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Abstract

Metabolic syndrome (MetS) is a disease composed of different risk factors such as obesity, type 2 diabetes or dyslipidemia. The prevalence of this syndrome is increasing worldwide in parallel with the rise in obesity. Nonalcoholic fatty liver disease (NAFLD) is now the most frequent chronic liver disease in western countries, affecting more than 30% of the general population. NAFLD encompasses a spectrum of liver manifestations ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis, which may ultimately progress to hepatocellular carcinoma. There is accumulating evidence supporting an association between NAFLD and MetS. Indeed, NAFLD is recognized as the liver manifestation of MetS. Insulin resistance is increasingly recognized as a key factor linking MetS and NAFLD. Insulin resistance is associated with excessive fat accumulation in ectopic tissues, such as the liver, and increased circulating free fatty acids, which can further promote inflammation and endoplasmic reticulum stress. This in turn aggravates and maintains the insulin resistant state, constituting a vicious cycle. [...]

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Review

Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link?



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ABSTRACT

Metabolic syndrome (MetS) is a disease composed of different risk factors such as obesity, type 2 diabetes or dyslipidemia. The prevalence of this syndrome is increasing worldwide in parallel with the rise in obesity. Nonalcoholic fatty liver disease (NAFLD) is now the most frequent chronic liver disease in western countries, affecting more than 30% of the general population. NAFLD encompasses a spectrum of liver manifestations ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis, which may ultimately progress to hepatocellular carcinoma. There is accumulating evidence supporting an association between NAFLD and MetS. Indeed, NAFLD is recognized as the liver manifestation of MetS. Insulin resistance is increasingly recognized as a key factor linking MetS and NAFLD. Insulin resistance is associated with excessive fat accumulation in ectopic tissues, such as the liver, and increased circulating free fatty acids, which can further promote inflammation and endoplasmic reticulum stress. This in turn aggravates and maintains the insulin resistant state, constituting a vicious cycle. Importantly, evidence shows that most of the patients developing NAFLD present at least one of the MetS traits. This review will define MetS and NAFLD, provide an overview of the common pathophysiological mechanisms linking MetS and NAFLD, and give a perspective regarding treatment of these ever growing metabolic diseases.

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1. Introduction

Obesity is now a worldwide pandemic and is expected to affect 10% of the global population by 2030 if the current trend is maintained (Webber et al., 2014). In the United States, national surveys

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have observed a marked increase in the prevalence of obesity over time. For instance, ~20% of men and ~25% of women in the adult population and more than one-sixth of children are obese (Ogden et al., 2012). However, this increased prevalence of obesity is not confined to western countries. Notably, obesity is recognized as a major health problem in the United Arab Emirates (Hodge et al., 1995; Musaiger, 1996; Popkin, 1994). Moreover, obesity increases at an alarming rate in all Arabic-speaking countries with a prevalence of 2% to 55% in adult females and 1% to 30% in adult males (Badran and Laher, 2011). Other populations are also affected by this disorder, such as Indians, with a recent study reporting a prevalence of overweight and obesity in children up to 23% and 36%, respectively (Hoque et al., 2014). Obesity results from an imbalance between caloric intake and energy expenditure, leading to an excess of energy, which is stored as fat mainly in white adipose tissue (Chugh and Sharma, 2012; McKenney and Short, 2011). Importantly, obesity increases or exacerbates several health problems including cardiovascular diseases and type 2 diabetes (Kopelman, 2000). Metabolic alterations associated with obesity have been recognized and grouped to define the metabolic syndrome (MetS). MetS is a leading cause of mortality and morbidity in industrialized countries (Simons et al., 2011). It is characterized by the combination of multiple disorders including obesity, dyslipidemia, increased blood pressure, insulin resistance and a proinflammatory state (Reaven, 2002). The prevalence of MetS correlates with the global epidemic of obesity and is growing at an alarming rate, affecting more than 20% of the global adult population (Onat, 2011).

The rising epidemic of MetS and its related complications, such as cardiovascular diseases, has been accompanied by an increase in liver alterations including nonalcoholic fatty liver disease (NAFLD) (Angulo, 2002; Marchesini et al., 2003). In particular, it has been proposed that NAFLD may be the hepatic manifestation of MetS (Marchesini et al., 2003). NAFLD encompasses a wide spectrum of manifestations ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis (Tarantino et al., 2012). One of the major hallmarks of this disease is the consistent association with one of the characteristics of MetS, for instance type 2 diabetes mellitus, dyslipidemia or obesity (Vanni et al., 2010). Moreover, NAFLD is clearly associated with insulin resistance, which is a key risk factor for the development of type 2 diabetes (Jornayvaz and Shulman, 2012). Therefore, the aims of this review are 1) to discuss how MetS and NAFLD impact each other; 2) to describe the common mechanisms between these disorders; and 3) to provide an overview of the current diagnosis and treatment of both MetS and NAFLD.

2. Diagnosis and pathogenesis of MetS and NAFLD

MetS has received several definitions during the last decade. Therefore, it was important to find a consensus between different medical associations such as the World Health Organization (WHO), the European Group for the study of Insulin Resistance (EGIR), the National Cholesterol Education Program (NCEP), and finally the Third Adult Treatment Panel (ATPIII) (Alberti et al., 2006). The main aim of this consensus was to identify common criteria in order to use them in the clinical diagnosis of MetS worldwide. Using the ATPIII definition as a basis, the committee came with a new definition. Although insulin resistance is a critical feature of MetS, it remains difficult to measure in day-to-day clinical practice and thus was not included in the new definition. Central obesity, which is much easier to measure, was considered. Therefore, the new definition classified patients with MetS as subjects having central obesity and one of the following factors: raised triglycerides ≥ 1.7 mmol/l; reduced HDL-cholesterol

<1.03 mmol/l in males and <1.29 mmol/l in females; raised systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg; raised fasting plasma glucose, with fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed type 2 diabetes (Alberti et al., 2006). The major characteristic of this definition is that central obesity is required to diagnose MetS. As insulin resistance is frequently associated with obesity, the consensus considered obesity as a surrogate marker of insulin resistance in the definition of MetS. However, this definition of MetS is still a matter of debate (Jornayvaz et al., 2010b).

