Non-alcoholic fatty liver disease and the metabolic syndrome: An update

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INTRODUCTION
Sedentary lifestyles and poor dietary choices are contributing to a weight gain epidemic in westernized societies. Recent epidemiological studies suggest an increased risk of cardiovascular disease (CVD) and type II diabetes in overweight and obese individuals. Unfortunately, incidence of the metabolic syndrome and nonalcoholic fatty liver disease (NAFLD), which can precede the development of CVD and type II diabetes, are also increasing.

The metabolic syndrome, a cluster of metabolic abnormalities with abdominal adiposity and insulin resistance as its central components, affects approximately 25% of the American adult population[3] and is associated with an increased risk of CVD and type 2 diabetes[6]. NAFLD, hepatic steatosis not due to alcohol consumption, is the most common cause of chronic liver disease[13]. It is estimated that about 30% of the general US population has excessive fat accumulation in the liver[14], reaching levels as high as 75%-100% in obese and morbidly obese individuals[3].

The relationship between NAFLD and the metabolic syndrome is becoming increasingly recognized. Approximately 90% of patients with NAFLD have ≥ 1 characteristic feature of metabolic syndrome and about 33% have the complete diagnosis[8], placing NAFLD as the hepatic representation of the metabolic syndrome[5,6]. In addition, presence of the metabolic syndrome predicts higher risk for the development of NAFLD in both men and women[8]. NAFLD encompasses a histological spectrum ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis. The majority of individuals with NAFLD have no symptoms with a normal physical examination; however, about 2%-6% of adult Americans and 20% of those who are obese may develop steatosis with inflammation (NASH), advanced fibrosis, and cirrhosis[9]. Furthermore, there appears to be a close link between the metabolic syndrome, low grade inflammation, and oxidative stress[10].

In this editorial, we will review the clinical links between the components of the metabolic syndrome with...
the manifestations of NAFLD and the progression to nonalcoholic steatohepatitis (NASH). In addition, we will discuss the potential role of adipocytokines and oxidative stress in the exacerbation of these conditions. Further, we will briefly discuss commonly prescribed therapeutic treatments.

DIAGNOSIS AND PREVALENCE OF THE METABOLIC SYNDROME

Although an association between different metabolic abnormalities had been noted for several years, the metabolic syndrome was first publicly described in 1988 by Reaven[11]. Then called Syndrome X, the metabolic syndrome consisted of a cluster of metabolic abnormalities, including obesity (especially abdominal obesity), insulin resistance, impaired glucose metabolism, dyslipidemia, and elevated blood pressure[11]. The current definition of the metabolic syndrome varies depending on the position of different regulating bodies[12], and it is not within the scope of this review to delineate the best available definition.

The metabolic syndrome is estimated to affect about 25% of the American adult population[1], and the prevalence will continue to increase dramatically. Regardless of the precise definition, the hallmark features of the metabolic syndrome are impaired glucose tolerance/insulin resistance, hypertension, central adiposity, and dyslipidemia consisting of low-density lipoprotein-cholesterol (HDL-C) and elevated plasma triglycerides (TG). It is largely believed that insulin resistance is the central feature in the development of the metabolic syndrome.

DIAGNOSIS AND PREVALENCE OF NAFLD

It is estimated that about 30% of the US adult population has NAFLD, approximately 16%-20% of non-obese individuals and as high as 76% and 100% prevalence in the obese and morbidly obese individuals, respectively[4]. Overall, NAFLD is estimated to affect over 90 million Americans. NAFLD is characterized by increased accumulation of triglycerides (TG) in hepatocytes (hepatic steatosis). The diagnosis of NAFLD requires that hepatic steatosis be ≥5% by weight in the absence of excess alcohol consumption (>20 g/d)[13]. Most people with NAFLD, especially those with fatty liver but no inflammation, experience little to no problems from the condition. The diagnosis usually is first suspected in an overweight or obese person who is found to have mild elevations in specific liver enzymes measured in circulation including elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), with ALT levels being greater than AST levels.

Currently, the liver biopsy is considered the “gold standard” for the direct measurement of hepatic fat and is the only reliable method for diagnosing fatty liver and/or NASH. However, the liver biopsy has limited applicability in epidemiological and clinical studies and is not routinely performed because of the invasiveness of the procedure. Several non-invasive methods are currently available, which include ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and 1H-magnetic resonance spectroscopy (1H-MRS). Each has its limitations and is only semi-quantitative, but is more easily employed for routine screening.

