Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance

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Abstract

Nonalcoholic fatty liver disease (NAFLD) has become a major health problem in developed countries. It has affected more than 30% of the general population and is commonly associated with insulin resistance, which is a major risk factor for the development of type 2 diabetes and a central feature of the metabolic syndrome. Furthermore, accumulating evidences reveal that NAFLD as well as insulin resistance is strongly related to inflammation. Cytokines and adipokines play a pivotal role in inflammatory processes. In addition, these inflammatory mediators regulate various functions including metabolic energy balance, inflammation, and immune response. However, their role in modulating ectopic lipids involved in the development of insulin resistance, such as diacylglycerols and ceramides, remains unknown. The aim of this review is first to describe the pathophysiology of insulin resistance in NAFLD. In particular, we discuss the role of ectopic lipid accumulation in the liver. Secondly, we also summarize recent findings emphasizing the role of main inflammatory markers in both NAFLD and insulin resistance and their potential role in modulating hepatic fat content in NAFLD and associated hepatic insulin resistance.

Key Words

- liver
- insulin resistance
- inflammatory diseases
- lipid
- obesity

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major health problem considered to be the most common chronic liver disorder in the Western countries. NAFLD is estimated to affect at least 30% of the general population (Ratziu *et al.* 2010, Younossi *et al.* 2011). It is histologically characterized by hepatic triglyceride (TG) accumulation of more than 5%, resulting in steatosis and hepatic inflammation (Tarantino *et al.* 2010). NAFLD includes a complex spectrum of disorders ranging from simple fatty liver to nonalcoholic steatohepatitis (NASH) and cirrhosis (Adams *et al.* 2005). NAFLD has been first considered to be a benign disease, but it is now being recognized as a leading cause of liver-related mortality and morbidity in the Western

countries. This consideration of NAFLD as a benign disease can be explained by the fact that simple actions such as lifestyle interventions, i.e. dietary changes and physical activity, can reverse simple hepatic steatosis. However, when disease-promoting factors such as eating an unhealthy diet persist, simple steatosis progresses to NASH, which is characterized by inflammation and fibrosis, and can then evolve into cirrhosis and to a lesser extent to hepatocellular carcinoma (Gariani *et al.* 2013). Whether NAFLD should be considered as a progressive disease encompassing different stages, or each stage should be distinguished as a single disease, is still controversial. Importantly, NAFLD is strongly associated

with obesity, insulin resistance, hypertension, and dyslipidemia, suggesting that NAFLD might be considered as the liver manifestation of the metabolic syndrome (Marchesini *et al.* 2001, Adams *et al.* 2005).

The metabolic syndrome is a leading cause of mortality and morbidity in industrialized countries and is characterized by the combination of multiple disorders including insulin resistance, abdominal obesity, dyslipidemia, increased blood pressure, hypercholesterolemia, and a pro-inflammatory state (Eckel *et al.* 2005, Alberti *et al.* 2009). One of the central features of this syndrome is obesity, probably the fastest growing problem in the Western world. Obesity, which is often associated with insulin resistance, represents a chronic low-grade inflammatory state, characterized by elevated circulating levels of cytokines and activation of pro-inflammatory signaling pathways (Shoelson *et al.* 2006). In contrast, others have revealed controversial data showing that obesity is not necessarily associated with an inflammatory state (Apovian *et al.* 2008, Stefan *et al.* 2008). Therefore, it is not surprising that several studies have documented the role of inflammation in the development of insulin resistance. Similarly, others have revealed a link between inflammation and NAFLD. Despite these correlations, it remains unclear whether inflammation could be a common link between insulin resistance and NAFLD. Thus, in this review, we will first update and summarize current knowledge about the cellular mechanisms involved in the development of insulin resistance associated with NAFLD, focusing on the role of ectopic lipid deposition in this process, i.e. deposition of lipids outside of the adipose tissue, such as in the liver or skeletal muscle. As an inflammatory state is frequently associated with NAFLD and insulin resistance, several studies focused on the role of numerous inflammatory mediators in these disorders and have been reviewed elsewhere (Tilg 2010). However, tumor necrosis factor α (TNF α), interleukin 6 (IL6), IL10, and adiponectin are considered to be the major inflammatory mediators found in NAFLD and insulin resistance and will thus be discussed here in more detail. Therefore, we also aim at updating and discussing recent studies describing how these molecules impair insulin responsiveness and concomitantly contribute to NAFLD.

