Insulin Resistance in Nonalcoholic Fatty Liver Disease

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Abstract: Nonalcoholic fatty liver disease (NAFLD) refers to a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis. NAFLD is considered the hepatic component of the metabolic syndrome and insulin resistance represents its pathophysiological hallmark. Insulin resistance in NAFLD is characterized by reduced whole-body, hepatic, and adipose tissue insulin sensitivity. The mechanism(s) underlying the accumulation of fat in the liver may include excess dietary fat, increased delivery of free fatty acids to the liver, inadequate fatty acid oxidation, and increased de novo lipogenesis. Liver fat is highly correlated with all the components of the metabolic syndrome, independent of obesity, and NAFLD may increase the risk of type 2 diabetes and atherosclerosis. Overproduction of glucose, very low-density lipoproteins, C-reactive protein and coagulation factors by the fatty liver could contribute to the excess risk of cardiovascular disease. The reason(s) why some patients will develop NASH are poorly understood. Circulating free fatty acids may be cytotoxic by inducing lipid peroxidation and hepatocyte apoptosis. Insulin resistance is often associated with chronic low-grade inflammation, and numerous mediators released from immune cells and adipocytes may contribute liver damage and liver disease progression. Understanding the molecular mediators of liver injury would promote the development of mechanism-based therapeutic interventions. This article briefly summarizes the recent advances in our understanding of the relationship between NAFLD/NASH, insulin resistance and the metabolic syndrome.

Keywords: Nonalcoholic fatty liver disease, metabolic syndrome, insulin, insulin resistance, cardiovascular disease.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a wide spectrum of liver damage, ranging from simple fatty liver (pure steatosis), to non-alcoholic steatohepatitis (NASH), characterized by hepatocellular injury/inflammation with or without fibrosis, to cryptogenic cirrhosis, liver failure, and eventually to hepatocellular carcinoma [1]. A central role for insulin resistance (IR) in its pathogenesis is supported by physiopathologic considerations and laboratory investigations, as well as by clinical association. According to several studies, NAFLD is now considered the hepatic manifestation of metabolic syndrome (MetS) [2], associated with IR and its clinical features, namely obesity, type 2 diabetes (T2DM), dyslipidemia and hypertension. The definition of MetS, as proposed in 2001 by the National Cholesterol Education Program, Adult Treatment Panel III (ATPIII), was based on the presence of three out of five very simple criteria [3]; it was later challenged by the definition of the International Diabetes Federation, which considered the presence of low-grade visceral adiposity as an essential feature [4]. Only recently, the 2 classifications have been harmonized and a consensus was reached by several international agencies (Table **1**) [5]. Using the original ATPIII definition, approximately 90% of patients with NAFLD have one or more characteristic features of MetS and about 33% fulfil the diagnostic criteria of MetS, but the prevalence increases with obesity grade and T2DM. The presence of multiple metabolic disorders, such as diabetes mellitus, obesity, dyslipidemia and hypertension, is also associated with a potentially progressive, more severe liver disease [2, 6].

Any attempt to disentangle the relation between NAFLD, IR and MetS should first consider a definition of IR and its quantitative measurement in different organs and on various substrates.

THE MEASUREMENT OF INSULIN RESISTANCE IN RESEARCH AND IN CLINICAL SETTINGS

IR is defined as a condition where normal insulin levels fail to achieve a normal metabolic response, or a condition where higher-than-normal insulin concentrations are needed to achieve a normal metabolic response. This definition does not provide any insight on the type of tissue where insulin activity is measured (muscle, adipose tissue, hepatocytes, etc.) and on the substrate that is tested (glucose, lipids, proteins, etc). Insulin sensitivity/resistance is usually tested on glucose metabolism, but also in this case the ability of insulin to control blood glucose levels by stimulating glucose uptake (in peripheral tissue, mainly skeletal tissue) and suppressing its production (mainly in liver) should be separately defined. In epidemiological studies the homeostasis model assessment of IR (HOMA-IR) or the quantitative insulin sensitivity check index (QUICKI) are largely used because they only require the measurement of fasting insulin (FPI) and glucose (FPG). HOMA-IR [7] is calculated as the product of insulin and glucose concentration (FPIxFPG/22.5) and QUICKI [8] as the log transformation of the product of insulin and glucose [insulin-sensitivity = 1/log(FPIxFPG)]. Both tests have limits, particularly in subjects with overt diabetes and beta-cell failure, but have been validated against the euglycemic hyperinsulinemic clamp technique [9], which remains the gold standard for the measurement of peripheral IR; when coupled with tracer techniques or low-dose insulin infusion, the clamp technique may also provide separate data on glucose uptake and suppression of hepatic glucose output, as well as on the peripheral and hepatic insulin sensitivity.

In clinical practice, indices derived from the oral glucose tolerance test (OGTT) are also commonly used for the measurement of peripheral insulin sensitivity, because they provide a simultaneous assessment of glucose tolerance, IR and beta cell function (from the insulin profile). The oral glucose insulin sensitivity (OGIS) [10] and the Matsuda index [11] have been validated in diabetic and non diabetic subjects against the euglycemic hyperinsulinemic clamp. They are calculated on the basis of glucose and insulin in the 2 hours following the glucose ingestion (75 g). Many other indexes have been developed, including clinical and anthropometric parameters such as age, gender, BMI, but they were either validated only in non-diabetic subjects, or were not superior to the above tests and are scarcely used in clinical practice.

