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Pathogenesis of MASLD and MASH – role of insulin resistance and lipotoxicity

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Summary

Background: Insulin resistance and lipotoxicity are extremely interconnected but fundamental in setting the stage for the development of MASLD/MASH.

Aim/Methods: A comprehensive literature search was performed and key themes were synthesised to provide insight into the underlying molecular mechanisms of insulin resistance and lipotoxicity in the liver, muscle, pancreas and adipose tissue and how organ cross-talk is fundamental to driving disease pathogenesis.

Results: Classical thinking postulates that excess FFA load exceeds the storage capacity of adipose tissue, which is predicated upon both genetic and environmental factors. This results in insulin resistance and compensatory hyperinsulinaemia by pancreatic beta cells to overcome target organ insulin resistance. As adipocyte dysfunction worsens, not only are excess FFA delivered to other organs, including skeletal muscle, pancreas and liver but a pro-inflammatory milieu is established with increases in IL-6, TNF-α and changes in adipokine levels (increased leptin and decreased adiponectin). With increased intramuscular lipid accumulation, lipotoxic species decrease insulin signalling, reduce glucose uptake by downregulation of GLUT4 and decrease glycogen synthesis. With this additional reduced capacity, hyperglycaemia is further exacerbated and increased FFA are delivered to the liver. The liver has the largest capacity to oxidise fat and to adapt to these stressors and, therefore, has become the last line of defence for excess lipid storage and utilisation, the capacity of which may be impacted by genetic and environmental factors. However, when the liver can no longer keep up with increasing FFA delivery and DNL, lipotoxic species accumulate with ensuing mitochondrial dysfunction, increased ER stress, oxidant stress and inflammasome activation, all of which drive hepatocyte injury and apoptosis. The resulting wound healing response, marked by stellate cell activation, drives collagen accumulation, progressive fibrosis, and, ultimately, end organ failure and death. This vicious cycle and complex interplay between insulin resistance, hyperinsulinaemia, lipotoxicity and multi-directional cross-talk among different target organs are critical drivers of MASLD/MASH.

Conclusions: Targeting tissue-specific insulin resistance and hyperinsulinaemia while decreasing FFA load (lipotoxicity) through dietary and lifestyle changes remain the best upstream interventions.

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1 | MASH/MASLD OVERVIEW

1.1 | Brief overview MASLD/MASH

Metabolic-dysfunction-associated steatotic liver disease (MASLD) is the most common cause of chronic liver disease[1] and is characterised by excessive accumulation of fat in the liver, also known as steatosis, in individuals with little to no alcohol consumption.[2] It consists of two clinical entities: simple steatosis and metabolic-dysfunction-associated steatohepatitis (MASH). While simple steatosis does not often progress, patients with MASH are at risk of progressive liver injury that can advance to cirrhosis and the development of hepatocellular carcinoma. Progressive MASH is characterised histologically by the presence of steatosis, lobular inflammation and ballooning with varying degrees of fibrosis.

1.2 | Metabolic dysfunction central to pathogenesis of MASLD/MASH

The pathogenesis of MASLD is due to multiple mechanisms, including environmental and metabolic factors superimposed on genetic factors (Figure 1). From a metabolic perspective, MASLD is caused by an imbalance in energy metabolism in the liver. In the presence of excess energy in the form of carbohydrates and fat, the liver is unable to oxidise it all, resulting in an accumulation of this energy as triglycerides. In the liver, insulin functions to increase the uptake of glucose into hepatocytes, promote glycogen synthesis for storage and decrease gluconeogenesis. In the absence of a response to insulin, hepatocytes instead shunt the excess glucose into lipogenic pathways, further exacerbating the lipid build-up that is central to MASLD.

1.3 | Nomenclature change

Due to this recognition in the pathogenesis of MASH and MASLD, new nomenclature has been introduced to reflect the multisystem nature of MASLD and MASH and its link to metabolic dysfunction. What was previously known as non-alcoholic fatty liver disease (NAFLD) was changed to metabolic dysfunction-associated steatotic liver disease (MASLD) and MASH, previously known as non-alcoholic steatohepatitis (NASH), was changed to metabolic dysfunction-associated steatohepatitis.[3]

2 | OVERVIEW OF THE OBESITY-HYPERINSULINAEMIA-INSULIN RESISTANCE AXIS

2.1 | Insulin resistance development

2.1.1 | Normal function of insulin

Insulin is a peptide hormone that is secreted by pancreatic beta-islet cells in response to elevated blood glucose levels and functions to regulate systemic glucose homeostasis. Three major organs are involved in this glucose homeostasis: the liver, muscle and adipose tissue. The insulin receptor (INSR) is composed of both alpha (α) and beta (β) subunits, wherein tyrosine phosphorylation of the β subunit is more specific to insulin binding. This β subunit is highly expressed in differentiated liver, muscle and white adipose tissue (WAT)[4] and thus are the organs most impacted by hyperinsulinaemia and insulin resistance.

In these target organs, insulin binds to cellular receptors on their plasma membranes and coordinates anabolic responses in response