Update on the molecular mechanisms of insulin resistance in NAFLD

The molecular etiology of NAFLD involves multiple genetic and nongenetic mechanisms contributing to the final phenotype (Amarapurkar *et al.* 2007, Das *et al.* 2010).

However, this pathophysiology is far from being completely elucidated. Besides the genetic susceptibility to develop the disease (Gariani *et al.* 2013), it appears that promoting factors notably include i) lipid intermediate accumulation, ii) altered expression of pro-inflammatory cytokines, and iii) mitochondrial dysfunction (Day & James 1998, Diehl *et al.* 2005, Begriche *et al.* 2006).

Under physiological conditions, fatty acids (FAs) are transported to various organs including liver and skeletal muscle; thereafter, FAs undergo either β -oxidation in the mitochondria or are stored as TGs. Hepatic TG storage mainly originates from lipolysis of TGs released from white adipose tissue (Donnelly *et al.* 2005). The rest of the lipid stores derive from dietary FAs and *de novo* lipogenesis. Imbalances between these pathways lead to excessive FA flux and accumulation, which not only induces hepatic (and skeletal muscle) insulin resistance but also impairs insulin responsiveness at the level of the whole organism (Svedberg *et al.* 1991, Wiesenthal *et al.* 1999, Carpentier *et al.* 2000, Balent *et al.* 2002). This altered lipid metabolism is believed to be a central mechanism in the development of insulin resistance. Most of the adverse effects induced by FA accumulation are likely to be mediated by lipid intermediates, notably diacylglycerols (DAGs) and ceramides (Jornayvaz & Shulman 2012). Lipid intermediates can induce insulin resistance by activating different kinases such as mammalian target of rapamycin (mTOR), inhibitor of κ B kinase (IKK), Jun N-terminal kinase (JNK), and novel protein kinase C (nPKC) that are known to exert negative feedback on proximal insulin signaling (Fig. 1; Petersen & Shulman 2006, Schenk *et al.* 2008). Together, this contributes to the insulin-resistant state frequently encountered in patients with NAFLD (Bugianesi *et al.* 2005a, Kotronen *et al.* 2008).

Recently, it has been observed that under hyperinsulinemic conditions, hepatocytes present a paradoxical response to insulin stimulation. Indeed, although insulin failed to inhibit FOXO protein, a master regulator of gluconeogenesis, it is still able to promote *de novo* lipogenesis (Brown & Goldstein 2008). A potential mechanism to explain this situation is that insulin activates sterol regulatory element-binding protein-1c (SREBP1c), a master regulatory transcription factor in lipid synthesis, through stimulation of the mTOR complex 1, which results in increased lipogenesis (Laplane & Sabatini 2010, Yecies *et al.* 2011). Therefore, insulin resistance characterized by a hyperinsulinemic state as observed in patients with NAFLD increases *de novo* lipogenesis, which further exacerbates hepatic lipid deposition and accelerates the development of the disease.

Figure 1

Schematic representation of cellular pathways involved in the development of nonalcoholic fatty liver disease. Under physiological conditions fatty acids (FA) enter the cell and are either oxidized or stored. When the influx/outflow ratio is altered, FA accumulate, leading to hepatic steatosis, inflammation, altered mitochondrial function and increased lipid intermediates [LPA: lysophosphatidic acids; PA: phosphatidic acid, DAG: diacylglycerol and TG: triglycerides]. In turn, these lipid intermediates activate various kinases [mammalian target of rapamycin (mTOR), inhibitor

of kappa-B kinase (IKK), Jun N-terminal kinase (JNK), and novel protein kinase C (PKC)] that inhibit proximal insulin signaling, mainly through insulin receptor substrate (IRS) inhibition. Increased insulinemia stimulates *de novo* lipogenesis via activation of sterol regulatory element-binding protein-1c (SREBP1c), hence exacerbating steatosis. In addition, under insulin resistant conditions, insulin fails to inhibit FOXO pathway that promote gluconeogenesis. Altogether, these intracellular pathways finally promote NAFLD and insulin resistance.