There is no gold standard measurement of the *hepatic IR*, but a hepatic IR index can be obtained during fasting as the product of hepatic glucose output (HGO) and fasting plasma insulin (Hepatic IR index = HGOxFPI). Unfortunately, the measurement of HGO

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Table 1. Definition of the Metabolic Syndrome, According to the Most Recent Classifications

Feature	National Cholesterol Education Program, ATPIII [3]	International Diabetes Federation [4]	Joint Statement of IDF, NHLBI, AHA, WHF, IAS, IASO [5]
Visceral obesity	>102 cm (males), >88 cm (females)	≥94 cm (males), ≥80 cm (females)(ethnic differences)	≥94 cm (males), ≥80 cm (females)(ethnic differences)
Lipid levels	TG ≥150 mg/dL or treated for dyslipidemia	TG ≥150 mg/dL or treated for dyslipidemia	TG ≥150 mg/dL or treated for dyslipidemia
	HDL-Chol <40mg/dL (males); <50 mg/dL (females)	HDL-Chol <40mg/dL (males); <50 mg/dL (females)	HDL-Chol <40mg/dL (males); <50 mg/dL (females)
Arterial pressure	≥130/85 mmHg or treated fot Htx	≥130/85 mmHg or treated for Htx	≥130/85 mmHg or treated for Htx
Blood glucose	≥110 mg/dL or treated for DM	≥100 mg/dL or treated for DM	≥100 mg/dL or treated for DM
Notes	3 of the above	Visceral obesity + 2 of the above	3 of the above

Abbreviations: ATPIII, Adult Treatment Panel-III; IDF, International Diabetes Federation; NHBLI, National Heart, Blood and Lung Institute; AHA, American Heart Association; WHF, World Heart Federation; IAS, International Atherosclerosis Society; IASO, International Association for the Study of Obesity.

can only be obtained by tracer techniques. During the hyperinsulinemic clamp procedure, the hepatic-IR can be evaluated as the residual HGO or the percent suppression of HGO. Under these conditions, the use of different substrates may provide a measure of the effects of insulin on lipids and glycerol.

INSULIN-RESISTANCE, NAFLD AND THE METABOLIC SYNDROME

The relation between NAFLD and MetS is bidirectional; NAFLD is a strong predictor of the MetS; in turn, liver fat content is significantly increased in subjects with the MetS as compared with those without the syndrome, independently of age, gender, and body mass index (BMI) [6]. The presence of MetS has been also associated with a higher risk of prevalent [12] and incident NAFLD in both men and women, independent of weight gain, and the individuals with MetS are less likely to experience regression of NAFLD [13]. Also high serum levels of liver enzymes, namely alanine and aspartate aminotransferases (ALT and AST), commonly used as markers of NAFLD, are associated with the presence of multiple features of MetS and its individual components [14], whereas obesity and the presence of IR are strong predictors of increased ALT activity in NAFLD. Data from the NHANES III study confirmed a significant association between increased ALT and IR, T2DM, and MetS [15].

IR is more severe in individuals with NASH compared with subjects with simple fatty liver [2, 16], and predicts NASH also in subjects with normal liver enzymes [17]. MetS is intimately linked to inflammation and oxidative stress [18]; individuals with MetS have increased lipid peroxidation [19], but hepatic inflammation and fibrosis are associated with the presence and the severity of MetS [20]. When tested by histology, the presence of NASH with fibrosis was associated with increased waist circumference and body mass index [21].

Prospective cohort studies suggest that markers of NAFLD are also associated with future risk of T2DM, independent of other established risk factors. A systematic review and a meta-analysis of 21 prospective, population-based studies in different ethnic groups, found that ultrasonography-diagnosed NAFLD and raised liver enzymes (ALT and GGT) were associated with an increased risk of incident diabetes [22]. However, in selected non-diabetic populations with biopsy-proven NAFLD, progression to T2DM has never been established.

Finally, NAFLD is associated with cardiovascular disease (CVD). Increased GGT predicts incident coronary artery disease, stroke and any cardiovascular disease, independently of ethnic group [23]. ALT levels are less predictive, but this association has been strengthened by more recent data [24]. Also steatosis *per se* is associated with an increased prevalence of CVD [25, 26], as well as incident non-fatal CVD events [27] and CVD mortality [28]. In most cases, the association was found to be independent of classical CVD risk factor and, in a few cases, of the diagnosis of MetS [24, 29]. In biopsy-proven NAFLD, the presence of hepatic fat accumulation has been associated with increased carotid artery intima-media thickness and the presence of carotid plaques [29], with significant carotid atherosclerosis occurring approximately 5-10 years earlier in subjects with NAFLD [30], independently of T2DM, and endothelial dysfunction (endothelium-dependent flow-mediated vasodilation) [31]. In subjects with T2DM, the presence of NAFLD further increases the risk of incident CVD and the presence of diabetes complications [32, 33].

SYSTEMIC EFFECTS OF INSULIN RESISTANCE IN NAFLD

Obesity is certainly the most common factor associated with NAFLD, is the likely cause of IR, and may be considered a clinical surrogate of IR. The adipose tissue contributes to increased free fatty acid (FFA) turnover and IR. This pathophysiological mechanism appears to be specifically operative in the presence of enlarged visceral adipose tissue, as visceral adipocytes are more sensitive to catecholamine-stimulated lipolysis than subcutaneous adipocytes [34]. Because the venous drainage of visceral adipose tissue is directly into the portal system, IR in visceral obesity might be the direct consequence of free fatty acid overflow into the liver (portal theory) [35]. Furthermore, accumulation of visceral fat has also been positively correlated with liver fat [36, 37] and hepatic IR in both men and women [38]. There is however evidence that liver fat may be associated with IR, independent of intra-abdominal fat [36, 39]. Lean non diabetic men with increased liver fat, quantified by magnetic resonance spectroscopy, have both hepatic and adipose tissue IR, expressed by impaired insulin suppression of glucose production and serum FFAs, when compared with subjects well matched for both BMI and intra-abdominal fat, but with low level of hepatic fat [39]. Finally, ectopic fat accumulation, wherever located, may contribute to the pathophysiology of NAFLD *via* excessive release of proinflammatory cytokines, but a subgroup of obese subjects has been identified (obese, metabolically healthy), where obesity is not associated with any of the features of MetS, including IR. Stefan *et al.* [40] found that obese, but insulin-

sensitive subjects have less fat accumulation in the liver and muscle tissues compared with obese insulin-resistant individuals, despite comparable amounts of visceral and subcutaneous abdominal fat in both groups.

Animal studies confirmed that the inflammation of adipose tissue might be pathogenically related to IR [41, 42], but also that hepatic steatosis might be involved in hepatic IR. In ob/ob mice, the specific inhibition of hepatic endoplasmic reticulum stress reduces hepatic triglyceride content and increases IR both in the liver and in peripheral organs, with no effects in the adipose tissue [43].