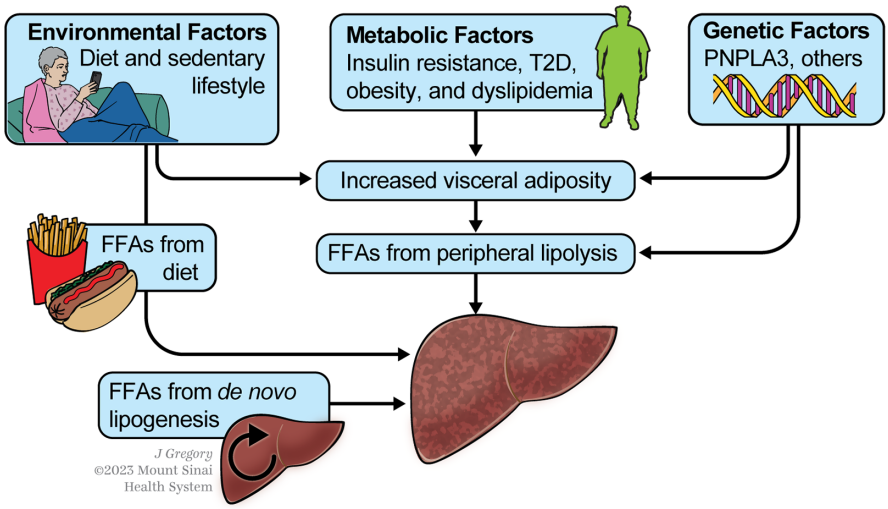

FIGURE 1 Dysregulated lipid metabolism in MASLD/MASH: convergence of genetic, environmental and metabolic factors. Environmental factors, such as high fructose diet and sedentary lifestyle, along with metabolic factors such as insulin resistance, T2D, obesity and dyslipidaemia, superimposed genetic factors conspire to promote increased visceral adiposity and increased delivery of FFA to the liver by peripheral lipolysis. Fructose and glucose from the diet also promote de novo lipogenesis. Normally mitochondrial β-oxidation and esterification to form triglycerides is adequate to maintain cellular homeostasis but when the system is overwhelmed, surplus of FFAs causes progressive hepatic steatosis, inflammation and fibrosis.

to increased glucose and nutrient availability. In all cell types, the activation of INSR is mediated by the recruitment of phosphotyrosine-binding scaffold proteins (PTB), initiating a cascade of downstream signalling pathways.[5,6] The INSR receptor substrates include IRS1 and IRS2, Src homology collagen (Src), adaptor protein (APS), pleckstrin homology (PH) and Src homology 2 (SH2). Once phosphorylated, the substrates bind and activate kinases that mediate effects of insulin on the cell. Even though there are six isoforms of IRS, IRS1 and IRS2 mediate most of the effects of insulin receptor signalling.[7,8] Once this cellular cascade is activated, insulin promotes glucose uptake into muscle and adipocytes via GLUT4 transporters, inhibits hepatic glucose release and fat cell lipolysis and promotes lipid accumulation in hepatocytes and adipocytes. Ultimately, glucose is removed from the blood and both glucose and lipids are stored in these tissues. Hepatic-specific insulin signalling will be discussed in greater detail below, but we will first briefly review how insulin resistance and lipotoxicity converge to disrupt these normal physiologic functions in other target organs to promote the development of MASLD/MASH.

2.2 | Insulin resistance definition and overview

Insulin resistance is defined physiologically as a state of reduced responsiveness in insulin-targeting tissues to high physiological insulin levels and is considered the pathogenic driver of many modern diseases, including metabolic syndrome, MASLD, atherosclerosis and T2DM.

Diminished response to insulin results in the failure of target organs to dispose of blood glucose and thus hyperglycaemia, loss of inhibition of lipolysis resulting in increased circulating free fatty acids and increased fatty acid intermediates within cells (lipotoxicity), decreased glycogen synthesis and increased hepatic glucose output.[9] As will be discussed further below, lipotoxicity itself then further promotes insulin resistance creating a complicated vicious cycle with common themes emerging across several affected organs.

2.3 | Development of hyperinsulinaemia and insulin resistance: The chicken or the egg?

There is no question that insulin resistance and hyperinsulinaemia are central drivers of the pathogenesis of MASLD/MASH. The key debate is: which comes first? The classic prevailing theory is that insulin resistance, the reduced response to insulin, results in increased insulin production by beta-cells in the pancreas in an effort to overcome peripheral insulin resistance and maintain normal glucose homeostasis. This feed-forward notion suggests that insulin resistance then stimulates hyperinsulinaemia, and hyperinsulinaemia worsens obesity and insulin resistance until β-cells fail, marking the onset of type 2 DM.[10] Eventually, the pancreas fails to supply enough insulin to overcome the resistance and homeostasis can no longer be maintained, leading to hyperglycaemia and glucose intolerance.

While this has been the classical thinking, some have argued that hyperinsulinaemia drives insulin resistance as target tissues work to 'protect themselves' from excess glucose uptake.[11] From an evolutionary perspective, insulin promotes energy storage so that reserves exist in times of fasting/starvation. Growth hormone (GH) promotes the lipid mobilisation and oxidation for energy when needed. With the excess calories and particularly the shift in diet to high carbohydrate/high fructose/high fat foods instead of fruits and vegetables, higher insulin levels are observed. Insulin and insulin-like growth factor (IGF-1) show high homology and are coordinately regulated by nutrient intake but bind to distinct receptors. Insulin, produced in the pancreas, and growth hormone, produced in the pituitary gland, both stimulate IGF-1 production in the liver. IGF-1, then, in turn, negatively inhibits the further secretion of insulin and GH. Therefore, in the setting of hyperinsulinaemia, increased IGF-1 secretion by hepatocytes causes a decrease in GH levels and thus less mobilisation of lipids.[12] Furthermore, increased insulin causes downregulation of insulin receptors on target tissues.[13] In addition to the changing dietary patterns, there a number of factors that may predispose one to hyperinsulinaemia including (1) hyperresponsiveness of β cells[14]; (2) fetal programming or epigenetic factors due to intrauterine environment[15]; (3) endocrine disrupting chemicals[16]; and (4) reduced hepatic clearance of insulin[17] which may be increased in African Americans.[18] Further discussion on this debate is beyond the scope of this review. However, it is important to point out that this alternative hypothesis exists and further research and clinical data will bring greater clarity. Despite which came first, hyperinsulinaemia and insulin resistance coalesce to drive physiologic changes in muscle, fat and the liver, ultimately driving the development and progression of MASLD/MASH.