LINKING THE METABOLIC SYNDROME AND NAFLD

Hyperinsulinemia and insulin resistance play a major role in the pathogenesis of NAFLD[14]. Because insulin resistance is the key component of the metabolic syndrome (often referred to as the insulin resistance syndrome or pre-diabetes) and is also commonly found with obesity, and because these same factors are commonly associated with NAFLD, there is a plethora of accumulating evidence supporting an association between obesity, the metabolic syndrome, and NAFLD[6,7]. In fact, NAFLD is now considered to be the hepatic representation of the metabolic syndrome[8,15].

The metabolic syndrome, in part through glucose intolerance and insulin resistance, is strongly associated with steatosis, fibrosis, and cirrhosis of the liver in severely obese adults[16]. In addition, central fat distribution, fatty liver, and glucose intolerance are noted in mildly obese and in normal weight subjects[17,18]. Further, numerous studies have demonstrated that obesity, type 2 diabetes, dyslipidemia, hypertension, and insulin resistance are strongly associated with NAFLD[18-22]. NAFLD also is strongly associated with hepatic adipose tissue, and whole body reductions in insulin sensitivity, increased rate of gluconeogenesis, impaired insulin response to suppress gluconeogenesis, and impaired fatty acid oxidation[23-25]. However, the question about whether hepatic insulin resistance is a cause or a consequence of hepatic steatosis is unresolved[26-28].

The metabolic syndrome predicts higher risk of NAFLD in men and women, independent of weight gain[8], and those individuals with the metabolic syndrome are less likely to experience regression of NAFLD[9]. In addition to insulin resistance, body mass index and waist circumference, indices of obesity and central adiposity, also are associated with the metabolic syndrome, insulin resistance, fibrosis, and steatohepatitis[29], and both liver fat and visceral adipose tissue stores are associated with metabolic risk[9]. Moreover, HDL-C concentrations are significantly reduced[30] and serum TG and cholesterol levels are elevated with NAFLD[31,32]. Collectively, it appears that insulin resistance is not the only contributing factor in the development of NAFLD, but rather, a combination of multiple stimuli.

IS THE METABOLIC SYNDROME MEDIATING THE PROGRESSION OF NAFLD?

Physical inactivity and poor nutritional choices are leading to a surge in obesity, insulin resistance, and metabolic syndrome in industrialized nations. Day and James[31] proposed the “two-hit hypothesis” to explain the presence of bland steatosis and the progression to inflammation...
(NASH), fibrosis, and cirrhosis. Recent findings suggest that components of the metabolic syndrome are integrally involved in the pathogenesis of this two-hit model.

The first “hit” is hepatic steatosis, NAFLD in its simplest form, develops from an imbalance in triglyceride formation vs turnover. We and others have shown that mitochondrial dysfunction (i.e. impaired mitochondrial β-oxidation) is a primary cause of hepatic steatosis (discussed in detail by Wei et al in this issue)\cite{21,22,32}. Insulin resistance is a near essential requirement and is believed to influence the first “hit” of NAFLD in the following ways: (1) activate the secretion of harmful adipocytokines from adipocytes, (2) alter rates of hepatocyte TG synthesis and transport, (3) and finally increases lipolysis [hydrolysis of TG to free fatty acids (FFA)] rates in central adipose which are dumped into the portal vein where they are processed in the liver, exposing the liver to excessive FFA levels\cite{23}. In addition to impaired mitochondrial function and conditions exacerbated by insulin resistance, elevated hepatic \textit{de novo} lipogenesis and TG synthesis strongly contributes to hepatic steatosis. In fact, a recent study found that about 26% of hepatic TG accumulation in NAFLD patients could be accounted for by \textit{de novo} lipogenesis\cite{34}.

Once hepatic steatosis is present, other factors, such as inflammation and oxidative stress, are thought to promote progression to NASH, fibrosis, and necrosis. The metabolic syndrome is linked to inflammation and oxidative stress\cite{10}, and it has been demonstrated that individuals with the metabolic syndrome have increased lipid peroxidation\cite{35}. The second “hit” likely is comprised of a secondary insult, particularly by adipocytokines and reactive oxygen species, which activate stellate cells and increase fibrogenesis and lipid peroxidation. Hepatic inflammation and fibrosis are associated with the presence and severity of the metabolic syndrome\cite{36}. Furthermore, the presence of steatohepatitis with fibrosis, confirmed with liver biopsy, is associated with increased waist circumference and body mass index\cite{29}. Hyperglycemia and hypertriglyceridemia also are associated with NASH\cite{37}, and insulin resistance is more severe in individuals with NASH vs simple fatty liver\cite{38}. If the insults are great enough and the patient develops cirrhosis, about 33% of these patients will die or develop morbid conditions\cite{39}.