However, it is important to emphasize that TG pool derived from *de novo* lipogenesis only accounts for a minor part of hepatic TG accumulation related to NAFLD (Donnelly *et al.* 2005).

Because of the major role of FA in the development of NAFLD, several strategies have targeted lipid synthesis to reduce hepatic steatosis (An *et al.* 2004, Samuel *et al.* 2004, Savage *et al.* 2006). Acyl-coenzyme A:DAG acyltransferase (DGAT) is an enzyme involved in the final step in TG synthesis and has therefore been proposed as a potential target to reduce TG accumulation and hepatic steatosis. Indeed, inhibition of DGAT2 expression by antisense oligonucleotides reduced hepatic steatosis and improved insulin sensitivity in diet-induced obese and leptin-deficient mice with early stage of NAFLD (Yu *et al.* 2005, Choi *et al.* 2007). Surprisingly, DGAT2 inhibition worsened liver injury and fibrosis. This study revealed a protective role for TG accumulation that appears to buffer the harmful effects of FA-induced lipotoxicity (Choi & Diehl 2008).

As mentioned earlier, FA influx to the liver leads to hepatic steatosis but also induces insulin resistance through the accumulation of toxic lipid intermediates, mostly DAG and ceramides. One hypothesis suggests that increased levels of DAG promote insulin resistance by interfering with the proximal insulin signaling.

This hypothesis has been confirmed in numerous rodent models of hepatic insulin resistance (Jornayvaz *et al.* 2010, 2011, 2012, Birkenfeld *et al.* 2011a,b, Lee *et al.* 2011, Jurczak *et al.* 2012, Camporez *et al.* 2013, Cantley *et al.* 2013). Surprisingly, an important study by Monetti *et al.* did not confirm the role of DAG in mediating insulin resistance. These authors generated transgenic mice over-expressing DGAT2 in the liver. A decrease in hepatic DAG content with concomitant improved insulin sensitivity could be expected. However, the authors reported increased hepatic DAG content associated with the development of NAFLD. Owing to the key role of DAG in mediating hepatic insulin resistance and the link between NAFLD and insulin resistance (Jornayvaz & Shulman 2012), one may expect worsened insulin sensitivity in this animal model. Paradoxically, the authors found a dissociation of hepatic steatosis and insulin resistance in this model (Monetti *et al.* 2007). In contrast to these results, Jornayvaz *et al.* (2011) found severe hepatic insulin resistance associated with NAFLD concomitant with DAG accumulation using the same animal model. These controversial findings point to different possibilities to explain the discrepancies between these two studies: i) a difference in the diet? ii) a different genetic background? or iii) different methods used to assess insulin sensitivity? Because the critical difference

between these studies was the hyperinsulinemic–euglycemic clamp technique used to assess hepatic and peripheral insulin response, [Jornayvaz & Shulman \(2012\)](#) suggested this as a possible explanation to the discrepancy. Although these findings remain a question of debate, this emphasizes the need for standard operating procedures when performing the gold standard hyperinsulinemic–euglycemic clamp technique in mice ([Ayala *et al.* 2010](#)).

Also, ceramides appear to play a role in the development of insulin resistance and have recently drawn the attention of the scientific community. Ceramides are a family of lipids composed of a sphingosine and FA group. Their production was believed to be mainly dependent on the availability of palmitate, which is the initial substrate. However, recent studies reported that tissue ceramide synthesis is modulated by several factors including hormones, inflammatory molecules, and saturated FAs. Together, these factors can lead to ceramide accumulation and further insulin resistance ([Bikman & Summers 2011](#)). The involved mechanisms are far from being completely eluded; nevertheless, it is proposed that ceramides interfere with insulin signaling through activation of the PKC ζ isoform, which in turn inhibits Akt and leads to increased levels of protein phosphatase 2A, which finally results in mitochondrial dysfunction and endoplasmic reticulum stress ([Gariani *et al.* 2013](#)). In summary, accumulation of ceramides alters a large number of intracellular signaling events, leading to metabolic dysfunctions. Thus, modulating ceramide synthesis could be considered as a potential therapeutic approach to prevent the development of insulin resistance.