2.4 | Factors promoting the development of insulin resistance: Nature versus nurture

2.4.1 | Genetic predisposition to insulin resistance

There exists a clear genetic predisposition to IR but it is complex and not fully understood.[19] The role of genetics in IR is underscored by studies in non-obese healthy subjects with a strong family history of T2D, where the presence of insulin resistance without clinically apparent features of metabolic syndrome is seen.[20] When studied carefully, these patients have clear defects in liver, adipose and skeletal muscle insulin resistance, and subtle defects in pancreatic beta-cell function. However, because these patients are not obese and their systems are not stressed by increased fatty acids, they are able to maintain reasonable metabolic homeostasis. These genetic predispositions are different than those discussed specifically in the context of MASLD/MASH, where strong family clustering and increased risk in first-degree family members suggest both shared genetic and epigenetic factors.[21,22] Moreover, a number of SNPs that are more common in certain ethnic groups have emerged as predictors of disease severity, bringing together both steatosis

and fibrosis and are reviewed extensively elsewhere.[23] One such example is PNPLA3-rs738409, which is important in the regulation of hepatocyte lipid homeostasis. The I148M variant is resistant to ubiquitylation and proteasomal degradation, thus promoting triglyceride accumulation through decreased breakdown.[24] In addition, there may be independent pro-fibrogenic effects through enhanced stellate cell activation[25] though the exact mechanisms by which this SNP drives MASH still require further clarification. This I148M variant is particularly enriched in the Hispanic population and has been proposed as a reason for increased rates of MASH in this group.[26] Interestingly, the risk of MASH in patients with this variant is maximised only if it coexists with adiposity, illustrating again the concept of genetic predisposition surfaced by environmental drivers.[27] Other SNPs that have been shown to be associated with an increased risk of MASH include TM6SF2-rs58542926[28] and glucokinase regulator gene [GCKR]-rs780094 or [GCKR]-rs1260326.[29] TM6SF2 rs58542926 variant (E167K) confers significantly greater MASLD-related hepatic fibrosis independent of gender, age at biopsy, BMI, T2DM and PNPLA3 rs738409 genotype.[28] The association between the E167K variant and fibrosis in the context of steatosis has been shown by others.[30] It may be related to increased hepatic triglyceride content due to decreased VLDL-TG secretion[31] but direct effects on fibrogenic pathways are not clear. Of note, carriers of the *TM6SF2* variant also have favourable cardiovascular outcomes associated with improved serum lipid profile. Thus, any therapeutic target must preserve this cardioprotective effect.

Conversely, loss-of-function variation in hydroxysteroid 17-β dehydrogenase 13 (HSD17B13) gene, which inhibits pyrimidine catabolism, may confer protection against chronic liver injury and mitigates progressive NASH[32] independent of effects on de novo lipogenesis[33] and insulin resistance. Only pyrimidine metabolites correlated inversely with histological fibrosis stage in the liver highlighting pyrimidines as a potential mediator of HSD17B13-mediated hepatoprotection. As HSD17B13 is thought to be hepatocyte-specific, effects on stellate cells and fibrogenesis may be indirect and require further investigation.

From a clinical perspective, the key question is whether genetic risk factors can be incorporated with clinical factors in our risk stratification models to predict those that have advanced fibrosis in MASH. When patients with biopsy-proven MASH were genotyped for the PNPLA3-rs738409 (minor allele: G), TM6SF2-rs58542926 (minor allele: T) and HSD17B13-rs72613567 (minor allele: TA) variants, it was found that the addition of these genetic markers into the prediction of advanced fibrosis (baseline model: age, sex, BMI, diabetes: AUC 0.777) resulted in a higher AUC if PNPLA3 (AUC 0.789), and TM6SF2 (AUC 0.786) but not if HSD17B13 (0.777) was added.[32] Recent studies demonstrated that polygenic risk scores which included unfavourable mutations in PNPLA3, TM6SF2, GCKR and MBOAT4 augmented prediction of significant fibrosis in those with metabolic risk factors. This further underscores the relationship between metabolic risk factors, genetics and MASH and the potential role of genetics in refining our risk stratification models.[34]

2.5 | Obesity: exploiting genetic predisposition and stressing the system

For those with a genetic predisposition, healthy lifestyle and diet can suppress manifestation of insulin resistance. With progressive obesity, these inherent systems are now under physiologic stress, resulting in the earlier development of IR and ultimately adipose tissue insulin resistance and subsequent lipotoxicity.

2.6 | Environmental endo-disruptors

While environmental factors such as diet and lifestyle are discussed frequently, the role of environmental toxins is emerging as an important driver of insulin resistance[35] and may contribute to in health disparities between patients with MASLD/MASH. Current knowledge supports the notion that endocrine-disrupting chemicals (EDCs) interfere with human metabolism and hormonal balance, contributing to the conventionally recognised lifestyle-related metabolic syndrome risk factors.[36] PFAS (per- and polyfluoroalkyl substances) are ubiquitous man-made chemicals found in consumer products including fabrics, food packaging, non-stick coatings and aqueous film-forming foams. PFAS are stable and extremely resistant to degradation, resulting in high persistence throughout the environment as well as in human blood.[37,38] Circulating PFAS have been associated with disruptions in key metabolic pathways involved in MASLD/MASH. Interestingly, stronger associations between the liver metabolome, chemical exposure and MASLD-associated clinical variables (liver fat content, HOMA-IR) were observed in females than males, with are larger impact of lipotoxic lipids, such as triacylglycerols and ceramides, in driving dysregulated glucose metabolism in females.[39]

Therefore, while genetics clearly play a role, they may not manifest in clinically apparent insulin resistance until stressed by lifestyle or environmental factors.

3 | EFFECTS OF INSULIN RESISTANCE

In the next few sections, we will discuss the various effects of insulin resistance in each affected organ system, the bidirectional interplay between lipotoxicity and insulin resistance and the complex interaction between these affected organs (Figure 2).