Whole-body and hepatic IR of NAFLD is considered the common soil responsible for the early atherosclerosis and for the increased prevalence and incidence of CVD, independent of underlying metabolic risk factors. This suggests that NAFLD is not merely a marker of CVD, but may be also an early mediator of atherosclerosis. The possible mechanisms linking NAFLD and accelerated atherosclerosis is complex and remains poorly understood. Fatty liver is strongly associated with an atherogenic dyslipidemia, characterized by large VLDL, small dense LDL, and decreased HDL concentrations in a manner that is partly independent of the contribution of visceral adiposity [44]. This pro-atherogenic phenotype has been described also in obese adolescents with NAFLD [45], which might be at particular risk of future CVD events, because of the long exposure time. Accelerated atherogenesis might be favored by an abnormal lipoprotein metabolism during the post-prandial phase [44, 46]. The magnitude of post-prandial trygliceride and LDL conjugated diene responses after an oral fat load test is much higher in NAFLD patients than in healthy controls, and closely correlates with the severity of liver histopathology [47, 48]. Although there is evidence that IR is a strong underlying mechanism for this dyslipidemia, other studies suggested that fat accumulation in the liver might have an independent effect on dyslipidemia. As an example, plasma insulin was reported to be much higher in subjects with steatosis compared with controls, but the correlation between insulin and serum triglycerides was much weaker than that with hepatic steatosis [49]. However, in this study hepatic IR was not quantitatively measured, and the relation between liver fat, triglycerides and hepatic IR is not settled.

Systemic subclinical inflammation can be estimated by the measurement of circulating C-reactive protein (CRP), which, by itself, is probably involved in the pathogenesis of atherosclerosis [50]. An association has been demonstrated between IR, CRP and other markers of inflammation such as fibrinogen and cellular adhesion molecules in insulin-resistant states [51]. Several studies showed that CRP is positively correlated with liver fat [52, 53], and CRP levels are higher in patients with histologically proven NASH compared with individuals with simple steatosis [54].

Interestingly, hepatic steatosis is also associated with myocardial IR [55], as well as a reduced phosphocreatine/adenosine triphosphate ratio [56], a recognized *in vivo* marker of myocardial energy metabolism. In patients with T2DM, Lautamaki *et al*. reported that liver fat was the strongest predictor of insulin-stimulated myocardial glucose uptake, compared with other determinants such as visceral fat mass and whole-body glucose uptake [55]. It was also inversely associated with myocardial perfusion, which was affected by coronary artery disease [55]. It is still unclear whether fat accumulation in the liver induces myocardial IR *via* humoral mechanism(s) and/or it mainly reflects myocardial steatosis and abnormal cardiac metabolism [57]. Very recently, echocardiographic features of early left ventricular dysfunction [58] and impaired energy metabolism, measured by cardiac ^{31}P-magnetic resonance spectroscopy [56] have been reported in NAFLD patients in the absence of obesity, hypertension and T2DM.

SITES OF INSULIN RESISTANCE IN NAFLD

NAFLD is strongly associated with IR in the liver and in peripheral organs, such as skeletal muscle and adipose tissue (Fig **1**). Peripheral IR is characterized by a reduced glucose uptake from blood into skeletal muscle and by a decreased suppression of lipolysis in the adipose tissue [59]. Conversely, hepatic IR is associated with impairment of glycogenesis, and with an increase of gluconeogenesis and glycogenolysis [59, 60]. It is not clear whether IR causes hepatic steatosis or whether the accumulation of fat in the liver is the primary event leading primarily to hepatic and later to peripheral IR (in skeletal muscle and in adipose tissue). In any case, a close association exists between NAFLD and both hepatic and adipose tissue IR, as well as reduced whole-body insulin sensitivity. We found that glucose disposal, a measure of whole-body insulin sensitivity, was reduced by 45-50% in NAFLD [61, 62], together with an impaired ability of insulin to suppress endogenous glucose production, indicative of hepatic IR [62]. Additionally, subjects with NAFLD exhibited a defect in insulin suppression of FFA, in keeping with IR at adipocyte level. Compared with control subjects, subjects with NAFLD also demonstrated a blunted inhibition of fatty acid oxidation, reflecting the decreased uptake and use of glucose as a source of fuel [61].

However, a few data support a primary role of liver fat in the pathophysiology of skeletal muscle IR. In patients with type 2 diabetes, both the peroxisome proliferator activated receptor-γ (PPARγ) agonist rosiglitazone and metformin increase hepatic insulin sensitivity *via* AMP-activated protein kinase [63], but a decrease in liver fat is only seen in subjects receiving the thiazolidinediones, who also demonstrate a marked increase of insulin sensitivity on glucose disposal [64]. Because skeletal muscle is not a major target of PPARγ action [65], these data suggest that the increase in muscle insulin sensitivity may be mediated or favoured by a decrease in liver fat. In keeping with these data, Hwang *et al* [66] found a very close negative correlation between liver fat content and skeletal muscle insulin sensitivity, possibly mediated by factors released by the liver that regulate insulin sensitivity in skeletal muscle.

In summary, the notion that IR is closely related with liver fat accumulation is well proven, but there is also evidence that IR may promote the progression of simple steatosis to NASH and fibrosis. In NAFLD patients, IR is significantly associated with the severity of fibrosis [67]. Experimental data have highlighted that high glucose levels and hyperinsulinemia cause an up-regulation of connective growth factor [68], and that hyperinsulinemia can both directly induce oxidative stress [69] and stimulate hepatic stellate cells (HSCs) to proliferate and to secrete the components of the extracellular matrix implicated in fibrosis progression [70].