Whether TG storage in the liver is protective or not in NAFLD remains an open question. Therefore, further studies are required to clarify this issue. Nonetheless, it appears that accumulation of certain lipid intermediates, such as DAG and ceramides, plays a central role in the development of NAFLD-associated hepatic insulin resistance. As most of fat accumulation in NAFLD is due to the release of FA from white adipose tissue, this highlights the importance of a potential cross talk between white adipose tissue and liver and suggests that white adipose tissue plays an important role in the development of NAFLD and will therefore be discussed in the next section.

Role of white adipose tissue in insulin resistance

Insulin resistance is described as the inability of insulin to stimulate glucose uptake ([Bugianesi *et al.* 2005b](#)). It is now recognized that the molecular etiology of insulin

resistance involves multiple genetic and nongenetic mechanisms contributing to the final phenotype. Growing bodies of evidence suggest a role for white adipose tissue in the development of insulin resistance. It is proposed that insulin resistance is associated with hyperlipidemia, which is due to highly abnormal breakdown of the TG pool.

As discussed earlier, the release of substantial quantities of FA from the white adipose tissue gets accumulated as lipid droplets within different organs including the liver, the myocardium, and the skeletal muscle ([Unger 2001](#)). This pathological accumulation of FA has been associated with lipotoxicity and insulin resistance ([Unger 2002](#)). It appears that insulin resistance is not a direct result of exposure to FA but rather to their lipid-derived metabolites. These metabolites promote the activation of numerous kinases, including the nPKC isoforms (ϵ in the liver and θ in the skeletal muscle), MAPK, ERKs, and c-Jun, S6K, and IKK β ([Kim *et al.* 2001](#), [Boden & Shulman 2002](#), [Park *et al.* 2008](#)). In turn, these kinases phosphorylate the insulin receptor substrate (IRS), which has either positive or negative effects on the insulin pathway ([Tanti & Jager 2009](#)). In line with these results, several studies investigating the mechanisms of insulin resistance have reported that any defects in the IRS gene contribute to insulin resistance, therefore identifying IRS as a major target in this disorder ([Bandyopadhyay *et al.* 2005](#), [Hoehn *et al.* 2008](#)). Nevertheless, there are several limitations to this model: first, it does not take into account the process of translocation of the glucose transporters to the plasma membrane, which is the ultimate and main event of insulin-induced glucose uptake, notably in skeletal muscle. Secondly, defects in the activation of insulin-targeted substrates are not always associated with insulin resistance ([Nadler *et al.* 2001](#)). Thirdly, it has been shown that partial inhibition of IRS does not substantially affect metabolism ([Nadler *et al.* 2001](#), [Cleasby *et al.* 2007](#), [Hoehn *et al.* 2008](#)). Therefore, defects in IRS could be considered as another factor contributing to the development of insulin resistance but not as the main element causing insulin resistance.

In addition, increased FA oxidation has been shown to enhance reactive oxygen species production and oxidative stress. It is believed that oxidative stress impairs the trafficking of the insulin signaling components, hence leading to insulin resistance ([Cleasby *et al.* 2007](#)). Furthermore, it is also argued that the ERK1/2, which belongs to the family of MAPKs, plays a pivotal role in metabolic regulation. Indeed, it has been shown that skeletal muscle samples from insulin-resistant patients have increased ERK activation compared with healthy

patients (Bandyopadhyay *et al.* 2005). In line with these findings, others found that mice deficient in the signaling adapter p62, an ERK inhibitor protein, develop obesity and insulin resistance associated with enhanced basal level of ERK activity (Rodriguez *et al.* 2006).

Moreover, there is also an evidence indicating that white adipose tissue not only stores excessive FAs but also synthesizes and secretes a cohort of active molecules (Boden & Shulman 2002). In fact, it has now become evident that white adipose tissue is capable of secreting hormones as well as cytokines. Indeed, Hotamisligil *et al.* (1993) have shown that adipocytes have immune cell-like properties such as production and secretion of pro-inflammatory cytokines. Others indicated that hormones and cytokines secreted by adipocytes are capable of modulating inflammation as well as glucose and lipid metabolism homeostasis (Wellen & Hotamisligil 2005).

