted by Mucinski *et al.*[57] supported the idea that ceramides, DAGs, and TAGs are packaged together in the liver in VLDL and secreted together into the bloodstream. Indeed, the serum concentration and composition of ceramides not only correlate with IHTG[58] but mirror some hepatic ceramides, *i.e.* C14:0, C18:0, C20:0, and C24:1. In contrast, only C24:1 ceramide correlated positively between VLDL and the liver.[59]

The synthetic rate of VLDLs is mainly regulated by the amount of hepatic TAGs, which increase their secretion[60] and by the insulin signal, which leads to the degradation of apolipoprotein-B100.[61] The increase in intrahepatic TAGs and IR cause an increase in VLDL secretion, which counteracts the accumulation of hepatic lipids. However, this adaptation is limited as VLDL secretion reaches a plateau, which occurs when IHTG is >10% in individuals with obesity according to a study conducted by Fabbrini *et al.*.[62] A reduction in the synthesis of PCs, such as the lack of choline from the diet, leads to an accumulation of intrahepatic TAGs due to a reduction in VLDL secretion.

IHTG is also associated with VLDL enlargement due to high TAG content,[63] which results in hypertriglyceridaemia (Fig. 2). In this condition, VLDL-TAGs are transferred to HDL or LDL, becoming a suitable substrate for the hormone lipase, which hydrolyses TAGs, forming small HDL, which is excreted by the kidneys, and small LDL, which is more atherogenic.[63,64] However, in individuals with the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*)-148M variant, less VLDL is produced than in those homozygous for *PNPLA3*-148I,[65] while those with the *TM6SF2* (transmembrane 6 superfamily member 2) E167K genetic variant have a defect in VLDL secretion and hepatic lipid export,[66,67] explaining their lower plasma TAG and LDL concentrations, their less atherogenic lipid profile and their lower risk of atherosclerosis and cardiovascular disease.[66,67]

Reduction of hepatic steatosis and regression of MASH

Reduction in IHTG and resolution of MASH are associated with weight loss achieved with lifestyle intervention, bariatric surgery (in individuals with obesity) or pharmacological treatment, though some drugs act independently of weight loss.

Diet and physical activity

The main therapeutic approach in the management of MASLD/MASH is represented by lifestyle interventions, as highlighted in the recent guidelines for MASLD(17). Several randomised clinical trials showed how weight loss leads to significant improvements in MASLD.[17]

The clinical trial by Vilar-Gomes *et al.* is one of the largest and involved 293 individuals with histologically proven MASH treated for 52 weeks with a hypocaloric diet (750 kcal/day less than their daily energy need, with carbohydrates, fats, and proteins accounting for 64%, 22% [including <10% of total calories from SFAs], and 14% of total daily calories, respectively) and physical activity (encouraged to walk for at least 200 min/week).[68] The primary outcome, MASH resolution with no fibrosis worsening, was achieved by 25% of participants (Fig. 2A); the degree of weight loss was independently associated with improvements in all MASH-related histologic parameters. The study also suggests that in adults with MASLD

