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THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE IS CLOSELY RELATED TO THE METABOLIC SYNDROME COMPONENTS

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is closely associated with all features of the metabolic syndrome (MS). This strongly supports the notion that NAFLD may be the hepatic manifestation of the MS. NAFLD is currently the most common cause of abnormal liver function tests and affects approximatively 15-25% of the general population. NAFLD covers a spectrum of liver disease, from steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. Insulin resistance (IR) has central etiologic roles in the development of MS and NAFLD, usually related to obesity. MS is frequently associated with chronic inflammation, having as principal mediators the adipocytokines and free fatty acids (FFA), but also CRP, TNF-a and IL-6. Chronic inflammation results in more IR and lipolysis of adipose tissue triglyceride stores, in enhanced hepatic glucose and VLDL production. The steatotic liver is thought to be vulnerable to secondary injuries including adipocytokines, mitochondrial dysfunctions, oxidative stress which lead to hepatocellular inflammation and fibrosis.

key words: *non-alcoholic fatty liver disease, metabolic syndrome, insulin resistance*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is closely associated with abdominal obesity, dyslipidemia, hypertension, insulin resistance (IR) and type 2 diabetes, all features of the metabolic syndrome (MS) [1,2]. This strongly supports the notion that NAFLD may be the hepatic manifestation of the MS and suggests its possible role in the development of atherosclerosis [2]. NAFLD is currently the most common cause of abnormal liver function tests, affecting approximatively

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15-25% of the general population and close to 70-90% of obese or type 2 diabetic patients [1-3].

NAFLD covers a spectrum of diseases that occurs in the absence of significant alcohol consumption; most patients with NAFLD have simple steatosis, only a small proportion of them (approximatively 10%) also have features of liver cell injury or fibrosis – non-alcoholic steatohepatitis (NASH) [4]. The distinction between simple steatosis and NASH is important because the natural history of these categories differs substantially. Patients with simple steatosis usually have a benign prognosis; by contrast, up to 20% of patients with NASH may ultimately develop advanced liver disease and the prognosis of NASH-related cirrhosis is poor, resulting in liver failure or liver-related death in approximately one third of cases [4,5]. Even if only a relative small percent of NAFLD patients develops advanced liver disease, its marked prevalence is alarming [5]. MS is identified by the presence of three or more of the following components: increased waist circumference, with ethnicity specific values, elevated triglycerides (≥150 mg/dl or hypolipemiant treatment), reduced HDL-C level (<40 mg/dl in men, <50 mg/dl in women or drug treatment for reduced HDL-C), hypertension (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or antihypertensive drug treatment) and increased fasting glucose (≥ 100 mg/dl) [6, 49].

Many studies have documented that IR predicts cardiovascular disease (CVD) and plays a pivotal role in the development of poor clinical outcomes in NAFLD patients [7,8]. Additionally, the more advanced forms of NAFLD might act as a stimulus for further increased whole-body IR and dyslipidaemia, leading to accelerated atherosclerosis. This hypothesis is sustained by several studies demonstrating that increased liver enzymes independently predict the development of the MS [9]. Although pathophysiology of NAFLD still has not been completely clarified, IR plays a fundamental role in the pathogenesis of fatty liver [9].

“Classical” Risk Factors

Insulin resistance. IR and compensatory hyperinsulinemia have central etiologic roles in the development of MS and NAFLD, usually related to obesity [6,10]. IR may be defined as a condition in which higher than normal insulin concentrations are needed to achieve normal metabolic responses or normal insulin concentrations fail to achieve a normal metabolic response [6,11]. Fasting serum insulin correlates closely with liver fat content independent of age, gender and body mass index (BMI) [12,13]. Because C-peptide is closely correlated with liver fat content, the association between the fat in the liver and fasting serum insulin is not only attributable to diminished hepatic insulin clearance [11,14]. The fatty liver is resistant to the action of insulin to suppress hepatic glucose production, which results in hyperglycemia and hyperinsulinemia [6,11].

In humans, exogenous insulin therapy decreases liver fat content significantly, suggesting that hyperinsulinemia may be a consequence rather than a cause [14]. However, during insulin therapy, other changes, such as decreases in glucose and free fatty acids (FFA), could decrease the amount of fat in the liver. These data do not exclude the possibility that hyperinsulinemia *per se* could promote liver fat accumulation, but, once the liver is fatty, the ability of insulin to inhibit hepatic glucose production is impaired, leading to an increase in the fasting plasma glucose concentration [11]. This in turn stimulates insulin secretion resulting in mild hyperinsulinemia and lowering of glucose to near-normal levels [12]. Even in non-diabetic individuals a fatty liver has been associated with causes of IR such as obesity and with consequences of IR such as hyper-triglyceridemia and hyperinsulinemia. ALT levels correlate significantly with fasting insulin levels, suggesting that hyper-insulinaemia is an important contributor to the development of fatty liver, more crucial to the pathogenesis than anthropomorphic data, blood glucose or serum lipids [10,12].