### The Metabolic Syndrome and Common Liver Markers

Circulating concentrations of the liver transaminases, ALT, AST, and to less extent γ-glutamyltransferase (GGT) are commonly used as markers of NAFLD for many years. Elevated levels are considered a consequence of liver damage due to fatty acid infiltration and inflammatory stimuli, and recent findings indicate that serum levels of these enzymes are associated with multiple components of the metabolic syndrome.

Increases in ALT are positively associated with each component of the metabolic syndrome, increased TG, glucose, waist circumference, diastolic blood pressure, and reduced HDL-C levels\cite{19}. In addition, insulin resistance, the prevalence of severe liver steatosis, and ALT values have been found to be significantly higher in subjects with the metabolic syndrome compared to those with less than three of the five clinical features considered for its diagnosis\cite{39}. Recent data published from NHANES III found significant association with increased ALT and insulin resistance, type II diabetes, and the metabolic syndrome\cite{40}. GGT, a less specific marker of liver function, is linked to obesity, hypertension, sedentary lifestyle, hyperinsulinemia, dyslipidemia, inflammation, and oxidative stress\cite{41-45}. Furthermore, GGT concentrations have been found to be associated with hypertension in individuals with central adiposity\cite{46}, suggesting the potential for a pathogenic link among fatty liver disease, endothelial dysfunction, and cardiovascular risk.

### Role of Adipose Tissue, Free Fatty Acids, and Adipocytokines

#### Adipose tissue

Adipose tissue is now recognized as not simply a storage depot for excess energy, but rather, an active endocrine organ that secretes a number of molecules termed adipocytokines. A number of these adipocytokines have been linked to alterations in insulin sensitivity, including adiponectin, leptin, resistin, and tumor necrosis factor-α (TNF-α). In addition, many of the same signaling molecules have been shown to be associated with suppressed hepatic insulin sensitivity\cite{47,48}, and it is thought that adipocytokines may contribute to the development of liver fibrosis.

Visceral adipose tissue is more biologically active than subcutaneous adipose tissue and is known to release greater quantities of adipocytokines. Visceral adipose tissue is a better predictor of altered liver function and insulin resistance than obesity defined by body mass index\cite{49,50}. FFAs lipolyzed from visceral adipocytes are dumped directly into the portal vein where they circulate through the liver and with suppressed oxidative capacity seen in insulin resistance will overload hepatocytes with lipid and promote hepatic TG storage.

#### Free fatty acids

The liver represents a major site of glucose uptake and storage and the major site for insulin clearance. Under normal insulin sensitive conditions, insulin inhibits glycogenolysis and gluconeogenesis by the liver, suppressing glucose production. However, both the metabolic syndrome and NAFLD commonly lead to hepatic insulin resistance in which the most common feature is the inability of insulin to cease hepatic glucose production. Boden \textit{et al}\cite{51} demonstrated that hepatic insulin resistance could be induced in rats by infusing lipids during a euglycemic-hyperinsulenic clamp, in part by elevating hepatic diacylglycerol concentrations and increasing the activation of protein kinase C, IKsB, and NF-κB. Moreover, free fatty acids (FFA) and lipid intermediate flux from adipocyte lipolysis or from excess dietary intake activate protein kinase C and phosphorylate serine residues on the insulin receptor and insulin receptor...
substrates, impairing tyrosine phosphorylation and increasing hepatic insulin resistance which likely lead to elevated glucose production and persistent hyperglycemia.

The liver also responds to elevated FFA by increasing cholesteryl ester synthesis, production of very low density lipoproteins[52], and de novo TG synthesis, exacerbating dyslipidemia. In addition, elevated FFA also have toxic effects on the liver due to lipid peroxidation, resulting in fibrogenesis and progression to cirrhosis[53]. Furthermore, lipid-laden hepatocytes also can swell and this architectural distortion can influence hepatocyte health[54].

Leptin
The role of leptin and leptin resistance in NAFLD has been under recent investigation. Under normal, healthy conditions, leptin is thought to play a role in regulation of body weight. However, in human obesity, leptin concentrations are elevated but there is reduced responsiveness to available leptin presumably from reduced leptin receptor expression, resulting in leptin resistance either centrally or locally at the level of the liver. Leptin is thought to play a major role in hepatic TG accumulation[55], and hepatic steatosis is observed in ob/ob and db/db mice that have leptin and leptin receptor mutations, respectively. Elevated leptin levels are associated with increased serum ALT levels and could be involved in promoting hepatocellular injury[60]. However, leptin therapy has been shown to reverse insulin resistance and hepatic steatosis in individuals with lipodystrophy[57]. In addition, existing data from animal studies also support the role of leptin replacement in reducing TG synthesis and inducing β-oxidation[58]. Future studies should focus on leptin receptor expression and the bioavailability of leptin and associations with NAFLD and the metabolic syndrome.