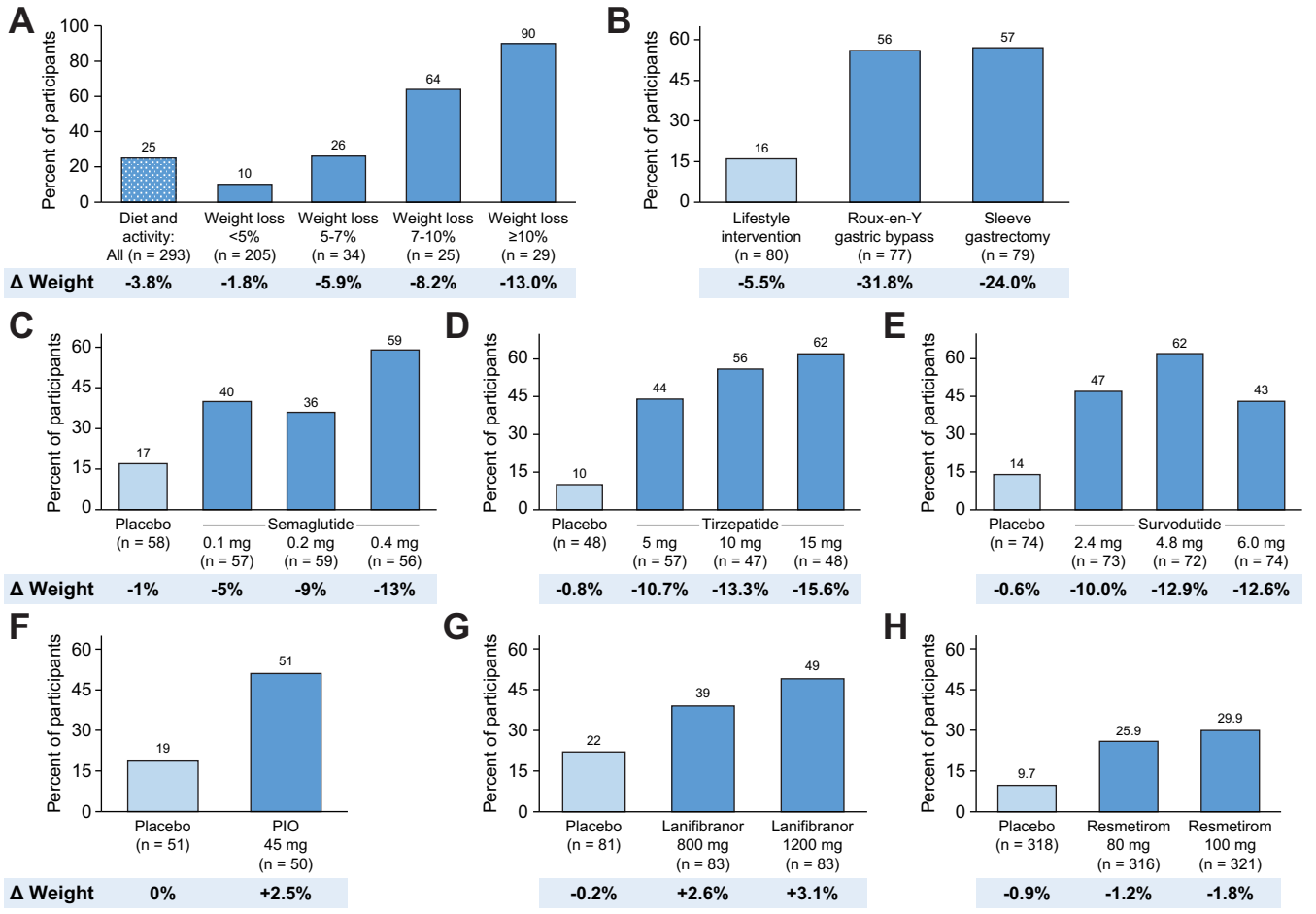

Fig. 2. Percentage of participants with resolution of MASH and no worsening of fibrosis (defined as no increase in the fibrosis stage) in clinical trials with different treatments. (A) Diet and physical activity for 1-year[68]; (B) bariatric surgery for 1 year[69]; (C) GLP-1 receptor agonist semaglutide for 72-weeks[71]; (D) pioglitazone for 18 months[80]; (E) lanifibranor for 24 weeks[83]; (F) the THRβ agonist resmetirom for 52 weeks[91]; (G) the dual GLP-1-GIP agonist tirzepatide for 52 weeks[73]; (H) dual GLP-1/glucagon agonist survodutide for 48 weeks.[74] GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; MASH, metabolic dysfunction-associated steatohepatitis; THR, thyroid hormone receptor.

and overweight/obesity, dietary and behavioural therapy-induced weight loss should aim at a sustained reduction of ≥5% to reduce liver fat, but at least 7–10% to improve liver inflammation and ≥10% to improve fibrosis.[68]

Bariatric surgery

Bariatric surgery is a potential option for individuals with obesity who failed to lose weight with lifestyle interventions. Bariatric surgery is an indication to improve liver histology, including advanced fibrosis.[17] Verrastro *et al.* conducted the first large randomised clinical trial to study the effect of the two main bariatric surgical procedures, Roux-en-Y gastric bypass and sleeve gastrectomy, *vs*. lifestyle modification plus best medical care in the BRAVES cohort that included 288 individuals (44% women) with histologically confirmed MASH and obesity (BMI 30–55 kg/m^2), with or without T2D.[69] At 1 year, MASH resolution with no fibrosis worsening was achieved in 56-57% of patients after surgery, compared to 16% of those assigned to lifestyle intervention (Fig. 2B). The same study showed that the proportion of individuals with resolution of MASH was above 50% in those that lost at least 15% of their initial body weight, which was more pronounced with surgery than lifestyle intervention (Fig. 2B). Lassailly *et al.*[70] also showed that resolution of MASH 5 years after bariatric surgery was associated with weight loss but not baseline histology.

Bariatric surgery is also associated with histological improvement of liver fibrosis.[17,69,70] In the BRAVES study, the improvement of liver fibrosis without worsening of MASH was reached in 37-39% of surgical patients *vs.* 23% of the lifestyle intervention group (intent-to-treat population), and was also more pronounced in those with greater weight loss. One year is likely insufficient to observe significant histological improvement in the fibrosis stage, despite the resolution of MASH.[70]

Pharmacological treatment

Several drugs are currently under investigation for the treatment of MASH, but the only approved (under the accelerated approval pathway) option is resmetirom.

Semaglutide and other single GLP-1RAs

Glucagon-like peptide 1 receptor agonists (GLP-1RAs), like exenatide, liraglutide, dulaglutide and semaglutide, initially developed for the treatment of diabetic hyperglycaemia, have been shown to reduce IHTG. Liraglutide and semaglutide have been investigated in phase II trials for the treatment of MASH,[71,72] but only the semaglutide trial has continued to phase III. In the phase II trial, 320 patients (72% with F2 or F3 fibrosis) were randomly assigned to receive semaglutide at a dose of 0.1 mg, 0.2 mg, or 0.4 mg or placebo for 72 weeks.[71] The highest dose of semaglutide was superior to placebo with respect to resolution of MASH (59% *vs*. 17%, respectively, Fig. 2C); however, no significant difference between treatment and placebo was observed with regard to improvement in fibrosis.

Semaglutide also significantly affects weight loss. Although there are no GLP-1 receptors in the liver or adipose tissue, the reduction in body fat and the improved glycaemic and lipid profiles are undoubtedly critical factors in the regression of MASLD and related cardiometabolic risk factors.

Dual and triple GLP-1RA treatment

New drugs are under evaluation for the treatment of MASH, like the dual GLP-1/gastric inhibitory polypeptide (GIP) RA (tirzepatide[73]) and dual GLP-1/glucagon RAs (*e.g.* survodutide[74] and efinopegdutide[75]). Recently, the data on liver histology from the phase II studies have been published. Both the MASH trials with tirzepatide[73] and survodutide[74] showed an excellent effect on histological parameters and significant response regarding the resolution of MASH without worsening fibrosis (Fig. 2D,E). All these compounds also have a substantial impact on weight loss, and a significant decrease of liver fat measured by MRI-proton density fat fraction was shown for the dual GLP-1/GIP RA tirzepatide,[76] the dual GLP-1/glucagon RA efinopegdutide[75] and the triple GLP-1/GIP/glucagon RA retatrutide.[77] Of these drugs, only tirzepatide is currently approved for the treatment of diabetes and obesity.

PPAR agonists

Peroxisome proliferator-activated receptor (PPAR)-γ agonists, like thiazolidinediones, have been approved for the treatment of diabetic hyperglycaemia, and have also been evaluated for the treatment of MASH in small trials, showing improvement in liver histology.[78–80] Cusi *et al.*[80] investigated the effect of pioglitazone, 45 mg/day or placebo plus hypocaloric diet for 18 months in 101 patients with prediabetes or T2D[80]; 51% of patients treated with pioglitazone achieved resolution of MASH *vs.* 19% in the placebo group (Fig. 2F). PPAR-γ agonists accomplish a reduction in IHTG[78–80] and in VAT at the expense of SAT[81,82] and despite weight gain (+2.5 kg when combined with a hypocaloric diet[80]). Thus, adipose tissue remodelling, and not just weight loss, is a potential mechanism for the reduction of IHTG and resolution of MASH.

Recently, other multiple PPAR agonists, like the PPAR-α/γ agonist saroglitazar and the pan PPAR-α/γ/δ agonist lanifibranor, have been tested for the treatment of MASH.[81] The phase II trial of lanifibranor enrolled 247 individuals randomised to a dose of 800 mg or 1,200 mg *vs.* placebo.[83] Lanifibranor was superior to placebo for resolving MASH without worsening fibrosis (51% and 49% *vs.* 22% in placebo, Fig. 2G), and was also associated with weight gain (+2.7 kg at the highest dose).