In obese persons, insulin binding to its receptor is reduced, in both muscle cells and adipocytes; an increased adipose energy storage generates an increased FFA flux to other tissues and increased ectopic triglyceride storage, which promotes IR. Accumulated visceral adipose tissue produces and secretes a number of adipocytokines, such as TNF-α and IL-6, which induce higher rates of sodium and water resorption at the proximal tubular level and development of hypertension [15,16]. Insulin promotes endothelin-1 (ET-1) production from endothelial cells, which acts on vascular smooth muscle cells, causing vasoconstriction and increased cell proliferation [16]. The MS is frequently associated with chronic inflammation, having as main mediators the adipocytokines and FFA, but also CRP, TNF-a and IL-6. Chronic inflammation results in more IR and lipolysis of adipose tissue triglyceride stores, in enhanced hepatic glucose and VLDL (Very Low Density Lipoproteins) production, increased fibrinogen and plasminogen activator inhibitor-1 (PAI-1) hepatic production, inducing a pro-thrombotic state [6].

Lipids metabolism abnormalities. The dyslipidemia in MS is associated with increased triglycerides, decreased HDL2 cholesterol and increased small dense LDL particles [17]. NAFLD patients have significantly higher plasma markers of lipid peroxidation, inflammation, and endothelial dysfunction than those without NAFLD [18]; hepatic synthesis of apolipoprotein B (apo B) is markedly reduced and postprandial responses are flat and dissociated from the concomitant increases of postprandial triglycerides [1,18]. The liver makes triglyceride rich VLDL particles, each containing one apo-B molecule, with subsequent processing by lipoprotein lipase to remnant lipoprotein particles containing less triglyceride and further processing by hepatic lipase to LDL and then to small and dense LDL particles [19]. In addition, triglyceride-rich VLDL interacts with LDL to exchange triglycerides for cholesterol ester via cholesterol ester transfer protein (CETP) to produce atherogenic small LDL. CETP also mediates an interaction of VLDL with HDL2 to produce less anti-atherogenic HDL3. Insulin-resistant persons and persons with type 2 diabetes, have increased VLDL-cholesterol, a shift in LDL-cholesterol to more dense particles and a decrease in HDL-cholesterol [17,19].

Role of adipose tissue. MS is associated with misdistribution of body fat, increased FFA and IR, leading to type 2 diabetes, hypertension, dyslipidemia, hypercoagulability. The effects of IR are mediated by increased portal FFA, hepatic or muscle fat, decreased adiponectin, and other factors [17,20,21].

Ectopic fat. The role of several ectopic fat depots, including the visceral depot, is that of a "spill-over" site for lipids when the subcutaneous sites become insufficient. But, visceral adipose cells have an increased lipolytic rate and they respond poorly to the anti-lipolytic effect of insulin. There is a high correlation between insulin sensitivity and intra-abdominal fat [20]. Visceral (intra-abdominal) fat is considered both a marker and an inducer of many of the metabolic abnormalities associated with obesity [20]. Abdominal circumference, used as a surrogate marker of visceral fat, is an easily available clinical measurement of both cardiovascular risk and type 2 diabetes risk in obesity, being a good predictor of IR, dyslipidemia, low-grade inflammation [6,8]. Excess of intra-abdominal fat in particular may be a key determinant in the pathogenesis of NAFLD, because of its strong association with IR and possibly as a source of serum FFA [6,21]. IR effects are mediated by increased portal FFA, hepatic or muscle fat, decreased adiponectin, cytokines and chemokines, which may exert local effects (e.g. endothelial dysfunction if located around the vessels), as well as contribute to systemic IR [22]. Obesity with excess visceral fat is associated with reduced adiponectin, whereas obese persons with normal visceral fat have normal adiponectin levels. In addition, the fact that the portal vein drains the visceral fat makes the liver an important target for the released adipokines [17].