3.1 | Insulin resistance and adipose tissue

Both brown adipose tissue (BAT) and white adipose tissue (WAT) together constitute our total adipose tissue mass. While both are composed of adipocytes, their functions are quite different. While WAT stores energy, BAT generates body heat and thus expends energy. BAT is present in high degrees in newborns in the interscapular space, neck and shoulders to generate heat by non-shivering thermogenesis. It declines rapidly after puberty and becomes

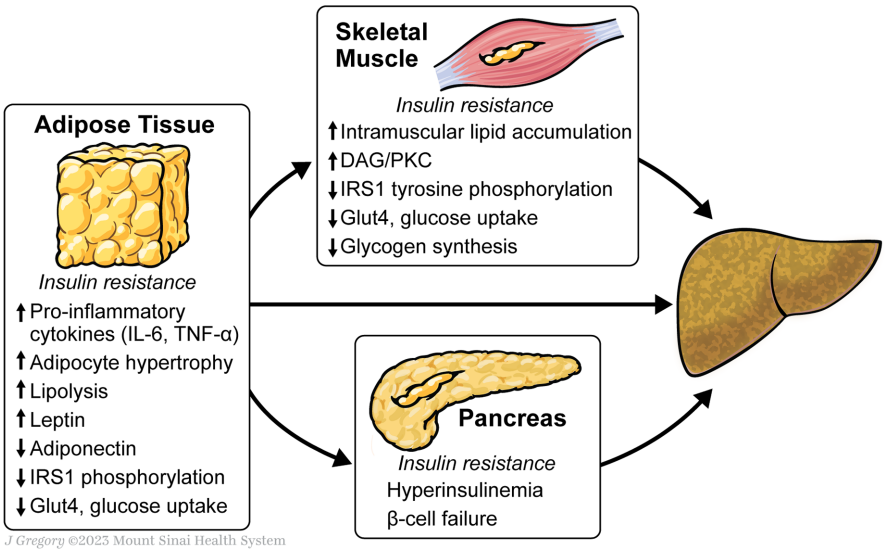

FIGURE 2 Changes related to insulin resistance and lipotoxicity in multiple target organs. Changes in the adipose tissue accompanied by insulin resistance and lipotoxicity collaboratively cause increased lipolysis, decreased storage capacity with increased delivery of FFA to other organs such as muscle and pancreas, decreased glucose uptake, secretion of pro-inflammatory cytokines and worsening insulin resistance. In skeletal muscle, increased lipid accumulation drives concomitant increase in lipotoxic lipids and worsening insulin sensitivity with resultant decrease in glucose uptake and glycogen stores. Pancreatic β-cell failure either as a primary event (alternate hypothesis) or secondary due to burnout (classic hypothesis) ultimately causes inability to maintain glucose homeostasis. Ultimately, excess FFAs are driven to the liver compounded by hepatic insulin resistance.

redistributed predominantly in neck, kidneys, adrenal glands, aorta and mediastinum. BAT has greater vascularity and is innervated by the sympathetic nervous system. The exact amount (volume) of active BAT in adult humans remains highly variable and was estimated at approximately 7% based on results from a systematic review and meta-analysis.[40] BAT is activated by cold exposure and oxidises lipids. The unique expression of uncoupling protein 1 (UCP-1), which is located in the inner mitochondrial membrane, allows for uncoupling of respiration to the production of ATP, resulting instead of the dissipation of energy in the form of heat. Therefore, 'browning of adipocytes' or increasing BAT volume represents a novel therapeutic tool to combat obesity and metabolic diseases.[41]

Deposits of WAT are localised mainly beneath the skin [subcutaneous adipose tissue (SAT)] and around internal organs [visceral adipose tissue (VAT)]. However, a small amount of WAT is found in the perivascular and epicardial regions, the mediastinal retro-optical space and bone marrow.[42]

From a clinical perspective, the total amount of adipose tissue and skeletal muscle can be measured by bioelectrical impedance analysis (BIA), where a weak electric current flows through the body and voltage is used to calculate impedance. As more body water is stored in muscle, adipose tissue has a higher impedance. Given the role of VAT in insulin resistance, as will be detailed further below, interest in measuring VAT, specifically, has garnered attention. MRI and CT can measure VAT but given cost and availability, alternative approaches need to be considered. DXA Scans have long been known to measure fat and muscle content but are underutilised clinically[43]; a recent study showed that BIA and ultrasound can also be used to estimate VAT.[44] Other than classical differences in location, exact quantification of BAT compared to WAT requires 18FDG-PET/ CT or MRI imaging and is limited to research use at this time.[45] It is anticipated that this will be an area of active research as we focus on increasing muscle mass, decreasing VAT/WAT and increasing BAT for the management of obesity and insulin resistance.

In white adipose tissue (WAT) with normal insulin response, insulin activates the pathways for de novo lipogenesis and inhibits the pathways for lipolysis favouring storage of excess lipid in adipose tissue. In both adipose and muscle tissue, insulin is responsible for activating glucose transport into these cells via GLUT4 glucose transport system.

Adipocytes play a special role in insulin resistance (IR) in that their dysfunction is not only an effect of IR but also may be a contributing trigger for its development. IR is caused by the interplay between multiple genetic and environmental factors that affect these cells. Certain genes involved in fat cell function, in the setting of overnutrition and excess of visceral fat, cause an inability of adipocytes to adequately store lipids. Typically, adipose tissue can expand via hyperplasia or hypertrophy. However, in the setting of dysfunctional lipid storage, adipocytes are unable to undergo hyperplasia and solely rely on hypertrophy. This hypertrophy has multiple important consequences. First, lipids accumulate in macrophages, with the percentage of M1 polarised pro-inflammatory resident macrophages increasing. These M1 pro-inflammatory macrophages release certain cytokines (IL-6 and TNF-α) and pro-inflammatory molecules that further antagonise insulin's actions, promote adipose tissue growth and systemic inflammation.[46] For example, M1 macrophages release TNF-α which promotes adipocyte lipolysis and

activates a variety of stress-related protein kinases, including c-Jun N-terminal kinase (JNK) and inhibitor of kappa-B-kinase beta (IKKB). These then induce serine/threonine-mediated phosphorylation of insulin receptor substrate 1 (IRS-1) which decreases IRS-1-mediated insulin signalling.[47] Furthermore, the increase in adipocyte mass results in hypoxia and eventual death. The death of adipocytes leads to the arrival of immune system cells, mainly macrophages, into the adipose tissue, followed by the further release of proinflammatory cytokines that further enhance lipolysis.[48]

This pro-inflammatory milieu within adipose tissue further promotes insulin resistance while failure of insulin-mediated suppression of lipolysis results in excess delivery of free fatty acids into the bloodstream. These pro-inflammatory cytokines are elevated and can have systemic effects on other organs such as muscle and liver.