In summary, white adipose tissue not only releases FA that can accumulate in peripheral organs such as the liver, leading to NAFLD and hepatic insulin resistance, but also secretes inflammatory molecules, cytokines, that can further modulate insulin resistance. The role of main cytokines in NAFLD and hepatic insulin resistance will be discussed in the next section.

Role of cytokines in NAFLD and insulin resistance

Chronic excessive caloric intake along with sedentary lifestyle not only promotes insulin resistance development but also leads to obesity. As mentioned earlier, obesity is now recognized as a chronic inflammatory disease that contributes to the development of various pathologies including cardiovascular diseases, type 2 diabetes, and NAFLD (Colicchio *et al.* 2005, Flegal *et al.* 2007, Freedman 2011, Tarantino & Caputi 2011). Whether obesity precedes insulin resistance or *vice versa* is however still a matter of debate. Weight loss is particularly important in improving NAFLD but also glucose metabolism and cardiovascular risk (Musso *et al.* 2012). Notably, in patients with type 2 diabetes and NAFLD, Petersen *et al.* have shown that moderate weight reduction (about 8 kg) was sufficient to improve NAFLD and reverse hepatic insulin resistance (Petersen *et al.* 2005).

Importantly, inflammation is one of the major risk factors associated with obesity and is related to white adipose tissue dysfunction, i.e. functions other than TG storage. Indeed, white adipose tissue was first considered as a passive non-secretory, inert tissue whose exclusive function is to store TGs in excess and to release them back

during caloric restriction in order to meet the energy body needs. However, recent findings unveiled this concept and described white adipose tissue as an active endocrine organ capable of synthesizing and secreting a multitude of hormones, cytokines, chemokines, and enzymes collectively known as adipokines (Halberg *et al.* 2008, Lumeng & Saltiel 2011). The adipokine profile secretion is a dynamic process depending on fat mass status. In fact, pro-inflammatory cytokine expression (e.g. TNF α and IL6) is increased, whereas anti-inflammatory protein expression (e.g. adiponectin and IL10) is decreased during weight gain and therefore fat mass expansion (Hotamisligil *et al.* 1995, Yang *et al.* 2001, Jung *et al.* 2008). Moreover, altered adipokine pattern associated with obesity is also observed in patients with NAFLD (Tilg & Diehl 2000) as well as in patients developing insulin resistance (Odegaard & Chawla 2013). As there is a close link between NAFLD, insulin resistance, and inflammation, it is not surprising that extensive efforts have been taken to understand the role of adipokines in the development of NAFLD and insulin resistance. Thus, in the following section, we will provide an overview of the pathophysiological role of major adipokines (TNF α , IL6, IL10, and adiponectin) in NAFLD and insulin resistance (Table 1).

Tumor necrosis factor α

Almost two decades ago, TNF α was identified as the first inflammatory molecule linking obesity with insulin resistance (Lang *et al.* 1992). TNF α is produced by a variety of cells including adipocytes. Nevertheless, under physiological conditions, TNF α is expressed at low levels. In contrast, TNF α is overexpressed in white adipose tissue of obese rodent models (Xu *et al.* 2002). Furthermore, it was observed that obese individuals with insulin resistance exhibit a higher adipose TNF α mRNA level relative to lean patients (Arner 2003), suggesting that TNF α may play a role in the pathophysiology of insulin resistance. Therefore, several approaches targeting TNF α were developed to treat insulin resistance. For instance, neutralization of TNF α with chimeric antibody increased insulin sensitivity (Hotamisligil *et al.* 1993). Consistently, it was shown that TNF α or TNF α receptor knockout obese mice exhibit improved insulin sensitivity compared with wild-type obese mice (Uysal *et al.* 1997). In contrast to these studies using animal models, injection of an engineered antibody against TNF α in type 2 diabetic patients had no effect on insulin sensitivity (Ofei *et al.* 1996), thus suggesting that insulin resistance is a multifactorial disorder involving some factors that are human specific. Indeed, others evaluating