Subcutaneous fat. Although most studies have found intra-abdominal fat to be more strongly associated with whole-body IR, subcutaneous fat is also important in NAFLD. Subcutaneous fat, because of its greater overall mass, contributes more than intra-abdominal fat to circulating FFA. The subcutaneous cells are inappropriately large in non-obese but insulin-resistant individuals, which may be an indication of an inability to recruit new pre-adipocytes to store the lipids in the subcutaneous tissue [6]. Induction of inflammation in the subcutaneous adipose tissue in obesity impairs the development of pre-adipocytes to adipocytes [23]. Adipose tissue is not simply a storage depot for excess energy but rather an endocrine organ that secretes adipokines – inducing insulin sensitivity and an anti-inflammatory effect (adiponectin, leptin) or insulin resistance and inflammation (resistin) [24,25].

Causes of Liver Fat Accumulation

The liver plays a central role in lipid metabolism, importing FFA and manufacturing, storing and exporting lipids and lipoproteins. The pathophysiology that leads to NAFLD is not well understood and the factors that lead to progressive hepatocellular damage after triglyceride accumulation are not well elucidated. It appears that alteration of local and systemic factors (particularly IR) that control the balance between the influx or synthesis of hepatic lipids and their export or oxidation lead to hepatic triglyceride accumulation. The steatotic liver is then thought to be vulnerable to secondary insults - a second "hit"- by various factors, including hormones derived from adipose tissue (adipocytokines), oxidative stress and gutderived bacterial endotoxin, which lead to hepatocellular inflammation and fibrosis [26].

Liver fat accumulation results from a disturbance in the balance between supply, formation, consumption and hepatic oxidation or disposal of triglycerides. It is not determined yet whether NAFLD actually causes or is simply a consequence of IR. In fact, it is possible that excessive intra-hepatic triglycerides (IHTG) are both a cause and a manifestation of IR, resulting from a sequence of events initiated by adipose tissue IR [27]: 1) increased release of FFA into the bloodstream and increased FFA delivery to the liver, 2) inadequate hepatic oxidization and/or secretion (as VLDL-triglyceride) of the increased fatty acid load results in fatty acid esterification and IHTG accumulation, 3) hyperinsulinemia and skeletal muscle IR also increase IHTG content by stimulating hepatic *de novo* lipogenesis and hepatic triglyceride synthesis, 4) excessive IHTG release fatty acids into the cytoplasm, which can cause hepatic IR and inflammation and 5) localized intrahepatic inflammation can contribute to peripheral IR [19]. Lipids are normally exported from the liver into VLDL, which are formed by microsomal triglyceride transfer protein (MTP) incorporating triglycerides into apo-B containing particles. A reduction in MTP activity and apo-B synthesis and secretion may also impair hepatic lipid export and favor hepatic triglyceride accumulation [26]. The amount of IHTG in obese persons without diabetes is directly correlated with impaired insulin action in liver, skeletal muscle and adipose tissue, independent of percent of body fat and intra-abdominal adipose tissue (IAAT) volume [27,28]. These results suggest that NAFLD should be considered part of a multi-organ system derangement in insulin sensitivity, and help explain why NAFLD is so closely linked with diabetes and MS and is an important risk factor for coronary heart disease [29,30].

Recent studies favor the concept that the accumulation of intra-hepatic lipids precedes the state of IR, while others have tried to show that hepatic triglycerides themselves are not toxic and may actually protect the liver from lipotoxicity (buffering the accumulation of fatty acids) [31], suggesting that hepatic steatosis is not necessarily associated with IR [32]. But, from what was proven until now, IHTG are correlated with insulin sensitivity in multiple tissues, independent of BMI and percent of body fat. In addition, IHTG content correlated directly with IAAT volume in various populations, including patients with type 2 diabetes [33], non-diabetic overweight and obese adults and helps explain the metabolic inter-relationships often observed between subjects with abdominal obesity and NAFLD [29].

“Non-Classical” Risk Factors

Inflammation and adipocytokines. Obesity is associated with subclinical inflammation and IR, related to macrophage infiltration of adipose tissues. This might be explained by the action of factors produced by adipocytes themselves and by macrophages production of some of the typically inflammatory cytokines such as IL-6, IL-1, and TNF-α, leading to hepatic production of acute-phase reactants including fibrinogen, CRP, lipoprotein(a), PAI-1 [34]. Chronic hyperstimulation by over-nutrition may lead to a state of chronic macrophage activation, chronic increase in cytokines, with central obesity, IR, defective insulin secretion and overproduction of hepatic products of cytokine activation [17,34].