INSULIN RESISTANCE, METABOLIC SYNDROME AND PROGRESSION FROM FATTY LIVER TO NASH

The factor(s) responsible for progression from steatosis to NASH have been extensively investigated, but remain a matter of speculation. The proposed "two-hit" model [71] was the first attempt to provide a patho-physiological rationale to the progression of liver damage, claiming that the reversible intracellular deposition of triacylglycerols ("first hit") could lead to metabolic and molecular alterations sensitizing the liver to a "second hit", usually referred to as oxidative stress and cytokine-induced liver injury. Recent evidence has challenged this theory by demonstrating that the mechanism(s) responsible for IR might also be involved in the pathogenesis of NASH, in both overweight and lean individuals [59, 72]. Severe liver damage has been associated with decreased insulin sensitivity measured by the OGTT-derived OGIS [73] and with the clustering of the clinical and biochemical features of MetS [2]. In diabetic patients, insulin sensitivity measured by the euglycemic clamp is markedly diminished in relation to fatty liver [36] and correlates with the degree of fat infiltration. In 109 NASH patients who underwent a second liver biopsy at least 3 years after the first histological assessment, progression of liver fibrosis was

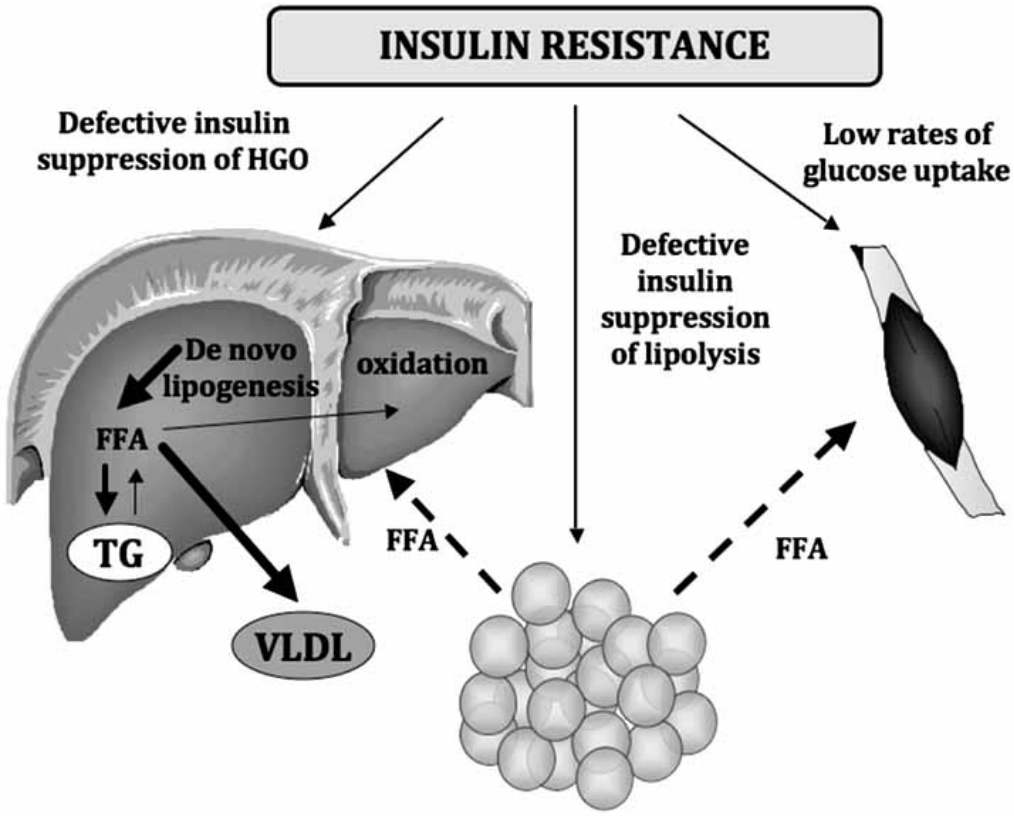

Fig. (1). Metabolic effects of insulin resistance in the liver and in peripheral organs.
Abbreviations: HGO, hepatic glucose output; FFA, free fatty acids; TG, triglycerides; VLDL, very low-density lipoprotein.

found in one third of patients [74], and obesity or high BMI were the only associated variables.

In unravelling the complex relationship between IR and hepatic damage, it is important to consider that insulin also regulates non-metabolic processes such as cell growth and differentiation and that not all insulin-regulated processes and tissues become equally resistant to insulin. Normal insulin signalling is triggered by the binding of insulin to its receptor in the cell membrane, promoting the autophosphorylation of the receptor and the subsequent tyrosine phosphorylation of insulin-receptor substrate (IRS) proteins (namely IRS-1 and IRS-2). This initiates a cascade of events finally leading to translocation of a specific glucose transporter (Glucose Transporter-4) from its intracellular pool to the cell membrane [59].

The most likely mechanism of IR within the muscle cell is a cytokine-induced serine rather than tyrosine phosphorylation of IRS-1 [75]. A similar abnormality of IRS-2 in the hepatocytes is an important mediator of IR within the liver [76, 77]. While glucose uptake is always impaired due to a dramatic decrease in phosphatidyl-inositol-3 kinase activity, activation of Akt and mitogen-activated protein-kinase (MAP-K), which are associated with the proliferative effects of insulin, is normal and stimulated by hyperinsulinemia [78]. Similarly, hyperinsulinemia acting on the liver stimulates *de novo* lipogenesis leading to the increased production of VLDL commonly observed in insulin resistant states.

MECHANISMS OF STEATOHEPATITIS

Available data suggest that the hepatic injury is mediated by oxidative stress and by an imbalance in the relative proportion of pro-inflammatory/anti-inflammatory cytokines, ultimately leading to lipoperoxidation, apoptosis and fibrogenesis (Fig. **2**). The role of steatosis *per se*, once considered the main culprit of the progression to NASH, needs to be reassessed in view of the recent findings that triglycerides represent a non-toxic form of lipid accumulation. In an animal model, the knock-down of diacylglycerol acyltransferase-2 gene decreased hepatic fat content by inhibiting the final step in triglyceride synthesis, but worsened liver injury and fibrosis. Liver disease progression correlated with increased hepatic FFA content, induction of cytochrome P450 2E1, and evidence of oxidative stress and lipid peroxidation [79]. Intra-hepatocellular triglyceride storage might thus represent a mechanism protecting the liver from FFA cytotoxicity, mediated by individual, unknown factors leading to different FFA partitioning. This might explain the poor correlation between the severity of steatosis and liver injury in NAFLD.