In addition to a pro-inflammatory milieu, these changes in adipose tissue result in altered expression of major adipokines, leptin and adiponectin. Leptin is a hormone, primarily produced by adipose tissue that serves as a negative feedback signal to suppress central hunger, resulting in decreased fat mass in adipocytes.[49] Leptin resistance, and thus a decreased ability to suppress hunger in the face of excess calories, may predispose to the development of obesity.[50] Indeed, higher levels of leptin are associated with obesity. Moreover, hyperinsulinaemia may promote leptin resistance, and hence obesity, demonstrating the interconnectedness of hyperinsulinaemia, insulin resistance, leptin resistance and obesity.[51,52]

Secreted adiponectin promotes fatty acid β-oxidation (FAO), glucose utilisation and fatty acid synthesis suppression. Thus, a reduction in adiponectin favours accumulation of lipotoxic species. As the storage capacity of adipose tissue is exceeded, there are increased levels of circulating free fatty acids that deposit in muscle, liver and the pancreas, thereby promoting systemic lipotoxicity. Lipotoxicity is a type of cellular stress induced by the accumulation of lipid intermediates such as diacylglycerols (DAGs), ceramides and triglycerides that facilitate the development of insulin resistance in muscle, liver and adipose tissue. At the same time once insulin resistance develops, adipocyte dysfunction worsens.

3.2 | Visceral versus peripheral adipose tissue: Does location matter?

While we often discuss the association of visceral adiposity with MASLD/MASH, whether it serves as a pathogenic driver or is simply a reflection of ectopic lipid deposition when the capacity of subcutaneous white tissue is overwhelmed is not entirely clear. Support for association rather than causation comes from a few key observations: (1) although rates of VAT lipolysis are increased in obesity, lipolysis in subcutaneous WAT from upper body quantitatively accounts for the bulk of FAAs delivered to the liver,[53,54] (2) intrahepatic triglyceride content itself, not VAT, is associated with insulin resistance in obese individuals[55] and (3) the removal of VAT does not restore insulin sensitivity.[56]

3.3 | Insulin resistance and muscle tissue

Skeletal muscle is responsible for 80% of postprandial glucose disposal through glycolysis and glycogen synthesis and is therefore critical in maintaining glucose homeostasis.[10] In response to insulin, signalling pathways in myocytes result in the translocation of GLUT4 to the plasma membrane, which allows for an increase in insulin-stimulated glucose uptake. In order to sustain normal insulin-stimulated glucose uptake, the IRS1/PI3K/Akt pathway must be maintained.[57] Continuous exposure to high insulin has been shown to promote both serine/threonine phosphorylation, thereby decreasing IRS1 and GLUT4 translocation to the plasma membrane, both of which promote insulin resistance in skeletal muscle.[58] While numerous studies have been performed examining exact molecular mechanisms, defects at the proximal level of insulin signalling that involve INSR, IRS1, PI3K and Akt pathways are emerging as the most relevant in causing decrease in skeletal muscle insulin-mediated glucose uptake.[10]

3.4 | Evidence for lipotoxicity in driving insulin resistance in muscle tissue

Skeletal muscle is composed primarily of two types of fibres: slow-twitch type I fibres and fast-twitch type II fibres. Type I fibres have high GLUT4, hexokinase II and increased mitochondria and thus are thought to have better glucose-handling capacity. Many studies have proposed that lipid accumulation in muscle may promote insulin resistance. Patients with T2DM store lipid droplets in the subsarcolemmal region of type II fibres while athletes store lipid droplets in the myofibrillar region of type I fibres, suggesting that specific lipid morphology and storage have implications for the development of insulin resistance.[59] While detailed mechanistic studies are outlined elsewhere[10] the following themes are emerging: (1) lipotoxicity in muscle prompts excessive mitochondrial fission resulting in impaired insulin-stimulated glucose uptake[60]; (2) increased lipid-derived toxic intermediates from incomplete beta-oxidation such as acylcarnitine[61] and long-chain[62] fatty acyl CoAs,[61] ceramides,[63,64] and DAGS[65] may contribute to mitochondrial dysfunction and impair insulin signalling; (3) lipotoxicity induced activation of intramuscular inflammatory pathways including PKC isoforms and IkB/NFkB are important drivers of insulin resistance in muscle[9,66]; (4) response to circulating pro-inflammatory cytokines, such as IL-6 and TNF-α, and infiltrating pro-inflammatory macrophages (M1) from adipose tissue.[67]

Therefore, adipose insulin resistance has a domino effect on promoting skeletal muscle insulin resistance and, indeed, both obese[68] and non-obese[69] patients with MASLD/MASH have adipose insulin resistance accompanied by muscle insulin resistance.[70]

3.5 | Sarcopenic obesity and insulin resistance

Fat accumulation in muscle tissue promotes a proinflammatory cascade and oxidative stress, leading to mitochondrial dysfunction,

impaired insulin signalling and muscle atrophy. To compound the problem, decreased muscle mass aggravates insulin resistance. In addition, the crosstalk between myokines and adipokines leads to negative feedback, which in turn exacerbates sarcopenic obesity and insulin resistance – this is reviewed in detail elsewhere.[71] As muscle insulin resistance perpetuates with decreased glucose uptake and glycogen synthesis, increased FAA beta oxidation increases ROS causing myocyte toxicity and development of sarcopenia.[62] The less the ability of muscle to store lipid, the more that is delivered to the liver.

3.6 | Measuring sarcopenia in clinical practice

Given the critical importance of muscle in insulin resistance, clinicians need to be able to easily assess muscle mass and then determine whether interventions (physical or pharmacological) have a positive or negative impact. As mentioned above, CT, DXA and BIA can be leveraged to measure both adipose and muscle mass; however, they are expensive and difficult to access. Point-of-care assessment would be ideal and the role of grip strength, where patients squeeze a hand dynamometer three times in each hand, has emerged as a reliable and easy way to assess changes in muscle strength/mass. More recently the role of point-of-care ultrasound is being explored as an appealing way to not only assess muscle mass but lipid deposition, as reflected by echogenicity, within muscle which drives insulin resistance.[72,73]

3.7 | Lipotoxicity as a driver of pancreatic β-cell failure

The term lipotoxicity was initially coined by Lee in 1994, where they described how lipid overload in pancreatic β-cells led to the loss of pancreatic β-cell function and the onset of DM2 in rats.[74] Therefore, the combination of hyperinsulinaemia causing β-cell burnout and the impact of lipid overload ultimately drive beta-cell failure and the inability to regulate glucose homeostasis, hyperglycaemia and type II diabetes.