NAFLD is a pathological entity encompassing a whole histological spectrum ranging from simple steatosis to steatohepatitis, with inflammation and fibrosis, to cirrhosis (Contos et al., 2004). Cirrhosis, which is irreversible, can further progress to hepatocellular carcinoma (Farazi and DePinho, 2006). The development of NAFLD is notably based on the concept of the “two hits” hypothesis (Day and James, 1998). The first hit is the accumulation of triglycerides, leading to hepatic steatosis, while the second hit is the production of free radicals and inflammatory mediators giving rise to NASH. However, more recently, it has been argued that multiple hits derive simultaneously from adipose tissue and gut to promote liver inflammation, suggesting that cellular inflammation and insulin resistance are acting at the same time (Tilg and Moschen, 2010). Others have proposed that hepatic insulin resistance may be at the origin of the development of NAFLD. Indeed, mice specifically lacking the insulin receptor in the liver (LIRKO mice) develop hepatic insulin resistance associated with hyperglycemia, suggesting that hepatic function is critical to control peripheral insulin responsiveness (Michael et al., 2000). Additionally, this insulin resistance leads to altered liver function. Thus, one could propose that hepatic insulin resistance is the first step in the development of peripheral insulin resistance and NAFLD. In accordance with this hypothesis, deletion of the insulin receptor in skeletal muscle (MIRKO mice) does not impair peripheral glucose levels (Bruning et al., 1998). In addition to insulin resistance, other aspects of MetS such as obesity have been related to NAFLD. For instance, 85% of the patients developing NAFLD present at least one of the MetS characteristics (Gariani et al., 2013).

Clinically, NAFLD does not manifest with specific symptoms and is commonly silent. Currently, the diagnosis of NAFLD is based on exclusion criteria. For instance, causes such as alcohol consumption (more than 20 g/day for women and 30 g/day for men), autoimmune liver disease, viral hepatitis infection, hemochromatosis, Wilson's disease, or drug consumption, must be excluded before considering NAFLD. Liver biopsy remains the gold standard to diagnose NAFLD, but this approach is associated with potential risks, such as bleeding. Therefore, patients need to be well selected before undergoing liver biopsy, although data are currently lacking regarding specific criteria. Alternative methods to assess NAFLD, such as non invasive imagery, are beyond the scope of this review and have been discussed elsewhere (Musso et al., 2011).

3. NAFLD: the liver manifestation of MetS

NAFLD is frequently associated with central obesity, insulin resistance and dyslipidemia, all of which are features of MetS. Therefore, NAFLD has been identified as the liver manifestation of MetS, with obesity as the main common component. Nevertheless, it should be noted that NAFLD also develops in non-obese patients, even though it is less common. For instance, several studies have shown that NAFLD could be detected in non-obese subjects with metabolic alterations (Kim et al., 2004; Musso et al., 2008; Sinn et al., 2012). Furthermore, these studies reported that NAFLD could be considered as an independent predictor of insulin resistance in non-obese patients (Kim et al., 2004; Musso et al., 2008;

Sinn et al., 2012). Although NAFLD is found in non-obese subjects, it remains closely related to central (or visceral) obesity. Indeed, Jeong and coworkers have reported a relationship between visceral fat and the prevalence of both NAFLD and MetS (Jeong et al., 2008). In their prospective study, they included 224 hospital workers and assessed the prevalence of NAFLD and MetS, as well as visceral fat thickness. MetS was diagnosed according to the ATP III guidelines, and NAFLD was diagnosed by ultrasonography. Altogether, their data revealed that 73.1% of the subjects with MetS had NAFLD, indicating a strong correlation between these two disorders. Additionally, visceral fat thickness, assessed by ultrasonography with a 3.5-MHz convex probe, was significantly increased by both MetS and the severity of NAFLD, indicating a direct association and suggesting that visceral fat could be considered as a common denominator of both MetS and NAFLD (Jeong et al., 2008). In line with these results, Kwon et al., studied a total of 29,994 adults who underwent routine comprehensive health evaluations, and found that NAFLD is associated with components of MetS such as insulin resistance or type 2 diabetes. Interestingly, this association was stronger in non-obese than in obese individuals (Kwon et al., 2012). Furthermore, Kwon et al., found that the differences in the amount of visceral fat between individuals with NAFLD and those without NAFLD may be greater in the non-obese group than in the obese group (Kwon et al., 2012). This suggests that visceral fat could be used as an important predictive factor for the development of NAFLD (Eguchi et al., 2006).

4. Common pathophysiological mechanisms involved in MetS and NAFLD

The increased prevalence of both MetS and NAFLD is mainly due to over-nutrition and a sedentary lifestyle. However, the contribution of MetS to NAFLD involves different factors among which insulin resistance, central obesity, inflammation, oxidative stress and genetic predispositions. Nevertheless, several controversies remain regarding which of these mechanisms comes first and how they drive each other to promote MetS and NAFLD. Therefore, this section will discuss studies highlighting these mechanisms.

4.1. Insulin resistance: a critical node between MetS and NAFLD

Insulin resistance, defined as the failure of insulin to stimulate glucose transport into its target cells, plays a central etiological role in MetS and is harmful for the liver since it likely promotes NAFLD (Bugianesi et al., 2005). Insulin is a pleiotropic hormone that regulates several cell functions among which stimulation of glucose transport, cell growth, energy balance and regulation of gene expression (de Luca and Olefsky, 2008). Insulin initiates its action through binding to the insulin receptor which results into dimerization and autophosphorylation of this insulin receptor. This in turn recruits and phosphorylates the insulin receptor substrate (IRS), IRS2 being the most represented isoform in the liver. Downstream, this actor modulates two different signaling pathways: the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB/AKT) pathway, which promotes the metabolic actions of insulin, and the mitogen-activated protein kinase (MAPK) pathway, which regulates the expression of genes involved in cell growth and differentiation (Taniguchi et al., 2006). Alteration of these signaling pathways by various factors could therefore lead to insulin resistance. Among these, free fatty acids are recognized as a significant risk factor contributing to the development of insulin resistance in several organs. Notably, hepatic insulin resistance correlates with increased liver fat content, the latter mainly originating from plasma free fatty acids (Donnelly et al., 2005). Plasma free fatty acids are largely released by white adipose tissue when insulin

resistance develops. Indeed, insulin resistance in this tissue results in increased lipolysis, which leads to high levels of circulating fatty acids (Fig. 1) (Eguchi et al., 2006). Altogether, hepatic fat accumulation causes NAFLD, subsequently leading to hepatic insulin resistance. Therefore, insulin resistance could be considered as the missing link between MetS and NAFLD, even in the absence of obesity. However, the mechanisms of hepatic insulin resistance remain poorly characterized in NAFLD. Several hypotheses regarding the development of hepatic insulin resistance in NAFLD can be considered and will be further discussed, such as ectopic fat accumulation, inflammation, endoplasmic reticulum (ER) stress and gut microbiota.