Table 1 Summary of the most relevant cytokines involved in NAFLD and insulin resistance and their roles in these diseases

Cytokines	References	Findings and role in insulin resistance and NAFLD
TNF α	Xu <i>et al.</i> (2002) Arner (2003) Hotamisligil (2003) Uysal <i>et al.</i> (1997) Meyer & de Groot (2003) Tilg & Diehl (2000) Lesmana <i>et al.</i> (2009) Zhou <i>et al.</i> (2010) Lee <i>et al.</i> (2008)	TNF α is present in white adipose tissue and its production is significantly increased in different rodent obesity models TNF α level correlates with insulin resistance Blocking TNF α with an antibody increases insulin sensitivity Mice lacking TNF α function are protected against obesity-induced insulin resistance TNF α induces cytotoxicity through regulation of ceramide synthesis Increased TNF α level in NAFLD patients Correlation between TNF α and degree of liver fibrosis in NAFLD subjects High prevalence of certain TNF α polymorphisms in patients with NAFLD Pharmacological inhibition of TNF α reduces aminotransferase levels in patients with NAFLD
IL6	Bastard <i>et al.</i> (2002) El-Assal <i>et al.</i> (2004) Wallenius <i>et al.</i> (2002) Wieckowska <i>et al.</i> (2008) Yamaguchi <i>et al.</i> (2010)	IL6 produced by white adipose tissue contributes to insulin resistance observed in obese humans IL6 protects against ethanol-induced liver injury IL6-deficient mice develop mature onset obesity and associated insulin resistance Hepatic IL6 expression correlates with the severity of NAFLD Blocking IL6 prevents liver damage and enhances liver steatosis
IL10	Marra & Bertolani (2009) Cintra <i>et al.</i> (2008)	IL10 ameliorates hepatocytes damage IL10 prevents liver steatosis in mice models of liver disease, although it does not protect against insulin resistance
Adiponectin	Arita <i>et al.</i> (1999) and Hotta <i>et al.</i> (2000) Kumada <i>et al.</i> (2004) and Wolf <i>et al.</i> (2004) Hui <i>et al.</i> (2004)	Positive correlation between adiponectin levels and insulin resistance Adiponectin induces anti-inflammatory effects through IL10 expression Low levels of adiponectin in patients presenting hepatic steatosis

the effect of salsalate, an anti-inflammatory drug, on insulin resistance revealed that several markers of glycemia were lowered in treated subjects compared with controls (Goldfine *et al.* 2010). However, this study presents some limitations. Notably, the levels of relevant markers of inflammation related to insulin resistance (such as IL6 and TNF α) were not reported. As insulin resistance can be considered as a chronic inflammatory disease, it would have been worthy to pursue the study more than 14 weeks. Taken together, these results indicate that treatments using anti-inflammatory molecules to prevent insulin resistance are still debatable and need further research.

Nevertheless, the mechanisms involved in TNF α -induced insulin resistance are not fully understood, although it is believed that TNF α inhibits insulin signaling mainly by affecting the IRS protein (Hotamisligil 2003). First, TNF α binds to a classical cytokine receptor and activates various MAPKs including JNK and p38 MAPK in addition to PKC and IKK activation. These proteins in turn phosphorylate IRS and the insulin receptor, leading to inhibition of insulin signaling (Weickert & Pfeiffer 2006). Secondly, TNF α can activate *de novo* ceramide synthesis, likely via induction of sphingomyelinase that converts sphingomyelin to ceramides (Chatterjee 1993, Meyer & de Groot 2003). In turn, ceramides, concomitantly with DAG, activate different kinases interacting with IRS

phosphorylation, finally inhibiting insulin signaling (Griffin *et al.* 1999, Itani *et al.* 2002, Summers 2006).