Resistin
Resistin has recently been identified as a signaling molecule that is induced during adipogenesis and secreted by adipocytes, particularly visceral adipose tissue stores[59,60]. Resistin was identified on the basis of an adipocyte specific protein that played a role in insulin resistance. Little attention has been given to the role of resistin in the metabolic syndrome and NAFLD, but available findings targeting resistin appear promising. Resistin levels are increased in NAFLD patients and levels are related to the histological severity of the disease[60]. Resistin gene expression and protein secretion are markedly reduced by anti-diabetic thiazolidinediones (TZDs), and administration of blood glucose and insulin action in mice with diet-induced obesity[61]. In addition, weight loss interventions in humans have demonstrated significant reductions in circulating concentrations of resistin[61].

TNFα
Adipose tissue represents a site for significant macrophage accumulation, the major source of local TNFα expression[61]. TNFα is an adipocytokine with a well-known role in antagonizing the effects of adiponectin and contributing to insulin resistance[62]; however, recent observations suggest that it also is involved in the metabolic syndrome and progression of NAFLD. In particular, TNFα has been shown to play an important role in mouse models of obesity and insulin resistance, and there also appears to be a link between TNFα levels and liver mitochondrial dysfunction that contributes to NAFLD and NASH. TNFα is elevated in individuals with central adiposity and other components of the metabolic syndrome[58]. It is thought that overproduction of TNFα in liver plays a pathogenic role in NAFLD, as TNF mRNA and TNF receptor 1 mRNA levels are increased in patients with NASH[63]. Furthermore, TNFα alters apolipoprotein metabolism by suppressing apoE secretion and apoA1 expression in HepG2 cells[64] and rapidly down-regulates the anti-oxidative protein associated with high-density lipoprotein, paraoxonase-1 (PON1)[64].

Adiponectin
Adiponectin is an adipocyte-specific secretory protein that is found in relatively high circulating levels (5-10 mg/L), but decreased concentrations predict the incidence of CVD, the metabolic syndrome, and NAFLD[56,71]. Adiponectin concentrations are decreased in patients with obesity, insulin resistance, type 2 diabetes, and NAFLD[67,72] and correlate negatively with hepatic fat content[28]. In addition, hyperinsulinemia down regulates adiponectin receptor expression[73], reducing overall biological activity. Adiponectin has antilipogenic effects that may protect non-adipocyte tissues like liver and muscle. Adiponectin stimulates mitochondrial β-oxidation by activating AMP-dependent protein kinase (AMPK)[74] and down regulates a key transcription factor in de novo fatty acid synthesis, sterol regulatory element binding protein 1-c (SREBP-1c)[75]. This, in turn, reduces malonyl-CoA levels and the inhibition on carnitine palmitoyl transferase-1, leading to increased fatty acid oxidation and reduced hepatic TG accumulation[76].

Adiponectin also displays anti-oxidative/inflammatory properties as it may antagonize the effects of inflammatory mediators like TNFα and attenuate the progression of NAFLD by reducing hepatic stellate cell proliferation and increases apoptosis[77]. Furthermore, administrations of recombinant adiponectin in ob/ob mice, a model of NASH, attenuates serum ALT levels and drastically reduces hepatic steatosis[78].

Regardless whether adipocytokines are merely markers of the presence of NAFLD or are playing pivotal roles in the pathogenesis of the disease, existing evidence supports further study of adipocytokines as possible therapeutics for human NAFLD. It appears that alterations in adipocytokines secretion are exacerbating insulin resistance in skeletal muscle and liver and promoting hepatic steatosis and the development of NASH.

THERAPIES OF NAFLD AND THE METABOLIC SYNDROME
With no proven treatment for NAFLD/NASH, the focus of previous investigations has been on the treatment of components of the metabolic syndrome (obesity, hypertension, dyslipidemia, and diabetes). This strategy has
been met with some success and available therapies appear to slow NAFLD development and progression.