3.8 | Converging on the liver: Development of hepatic insulin resistance and lipotoxicity

The increased delivery of fatty acids to the liver results in increased expression of hepatic proteins involved in triacylglycerol uptake, such as fatty acid transport proteins and fatty acid translocase/CD36. Once inside the hepatocyte, FFAs are converted to triacylglycerol by the glycerol-3-phosphate pathway. Adiponectin derived from adipose tissue normally inhibits this pathway, but with adipose insulin resistance, adiponectin levels are decreased. In addition to excess delivery of FFA to the liver, increased consumption of mono and disaccharides prevalent in processed foods, especially fructose, sucrose and high-fructose corn syrup, can activate programs of de novo lipogenesis (DNL). Fructose, in particular, is almost exclusively metabolised by the liver so disproportionately contributes to the triglyceride pool by hepatic de novo lipogenesis.[75,76] Within hepatocytes, free fatty acids (FFA) primarily undergo one of two major pathways: mitochondrial beta-oxidation to CO_2 or re-esterification to form triglycerides. These triglycerides can either be released into the bloodstream as VLDL or stored within lipid droplets inside hepatocytes. The lipid droplet triglycerides are subject to regulated lipolysis, releasing fatty acids back into the hepatocytes FFA pool. Therefore, the development of hepatic steatosis is a highly intricate process, driven by the excessive delivery of free fatty acids to the liver, accompanied by increased DNL primarily fuelled by an overabundance of dietary carbohydrates, especially fructose.[22,77] Insulin resistance and hyperinsulinaemia further exacerbate lipid accumulation by diverting glucose away from glycogen synthesis, redirecting it instead to lipogenic pathways. Insulin resistance in skeletal muscle results in decreased glucose uptake and glycogen storage, further promoting hepatic DNL (Figure 3).[78–80]

Insulin binds tyrosine receptors on hepatocellular cell membranes, which causes receptor auto-phosphorylation, followed by phosphorylation of insulin receptor substrate 1 (IRS-1) and insulin receptor substrate 2 (IRS-2). Insulin resistance selectively inhibits the hypoglycaemic effects of insulin, while allowing de novo lipogenesis to continue, via activation of sterol regulatory element binding protein (SREBP1). It is hypothesised that the increased production of lipid intermediates, such as ceramides and diacylglycerols (DAGs), activate numerous pathways which influence signalling by IRS. These pathways include mTOR (mammalian target of rapamycin), c-Jun N-terminal kinase (JNK), protein kinase C (PKC), MAPK and ERK.[81–83] A key role for the plasma membrane sn-1,2-diacylglyceral-protein kinase C epsilon (PKCε)-insulin receptor kinase threonine^{1160} has been implicated in the development of hepatic insulin resistance.[84] More specifically, DAGs inhibit insulin signalling by increasing protein kinase Cε (PKCε), which inhibits IRS1 and 2.[85] Moreover, hepatic DAG content and PKCε activation were the strongest predictors of hepatic insulin resistance in individuals with MASLD undergoing bariatric surgery.[86]

Thus, if the ability of the mitochondria to oxidise the fatty acids is overwhelmed, increased toxic lipid intermediates affect insulin pathways to drive insulin resistance in the liver. At the same time, increased mitochondrial dysfunction causes oxidative stress[87] which is exacerbated by hyperglycaemia. Oxidative stress can inhibit insulin signalling via activation of inhibitor of nuclear factor kappa-B (I-κB) kinase subunit beta (IKKβ) and c-Jun N-terminal kinase (JNK).

Inflammatory cytokines, such as IL-6 and TNF-a, can also activate (IKKβ), JNK and additionally, suppressor of cytokine signalling (SOCS), which can phosphorylate IRS1 and IRS2 to inhibit insulin signalling. IKKβ and JNK can activate nuclear factor kappa-B (NF-κB), causing translocation to the nucleus. Glucose can also enter cells via the GLUT-4 transporter protein. An increase in intracellular glucose causes increased activation of carbohydrate response element binding protein (ChREBP), which is involved in lipogenesis from glucose. The net effects of these converging processes are inflammation, a reduction in glycogen synthesis, an increase in lipogenesis and an increase in blood insulin and glucose levels.