4.1.1. Ectopic fat accumulation promotes hepatic insulin resistance

As previously discussed, insulin resistance, a key feature of MetS, plays a critical role in the development of NAFLD. Indeed, numerous studies in animal models of NAFLD reveal a clear link between NAFLD and hepatic insulin resistance (Birkenfeld et al., 2011b, Camporez et al., 2013a, 2013b; Cantley et al., 2013; Jornayvaz et al., 2010a, 2011, 2012; Lee et al., 2011). Notably, evidence suggests that certain lipid intermediates, such as diacylglycerols, activate novel protein kinase C ϵ (PKC ϵ) (Fig. 2), which further inhibits the insulin receptor and its downstream signaling (Samuel et al., 2004). Moreover, in obese non diabetic humans with NAFLD, Kumashiro et al., could show that hepatic diacylglycerol content was the best predictor of insulin resistance, when assessed by the HOMA index (Kumashiro et al., 2011). However, whether insulin resistance promotes hepatic lipid accumulation or whether NAFLD initiates insulin resistance remains unclear to date. Nevertheless, it appears that skeletal muscle insulin resistance can cause insulin resistance in other tissues (Jornayvaz and Shulman, 2012; Perry et al., 2014). For instance, deletion of the muscle insulin receptor in mice (MIRKO mice) results in the development of insulin resistance in other organs, possibly through substrates redistribution toward adipose tissue (Kim et al., 2000b). Similarly, mice lacking the insulin-responsive glucose transporter 4 (GLUT4) in skeletal muscle exhibit altered insulin sensitivity with redistribution of substrates to the liver, which promotes liver steatosis (Kotani et al., 2004). These results in animals were translated to humans, where skeletal muscle insulin resistance diverts ingested glucose away from muscle glycogen storage toward hepatic *de novo* lipogenesis, thus predisposing to the development of NAFLD (Petersen et al., 2007). Altogether, these findings suggest that peripheral insulin resistance leads to altered lipid metabolism, which may exacerbate liver steatosis. Interestingly, NAFLD and hepatic insulin resistance can develop in a very short time. Notably, Samuel and coworkers showed that hepatic insulin resistance as well as steatosis can occur in high fat fed rodents within three days (Samuel et al., 2004). In this model, hepatic insulin resistance was secondary to increased diacylglycerols synthesis, known to activate PKC ϵ (Fig. 2). Supporting these findings, inhibition of PKC ϵ using an antisense oligonucleotide prevented hepatic insulin resistance despite hepatic lipid accumulation (Samuel et al., 2007). Together, these studies showed that the regulation of hepatic lipid accumulation plays a major role in the development of NAFLD and associated hepatic insulin resistance.

Hepatic lipid uptake and oxidation also play an important role in the development of NAFLD. Notably, specific hepatic over-expression of lipoprotein lipase (LPL) in mice promoted hepatic lipid accumulation and hepatic insulin resistance (Kim et al., 2001). Impaired lipid oxidation may also lead to an imbalance leading to increased hepatic lipid content. Indeed, Fullerton et al., have shown that mutations in both acetyl-CoA carboxylase 1 and 2 (ACC 1, ACC2) promote lipogenesis and decrease lipid oxidation. These mutations led to the inability of AMPK-activated protein kinase

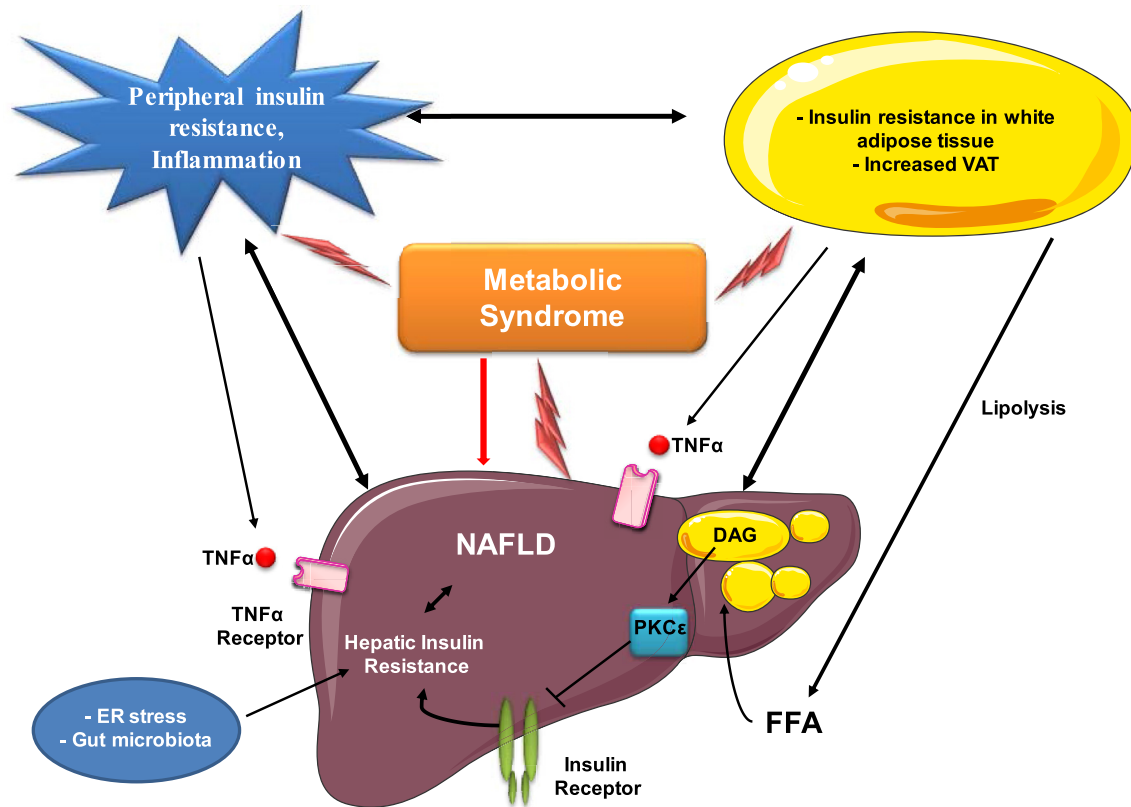


Fig. 1. Role of white adipose tissue and liver in the development of MetS and NAFLD.