Lipotoxic Oxidative Stress

The adipose tissue constitutes a reserve tissue, where energy can be stored in the most concentrated form (i.e. lipids). In other tissues (mainly the liver, muscle and pancreas) lipid accumulation may similarly occur, but fat accumulation is not physiological and produces cellular toxicity (lipotoxicity). Lipotoxicity may ensue as a consequence of dysfunctional fat. In insulin resistant individuals, fat cells tend to be larger and have a diminished capacity to store fat. When the storage capacity of adipocytes is exceeded, the lipids 'overflow' into the muscle tissue, the liver and the β-cells of the pancreas, exacerbating insulin resistance and further impairing insulin secretion. In addition, dysfunctional fat cells produce excessive amounts of insulin resistance-and atherosclerosis-inducing inflammatory cytokines, and fail to secrete the normal amount of insulin-sensitizing cytokines [80]. Lipotoxicity might also be a key factor in the progression to NASH and is generally attributed to the products of oxidative FFA metabolism, i.e., high levels of reactive oxygen species (ROS) and other toxic intermediates [81]. In obesity ROS may also arise from an expanded adipose tissue in subjects with decreased expression of antioxidant genes, contributing to the systemic oxidative stress of insulin resistant states [82].

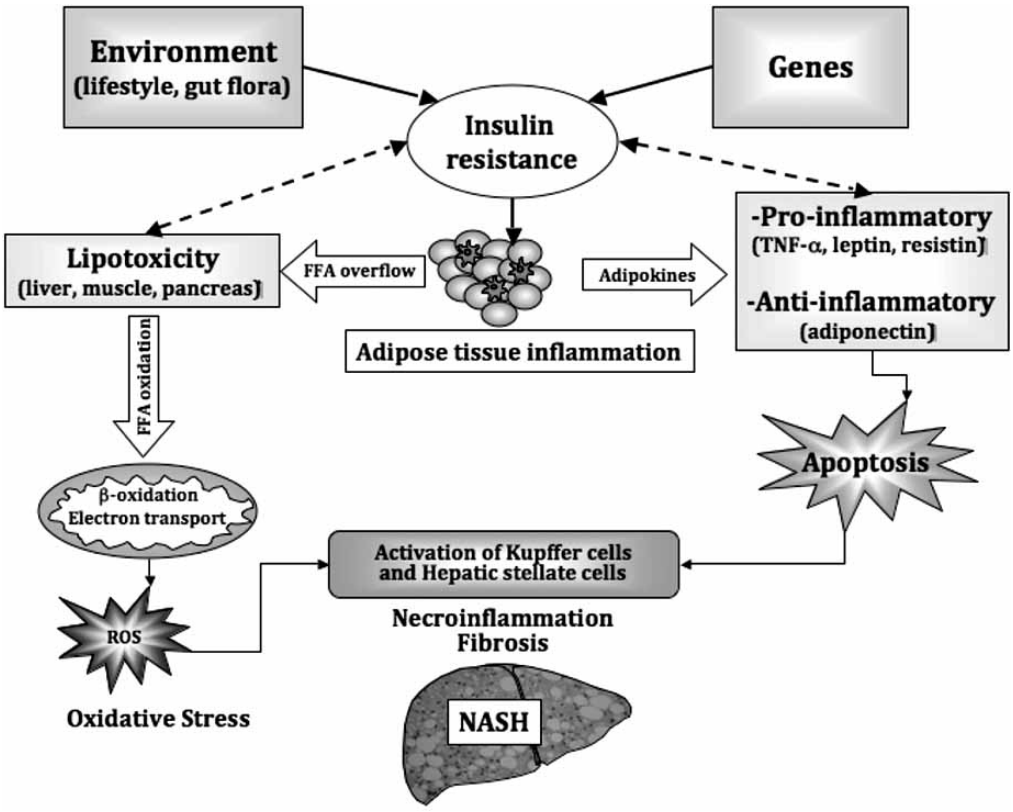

Fig. (2). Pathogenic mechanism linking insulin resistance with NAFLD progression.
Abbreviations: ROS, reactive oxygen species; FFA, free fatty acids.

The intrahepatic pathways of FFA oxidation are all capable of generating ROS. FFAs are mainly oxidized in the mitochondria, but the accumulation of FFAs in the cytosol increases their oxidation also in peroxisomes and in the endoplasmic reticulum (ER). The initial reaction in peroxisomal β oxidation is catalyzed by acyl-CoA oxidase that forms hydrogen peroxides through the donation of electrons to molecular oxygen. Microsomal β oxidation is catalyzed by cytochrome P450 enzymes 2E1, 4A10, and 4A14, which form ROS through flavoprotein-mediated donation of electrons to molecular oxygen. ROS-mediated lipid peroxidation results in the formation of aldehyde by-products such as *trans*-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA). ROS and aldehydes induce oxidative stress and cell death via ATP and nicotinamide adenine dinucleotide depletion, DNA and protein damage, and glutathione depletion. Additionally, they induce inflammation through proinflammatory cytokines, leading to neutrophil chemotaxis, and promote fibrogenesis by activating HSCs, which synthesize collagen and perpetuate the inflammatory response [81].

Both serum markers of oxidative stress [83] and intra-hepatic markers of lipid peroxidation [84] are increased in NASH and correlate with both the histological severity of liver disease and the extent of IR. The peroxidation of intracellular membranes impairs organelle functions and initiates a vicious circle leading to structural abnormalities in the mitochondria. Megamitochondria and para-crystalline inclusion bodies are more frequently observed in NASH than in simple fatty liver [85] and the activity of the mitochondrial respiratory chain complexes are markedly decreased leading to reduced ATP generation [86].

In situations of oxidative stress as well as of metabolic overload, key adaptive functions of ER are activated, collectively termed "the unfolded protein response". These processes serve to overcome stress stimuli; however, when faced with prolonged ER stress, apoptotic pathways are also activated, and ceramides are synthesized from sphingosine and a fatty acid moiety. Ceramides have a variety of metabolic and immuno-modulating functions, that can induce *per se* IR, inflammation, and apoptosis [87], potentially contributing to liver damage [88]. However, their final role in the pathogenesis of NASH is still unclear.

Apoptosis

All the mechanisms discussed above are capable of inducing apoptosis, considered the major mode of cell death in NASH. Briefly, hepatocytes can undergo apoptosis *via* either an extrinsic pathway activated by death ligands, Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or an intrinsic pathway, activated by intracellular stress of membrane-bound organelles, such as lysosomes, ER and mitochondria. FFAs can modulate both the extrinsic and the intrinsic pathways of hepatocyte apoptosis [89].