The improvement in adipose tissue metabolism through PPAR-γ activation is likely crucial for the resolution of MASH. PPAR-γ is mainly expressed in adipose tissue and regulates adipogenesis, lipid storage and fatty acid metabolism.[84] Mechanistically, this has been explained by the enhanced adipogenesis observed in SAT following treatment with pioglitazone.[82] At the same time, all PPAR-γ agonists have a general insulin-sensitising effect by improving adipose tissue insulin sensitivity,[80,85] promoting the suppression of peripheral lipolysis,[86] and thus decreasing the concentrations of plasma fatty acids and their efflux to the liver. Moreover, they have been associated with a reduced concentration of circulating pro-inflammatory adipokines and an increase of anti-inflammatory ones like adiponectin,[87,88] together with the promotion of anti-inflammatory pathways.[89] In the liver, in addition to the reduction of IHTG, thiazolidinediones promote the reduction of hepatic inflammation[78,79] and fibrosis,[90] also preventing the activation of hepatic stellate cells.[84]

Thyroid hormone receptor-β agonists

Resmetirom is the first compound approved for the treatment of MASH in conjunction with diet and exercise, showing the importance of combining lifestyle changes to pharmacological treatment.[91] Resmetirom was associated with resolution of MASH without worsening of fibrosis in 29.9% of patients *vs.* 9.7% in the placebo group, and fibrosis improvement by at least one stage was also significantly more frequent than with placebo (Fig. 2H). Resmetirom selectively activates thyroid hormone receptor-β, which is mainly expressed in the liver and increases hepatic fat oxidation but also decreases LDL concentrations (-16.3%), and although there was a tendency toward a decrease in TAG and non-HDL cholesterol levels, these changes were not significantly different from placebo in the phase III trial.[91] Resmetirom treatment showed no effect on weight (Fig. 2H).

Which lipids should be monitored in MASLD?

Dyslipidaemia and hepatic lipid dysfunction are diagnosed in the presence of high plasma concentrations of TAGs and total and LDL cholesterol. The advancement of mass spectrometry technologies has enabled the identification of hundreds (>800) of lipid species (TAGs, DAGs, ceramides, sphingomyelins, PCs, and lysophosphatidylcholines [LPCs]) in the liver and plasma/serum in recent years. These lipids were significantly altered at different stages along the spectrum of MASLD, from MASL to MASH, and we expect that new lipids will be used in the future to characterise dyslipidaemia. The most important studies are reported in Table 1.

Perakakis *et al.*[92] showed that serum lipids (mainly PCs, sphingomyelins, ceramides and DAGs) and lipid-related metabolites (*e.g.* fatty acids, leptin, and adiponectin) were predictive of the presence of liver steatosis, MASH and significant fibrosis. In the IMI-DIRECT study, Atabaki-Pasdar *et al.*[93] identified several variables with high discriminative power for MASLD; those with the best performance were associated with lipids, *i.e*. PCs and glycerophospholipids, in particular ceramides, and included lipoprotein lipase proteins and the *PNPLA3*-148M gene variant. McGlinchey *et al.*[94] investigated the circulating metabolomic signature across the full spectrum of MASLD, identifying lipids (mainly TAGs, PCs, sphingomyelins, ceramides, and FFAs) and metabolites (including ketone bodies

Table 1. Changes in lipidomic profile in serum or liver of subjects with MASLD/MASH.