Obesity, one of the central features of MetS, is associated with increased visceral adipose tissue (VAT) release of proinflammatory cytokines such as tumor necrosis factor α (TNF α) and free fatty acids (FFA), which promote hepatic diacylglycerols (DAG) accumulation and activation of inflammatory pathways. DAG in turn activate protein kinase ϵ (PKC ϵ), which inhibits insulin signaling, leading to hepatic insulin resistance. These processes in turn play a major role in the development of MetS and its hepatic manifestation, known as NAFLD. Other factors such as peripheral insulin resistance, inflammation, endoplasmic reticulum (ER) stress and gut microbiota also play a role in the development of NAFLD and hepatic insulin resistance.

(AMPK) to phosphorylate ACC1 and 2 and thus inactivate these enzymes (Fullerton et al., 2013). Therefore, in this case, impaired lipid oxidation led to increased hepatic lipid content and hepatic insulin resistance. In contrast, in another mouse model, increased AMPK activity promoted inactivation of ACC1 and ACC2, leading to increased lipid oxidation, lower hepatic diacylglycerol content and decreased PKC ϵ activity, leading to a protection from diet-induced hepatic insulin resistance (Birkenfeld et al., 2011a).

Importantly, fatty acids that promote hepatic insulin resistance and NAFLD mainly originate from white adipose tissue lipolysis. Lipolysis increases with total fat mass content (Mittendorfer et al., 2009; Pardina et al., 2009). However, the relationship between lipolysis and insulin responsiveness seems to be independent of body mass. For instance, insulin resistant obese adolescents present higher visceral fat content than their weight-matched, insulin sensitive counterparts (Weiss et al., 2005). Similarly, in rodents, obese mice lacking fatty-acid-binding protein (FABP) in adipocytes are more insulin sensitive than their wild-type littermates (Cao et al., 2006). Therefore, it seems that the increase in white adipose tissue lipolysis provides substrates for ectopic lipid accumulation and plays a facilitative role in the induction of hepatic insulin resistance. The importance of adipose tissue in the development of insulin resistance and NAFLD has been revealed by lipodystrophic models. Indeed, despite the absence of peripheral or visceral fat expansion in lipodystrophy, fatty acids accumulate in the liver and skeletal muscle, which leads to the development of insulin resistance. For instance, in a genetic mouse model expressing the dominant negative protein A-ZIP/F-1 ("fatless" mice) in adipose

tissue, there is a lack of visceral and peripheral fat, but an ectopic accumulation of fat in liver and skeletal muscle, leading to severe insulin resistance in these tissues (Kim et al., 2000a). Interestingly, transplantation of white adipose tissue from wild-type mice to these lipodystrophic mice leads to an improvement in insulin sensitivity (Kim et al., 2000a). Similar results are observed with leptin administration (Shimomura et al., 1999). In line with these studies, investigations in humans with lipodystrophy revealed that although visceral or peripheral fat were almost absent, these patients developed NAFLD and hepatic insulin resistance (Petersen et al., 2002; Savage et al., 2005). Leptin treatment of these subjects decreased hepatic lipid content and improved hepatic insulin sensitivity (Petersen et al., 2002). Altogether, these studies emphasize the important role of ectopic lipid accumulation in the development of insulin resistance. Notably, fatty acids derived from white adipose tissue play a critical role in the induction of NAFLD and hepatic insulin resistance. Further, reversion of hepatic steatosis in these models of lipodystrophy restores hepatic insulin sensitivity.

In addition to lipodystrophy, another important model exemplifying the major role of white adipose tissue in the development of liver steatosis and insulin resistance is ageing. Indeed, the prevalence of NAFLD and MetS in the general population increases with age (Choi and Diehl, 2008; Kagansky et al., 2004; Reynolds and Wildman, 2009). Moreover, body fat distribution shifts from subcutaneous adipose tissue to visceral adipose tissue (VAT) in elder subjects, leading to deleterious metabolic consequences such as the development of insulin resistance (Petta et al., 2010; Tran et al.,

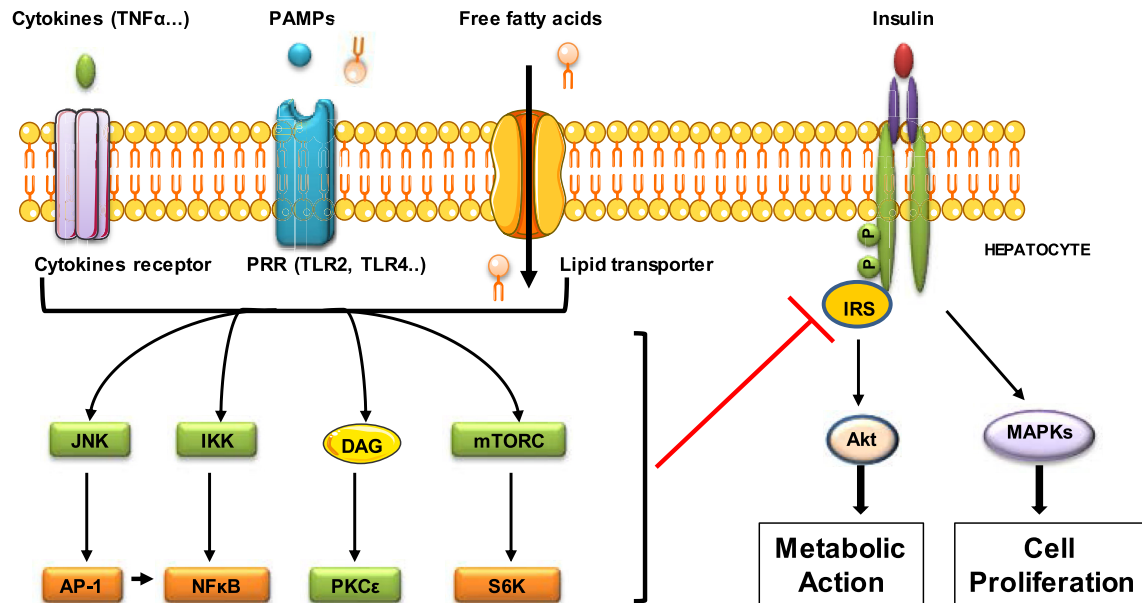


Fig. 2. Inhibition of insulin signaling pathways associated with both MetS and NAFLD.