Compared to patients with simple steatosis, subjects with NASH show higher rates of hepatocyte apoptosis, which correlate with liver injury and fibrosis. In NAFLD, FFAs have been shown to induce the lysosomal permeabilization and the release of the protease cathepsin B secondary to FFA-induced activation of Bcl-2 associated X protein and translocation to lysosomes [89]. In patients with NASH, lysosomal permeabilization and cathepsin B release correlate with the degree of inflammatory activity, and lysosomal permeabilization is associated with the activation of nuclear factor kB (NF-kB) and the generation of tumor necrosis factor-α (TNF-α), increased ROS production and mitochondrial dysfunction [90] (Fig. **3**). Following the activation of the apoptotic cascade, mitochondrial permeabilization leads to activation of caspases 3 and 7 and cleavage of cellular targets, such as cytokeratin 18 fragments (CK-18), whose serum levels are markers of epithelial cell apoptosis. A serologic assay of CK-18 can reliably identify NASH patients among NAFLD cases [91].

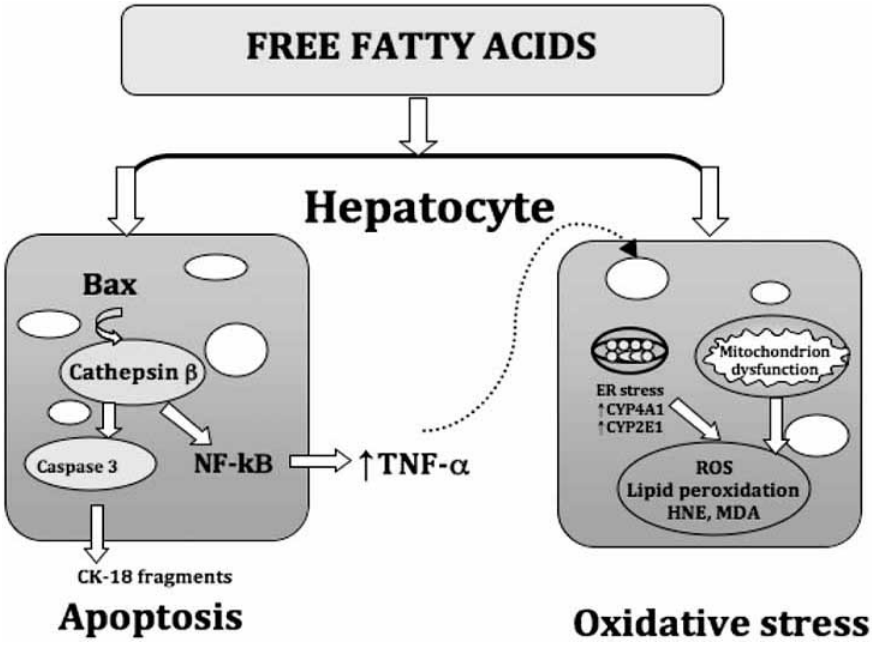

Fig. (3). Toxic effects of free fatty acids within the hepatocytes.
Abbreviations: ROS, reactive oxygen species; Bax, BCL-2 activated protein X; NF-kB, nuclear factor k-B; CK-18, Cytokeratin-18 fragments; ER, endoplasmic reticulum; HNE, *trans*-4-hydroxy-2-nonenal; MDA, malondialdehyde.

Inflammation and Insulin Resistance

Proinflammatory signaling pathways and insulin-mediated control of energy production are tightly interconnected processes. This concept has been initially expressed by Hotamisligil *et al.* [92] and has been explained in the light of mammalian evolution. Innate immunity, which provides the first line of defense against invading pathogens, and insulin action are two of the most primitive, phylogenetically-conserved regulatory systems. Mounting an immune response is an energetically costly process that requires a shift in energy away from anabolic functions [93]. Thus, during a bacterial infection, nutrient storage is inhibited and adipose tissue triglycerides are hydrolyzed and released after inhibition of IRS-1 and -2, through pathways involving c-Jun NH2-terminal kinase and NF-kB [94].

Adipose tissue, liver and immunocytes recognize the same ancestor in the "fat body" of the fruit fly *Drosophila*, the mammalian homologue of the above three tissues in insects. A recent study has demonstrated that the fat body of *Drosophila* adjusts the net energy balance in response to infection by activating the Toll-like receptor (TLR) pathway, a family of pattern recognition receptors, which activates the innate immune system but also inhibits insulin activity thus decreasing triglyceride storage and larval growth [95]. In humans these processes reside in adipocytes and macrophages, respectively, two cell types that display similar gene expression patterns and overlapping functional properties [18]. Although the mechanisms governing inflammatory reactions in the fat loaded adipose tissue are incompletely understood, macrophages appears to be important players in the development of IR and MetS. In addition to releasing large amounts of FFAs, adipocytes produce macrophage accumulation, an increased release of macrophage-derived chemokines (such as TNF-α, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α) and an altered pattern of circulating adipokines (leptin, adiponectin and resistin) [96]. A recent work also suggested that the macrophages migrating into the adipose tissue in response to high-fat feeding are pro-inflammatory and overexpress cytokines, such as TNF-α, differently from anti-inflammatory macrophages that normally reside in this tissue [97]. Weight loss reduced macrophage infiltration and the expression of genes involved in macrophage recruitment.

Gut Microbiota and Insulin Resistance

The gut microbiota is likely to promote liver damage by enhancing hepatic exposure to endotoxins. The possible mechanisms include bacterial overgrowth, the release of the lipopolysaccharides (LPS) constituent of the Gram-negative bacteria and the disrupted integrity of the intestinal barrier resulting in increased endotoxin absorption [98].

Evidence supporting a role for bacterial overgrowth in the pathogenesis of NAFLD has been recently provided by a study showing a higher prevalence of small intestinal bacterial overgrowth (SIBO) and increased intestinal permeability in patients with NAFLD [99]. SIBO and/or intestinal permeability were associated with the extent of steatosis and with an increased prevalence of MetS. The gut microbiota has been shown to affect fat storage and energy balance, playing a direct role in the development of IR and related metabolic diseases. The hypothesis is that, upon specific dietary conditions, the intestinal microflora may change, with an increased intestinal permeability and endotoxaemia that trigger inflammation and favor the occurrence of metabolic disorders. Dietary factors can lead to transient as well as long term changes in the composition of the gut microbiota [100]. In animal models, high-fat feeding increases endotoxemia and affects bacterial populations [101], while moderate fructose consumption leads to enhanced intestinal translocation of bacterial LPS, induction of TNF-α and liver steatosis [102].