There is an association of NAFLD with impaired fibrinolytic activity and increased CRP and fibrinogen concentrations in non-diabetic individuals, independent of age, BMI, blood pressure, lipids and IR [8, 18]. Although it may not be as predictive of CVD as cholesterol, hypertension, and cigarette use, CRP appears to be the strongest "novel" CV risk factor thus far identified, showing greater predictive power than homocysteine, IL-6, and other inflammatory markers, such as serum amyloid A (SAA) and intracellular adhesion molecule (ICAM)-1. CRP adds prognostic information at all levels of risk based on Framingham score [17,35]. CRP is increased by diabetes, obesity, smoking, chronic infections and chronic inflammatory diseases, while being decreased by lifestyle factors such as exercise and weight loss [35]. Insulin has an anti-inflammatory effect in inhibiting the hepatic response to cytokine stimulation. Insulin secretion does not itself increase CRP, although IR is associated with increased CRP levels and with other inflammatory markers, such as secretory phospholipase A2, E-selectin, and ICAM-1 [35-37]. In the context of IR, obese persons have improvements in insulin sensitivity and CRP levels with weight loss, while obese persons with normal insulin sensitivity, whose CRP level is lower, do not show further decrease following weight loss, suggesting that improvement in insulin sensitivity rather than weight loss mediates the reduction in CRP [17].

Adiponectin is a specific white adipose tissue derived protein, with anti-inflammatory/ antiatherogenic properties such as decreasing the expression of adhesion molecules, monocyte adhesion to endothelial cells, uptake of oxidized LDL, foam cell formation, proliferation and migration of vascular smooth muscle cells, hepatic glucose production and intracellular triglycerides. Adiponectin increases insulin sensitivity, smooth muscle glucose uptake and FFA oxidation. Despite being expressed exclusively by adipocytes, its levels are lower in obese individuals. At any level of adiposity, however, persons with higher levels of adiponectin have greater insulin sensitivity [38,39]. The decrease in adiponectin is associated with the histological severity of NAFLD independent of IR and MS components. Decreased adiponectin concentrations predict the incidence of CVD, type 2 diabetes and MS in large prospective studies, and appear to play a key role in the development and progression of NAFLD [40]. Adiponectin "is a key piece of the puzzle," potentially offering a link between overnutrition and IR, further implying that cytokines are side players rather than direct mediators (low adiponectin levels might explain the effect of CRP, IL-6, TNF-α, phospholipase A2, E-selectin, ICAM-1, and VCAM-1) [38-40]. The beneficial effects of recombinant adiponectin injection on obesity-associated hepatomegaly and fatty liver infiltration in *ob/ob* mice also provide support for the protective role of adiponectin [1,40]. Resistin, an IR promoting adipocytokine, has increased levels in patients with NAFLD and is related to the histological severity of the disease. The major source of resistin is probably from the peripheral blood macrophages and adipocytes, but resistin is expressed at higher levels in intra-abdominal than subcutaneous fat depots in humans [25]. Additionally, the elevation in circulating resistin levels induced by high-fat feeding (in animals) plays an important role in the etiology of hepatic, but not of peripheral, IR [41].

The increased secretion of TNF-α and other proinflammatory cytokines by adipocytes and infiltrating macrophages is thought to lead to chronic systemic inflammation as well as obesity-linked IR [17]. TNF-α stimulates hormone-sensitive lipase and induces uncoupling protein expression, down-regulates insulin-stimulated glucose uptake via effects on GLUT-4 and insulin receptor auto-phosphorylation; it also decreases the production and secretion of leptin by adipocytes [18,22]. TNF-α is increased in obese and diabetic individuals while weight loss decreases its levels. IL-6 promotes IR in both hepatic and adipose cells, has pro-inflammatory properties and causes activation of Kupffer cells resulting in fibrogenesis. In paracrine fashion, it decreases adiponectin secretion from the surrounding adipocytes, inhibits lipoprotein lipase (LPL) on endothelial cells and activates lipolysis. IL-6 seems particularly abundant in the visceral fat [18,22]. IL-6 and TNF-α also determine an increased hepatic expression of suppressors of cytokine signaling (SOCS). Overexpression of SOCS-1 and SOCS-3 in liver further promotes IR and an increase in sterol regulatory element binding protein-1c (SREBP-1c), the key regulator of fatty acid synthesis in liver [24].

Oxidative stress. In the case of NAFLD, IR is thought to be responsible for the “first hit”, with secondary hyperinsulinemia, mobilization of peripheral fat to the liver (peripheral lipolysis and increased hepatic uptake of fatty acids), increased fatty acid synthesis and decreased β-oxidation activity [22], resulting in steatosis. The “first-hit” is “preparing” the liver to progress to more severe liver pathologies when it will be exposed to subsequent metabolic or environmental stressors (dietary components, smoking, pollutants and metabolic stressors - hyperglycemia, hypertriglyceridemia, hyper-cholesterolemia, which are components linked to the MS). Genetic risk factors can also influence the susceptibility and severity of fatty liver disease [42,43].