Insulin receptor substrate (IRS) phosphorylation can be inhibited by various processes, in particular inflammatory or kinases proteins as well as lipid derived sub-products. For instance, inflammatory proteins such as cytokines (notably TNF α), and others such as PAMPs, bind to their receptor and activate downstream inflammatory signaling pathways including IKK, mTORC or JNK. In turn, these effectors recruit downstream molecules, respectively NF κ B, S6K and AP-1, which target the inhibition of IRS phosphorylation, thus leading to hepatic insulin resistance. Moreover, accumulation of toxic lipid metabolites in the liver such as diacylglycerols (DAG) can activate kinases such as protein kinase C ϵ (PKC ϵ), which is known to inhibit IRS phosphorylation, subsequently leading to hepatic insulin resistance. Therefore, multiple ways can induce insulin resistance in the liver.

2008). Expansion of VAT notably promotes the production of deleterious cytokines such as tumor necrosis factor α (TNF α). TNF α binds to its receptor and activates downstream inflammatory signaling pathways including IKK, mTORC or JNK. In turn, these effectors recruit downstream molecules, respectively nuclear factor-kappa B (NF κ B), S6 kinase (S6K) and activator protein-1 (AP-1), which inhibit IRS phosphorylation, subsequently impairing insulin signaling in the liver (Kamada et al., 2008; Lumeng et al., 2008; Ozes et al., 2001) (Fig. 2). Evidence is accumulating that ageing is associated with mitochondrial dysfunction, which leads to accumulation of ectopic lipids and finally insulin resistance (Petersen et al., 2003).

In addition to ectopic fat accumulation, NAFLD can also be associated with genetic predisposition. This hypothesis originates from the fact that NAFLD also develops in healthy subjects without criteria of MetS. For instance, healthy Indian and Asian men are more prone to develop hepatic steatosis and hepatic insulin resistance than other populations because of specific polymorphisms in the insulin-response element for the apolipoprotein C3 gene (Petersen et al., 2006, 2010). Confirming the role of the genome in the pathogenesis of NAFLD, another study revealed that Hispanic adults and children are at high risk for the development of NAFLD due to a missense mutation found in the Patatin-like phospholipase domain-containing protein 3 gene (*PNPLA3*), which is more present in this ethnic group compared to the general population (Romeo et al., 2008). Interestingly, however, patients with variants of the *PNPLA3* gene did not develop insulin resistance, suggesting that in this case NAFLD could be dissociated from insulin resistance (Kantartzis et al., 2009). However, in these studies on variants of the *PNPLA3* gene, control subjects were obese and probably had already some degree of insulin resistance, making it difficult to specifically assess insulin resistance in subjects with NAFLD-associated *PNPLA3* gene variants. Moreover, treatment of high-fat fed rats with specific antisense oligonucleotides reducing *PNPLA3* expression protected them from the development of NAFLD and hepatic insulin

resistance (Kumashiro et al., 2013).

4.1.2. Inflammation and ER stress induce insulin resistance in MetS and NAFLD

Inflammation is present in both MetS and NAFLD. For instance, several systemic inflammatory markers such as TNF α , interleukin-6 (IL-6) and C-reactive protein (CRP) are increased in MetS (Haffner, 2003). Moreover, high plasma levels of CRP have been used as a predictor for the development of NAFLD because patients with NASH are more insulin resistant and have remarkably higher plasma CRP concentrations than overweight nonsteatotic patients with similar visceral adipose tissue mass (Targher et al., 2008). In addition to elevated pro-inflammatory cytokines, it was observed that metabolic dysfunctions such as insulin resistance are associated with reduced protective adipokines (such as adiponectin (Hotta et al., 2000)), and increased macrophage accumulation in the liver and adipose tissue (Lanthier et al., 2010). In particular, CD4 (+) and CD8 (+) T cells infiltration increases in the liver (Gadd et al., 2013). Nevertheless, the mechanisms of the inflammatory response related to MetS are far from being completely elucidated.

Pattern Recognition Receptors (PRRs) are a family of receptors that plays a critical role in innate immune systems and are well known for their ability to sense pathogen associated molecular patterns (PAMPs). Of these PRRs, Toll like receptors (TLRs) received a particular attention. Several TLRs isoforms were identified but only TLR2 and TLR4 were involved in obesity and insulin resistance, two features of MetS (Fresno et al., 2011; Konner and Bruning, 2011). Moreover, most of the studies focused on TLR4 isoform because of its ability to bind to free fatty acids and initiate the proinflammatory signaling pathway NF κ B (Fig. 2) (Fessler et al., 2009). In line with these results, mice knockout for the *TLR4* gene is protected from obesity-induced inflammation and lipid infusion-induced insulin resistance (Shi et al., 2006). These results in animals were also confirmed in humans. Notably, obese patients and patients with type 2 diabetes have elevated TLR4 muscle

expression, which positively correlates with insulin resistance (Konner and Bruning, 2011). In addition to its role in obesity, TLR4 was identified as a critical effector in the development of NAFLD (Miura et al., 2010). Therefore, TLR4 could be considered as one of the critical nodes between obesity, which represents the central feature of MetS, and NAFLD.