Study	Study population	Diagnosis of MASLD	Lipidomic analysis		
			Method	Matrix	Main findings
Araya *et al.* 2004[97]	11 CT (BMI 27.8 kg/m^2); 10 MASL (BMI 41.7 kg/m^2); 9 MASH (BMI 49.9 kg/m^2)	Liver biopsy	GC	Liver	↑ **MASL** and **MASH:** MUFA, n-6:n-3 ratio, n-6 long-chain PUFA in phospholipids ↓ **MASLD** and **MASH:** long-chain PUFA, n-3 PUFA, n-6 PUFA
Allard *et al.* 2008[96]	17 CT (BMI 28.5 kg/m^2, T2D [21%]); 18 MASL (BMI 27.2 kg/m^2, T2D [20%]); 38 MASH (BMI 31.1 kg/m^2, T2D [26%])	Liver biopsy	GC	Liver	↑ **MASH:** MUFA, palmitoleic acid, and oleic acid ↓ **MASH:** long-chain PUFA
Puri *et al.* 2007[21]	9 CT (BMI 34.5 kg/m^2); 9 MASLD (BMI 37.5 kg/m^2); 9 MASH (BMI 34 kg/m^2)	Liver biopsy	GC	Liver	↑ **MASLD** and **MASH:** TAG, DAG, total cholesterol, SFA, n6: n3 ratio ↓ **MASLD** and **MASH:** TAG, FA (20:4, n-6), TAG FA (22:6, n-3) ↓ **MASLD:** PC, PE ↑ **MASH:** FC, LPC
Chiappini *et al.* 2017[98]	7 CT (BMI 21 kg/m^2); 39 MASLD (BMI 25 kg/m^2); 15 MASH (BMI 31 kg/m^2)	Liver biopsy	GC/LC-MS	Liver	↑ **MASH:** SFA (14:0, 16:0, 18:0) ↓ **MASH:** PC, PE, PI, PS PC/PE, SM
Peng *et al.* 2018[99]	16 CT (BMI 41 kg/m^2, T2D [6%]); 10 MASLD (BMI 45.5 kg/m^2, T2D [20%]); 32 MASH (BMI 48.4 kg/m^2, T2D [34%])	Liver biopsy	HPLC-MS	Liver	↑ **MASL:** TAG, DAG, PE (38:4), CE (14:0, 16:0, 16:1, 16:2, 17:1, 18:2, 18:3, 20:4, 20:5, 22:5, 24:6) ↑ **MASH:** TAG, DAG, acylcarnitine, dihexosylceramide, CER (18:0/24:1), GM1 (d18:1/16:0), SM (38:1), PE (36:1, 38:4, 18:1/22:6), LPE (18:0), CE (14:0, 15:0, 16:0, 16:1, 16:2, 17:0, 17:1, 18:0, 18:1, 18:2, 18:3, 20:3, 20:4, 20:5, 22.5, 24:5, 24:6) ↓ **MASL** and **MASH:** PC (35:2, 40:4, 40:7, 40:8), LPC (18:0, 18:1, 20:0, 22:6, 22:0)
Apostolopoulou *et al.* 2018[100]	7 CT (BMI 25 kg/m^2); 7 MASLD (BMI 51 kg/m^2); 7 MASH (BMI 56 kg/m^2)	Liver biopsy	LC-MS/MS	Serum	↑ **MASLD:** dhCER (20:0) ↑ **MASH:** total dhCER, dhCER (16:0, 22:0, 24:1)
				Liver	↑ **MASH:** total CER, LactCER (24:1), HexCER(22:0, 24:0, 24:1), dhCER (16:0, 22:0, 24:1)
Ooi *et al.* 2021[101]	50 CT (BMI 45 kg/m^2, T2D [14%]); 110 MASLD BMI 47 kg/m^2, T2D [23%]); 16 MASH (BMI 50 kg/m^2, T2D [33%])	Liver biopsy	LC-MS/MS	Plasma	↑ **MASLD:** CER (d18:0/16:0, d18:0/18:0, d18:0/20:0, d18:0/22:0, d18:0/24:0, d18:0/24:1), DAG SFA (16:0, 18:0), MUFA (18:1), PUFA (18:2), TAG SFA (16:0, 17:0, 18:0), MUFA (18:1), PUFA (18:2, 20:3, 20:4)
				Liver	↑ **MASLD and MASH:** dhCER, TAG, DAG, CER (d18:0/18:0, d18:0/20:0, d18:0/22:0, d18:0/24:0, d18:0/24:1), LPC (26:0), PI (18:0/22:5), CE (18:0), DAG, TAG ↓ **MASLD** and **MASH:** PC (15-MHDA/18:2), PC (15-MHDA/22:6), PC (17:1/18:2, 18:1/22:6), CE (22:5) ↑ **MASLD:** total CER, CE, THC, CER (d18:1/16:0, d18:1/18:0, d18:1/20:0, d18:1/22:0, d18:1/24:0), GM3 (d18:1/20:0), PC (28:0, 31:0), PC (O-40:7), PS (38:4), CE (18:3), FC ↓ **MASLD:** SM (37:2, d18:2/20:0), PC (17:0/18:2, 18:1/18:2, 39:5, 17:0/22:6), PC (P-38:5), PE (18:1/22:6), PE (P-18:1/22:4, 20:1/22:6) ↑ **MASH:** total dhCER, dhCER(d18:1/18:0, d18:1/22:0, d18:1/24:0), SM (d18:0/16:0), PC (36:0) ↓ **MASH:** PC (16:1/20:4, 38:6), PC (15-MHDA/20:4), PE (16:0/20:4, 38:5), PI (38:5)
Puri *et al.* 2009[110]	50 CT (BMI 21.2 kg/m^2, no T2D); MASLD (BMI 35.2 kg/m^2, T2D [28%]); 50 MASH (BMI 32.1 kg/m^2, T2D [31%])	Liver biopsy	GC-MS	Plasma	↑ **MASLD** and **MASH:** TAG, DAG, FFA and CE MUFA, CE, DAG, PC, PE with 18:3 or 20:3 FA ↓ **MASLD:** DAG, PC, PE, TAG, SFA ↓ **MASH:** 22:6n-3/22:5n-3 ratio in PC, PE
Oresic *et al.* 2013[112]	392 CT (BMI 34.7 kg/m^2); 287 MASLD (BMI 34.8 kg/m^2)	^{1}H-MRS/liver biopsy	LC-MS	Serum	↓ **MASLD:** LPC (especially C16:0 and C18:0)
Alonso *et al.* 2017[109]	353 MASL (BMI 44.5 kg/m^2); 182 MASH (BMI 45.2 kg/m^2)	Liver biopsy	LC-MS/MS	Serum	↑ **MASH:** PE (C20:4), CER ↓ **MASH:** PC (C22:6), PC (20:4)/PE (20:4) ratio
Sen *et al.* 2022[115]	206 MASH (BMI 31.3 kg/m^2, T2D [53%])	Liver biopsy	LC-QTOFMS	Serum	↑ **MASH (F3):** deoxyCER (42:0), CER (d18:1/24:0, d18:1/23:0, d18:1/25:0) ↓ **MASH (F3):** hexCER (d18:1/20:0, d18:1/22:0, d18:1/23:0, d18:1/24:1, d18:1/24:0)

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Table 1. (*continued*)