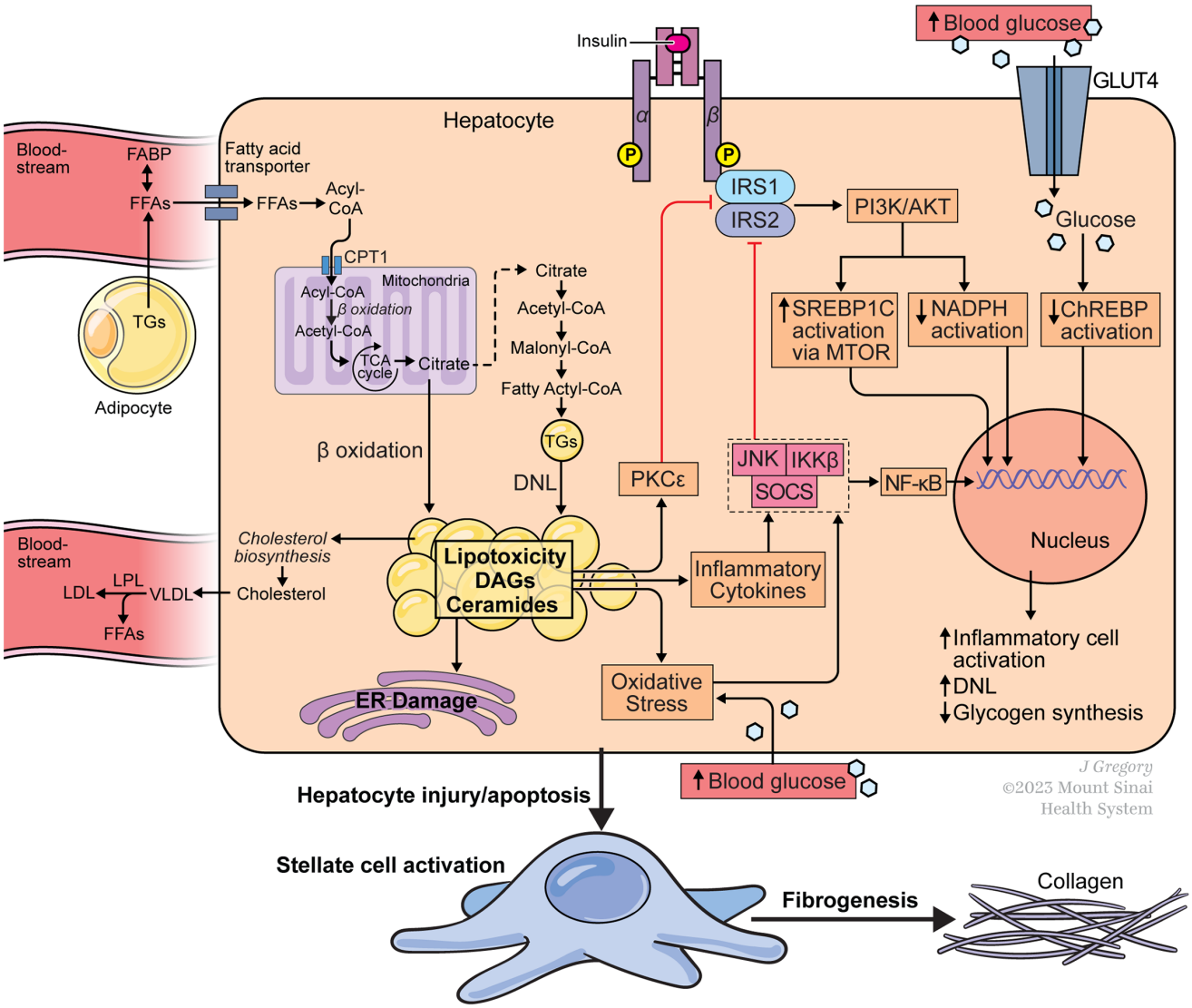

FIGURE 3 Converging on the liver: development of hepatic insulin resistance and lipotoxicity. Numerous concomitant and converging pathways perpetuate hepatic insulin resistance and lipotoxicity. Insulin binds its tyrosine receptor which causes auto-phosphorylation of Insulin Substrate 1 and 2 (IRS 1 and 2). Increased delivery of FFA and DNL increases lipotoxic species, such as DAGs. These, in turn, drive ER stress, oxidative stress, inflammatory cytokines. DAGs themselves increase PKCε which inhibits IRS 1 and 2, further promoting insulin resistance. Inflammatory cytokines increase JNK and SOCs, which along with oxidative stress induced Iκκβ, inhibit IRS 2. Inhibition of IRS 1 and 2 signalling pathways results in increased SREBP1 activation via mTOR, which drives DNL. Loss of NADPH inhibition from insulin resistance further increases ROS. Iκκβ causes increased NF-κβ activation which promotes pro-inflammatory signalling pathways. In addition to insulin resistance, increased intracellular glucose from increased GLUT4 expression causes increased ChREBP activation, which promotes lipogenesis from glucose. Collectively these cause increased inflammatory cell activation, increased DNL and decreased glycogen synthesis. Therefore, lipotoxicity promotes insulin resistance and insulin resistance promotes increased lipotoxicity. Once hepatocyte injury and apoptosis are ensured, stellate cells are activated and the fibrogenic process is perpetuated unless the upstream signals are decreased.

In addition to playing a role in promoting insulin resistance and oxidative stress, the formation of lipotoxic compounds leads to endoplasmic reticulum (ER) stress and inflammasome activation.[88] In the ER, proteins fold into their native conformation and undergo a multitude of post-translational modifications. Any disruption of these processes causes the accumulation of unfolded, aggregated proteins, which activate the unfolded protein response (UPR). This is an adaptive and protective mechanism aimed at restoring normal ER function. Three ER transmembrane protein sensors mediate UPR signals: (1) IRE1 (inositol requiring enzyme 1; ERN1—ER to nucleus signalling 1), (2) PERK (protein kinase RNA-like endoplasmic reticulum kinase) and (3) ATF6 (transcription factor activating transcription factor 6). These protein sensors detect unfolded protein loads in the ER lumen and transmit signals to downstream effectors.[89–91] FFAs are one of the agents able to activate UPR – this is reviewed elsewhere.[92] Lipotoxicity and subsequent FFA-induced ER stress are also thought to promote the development of MASLD, as observed in rat and mouse in vivo models.[93,94]

Given the importance of DNL in disease progression, the ability to measure DNL, both at baseline and in response to dietary or

pharmacologic interventions, would be highly valuable and informative. Outside of invasive liver biopsies, several non-invasive assessments include stable label isotype, fatty acid profiling or indices and indirect calorimetry. Of these, fatty acid profiling/indices, also known as lipidomics, are emerging as an option; however, currently are only used in the context of clinical research.[95] Other studies have attempted to correlate MASLD progression with toxic lipid intermediates, such as DAGs, by leveraging gas chromatography with mass spectrometry and magnetic resonance spectroscopy to determine intracellular fatty acid composition changes as MASLD progresses from simple steatosis to steatohepatitis (MASH). As the disease progressed, this study found that the amount of PUFA (polyunsaturated fatty acids) decreased, MUFA (monounsaturated fatty acids) increased and SFA (saturated fatty acids) remained the same.[96] The extent to which this becomes clinically relevant and surpasses the value of merely measuring triglyceride content via MRI-PDFF as a surrogate is yet to be determined. Clinical trial data suggest that MRI-PDFF reductions correlate with MASH resolution, contingent upon the disease's mechanism of action.[97]