Once steatosis is present, the liver becomes more susceptible to the “second-hit”; oxidative/nitrosative/nitrative stress and mitochondrial dysfunction are thought to be some of the stimuli for the progression from simple fatty liver to NASH and fibrosis [43]. The molecular mechanism underlying how steatosis predisposes liver to transition to steatohepatitis is not clear, but the hypothesis of a “lipotoxicity” of fatty acids themselves, initiating a pathological response, is evocated. Then, the oxidation and release of reactive lipid species (lipid peroxidation products) present in fat deposits may also contribute to liver injury. Increased reactive lipid species may be a critical factor predisposing liver to more severe injury when exposed to other insults or “hits” [44].

Two critical signaling pathways that increase lipogenesis in the liver are linked to development of steatosis induced by chronic alcohol consumption or type 2 diabetes – AMP kinase inhibition and SREBP-1 activation. AMP kinase inhibits lipogenesis and activates fatty acid oxidation. The inactivation of AMP kinase could result from allosteric modification by AMP and ATP [45];

mitochondrial reactive oxygen and nitrogen species balance may also be a critical controlling factor in the activation of AMP kinase. All these abnormalities may contribute to the progression of fatty liver disease to more severe forms [43,45].

Recent studies reported that obesity induced steatosis is associated with increased expression of uncoupling protein-2 (UCP-2) in liver, which promotes ATP depletion [42]. Decreased activity of all five oxidative phosphorylation complexes was found in liver biopsies from human patients with NASH as compared to normal liver. A discussed hypothesis is that up-regulation of UCP negatively impacts cellular energy conservation only when the availability of oxidizable substrates becomes limited (acute stress like ischemia, partial hepatectomy, lipopolysaccharide exposure) [46]. One other question continues to come up – the mitochondrial dysfunction and the inability to maintain hepatic ATP levels, contributes to, or is simply a consequence of fatty liver disease? The results of a few small studies suggested a NASH dependent bio-energetic defect and that BMI was inversely correlated with ATP recovery even in the healthy lean control subjects. This finding indicates that defects in energy conservation (mitochondrial dysfunction) may occur before NAFLD installation. More studies are nedeed to clarify the impact of obesity on hepatic mitochondrial physiology *in vivo* and to better understand the molecular mechanisms responsible for mitochondrial dysfunction in NAFLD [42,44].

Nitric oxide (NO) and other reactive nitrogen species are increased in response to chronic alcohol consumption, during inflammation and other hepatic pathologies, through induction of inducible nitric oxide synthase (iNOS) due to infiltrating inflammatory cells and via induction in Kupffer cells, hepatocytes, and biliary epithelial cells, whereas in normal healthy liver iNOS levels are very low or undetectable. It is postulated that this disruption in NO signaling in the liver contributes to alcohol and metabolic hepatotoxicity through inhibition of ATP synthesis, increased ROS, and inability to adapt to hypoxic stress [47]. On the other hand, it is also important to highlight the beneficial effects of circulating NO in the context of liver diseases. Decreased production of NO from endothelial NOS (eNOS) contributes to liver pathology via dysregulation of blood flow and oxygen delivery [48]. In support of this, NO-donor administration and eNOS overexpression have been shown to prevent liver injury in animal models of hepatotoxicity and to accelerate the restoration of liver function in transplantation patients [48].

Decreased NO from non-parenchymal (endothelial) cells (eNOS) and increased NO from hepatocyte (iNOS) have cumulative negative effects in chronic alcohol and obesity mediated liver injury. These data support the hypothesis that the source, site, and concentration of NO produced are critical for determining the functional consequence of NO (good or bad NO), but the complex inter-relationships between NO and mitochondria remain to be defined [47,48].

Conclusions

Pathophysiologic considerations, clinical associations, and laboratory investigations support the hypothesis that IR and hyperinsulinemia have a central role in pathogenesis of both MS and NAFLD. In the case of NAFLD, IR is thought to be responsible for the "first hit", with secondary hyperinsulinemia, mobilization of peripheral fat to the liver (peripheral lipolysis and increased hepatic uptake of fatty acids), increased fatty acid synthesis and decreased β-oxidation activity, resulting in steatosis. Once steatosis is present, the liver becomes more susceptible to the "second-hit"; a chronic inflammatory status, adipocytokine actions and further oxidative/nitrosative/nitrative stress and mitochondrial dysfunctions are thought to be the stimuli for the progression from simple fatty liver to NASH and fibrosis, resulting in liver failure or liver-related death in approximately one third of cases. Even if only a relatively small percent of NAFLD patients develops advanced liver disease, its marked prevalence is alarming.

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