Innate immunity has a pivotal role as a mediator of the link between gut microbiota and metabolic disturbances as well as hepatic damage. TLRs represent a major conduit for Kupffer cell activation by the innate immune system and this process may be perturbed at multiple steps in NAFLD. Nutritional fatty acids directly trigger the inflammatory response *via* TLR-4 in the adipocytes and macrophages [103], or indirectly by a modification of gut microbiota and increased LPS, once again highlighting the interconnection between metabolism and immunity. Fructose-induced NAFLD is associated with an increased intestinal translocation of

bacterial endotoxin, the formation of HNE adducts and the expression of TNF-α in the liver. In an animal model, hepatic steatosis and plasma ALT were markedly lower in fructose-fed mutant mice lacking TLR-4 than in wildtype controls exposed to fructose [104], suggesting that an increased intestinal translocation of bacterial endotoxin and the subsequent activation of Kupffer cells through a TLR-4-dependent mechanism might enhance the development of NAFLD by inducing IR.

TNF-α is one of the cytokines involved in the link between nutrient availability and innate immune activation and/or the development of fatty liver disease. The local/paracrine regulation of TNF-α release from plasma membranes through its ectodomain shedding is regulated by TNF-α-converting enzyme (TACE). TACE is naturally inhibited by the tissue inhibitor of metalloproteinase 3 (Timp3), and is activated by metabolic stumuli, namely by hyperinsulinemia. In an animal model [105], a high fat diet enhanced TACE activity, leading to impaired insulin-dependent phosphorylation of a variety of substrates. Furthermore, high TACE activity in Timp3(-/-) knock-out mice led to steatosis and ballooning degeneration compared with the limited microvesicular steatosis of wild-type mice, suggesting an important role of hepatic TACE over-activity in the development of NASH.

Hepatic Fibrogenesis and Insulin Resistance

Hepatic fibrosis is characterized by the deposition of high-density extracellular matrix protein, forming scarring tissue due to an imbalance between fibrogenesis and fibrolysis, a process largely regulated by HSCs.

Although the determinants of fibrosis in NASH are still poorly understood, IR *per se* might have a direct role in the pathogenesis of liver injury. Hyperglycemia and hyperinsulinemia cause an up-regulation of the tissue connective growth factor, thus promoting fibrogenesis [68]. In obese and diabetic rats, a high fat diet enhances IR and leads to NASH *via* up-regulation of genes modulating lipogenesis (sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase), inflammation (interleukin-6, TNF-α) and fibrogenesis (transforming growth factor-β) [106].

A possible link between IR and fibrogenesis is also provided by endocannabinoids (CB), endogenous lipid compounds that modulate a wide range of biological systems through their receptors (CB1 and CB2) on target tissues [107]. High-fat diets enhance endocannabinoid production, and an increased expression of cannabinoid receptors in hepatocytes has been found in NAFLD. CB1 receptors are expressed by activated HSCs, and their stimulation activates a profibrogenic response, *via* ROS generation. The genetic or pharmacological inactivation of CB1 receptors decreases fibrogenesis [108] and the CB1 receptor antagonist rimonabant has antifibrotic properties in diet-induced NASH *via* reduced proliferation and increased apoptosis of HSCs.

INTERACTION BETWEEN GENES AND ENVIRONMENT IN NAFLD

All the mechanisms discussed above mediate the interaction of genetic predisposition with environmental factors. Similarly to other metabolic disturbances, NAFLD is a polygenic disease, but several data now focus on the role of genetic factors in determining liver damage. Two studies reported a clustering of NASH and/or cryptogenic cirrhosis in the families of index cases with NASH [109, 110], and a Danish twin study suggested the heritability of elevated serum ALT concentrations, independent of BMI and alcohol consumption [111]. Recently, a familial aggregation study tested the hypothesis that NAFLD is a heritable condition using magnetic resonance imaging to assess hepatic steatosis in families of overweight children with and without NAFLD [112]. Fatty liver was significantly more common in siblings (59 *vs.* 17%) and parents (78 *vs.* 37%) of children with NAFLD. The liver fat fraction correlated with BMI, although the correlation was significantly stronger for families of children with NAFLD than those without NAFLD. After adjustment for confounding factors (age, sex, race, and BMI), the heritability of NAFLD as a dichotomous trait was very high.

Family clustering could simply reflect the well established heritability of risk factors for NAFLD (obesity and IR); however, studies examining ethnic differences in the prevalence of NAFLD and NAFLD-related 'cryptogenic' cirrhosis strongly suggest that susceptibility to NAFLD rather than to its risk factors may have genetic components. In the Dallas study, the prevalence of cryptogenic cirrhosis in Hispanic and African-Americans was threefold higher and fourfold lower, respectively, compared with European-American patients despite a similar prevalence of type 2 diabetes mellitus and obesity [113]. In this study a genome-wide association scan revealed that the rs738409 single nucleotide polymorphisms (SNP) in the adiponutrin gene is strongly associated with increased liver fat content. Similarly, another study showed that the adiponectin SNPs 45GT and 276GT predicted the severity of liver disease in NASH [114]. Defective antioxidant properties in patients with NAFLD and NASH are associated with an increased prevalence of a polymorphism in a T/C manganese superoxide dismutase linked to a decreased ability to detoxify superoxide anions [115]. Finally, the severity of fibrosis in NASH is associated with a functional polymorphism in the Kruppel-like factor-6 gene, encoding for a transcription factor known to play different roles in differentiation, cell growth and apoptosis [116].