In addition to the NF κ B pathway that can be activated through cytokine receptor or TLR, JNKs are other inflammation sensing proteins that are activated in obese patients (Solinas and Karin, 2010). These kinases appear to play an important role in the development of NAFLD and insulin resistance (Czaja, 2010; Donath and Shoelson, 2011). Although both JNK1 and JNK2 contribute to metabolic disorders, distinct functions for these proteins have been identified (Sabapathy et al., 2004). Indeed, JNKs were found to be increased in diet-induced and genetic models of obesity (Hirosumi et al., 2002). However, JNK1 plays a more prominent role in obesity and insulin responsiveness than JNK2. For instance, mice lacking JNK1, but not mice lacking JNK2, display a decreased weight gain, decreased glucose and insulin levels, and improved hepatic insulin sensitivity (Aguirre et al., 2000; Hirosumi et al., 2002). Based on these studies, one could suppose that JNK2 is not involved in metabolic alterations. Therefore, in order to address the question of the role of JNK2 in metabolism, Tuncman and coworkers generated *jnk2* null mice with one *jnk1* allele and found that these mice were protected from high-fat diet-induced obesity (Tuncman et al., 2006). These studies indicate that JNK1 and JNK2 are both involved in metabolic disorders such as obesity, and that JNK1 is able to compensate a loss of function of JNK2. Among metabolic alterations known in obesity, abnormal high levels of circulating cytokines activate the JNK pathway, which is considered as the molecular link between obesity and metabolic alterations (Solinas and Karin, 2010). For instance, IL-6 and TNF α , two proinflammatory cytokines present at high levels in obese patients (Asrih and Jornayvaz, 2013), have been shown to activate the JNK signaling pathway, leading to the development of insulin resistance (Hotamisligil et al., 1993; Spranger et al., 2003). Altogether, these studies suggest that the JNK pathway plays a major role in the development of MetS in obesity.

In addition to its function in obesity and insulin resistance, JNK has been shown to play a major role in the development of NAFLD (Schattenberg et al., 2006; Singh et al., 2009). For instance, Schattenberg et al., using a methionine- and choline-deficient (MCD) diet, induced the development of steatohepatitis in mice. In this case, the development of NAFLD was driven by an activation of the JNK-cJun-AP1 signaling pathway (Schattenberg et al., 2006). In line with this study and the one showing that *jnk1* plays a more prominent role in the development of insulin resistance than *jnk2*, Singh et al., revealed that *jnk1* but not *jnk2* null mice are protected from high-fat diet-induced steatosis and liver injury (Singh et al., 2009). Together, these data reveal that JNK1 plays a major role in the development of insulin resistance and NAFLD, but the exact mechanisms remain unclear. Moreover, JNK2 is likely involved in the development of NAFLD, although its exact function is still unresolved.

As previously discussed, different kinases including JNK-cJun could be activated by cytokines as well as by ER stress. This stress arises from an impaired synthesis capacity of the ER, which activates the unfolded protein response (UPR) (Xu et al., 2005). ER stress has been associated with obesity and was found to develop in liver and adipose tissue in genetic and diet-induced obese rodent models (Ozcan et al., 2004). In the latter study, the authors focused on a specific transcription factor named X-Box binding protein 1 (XBP1) known to modulate the ER stress response. They demonstrated that when deleted in mice, this leads to the development of insulin resistance through an activation of the JNK-cJun signaling

pathway (Ozcan et al., 2004), suggesting a role of XBP1 in the development of insulin resistance. Additionally, mice with liver-specific deletion of XBP1 have improved hepatosteatosis, liver damage, and hypercholesterolemia (So et al., 2012). Because these mice displayed lower plasma triglycerides compared to their wild-type littermates, insulin sensitivity was expected to be improved. However, insulin resistance was not investigated in this study (So et al., 2012). In contrast, Jurczak et al., challenged XBP1 liver depleted mice with a fructose diet to assess the respective roles of ER stress and liver lipids in the development of hepatic insulin resistance (Jurczak et al., 2012). They found that despite increased ER stress, XBP1 depleted mice showed increased hepatic insulin sensitivity along with decreased lipid accumulation in the liver (Jurczak et al., 2012). Therefore, these studies revealed controversies regarding the role of XBP1 in the development of NAFLD and insulin resistance, warranting further investigations to elucidate its exact functions. Finally, fibroblast growth factor 21 (FGF21), a major regulator of lipid and glucose homeostasis found to be upregulated in patients with obesity and NAFLD, has been shown to be regulated by XBP1 (Jiang et al., 2014). Indeed, increased XBP1 expression, along with the simultaneous elevation of FGF21 expression, were associated with the occurrence of NAFLD and type 2 diabetes in humans (Jiang et al., 2014). Therefore, it will be worthy to understand how XBP1 affects the development of obesity, insulin resistance and NAFLD in humans. Importantly, XBP1 is not the only factor involved in ER stress. Nevertheless, it is a central regulator of ER adaptive responses (Gregor et al., 2013).

4.1.3. Role of gut microbiota in the development of insulin resistance

In addition to ectopic fat accumulation, inflammation and ER stress, recent research findings revealed that gut microbiota plays an important role in the development of obesity and insulin resistance (Lee and Mazmanian, 2010; Ley et al., 2005; Palermo et al., 2014). Indeed, by comparing microbiota from lean and obese patients, Ley and coworkers found that gut microbiota differs in composition (Ley et al., 2006). In this study, the authors quantified *Bacteroidetes* and *Firmicutes* and showed that the relative proportion of *Bacteroidetes*, one of the major beneficial bacteria in human gut, was decreased in obese people compared to lean people (Ley et al., 2006). These results are corroborated by rodent studies revealing an increase of *Firmicutes* and decrease of *Bacteroidetes* in genetic obese mouse models compared to wild-type mice (Ley et al., 2005). However, although these results are promising, they are still a matter of debate (Arumugam et al., 2011; Schwierzt et al., 2010). Controversies about these results could be due to dietary variations in the different regions of the globe.