Study	Study population	Diagnosis of MASLD	Lipidomic analysis		
			Method	Matrix	Main findings
Barr *et al.* 2010[113]	9 CT (BMI 47 kg/m^2, no T2D); 24 MASLD (BMI 44.8 kg/m^2, no T2D); 9 MASH (BMI 43.2 kg/m^2, no T2D)	Liver biopsy	LC-MS	Serum	↑ **MASLD** and **MASH:** FFA (16:0), SM (18:0/16:0, 18:1/18:0), PC (28:0), LPC (20:2, 20:1), LPE (P-16:0) ↓ **MASLD** and **MASH:** FFA (18:3), (20:2, n-6), SM (18:1/12:0, 18:2/14:0, 18:2/16:0, 36:3), PC (18:0/22:6) ↑ **MASH:** PC (14:0/20:4, 16:0/20:3, P-18:0/20:4), LPC (18:1) ↓ **MASH:** FFA (20:4), LPC (P-24:0, P-22:0, O-20:0)
Anjani *et al.* 2015[108]	24 CT (BMI 47 kg/m^2, no T2D); 22 MASH (BMI 45 kg/m^2, T2D [86%])	Liver biopsy	LC-MS	Serum	↑ **MASH:** PC, PE, and PG, CER (d18:0/22:0, d18:1/16:0, d18:1/18:0, d18:1/20:0, d18:1/22:0, d18:1/23:0, d18:2/20:0, d18:2/18:0, d18:2/20:0, d18:2/21:0, d18:2/22:0, d18:2/23:0),SM(36:1), PC(32:0, 32:1, 34:1, 34:3.36:1, 36:3, 36:4, 36:5, 38:3, 38:4, 38:5, 38:6, 40:4, 40:5, 40:6), PE(34:1, 34:3, 36:1, 36:2, 36:4, 36:5, 38:3, 38:4, 38:5, 38:6, 40:4, 40:5, 40:6, 40:7), LPC(16:0, 16:1, 20:3, 22:5), PG (36:1, 36:2, 36:3, 38:3, 38:4); PI (32:1, 34:1, 36:4, 38:4, 40:4, 40:5) ↓ **MASH:** CER (d18:1/24:0), SM (42:3)
Gorden *et al.* 2015[102]	31 CT (BMI 40 kg/m^2); 17 MASLD (BMI 46 kg/m^2); 20 MASH (BMI 47 kg/m^2); 20 cirrhosis (BMI 32 kg/m^2)	Liver biopsy	LC-MS	Plasma	↑ **MASLD** and **MASH:** TAG, CE, CER, DAG (36:2), HexCER (d18:1/24:1), GlucCER (d18:/24:1, d18:1/26:1), PC (36:4, 38:4), PE (38:5, 38:4, 40:6, 40:5), LPC (16:0), PI (36:1, 38:4, 38:3) ↑ **MASH:** PC (C32:0 and C32:1), SM, dhCER ↑ **Cirrhosis:** hexCER (d18:1/24:1, d18:1/26:1), deoxyCER (18:1/16:0, 18:1/26:1), glucCer (d18:1/26:1, 18:1/26:0), PC (32:0), PI (36:1) ↓ **Cirrhosis:** CE (18:2, 20:4, 20:3), TAG (52:4, 52:3), DAG (36:2), CER (d18:1/18:0, 18:1/20:0, d18:1/22:0, d18:1/24:1, d18:1/24:0, d18:0/18:0, d180/24:1, deoxyCER(18:1/16:0, 18:1/26:1, 18:0/18:0, 18:0/20:0, 18:0/22:0, 18:0/24:0), hexCER (d18:1/24:1, d18:1/26:1), glucCER (d18:1/24:1, d18:1/26:1, 18:1/26:0), SM (d18:1/18:1, d18:1/18:0, d18:1/20:0, d18:1/22:0, d18:1/24:0), PC (32:0, 34:3, 36:4; 38:6, 38:5, 38:4, 38:3, 40:6), PE (36:4, 38:6, 38:5, 38:4, 40:6, 40:5), LPC 16:0, PI (36:1, 38:4, 38:3)
Tiwari-Heckler *et al.* 2018[111]	28 CT; 25 MASLD; 42 MASH	Liver biopsy	LC-MS/MS	Plasma	↑ **MASLD** and **MASH:** SM, PC ↓ **MASLD** and **MASH:** LPE ↑ **MASH:** PE
Sanders *et al.* 2018[105]	663 CT (BMI 25 kg/m^2, no T2D); 233 MASLD (BMI 31 kg/m^2, no T2D)	Ultrasonography	LC-MS	Plasma	↑ **MASLD:** TAG (54:2, 48:1, 48:2, 50:1, 50:2) ↓ **MASLD:** TAG (52:3, 52:4, 56:7, 56:6, 54:4, 56:8)
Yang *et al.* 2017[107]	23 CT (BMI 23.8 kg/m^2, no T2D); 42 MASLD (BMI 27.4 kg/m^2, no T2D); 17 CHB (BMI 21.8 kg/m^2, no T2D); 22 CHB with MASLD (BMI 25.5 kg/m^2, no T2D)	Liver biopsy	LC-MS/MS	Serum	↑ **MASLD:** TAG [lower carbon numbers (≤52) and double bonds (0–3)], DAG (34:1, 34:2, 36:2), CER, CE (20:4, 22:6) ↑ **MASH:** TAG [lower carbon numbers (≤52) and double bonds (0–3)] ↓ **MASLD:** PE-O (38:6, 38:7, 40:8, 42:7), PC-O (34:2, 34:3, 36:2, 36:3, 36:4, 38:5, 30:7, 40:5, 42:5, 42:6, 44:6) ↓ **CHB:** TAG [higher carbon numbers (>52) and double bonds (>3)], DAG (36:4), CER, PE-O (36:5, 36:6, 38:5, 38:6, 38:7, 40:7, 40:8), PC-O (32:0, 34:0, 34:2)

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Table 1. (*continued*)

Study	Study population	Diagnosis of MASLD	Lipidomic analysis		
			Method	Matrix	Main findings
McGlinchey *et al.* 2022[94]	627 MASLD (BMI 31.8 kg/m^2, T2D [42%])	Liver biopsy	LC-QTOFMS	Serum	↑ **MASL:** TAG (51:2, 54:1, 49:0, 56:2, 54:2, 54:1, 52:0, 51:1, 50:5, 50:3, 50:2, 50:1, 49:1, 48:3, 48:1, 48:0, 54:0, 47:1, 45:0, 47:0, 54:6, 18:1/12:0/18:1, 18:0/18:0/18:0, 16:0/16:0/16:0, 14:0/16:0/18:1), PE (16:0/18:1, 34:2), LPC (20:4, 32:1, 40:5), SM (d36:0), CER (d18:1/24:0, d18:1/23:0), CE (18:0) ↓ **MASL:** TAG (O-52:1/P 52:1), LPC (16:0, 22:6) ↑ **MASH:** TAG (49:0, 56:2, 54:2, 54:1, 54:6, 54:4, 54:3, 53:4, 53:2, 52:5, 50:0, 49:2, 58:9, 58:6, 56:9, 56:6, 56:4, 56:3, 55:5, 54:7; 54:5, 53:5, 52:6, 51:2, 52:0, 51:1, 50:5, 50:3, 50:2, 50:1, 49:1, 48:3, 48:1, 48:0, 54:0, 47:1, 47:2, 45:0, 47:0, 54:6, 18:1/12:0/18:1, 18:0/18:0/18:0, 16:0/16:0/16:0, 14:0/16:0/18:1), PE (16:0/18:1, 34:2, 38:6, 38:4, 36:4) ↓ **MASH:** LPC (20:4, 18:1, 18:2, 20:3, 16:0, 18:0, 22:6), SM (d42:2, d41:1, 18:1/24:0, d34:1) PC (O-38:5, 38:4, 36:3, 32:1, 34:1), PC (O-38:5, 36:5, 36:4, 36:3, 34:3, 34:2) ↑ **Fibrosis:** TAG (49:0, 56:2, 54:2, 54:1, 52:0, 51:1, 50:5, 50:3, 50:2, 50:1, 49:1, 48:3, 48:1, 48:0, 18:1/12:0/18:1, 18:0/18:0/18:0, 16:0/16:0/16:0, 14:0/16:0/18:1, 58:9, 58:6, 56:9, 56:6, 56:4, 56:3, 55:5, 54:7; 54:5, 53:5, 52:6, 51:2, 50:3, 50:1, 47:2), PE (16:0/18:1, 34:2, 38:6, 38:4, 36:4), LPC (20:4, 32:1, 40:5), SM (d36:0), CER (d18:1/24:0, d18:1/23:0), CE (18:0), PC (40:6, 32:1, 40:5) ↓ **Fibrosis:** LPC (20:4, 18:1, 18:2, 20:3, 16:0, 18:0, 22:6), SM (d38:2, d36:2, d36:1, d 41:1, d18:1/24:0), PC (35:4, 37:4, 18:0/19:1), PC (O-38:5, 36:5, 36:4, 36:3, 34:3, 34:2)