**Pharmacological interventions**
The strong relationship between insulin resistance and NAFLD suggests that insulin sensitizing therapies (TZDs and metformin) might be beneficial in the prevention or improvement in NAFLD. TZDs and metformin are oral glucose-lowering medications used to treat type 2 diabetes that enhance insulin sensitivity and signaling. TZDs bind to the peroxisome proliferator-activated receptors (PPARs) and improve insulin sensitivity, in part, by facilitating enhanced TG storage by adipocytes, suppressing the ectopic storage of lipids into liver and skeletal muscle. In addition, TZDs appear to have anti-inflammatory properties, inhibiting adipocyte gene expression and reduce circulating levels of TNFα and resistin[79], and increase adiponectin concentrations[80]. However, it should be noted that a common side effect of TZDs in clinical trials is weight gain.

Metformin is the drug of choice for treating obesity and type 2 diabetes, and additional findings support its use for treating NAFLD, although the precise mechanism(s) of action are not known. It is known, however, that metformin targets and activates AMPK[81], which has multiple beneficial effects. Administration of metformin reduces hepatic gluconeogenesis, decreases absorption of glucose from the gastrointestinal tract, and increases insulin sensitivity. It also has been shown to reduce hepatic lipogenesis, increase fatty acid oxidation, and reduce serum ALT levels, steatosis, and inflammation in the ob/ob mouse model of NAFLD[82]. Furthermore, metformin use in non-diabetic NAFLD patients has been shown to reduce liver fat by 50% and decrease liver inflammation and necrosis[83].

Lipid lowering agents also can lower risks of the metabolic syndrome and NAFLD. It is well known that statins combat dyslipidemia, a hallmark of the metabolic syndrome, by reducing serum TG and increasing HDL-C levels; however, statins also have been shown recently to improve liver enzymes and hepatic inflammation (reviewed in[84]). Fibrate administration also has been met with marginal success. Furthermore, the angiotensin II receptor antagonist losartan beneficially improved blood markers of hepatic fibrosis and serum ALT levels[85]. Large, randomized control trials are lacking, but future studies are warranted.

**Lifestyle modifications**
Lifestyle modifications targeted at increasing physical activity and reducing energy intake are recommended by health care providers for optimal health and are the most common prescribed therapy for individuals diagnosed with NAFLD. In addition, recent cross-sectional studies in humans provide evidence that increased habitual physical activity[86] and cardiorespiratory fitness[87] are inversely associated with NAFLD. However, there are relatively few prospective studies that have examined the effects of aerobic training on the development and prevention of hepatic steatosis in humans or animal models.

Managing body weight is likely the best overall method in the treatment of conditions in both the metabolic syndrome and NAFLD. Since insulin resistance and obesity play a central role in NAFLD, weight loss is the mainstay of treatment. The weight loss program should be centered on the caloric restriction rather than alteration of dietary contents, with a prescription of both aerobic and resistance training exercise. Initial goals from the patient should be a 10% reduction in body weight by the combination of exercise training and energy restriction. This should be targeted over a 6 mo period, and it is important that individuals continue exercising to maintain the lost weight and for overall cardiometabolic health.

Weight loss by energy restriction significantly reduces hepatic TG content, rates of endogenous glucose production, and increases insulin suppression of endogenous glucose production in type 2 diabetics[88]. Surgical weight loss has been reported to reduce prevalence of the metabolic syndrome, liver steatosis, inflammation, and fibrosis[89]. However, these findings are not universal and it has been suggested that rapid weight loss may in fact, significantly increase hepatic steatosis, presumably from increased FFA flux from elevated rates of lipolysis. Exercise training in combination with energy restriction significantly reduces fatty liver and serum ALT concentrations in obese individuals with NAFLD[90]. Furthermore, weight loss (10%) by diet and exercise significantly elevates adiponectin concentrations[72]. Moreover, we recently have shown that weight loss by diet and exercise significantly reduces circulating leptin concentrations[91] and improves insulin sensitivity and lipid peroxidation in overweight and obese adults with components of the metabolic syndrome[92].

**CONCLUSION**
Most experts consider NAFLD to be the hepatic manifestation of the metabolic syndrome. The progression from hepatic steatosis to steatohepatitis may worsen insulin resistance and dyslipidemia, or the worsening of peripheral factors may mediate alterations in the liver. Regardless of the initiating pathophysiological event(s), recent findings emphasize the important links between NAFLD and the metabolic syndrome. Future investigations targeting the components of the metabolic syndrome as they exacerbate conditions of NAFLD are warranted. Furthermore, weight loss and insulin-modulating pharmacologic agents are the most effective treatment thus far, but combination therapies of lifestyle modifications and pharmacological agents could prove to be more effective than the individual prescription of specific drugs.

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