3.9 | Measuring insulin resistance and impact of therapeutics on MASH

The gold standard for measuring systemic insulin resistance is euglycemic hyperinsulinemic clamp testing but this test is non-physiologic, operator-dependent and time-consuming, making it unpractical for clinical use. Oral or IV glucose tolerance tests can be done but have not been widely implemented due to required expertise and high variability of insulin level measurements across different laboratories. Surrogate or indirect single sample tests, such as HOMA-IR (Homeostatic Model Assessment for Insulin Resistance), can be done and include fasting insulin and glucose levels (fasting insulin (microU/L)×fasting glucose (nmol/L)/22.5). While classic teaching was that a value >2.5 was consistent with IR, cut-offs vary based on ethnicity, race, gender and co-morbidities. HOMA-IR levels can also vary due to the lack of standardisation of insulin assays across platforms and/or altered relationships of insulin concentration with insulin resistance in individuals with diabetes. Newer markers such as a nuclear magnetic resonance biomarker, lipoprotein insulin resistance (LPIR) index, reflects a non-glycaemic measure of insulin resistance and may be a predictor of DM development and complications.[9,98]

As the endocrine pancreas secretes insulin into the portal vein, the liver is exposed to double or triple the levels of insulin seen by other tissues.[90] Hepatic insulin resistance can occur in the absence of systemic IR and may be due to defective glycogen metabolism. Furthermore, selective hepatic insulin resistance may manifest solely in impaired glucose handling, without affecting lipogenic pathways. Chronic overnutrition can also activate multiple insulin-dependent lipogenic, as discussed elsewhere.[9] Serum fasting insulin levels may potentially be a crude surrogate of hepatic insulin resistance, but they are only useful in large epidemiologic studies.

Not all patients with insulin resistance develop MASH. The intricate interplay between genetics and environmental factors in determining steatohepatitis, and more significantly, fibrosis, will be discussed below. For those who develop MASH in the context of insulin resistance, the key question is: does treatment of insulin resistance improve MASH? Weight loss, whether it be by bariatric surgery or pharmacologic therapy, improves insulin resistance and steatosis. Bariatric surgery clearly results in resolution of MASH and fibrosis progression if there is a weight loss of 10% or more.[91] GLP-1 agonists have been shown to decrease steatohepatitis but have not yet shown an effect on fibrosis progression; phase 3 studies are currently underway.[99] However, drugs that improve insulin sensitivity without weight loss, such as Metformin, do not have an impact on MASH or fibrosis.[100] PPAR agonists with varying receptor activity (α, δ, γ) improve insulin sensitivity and cause lipid redistribution away from the liver. While some have shown decreased steatohepatitis,[26] the newer pan-PPAR agents' effects on fibrosis regression are pending Phase 3 results.[26] Therefore, based on current clinical data, one could speculate that without decreasing intrahepatic lipid load, improving insulin resistance in isolation will be inadequate.

3.10 | Progressive fibrosis: Most important determinant of outcomes but dependent on complex interplay of genes and environment

With the convergence of insulin resistance and lipotoxicity, the hepatocyte is unable to effectively handle the stress, resulting in

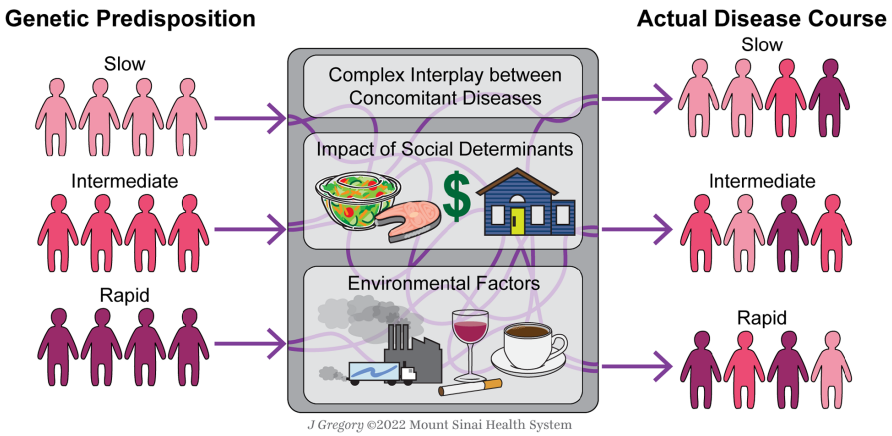

FIGURE 4 Complex interplay between genetic predisposition, medical comorbidities, environmental factors and social determinants of health in determining disease progression in individuals with MASH.

hepatocyte injury and apoptosis. This process, in turn, initiates the recruitment and activation of inflammatory cells and triggers the activation of hepatic stellate cells, which constitute the principal cellular source of collagen and fibrosis in response to chronic liver injury. The activation of these repair pathways signifies a transition from bland steatosis (MASLD) to the more aggressive and progressive form of steatohepatitis (MASH).[101] In its quiescent state, the hepatic stellate cell is rich in Vitamin A and primarily produces type IV collagen. However, when subjected to injury, it undergoes phenotypic changes characterised by increased proliferation, contractility, and a shift towards producing type I and III collagens, which are typical of cirrhotic liver tissue.[102] Progressive fibrosis is often categorised histologically on a scale ranging from F0 to F4. Importantly, fibrosis stands out as the single most important predictor of liver-related outcomes, and once a patient reaches Stage 2 fibrosis, their risk of liver-related mortality increases tenfold.[103] If the injury persists, progressive fibrosis leads to end organ failure and death without liver transplantation. It has been well established that patients have variable fibrosis progression rates; some patients will never develop cirrhosis while others will develop cirrhosis rapidly. Fundamentally, fibrosis accumulates when fibrogenesis outpaces fibrinolytic and reparative regenerative pathways. At the individual level, in the context of MASLD/MASH, patients are born with genetic predispositions which are then modulated by comorbid conditions, dietary and lifestyle effects and environmental/toxic exposures, ultimately determining those that will have faster fibrosis progression rates (Figure 4).

AUTHOR CONTRIBUTIONS

Shalini K. Bansal: Conceptualization; investigation; writing – original draft; writing – review and editing. **Meena Bansal:** Conceptualization; data curation; investigation; writing – original draft; writing – review and editing.

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