The beneficial effects of the microbiome in energy balance were further demonstrated in clinical studies (Kootte et al., 2012; Smits et al., 2013; Vrieze et al., 2012). For instance, insulin resistant male subjects with MetS receiving either feces infusion from lean donors exhibited improved peripheral insulin responsiveness (Vrieze et al., 2012). This was associated with increased butyrate-producing bacteria (mainly *Roseburia* and *Eubacterium halii*) in feces. This study was supported by others investigations that observed decreased butyrate-producing bacteria in the gut microbiota of diabetic patients (Karlsson et al., 2013; Qin et al., 2012). Butyrate is a short-chain fatty acids produced by the intestinal bacteria through a fermentation process (Cummings, 1981). Interestingly, oral administration of butyrate to mice improved insulin sensitivity and increased energy expenditure (Gao et al., 2009). Whether butyrate promotes similar effects in human is still under investigation. However, as insulin resistance is associated with NAFLD, one could propose that butyrate may improve NAFLD. Indeed, Mattace Raso and coworkers showed that sodium butyrate

Table 1

Potential treatments for nonalcoholic fatty liver disease (NAFLD).

Treatment	Results of the study	Relevance for NAFLD	Reference
Weight loss, lifestyle changes, adjuvant appetite suppressants	Reduction of waist circumference, systolic and diastolic blood pressure, triglycerides, total cholesterol, LDL-cholesterol, uric acid, fasting insulin, and HOMA index	Could lead to a reduction of hepatic lipid content and improve NAFLD	Park et al., 2004
Orlistat (lipase inhibitor)	Significant decrease in body weight, HbA1c, ALT and AST	A 10% reduction in body weight improved steatosis and fibrosis as well as HbA1c levels in the majority of patients	Harrison et al., 2004
Metformin	Improvements in liver histology and ALT levels in 30% of patients with NASH	Appears to be beneficial for NAFLD patients but not for non-obese patients developing NAFLD	Loomba et al., 2009
Pioglitazone (insulin sensitizing agent)	Improvement in biochemical and histological features of NASH	Could be used as a treatment for NAFLD	Promrat et al., 2004
Pioglitazone (insulin sensitizing agent)	Improvement of insulin resistance but not in hepatic fibrosis and ALT levels	Not adapted to treat NAFLD	Sanyal et al., 2010
Atorvastatin (statin, lipid lowering agent)	74.2% of patients normalized transaminases levels. Increased adiponectin and decreased TNF α levels. Decreased fatty acids. Steatosis and NAFLD were improved. However, 4 patients had progression of fibrosis. Thus, these results require further investigations	May represent a therapeutic approach but further investigations are required	Hyogo et al., 2008
Lifestyle advices and treatment for hypertension (mainly inhibitors of the renin-angiotensin system), impaired fasting glucose (metformin), obesity (orlistat) and dyslipidaemia (atorvastatin, fenofibrate)	At the end of treatment, 67% of patients on atorvastatin, 42% on fenofibrate and 70% on combination treatment had no longer evidence of NAFLD	Represents a multifactorial therapeutic approach	Athysos et al., 2006
PPAR α agonists	Prevented the development of liver fibrosis in mice	Potential to delay progression to NASH in humans	Pawlak et al., 2014
Salsalate (inflammation inhibitor)	Improvement in glucose levels, lipid homeostasis and NAFLD	Because inflammation is part of NAFLD, salsalate may represent a new therapeutic approach	Goldfine et al., 2008; Jung et al., 2013
Etanercept (TNF α inhibitor)	Improves β -cell function	Could be considered as a treatment for NAFLD but studies enclosing larger cohorts for an extended period are required to confirm the results	Dominguez et al., 2005 Campanati et al., 2013
4-Phenyl butyric acid and taurine-conjugated ursodeoxycholic acid (Chaperone proteins)	Normalization of hyperglycemia, restoration of systemic insulin sensitivity, resolution of fatty liver disease, and enhancement of insulin action in liver, muscle, and adipose tissue in diabetic mouse models	As ER stress is involved in NAFLD development, chaperones could represent an interesting option to treat NAFLD in human	Ozcan et al., 2006
FGF21 analogs	Decreased fasting plasma insulin and improved cholesterol panel, decreased body weight (humans). Improved hepatic steatosis (animal models)	Could be considered as a treatment for NAFLD, but requires further investigations in humans	Gaich et al., 2013, Huang et al., 2013
Low-fat or low-carbohydrate low-calorie diet	Increased beneficial gut bacteria such as <i>Bacteroidetes</i> and restored insulin sensitivity	Since insulin resistance is a major risk for NAFLD, these diets may improve NAFLD	Ley et al., 2006 Cotillard et al., 2013; Wu et al., 2011b

Fibroblast growth factor 21 (FGF21), endothelial reticulum stress (ER stress), glycated hemoglobin (HbA1c), alanine and aspartate transaminase (ALT, AST), Low density lipoprotein (LDL) cholesterol.

protected high-fat fed rats from NAFLD ([Mattace Raso et al., 2013](#)). Altogether, these studies reveal a potentially important role of gut microbiota in the development of MetS, NAFLD and insulin resistance.

5. Treatment of MetS and NAFLD

There is no specific therapy for MetS or NAFLD. However, due to their potential common trigger, insulin resistance, some therapies notably studied in NAFLD may be relevant for MetS as well. As MetS is also characterized by other cardiovascular risk factors, it is important to treat separately these associated diseases, such as hypertension and dyslipidemia. To date, there is no established therapy for NAFLD either. Weight loss, physical activity and lifestyle

changes remain the most effective approaches to improve NAFLD ([Asrih and Jornayvaz, 2014](#); [Park et al., 2004](#)). To achieve weight loss, the simplest solution is to change dietary habits. Also, dietary modifications have been shown to be beneficial for gut microbiota ([Wu et al., 2011b](#)). For instance, patients under a low-fat or low-carbohydrate low-calorie diet present an increase in beneficial gut bacteria such as *Bacteroidetes* and a decrease in deleterious bacteria such as *Firmicutes* ([Ley et al., 2006](#)). These data are in accordance with other studies showing that diet-induced weight loss reduces systemic inflammation in obese patients and enriches intestinal microbial diversity ([Cotillard et al., 2013](#); [Wu et al., 2011b](#)). Together, these studies show that dietary interventions stimulate the growth of beneficial gut bacteria, which may in part contribute to reverse NAFLD and hepatic insulin resistance.