CE, cholesteryl esters; CER, ceramides; CHB, chronic hepatitis B; CT, control; DAG, diacylglycerol; deoxyCER, deoxyceramide; DHC/Hex2Cer, dihexosylceramide; dhCER, dihydroceramides; ESI, electrospray ionization; FA, fatty acid; FC, free cholesterol; FFA, free fatty acids; GC-MS, gas cromatography-mass spectrometry; GlucCER, glucosylceramide; GM1, GM1 ganglioside; HexCER, hexosylceramides; LactCER, lactosylceramide; LC-MS, liquid chromatography-mass spectrometry; LPC, lysophosphatidylcholine; MASLD, metabolic associated steatotic liver disease; MASL, metabolic associated steatotic liver; MASH, metabolic associated steatoepatitis; MHDA, methylhexadecanoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PI, phosphatidylinositol; PS, phosphatidylserine; QTOFMS, quadrupole time-of-flight mass spectrometry; SFA, saturated fatty acid; SM, sphingomyelin; T2D, type 2 diabetes; TAG, triacylglycerol; THC, trihexosylceramide; UNSFA, unsaturated fatty acid.

Table 2. Changes in lipidomic profile after pharmacological treatment.

Study	Study population	Treatment	Lipidomic analysis		
			Method	Matrix	Main findings
Bagheri *et al.*[125]	104 individuals with obesity (SG: n = 77, RYGB: n = 27; BMI 45.6 kg/m^2)	12-months after bariatric surgery: SG and RYGB	UPLC-MS/MS	Serum	↓**TAGs**, ↓**DAGs**, ↓**CE**(16:1, 18:3, 18:4) ↓ **CER**(18:0, 22:1), ↓**PE**(O-16:0/20:4) ↑**PC**, ↑**PE**, ↑**CE**(22:1, 22:4), ↑**HexCER**(18:0, 20:0, 22:0, 24:0, 24:1), ↑**LactCER**(14:0, 16:0, 20:0, 22:0, 22:1, 24:0, 24:1), ↑**SM**(16:0), ↑**LPC**(17:0, 18:1, 18:2, 20:0, 20:1, 22:4, 22:51), ↑**LPE**(16:0, 18:0)
Zhang *et al.*[132]	35 patients with newly diagnosed T2D (BMI 30.9 kg/m^2)	12-week GLP-1RA exenatide	UPLC-QTOF-MS	Serum	↓**SM**(d18:1/18:0); ↓**SM**(d18:1/18:1), ↓**LPC**(16:0), ↓**LPE**(18:0)
Zobel *et al.*[133]	Liraglutide: 51 T2D (BMI 30.5 kg/m^2) Placebo: 51 T2D (BMI 29.3 kg/m^2)	26-weeks GLP1-RA liraglutide *vs*. placebo	UPLC-QTOF-MS	Plasma	↓**CER**(d38:1, d37:1), ↓**HexCER**(d34:1), ↓**PE**(38:6), ↓**PC**(36:5, 37:5, 40:7), ↓**PE**(O-34:2, 40:7), ↓**TAG**(54:6, 55:4, 56:4, 56:7, 58:6, 58:8, 58:10, 58:1, 60:9, 60:10, 60:1, 60:12)
Jendle *et al.*[134]	Liraglutide: 33 T2D (BMI 30.5 kg/m^2) Glimepiride: 29 T2D (BMI 29.0 kg/m^2)	18-weeks GLP1-RA liraglutide *vs.* glimepiride	UPLC-QTOF-MS	Plasma	↓**CE**, ↓**CER**(d18:1/16:0), ↓**HexCER**(d18:1/24:0), ↓ **PC**(35:4, 36:2, 36:4, 38:3, 38:4, 38:5, 38:6, 39:0, 40:4, 40:5, 40:8, 42:8), ↓**PC**(O-32:0, 34:0, 34:3, 36:3, 36:4, 36:5, 38:4, 38:5, 40:5), ↓**PE**(O-38:5, 38:6), ↓**PI**(38:3, 38:7, 44:4), ↓**LPC**(16:0, 18:0), ↓**TAG**(48:4, 48:3, 53:3, 54:5, 56:3, 56:4), ↓**CE**(16:0, 18:0, 18.2, 20:4, 20:5), ↓**SM**(37:1, d39:1, 39:2 d42:3, d34:2, d32:1, d33:1, d34:2, d36:1, d36:2, d38:1, d40:1, 40:2, d41:1, d41:2, d42:2)
Warshauer *et al.*[135]	Pioglitazone: 19 MetS (BMI 30.7 kg/m^2) Placebo: 18 MetS (BMI 36.1 kg/m^2)	6-month pioglitazone *vs.* placebo	UPLC-MS/MS	Plasma	↓**CER**(C18:0, C20:0, C24:1) ↓**dhCER**(C18:0, C24:1) ↓**LactCER**(C16:0), ↓**HexCER**(C16:0, C18 : 0, C22:0, C24:1).

BMI, body mass index; CE, cholesteryl esters; CER, ceramides; DAG, diacylglycerol; dhCER, dihydroceramides; GLP1-RA, glucagon-like peptide 1 receptor agonist; HexCER, hexosylceramides; LactCER, lactosylceramide; LPC, lysophosphatidylcholine; MetS, Metabolic syndrome; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; RYGB, Roux-en-Y Gastric Bypass; SG, sleeve gastrectomy; SM, sphingomyelin; T2D, type 2 diabetes; TAG, triacylglycerol; UPLC-MS/MS, ultra pression liquid chromatography tandem mass spectrometry; UPLC-QTOF-MS, ultra pression liquid chromatography-quadrupole time-of-flight -mass spectrometry.