In addition to its role in the development of insulin resistance, TNF α is believed to be a key mediator in NAFLD (Tilg & Diehl 2000). In fact, TNF α plasma levels have been shown to positively correlate with the degree of liver fibrosis when assessed by the ultrasound-guided liver biopsy method in patients with advanced stages of NAFLD (Lesmana *et al.* 2009). In line with these results, increased levels of TNF α were observed in patients with steatohepatitis compared with healthy subjects (Hui *et al.* 2004). Moreover, it has been established that certain TNF α polymorphisms increase the susceptibility to NAFLD (Zhou *et al.* 2010). Taken together, these results indicate that TNF α may be an adapted therapeutic target to treat NAFLD. In fact, pharmacological inhibition of TNF α with pentoxifylline in combination with diet and exercise significantly reduced aminotransferases levels in patients with NASH when compared with controls (Lee *et al.* 2008), suggesting that this treatment might prevent the development of NAFLD. However, this approach is very optimistic as NAFLD may develop without any changes in the circulating levels of TNF α (Lucero *et al.* 2011). Therefore, several controversies remain to be clarified regarding the pathological role of TNF α in NAFLD.

In summary, metabolic alterations leading to lipid accumulation promotes local TNF α production (e.g. liver) and kinase activation. In turn, TNF α activates various inflammatory signaling pathways that inhibit insulin signaling, resulting in insulin resistance, which represents an important risk factor for the development of NAFLD. Therefore, TNF α could be proposed as a common mediator of insulin resistance and NAFLD.

Interleukin 6

IL6 is a pleiotropic cytokine involved in the immune response and produced by various cell types including fibroblasts, endothelial cells, monocytes, and adipocytes (Gwechenberger *et al.* 1999). IL6 content in the adipose tissue correlates with insulin resistance assessed by a hyperinsulinemic-euglycemic clamp in obese subjects with and without diabetes (Bastard *et al.* 2002). Although the exact mechanism has not been clearly elucidated, it is suggested that IL6 binds to its receptor and thereby recruits signaling transducer proteins, such as the JAKs/STATs. Thereafter, JAK activation induces STAT phosphorylation, dimerization, and translocation to the nucleus, leading to transcription of several genes, such as the suppressor of cytokine signaling (SOCS), which is known to alter proximal insulin signaling through inhibition of IRS phosphorylation (Ueki *et al.* 2004). Therefore, these studies reveal that high levels of IL6 could contribute to insulin resistance.

As IL6 induces a plethora of effects including insulin resistance and regulation of inflammation, known to be risk factors for NAFLD, it has been proposed as a potential mediator leading to NAFLD (Kishimoto 2010). However, until now, the mechanisms driving IL6-induced NAFLD remain unclear. Preliminary studies found that IL6 plays a protective role in liver fibrosis by promoting hepatocyte proliferation and by protecting against oxidative stress and mitochondrial dysfunction (Cressman *et al.* 1996, El-Assal *et al.* 2004). On the other hand, mice lacking IL6 develop insulin resistance and mature onset obesity (Wallenius *et al.* 2002), which are major risk factors for the development of NAFLD. Furthermore, Wieckowska *et al.* (2008) showed a positive correlation between IL6 hepatic expression and the severity of NAFLD. Thus, although IL6 improves liver injury, it could not be excluded that it may participate to the development of NAFLD. For instance, blocking IL6 in mice presenting a diet-induced NASH prevents liver injury but concomitantly enhances steatosis (Yamaguchi *et al.* 2010). Altogether, these studies provide controversial results for

the role of IL6 in NAFLD and insulin resistance that needs to be further investigated.

Interleukin 10

In contrast to TNF α or IL6, IL10 is an anti-inflammatory cytokine that ameliorates hepatocellular damage (Marra & Bertolani 2009). This cytokine is mainly produced by the liver, which releases it massively after ectopic transplantation (Le Moine *et al.* 1994, Alfrey *et al.* 1995).