Because of the association between MetS and NAFLD, several therapeutic agents used in obesity and insulin resistance have been considered for NAFLD (Table 1). Notably, orlistat, which inhibits pancreatic lipases, prevents fat absorption in the digestive tract, leading to weight loss. Indeed, Harrison and coworkers found that orlistat was effective in reducing body weight, subsequently significantly improving aminotransferases levels and haemoglobin A1C in the majority of patients. These data suggest that orlistat could be used as a therapeutic drug against NAFLD (Harrison et al., 2004).

Most of current pharmacological approaches aimed at treating NAFLD consist in inhibiting either 1) fat absorption; 2) inflammation; 3) the endocannabinoid system; or 4) modulating the central nervous system (Carter et al., 2012). Notably, metformin was shown to improve liver injury, but this medication used in type 2 diabetes could not prevent fibrosis in patients with steatosis (Loomba et al., 2009). Also, glitazones, which are peroxisome proliferator-activated receptor γ (PPAR γ) agonists, were found to be efficient in managing NAFLD, by notably decreasing liver fibrosis (Promrat et al., 2004). In contrast, another study revealed that pioglitazone does not promote beneficial effects on liver fibrosis, although it diminished inflammation and steatosis (Sanyal et al., 2010). Therefore, further studies are required to elucidate these contradictory results.

In addition to PPAR γ agonists, several studies have investigated the role of PPAR α activation in NAFLD since it is involved in lipid and glucose metabolism (Auboeuf et al., 1997; Cariou et al., 2013; Fruchart, 2013; Shiri-Sverdlov et al., 2006). Recently, Pawlak et al., using a pharmacological approach, showed that activation of PPAR α inhibited hepatic inflammation and the transition from steatosis toward NASH through a direct, anti-inflammatory mechanism independent of its lipid handling properties (Pawlak et al., 2014). These findings highlight the potential of novel therapeutic strategies to limit the progression of chronic inflammatory liver diseases initiated by metabolic perturbations.

Atorvastatin, a lipid lowering agent of the statins class, led to improved liver steatosis and NAFLD score (Eslami et al., 2013; Foster et al., 2011; Hyogo et al., 2008). Another study including a larger cohort of patients revealed similar results with atorvastatin treatment (Athysos et al., 2006). Also, salsalate, a potential anti-diabetic drug under development, has been shown to improve glycemia in diabetic patients through a downregulation of the proinflammatory IKK β /NF κ B pathway (Goldfine et al., 2008). Additionally, this agent is likely improving NAFLD through an induction of adiponectin (Jung et al., 2013).

Further pharmacological agents inhibiting inflammatory signaling such as etanercept, which inhibits TNF α , and chaperone proteins, which inhibit ER stress, were used in obese patients with insulin resistance and resulted in metabolic benefits (Dominguez et al., 2005; Ozcan et al., 2006). However, Dominguez and coworkers revealed only benefit on β -cell function with etanercept treatment, suggesting that it may not be a valuable treatment for NAFLD. However, others found that etanercept reduced the risk of developing hepatic fibrosis in patients with MetS (Campanati et al., 2013). In this study, patients with psoriasis, which are at risk of developing NAFLD and MetS probably because of underlying insulin resistance, were treated with etanercept and compared to patients treated with UVA. The group receiving etanercept showed significant reductions in transaminases, CRP and fasting insulin levels, and improved HOMA index, indicating that etanercept may be efficacious in reducing the development of NAFLD and MetS in patients with psoriasis. However, there were some limitations in this study, such as the diagnosis of NAFLD, which was done by ultrasonography (Campanati et al., 2013).

Most Recently, FGF21, a potent metabolic regulator, has been

proposed as a therapeutic agent for obesity and type 2 diabetes. Notably, FGF21 has been shown to reverse hepatic steatosis in animal models of NAFLD and insulin resistance (Camporez et al., 2013b; Xu et al., 2009). In particular, Camporez et al. showed that these improvements were associated with reduced hepatocellular triglyceride and diacylglycerol contents, decreased activation of PKC ϵ in the liver, as well as improved insulin signaling (Camporez et al., 2013b). Interestingly, in humans, FGF21 plasma levels increase in situations of insulin resistance such as obesity, type 2 diabetes and NAFLD (Chavez et al., 2009; Dushay et al., 2010; Li et al., 2010; Zhang et al., 2008). This increase in FGF21 plasma levels may reflect a state of FGF21 resistance. Nevertheless, FGF21 action was investigated in obese human subjects with type 2 diabetes by the use of an analog, LY2405319 (Gaich et al., 2013). In this clinical trial, the FGF21 analog produced significant improvements in dyslipidemia, body weight and fasting plasma insulin decreased, but only at higher concentrations of the analog. Surprisingly, plasma glucose only modestly decreased, and this was not significant. This may be due to the short duration of the trial (28 days), but may also reflect FGF21 resistance (Fisher et al., 2010). Importantly, other FGF21 mimetic molecules are in development (Foltz et al., 2012; Huang et al., 2013; Veniant et al., 2012; Wu et al., 2011a). It would be of interest to investigate whether FGF21 analogs are able to reverse or stop the development of NAFLD in humans as they do in animals. If so, this may lead to a novel therapeutic approach targeting MetS and NAFLD.

6. Conclusions

MetS and NAFLD are always more prevalent in western countries, but are also increasing in emerging countries, making these diseases public health problems. The association between MetS and NAFLD is becoming increasingly clear, although the exact mechanisms linking these diseases remain only partly known. To date, many questions remain without answers. Notably, it is not clear whether MetS precedes NAFLD or if it the opposite. Also, why are some patients with MetS more susceptible to develop NAFLD than others? Are there unknown genetic susceptibilities to unravel? These questions require further investigations. Nevertheless, it appears that hepatic insulin resistance, which is notably due to ectopic lipid accumulation and inflammation, may be a central player in the development of both MetS and NAFLD. Current therapies for these metabolic diseases are currently disappointing and mainly rely on lifestyle measures and weight loss. These interventions may stimulate the growth or activity of beneficial bacterial species such as *Bacteroidetes*, thus modifying gut microbiota, potentially leading to improved hepatic insulin responsiveness and decreased NAFLD. Nevertheless, further research is warranted to better understand the pathophysiology of both MetS and NAFLD in order to better identify potential therapeutic targets in these ever growing diseases.

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