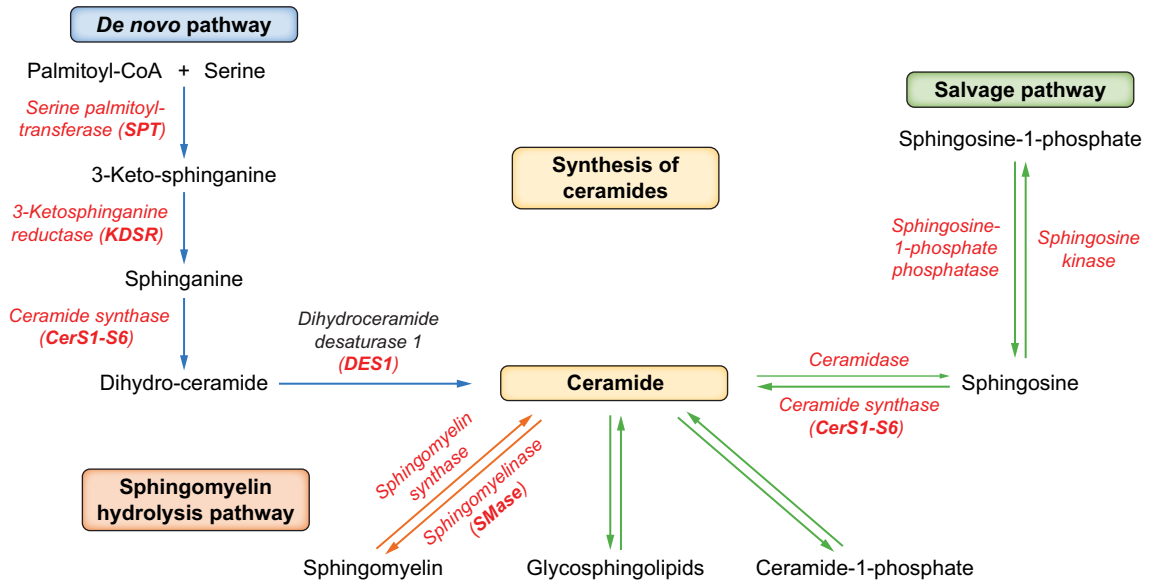

Fig. 3. Synthesis of ceramides. *De novo* synthesis of ceramide occurs in the ER where the condensation of palmitate and serine (by the enzyme SPT) forms 3-keto-dihydrosphingosine which is reduced to dihydrosphingosine and acetylated by the CerS to produce dihydroceramide. DES1 and DES2 insert an unsaturation into the sphingosine backbone to produce ceramides. Ceramides can also be synthesised via the salvage pathway from hydrolysis of sphingomyelins catalysed by SMase. Ceramides are transported from the ER to the Golgi by either vesicular trafficking or by the ceramide transfer protein and can be further metabolized to sphingomyelins, complex glycosphingolipids, phosphorylated into ceramide-1-phosphate or transformed into sphingosines and phosphorylated by sphingosine kinase to form sphingosine-1-phosphate. ER, endoplasmic reticulum.

derived from fatty acid β-oxidation) that uniquely changed with the progression of steatosis, MASH or fibrosis.

Despite some limitations regarding sample size, different clinical characteristics of the patients, and the methods of lipidomic profile detection, most of the studies were concordant in showing that individuals with MASLD have higher hepatic accumulation of TAGs, DAGs, ceramides, and dihydroceramides, and in general of lipids rich in SFAs.[95] In contrast, some lipid species like PCs and LPCs were significantly reduced compared to controls without MASLD. Individuals with MASH had significantly lower long-chain polyunsaturated fatty acids (PUFAs) and some species of phosphatidylethanolamine (PE), and they had significantly higher lysophosphatidylethanolamine *vs.* those with isolated steatosis.

Acyl glycerols and phospholipids in MASL/MASH

The first lipidomic studies showed that individuals with moderate[96] or severe[97] obesity and MASLD had phospholipids rich in monounsaturated fatty acids (palmitoleic, oleic acid) and long-chain PUFAs (n-6:n-3 ratio, and n-6) compared with controls. In contrast, long-chain PUFAs were decreased in MASH (Table 1). A more detailed lipidomic profile was given by Puri *et al.*,[21] who reported a significant increase in TAGs, DAGs, and SFAs in MASLD, with an increase in TAG/DAG and cholesterol/PC ratios and a decrease in PC and PE from MASL to MASH. These data were confirmed by Chiappini *et al.*,[98] who showed a significant reduction in PC, PE and SM in MASH. Peng *et al.*[99] showed that TAGs, DAGs, ceramides, PE, lysophosphatidylethanolamine and cholesterol esters were significantly increased, and PC and LPC significantly reduced, in MASH *vs.* controls (Table 1). Among ceramides, in a small cohort of seven patients, Apostolopoulou *et al.*[100] showed that total ceramides, as well as lactosyl-, hexosyl-, and dihydro-ceramides were significantly increased in MASH, and this finding was also confirmed by Ooi *et al.*[101] in a larger cohort, particularly with regard to total ceramides and dihydroceramide.

Plasma saturated/unsaturated fatty acid TAGs have been found to be elevated,[102,103] and associated with IR[11,104] in individuals with MASLD. Furthermore, specific TAGs containing palmitate or stearate, *i.e.* TAG 48:0, 50:0, 48:1 and 50:1, have been associated with the severity of MASLD and with an increased risk of developing T2D.[105,106]

In MASLD, a significant increase in TAGs with lower carbon numbers and double bonds,[94,102,105] DAGs,[99,102,107] and PE[94,108–111] has been reported.

Conflicting data regarding circulating PC and LPC are present (Table 1). In fact, PC and LPC, particularly PC(22:0, 20:4) and LPC(16:0,18:0), were found to be reduced in several studies,[107,109,110,112] while LPC (16:0, 16:1, 18:1, 20:0, 20:1) were increased in others[102,108,111,113] or increased in MASL and reduced in MASH.[94] Also, conflicting results were reported for sphingomyelins, which were found to be both increased[94,108,111,113] and decreased[94,113,114] in MASLD. Little evidence is available on circulating phosphatidylinositol and phosphatidylserine, which have been found to be higher in MASL(101, 102) or only in MASH (phosphatidylinositol).[108]

Ceramides in MASL/MASH

Ceramides are among the most studied lipids because of their lipotoxic activity in several organs and have been reported to be increased in the plasma of patients with MASLD.[94,99–102,107–109,115] Ceramides are recognised as key

mediators of hepatic IR.[7,104,116] They are part of sphingolipids and are components of membranes. However, ceramides are not only structural elements but also bioactive lipids that participate in various cellular functions such as signalling, proliferation, differentiation, apoptosis and inflammation.

A few studies have also investigated circulating lipidomic species in patients with advanced forms of MASH (F3), fibrosis, and cirrhosis, showing an increase in circulating levels of some ceramides (d18:1/20:0, d18:1/22:0, d18:1/23:0, d18:1/24:1, d18:1/24:0) and a decrease in hexosylceramide,[115] some glucosylceramide (d18:1/24:1, d18:1/26:1, 18:1/26:0), SM, PC, LPC, and phosphatidylinositol[94,102] (Table 1).