Nonetheless, controversies remain on the role of IL10 in high-fat diet-induced liver steatosis and insulin resistance. It was reported that endogenous IL10 does not protect against insulin resistance, although it prevents liver steatosis in mouse models of liver disease (den Boer *et al.* 2006). In this study, glucose and lipid metabolism was analyzed in *Il10*^{-/-} mice and wild-type mice fed a high-fat diet for 6 weeks to induce steatosis and insulin resistance. After 6 weeks on the high-fat diet, no differences in body weight, basal metabolism, or plasma levels of glucose, TGs, or cholesterol were observed between the two groups. However, hepatic TG content was increased by ~55% in *Il10*^{-/-} mice. In addition, no differences were observed in whole-body or hepatic insulin sensitivity between both groups. Therefore, the authors concluded that basal IL10 production protects against hepatic steatosis but does not improve hepatic or whole-body insulin sensitivity during high-fat feeding. Unfortunately, apart from hepatic TG content, which is usually considered inert, lipid intermediates involved in insulin resistance such as DAG and ceramides were not measured. Moreover, in another mouse model of NAFLD, IL10 inhibition using a neutralizing antibody or antisense oligonucleotides aggravated insulin resistance by increasing TNF α and IL6 expression in liver (Cintra *et al.* 2008). Again, the effect of IL10 inhibition on hepatic lipid content, particularly DAG and ceramides, was not assessed.

In summary, current evidence suggests a beneficial role of IL10 in hepatic steatosis, although its effects on insulin resistance remain controversial. Therefore, more studies are needed to assess the potential role of IL10 in decreasing hepatic lipid content by measuring not only hepatic TG content but also lipid intermediates involved in the development of insulin resistance such as DAG and ceramides.

Adiponectin

Adiponectin is an anti-inflammatory mediator secreted mainly by the white adipose tissue. Similar to other

cytokines, its exact function remains uncertain, although it appears to potentially stimulate ceramide catabolism (Holland *et al.* 2011). This in turn protects against apoptosis and may prevent the development of insulin resistance. Supporting this hypothesis, several research groups have found a positive correlation between adiponectin levels and insulin resistance as well as fat mass (Arita *et al.* 1999, Hotta *et al.* 2000).

Several reports revealed that adiponectin promotes its anti-inflammatory effects through the induction of IL10 (Kumada *et al.* 2004, Wolf *et al.* 2004). Furthermore, adiponectin has been shown to inhibit the IKK signaling inflammatory pathway. Altogether, these results suggest that in addition to its role on insulin resistance, adiponectin may be involved in liver diseases associated with insulin resistance and inflammation such as NAFLD. Indeed, Hui *et al.* (2004) found lower adiponectin levels in patients developing steatosis compared with control subjects. However, patients with cirrhotic liver disease (i.e. in the late stage of NAFLD) present increased adiponectin levels (Moschen *et al.* 2012).

Although these results need confirmation and further research, it appears that adiponectin could be a potential therapeutic target to prevent the development of NAFLD and its progression to cirrhosis. However, again, adiponectin levels should be correlated with different hepatic lipid intermediates involved in the development of insulin resistance to better delineate its role as a potential therapeutic target in NAFLD and hepatic insulin resistance.

Interaction between adipokines

In the previous sections, we deliberately decided to present major adipokines, but it is clear that the interaction and cross talk of all these molecules, including less-studied adipokines, should be kept in mind, notably when evaluating these molecules *in vivo*. For instance, it has been shown that adiponectin plasma levels are inversely associated with IL6 and TNF α (Bruun *et al.* 2003, Engeli *et al.* 2003, Kern *et al.* 2003). Together, these results suggest that adiponectin may be inhibited by endogenous cytokines (such as IL6) and this knowledge could be of major importance in targeting these molecules in order to prevent insulin resistance.

Conclusions

White adipose tissue is the main source of ectopic fat accumulation, notably in the liver, leading to NAFLD. NAFLD almost always results in insulin resistance through

the accumulation of toxic lipid intermediates such as DAGs and ceramides, which lead to inhibition of the insulin signaling cascade. This ectopic fat accumulation in the liver and the subsequent hepatic insulin resistance result in inflammation, which is secondary to cytokines release, either locally by the liver or systematically by the white adipose tissue. These cytokines may either exacerbate NAFLD and insulin resistance, such as TNF α or IL6 or try to compensate the situation, such as IL10 and adiponectin. However, their exact role in NAFLD and hepatic insulin resistance remains to be determined. Notably, modulation of lipid intermediates associated with insulin resistance such as DAGs and ceramides by these inflammatory mediators is unknown and warrants further research. In conclusion, understanding the role of cytokines in the modulation of hepatic fat content might provide new therapeutic targets for conditions associated with hepatic insulin resistance, such as NAFLD, obesity, and type 2 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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