Ceramides are composed of a sphingosine backbone, which consists of a long-chain amino alcohol and a variable-length fatty acid. These lipids can be synthesised through three pathways: 1) via hydrolysis of sphingomyelins catalysed by the enzyme sphingomyelinase; 2) via a "salvage" pathway in which sphingolipids are broken down to produce sphingosine that is reused to form ceramides; 3) via *de novo* synthesis (Fig. 3).

High levels of plasma ceramides are correlated with the severity of MASLD and reflect hepatic ceramide accumulation. Patients with MASH have 20% higher ceramide concentrations compared to healthy individuals.[100,102,104,117] Ceramides have been linked to hepatic IR, and they inhibit several mediators of the insulin signalling pathway, such as insulin receptor substrate 1, phosphatidylinositol 3-kinase, and Akt.[118] Furthermore, in adults with obesity, 3 weeks of a diet rich in SFAs, but not unsaturated fatty acids or sugars, increased plasma ceramide levels by 49%, worsening hepatic IR(52). Promrat *et al.*[117] showed that MASH was associated with lower hepatic expression of ceramide synthase 1 (CERS1), while 1 year of lifestyle intervention (with weight loss) reduced SPTCL1 expression and plasma ceramides.

In addition to the total content, the turnover of ceramides can be linked to their toxicity and give more information regarding their concentration. Different approaches for the measurement of ceramide synthesis *in vivo* have been proposed, *e.g.* the infusion of $U^{13}C$ palmitate as ceramide precursors, used in humans to measure *de novo* synthesis of ceramides in skeletal muscle[119–121] or labelled palmitate used to measure hepatic ceramide kinetics in rat models.[122,123] Recently, Mucinski *et al.*[124] measured the fractional synthesis of ceramides in the liver and mitochondria by administering $^{13}C_3{}^{15}N$-serine in water for up to 12 days in mice fed a chow or high-fat diet.

Effect of treatment on serum lipids and lipidomic profile

MASL/MASH are associated with a pro-atherogenic lipid profile with increased TAGs and LDL and reduced HDL. The reduction in IHTG and weight is linked with an improved lipidomic profile. Weight loss either through lifestyle intervention[68] or bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy)[69,70] improves the lipid profile by reducing total TAGs, and increasing PC, PE and HDL concentrations.[125]

The effect of semaglutide and other single GLP-1RAs on triglycerides and HDL is generally modest,[126] despite the significant weight loss, while it is better with tirzepatide.[127] GLP-1RAs have a positive effect on postprandial TAG and lipoprotein metabolism (mainly VLDL), with a reduction in TAG concentrations in the postprandial state due to the reduction in VLDL assembly and secretion[42,128,129] but also an action on chylomicron assembly and clearance.[128–131] Few clinical trials have investigated the effect of GLP-1RAs on lipidomic profiles only in individuals with diabetes and during fasting (Table 2). Zhang *et al.*[132] studied the effect of exenatide treatment on lipidomic profile in patients with newly diagnosed T2D and obesity, and despite the fact that several lipids were increased at baseline compared to healthy controls, only a few species were decreased after 12 weeks of treatment. Zobel *et al.*[133] investigated the effect of liraglutide for 26 weeks compared with placebo, showing a significant decrease in 21 lipid species, mainly ceramides, PCs, and TAGs. Similarly, Jendle *et al.*[134] showed that 18 weeks of treatment with liraglutide compared with glimepiride significantly reduced a more significant number of lipid species, including ceramides, PCs, phosphatidylinositol, cholesterol esters, and sphingomyelins. Considering the limited evidence available, at fasting, GLP-1RAs appear to reduce mainly TAGs, ceramides, PCs, and sphingomyelins, which are lipid species strictly associated with increased risk of cardiovascular disease; interestingly, this lowering effect remained after adjusting for body weight loss.

The reduction of serum concentrations of TAGs and the increase in HDL by pioglitazone is well documented[80] and is associated with a decrease in IHTG and similar effects to lanifibranor.[83] Warshauer *et al.* has investigated the effect of thiazolidinediones on lipidomic profiles[135] in patients with metabolic syndrome and reported a reduction in ceramides (Table 2).

Conclusion

MASLD is the most prevalent metabolic disease since it is associated with both obesity and T2D and with alterations in lipid metabolism not only in the liver but also in other organs, mainly adipose tissue and the intestine. The integration of multiple omics techniques has enabled the comprehensive profiling of individuals with metabolic diseases at various levels, contributing to the exploration of the path that links genotype to phenotype. The increase in MASLD prevalence calls for further efforts to increase the awareness and prevent the onset and progression of isolated steatosis to more severe forms of disease like MASH with advanced fibrosis through healthier lifestyles.

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Abbreviations

Adipo-IR, adipose tissue IR; ChREBP, carbohydrate-responsive-element-binding-protein; DGAT1/2, diacylglycerol acyltransferase 1/2; DNL, *de novo* lipogenesis; ER, endoplasmic reticulum; FFAs, free fatty acids; HDL, high-density lipoproteins; IHTGs, intrahepatic triglycerides; IR, insulin resistance; LDs, lipid droplets; LDL, low-density lipoprotein; LPCs, lysophosphatidylcholines; MASLD, metabolic dysfunction-associated steatotic liver disease; PCs, phosphatidylcholines; PE, phosphatidylethanolamine; PNPLA3, patatin-like phospholipase domain-containing

protein 3; PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids; SAT, subcutaneous adipose tissue; SFAs, saturated fatty acids; SREBP-1c, sterol regulatory element-binding protein-1c; THR, thyroid hormone receptor; VAT, visceral adipose tissue; VLDL, very low-density lipoprotein.

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Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the aforementioned parties. Neither of the aforementioned parties can be held responsible for them nor for any use that may be made of the information contained herein.

Conflicts of interest

A.G. has served as a consultant for: Boehringer Ingelheim, Eli Lilly and Company, Metadeq Diagnostics and Fractyl Health; has participated in advisory boards for: Boehringer Ingelheim, Merck Sharp & Dohme, Novo Nordisk, Metadeq Diagnostics and Pfizer; and has received speaker's honorarium and other fees from Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, and Pfizer. The other authors have no conflict of interest to declare regarding this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

FC and AG conceived the study. FC, GDP, SS, participated in literature review, designed figures and table, and wrote the first draft of the paper. TVP contributed to the discussion of content and reviewed/edited the manuscript before submission. AG coordinated the writing, and critically reviewed and edited the manuscript.

Supplementary data

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