All these polymorphisms should be put into the context of the environmental influences, exerting a primary role in the phenotypic expression of NAFLD in the individuals. In healthy subjects, fast-food overfeeding produced a 2.5-fold increase of liver fat in 4 weeks [117]. N the contrary, a 5 to 10% weight loss decreased liver fat by 40-80% in non-diabetic subjects and in patients with T2DM [118]. In cross-sectional studies, NAFLD subjects were reported to have a low intake of both polyunsaturated fatty acids and the antioxidant vitamins C and E, a high intake of saturated fats [47], of products with high glycemic index [119] and of soft drinks [120], and the daily intake of refined sugars was correlated with the extent of inflammatory changes at liver biopsy. In experimental models of obesity, polyunsaturated fatty acids reduced steatosis and improved insulin sensitivity by down-regulation of the SREBP-1c and by the activation of PPAR-α [121], while the effects of reduced antioxidants were compatible with the putative role of oxidative stress in the pathogenesis of NASH.

CONCLUSIONS

NAFLD is a complex condition resulting from the interaction between multiple genes and social, behavioural and environmental factors. As is the case for any complex metabolic diseases, a variable balance between environmental factors and genetics traits is likely to determine the phenotypic expression of NAFLD/NASH in the individual patient.

IR remains the common soil of the features of MetS, associated with an increased risk of fat accumulation and fibrosis not limited to the liver. Insulin signaling is involved in metabolic and immune modulations through different pathways, directly or indirectly affecting liver injury and the wound-healing response, possibly varying from patient to patient. A better understanding of the complex mechanisms underlying liver damage remains a primary target in the restoration of insulin sensitivity for a proper management of NAFLD/NASH.

ABBREVIATIONS

ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
ATPIII	=	Adult Treatment Panel III

BMI	=	Body mass index;
CB	=	Endocannabinoids
CK-18	=	Cytokeratin 18
CRP	=	C-reactive protein
CVD	=	Cardiovascular disease
CYP	=	Cytochrome P450
ER	=	Endoplasmatic reticulum
FFA	=	Free fatty acid
FPG	=	Fasting plasma glucose
FPI	=	Fasting plasma insulin
GGT	=	γ-glutamyl-transpeptidase
HDL-C	=	High-density lipoprotein cholesterol
HGO	=	Hepatic glucose output
HNE	=	*Trans*-4-hydroxy-2-nonenal
HOMA	=	Homeostatic model assessment
HSC	=	Hepatic stellate cell
IRS	=	Insulin receptor substrate
LPS	=	Lipopolysaccharides
IR	=	Insulin resistance
LDL-C	=	Low-density lipoprotein cholesterol
MAP-K	=	Mitogen-activated protein-kinase
MDA	=	Malondialdehyde
MetS	=	Metabolic syndrome
NAFLD	=	Nonalcoholic fatty liver disease
NASH	=	Non-alcoholic steatohepatitis
NF-kB	=	Nuclear factor kB
NHANES III	=	Third National Health and Nutrition Survey
OGTT	=	Oral glucose tolerance test
OGIS	=	Oral glucose insulin sensitivity
PPAR	=	Peroxisome proliferator-activated receptor
QUICKI	=	Quantitative insulin sensitivity check index
ROS	=	Reactive oxygen species
SIBO	=	Small intestinal bacterial overgrowth
SNP	=	Single nucleotide polymorphisms
SREBP-1c	=	Sterol regulatory element-binding protein-1c
T2DM	=	Type 2 diabetes
TACE	=	TNF-α-converting enzyme
Timp-3	=	Tissue inhibitor of metalloproteinase 3
TLR	=	Toll-like receptors
TNF-α	=	Tumor necrosis factor-α
TRAIL	=	Tumor necrosis factor-related apoptosis-inducing ligand
VLDL-C	=	Very low-density lipoprotein cholesterol

REFERENCES

[1] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346: 1221-31.

[2] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003; 37: 917-23.

[3] Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97.

[4] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet 2005; 366: 1059-62.

[5] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640-5.

[6] Kotronen A, Westerbacka J, Bergholm R, Pietilainen KH, Yki-Jarvinen H. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab 2007; 92: 3490-7.

[7] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from plasma fasting glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.

[8] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, *et al.* Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000; 85: 2402-10.

[9] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979; 237: E214-23.

[10] Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001; 24: 539-48.

[11] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22: 1462-70.

[12] Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, *et al.* Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2005; 90: 1578-82.

[13] Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, *et al.* The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005; 143: 722-8.

[14] Oh SY, Cho YK, Kang MS, Yoo TW, Park JH, Kim HJ, *et al.* The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. Metabolism 2006; 55: 1604-9.

[15] Liangpunsakul S, Chalasani N. Unexplained elevations in alanine aminotransferase in individuals with the metabolic syndrome: results from the third National Health and Nutrition Survey (NHANES III). Am J Med Sci 2005; 329: 111-6.

[16] Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol 2007; 102: 399-408.

[17] Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, *et al.* Risk of severe liver disease in NAFLD with normal aminotransferase levels: A role for insulin resistance and diabetes. Hepatology 2008; 48: 792-8.

[18] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005; 115: 1111-9.

[19] Palmieri VO, Grattagliano I, Portincasa P, Palasciano G. Systemic oxidative alterations are associated with visceral adiposity and liver steatosis in patients with metabolic syndrome. J Nutr 2006; 136: 3022-6.

[20] Ryan MC, Wilson AM, Slavin J, Best JD, Jenkins AJ, Desmond PV. Associations between liver histology and severity of the metabolic syndrome in subjects with nonalcoholic fatty liver disease. Diabetes Care 2005; 28: 1222-4.

[21] Rocha R, Cotrim HP, Carvalho FM, Siqueira AC, Braga H, Freitas LA. Body mass index and waist circumference in non-alcoholic fatty liver disease. J Hum Nutr Diet 2005; 18: 365-70.

[22] Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care 2009; 32: 741-50.

[23] Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. Arterioscl Thromb Vasc Biol 2007; 27: 2729-35.

[24] Schindhelm RK, Dekker JM, Nijpels G, Bouter LM, Stehouwer CD, Heine RJ, *et al.* Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the Hoorn Study. Atherosclerosis 2007; 191: 391-6.

[25] Lin YC, Lo HM, Chen JD. Sonographic fatty liver, overweight and ischemic heart disease. World J Gastroenterol 2005; 11: 4838-42.
[26] Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, *et al.* Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. World J Gastroenterol 2005; 11: 1848-53.
[27] Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, *et al.* Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. World J Gastroenterol 2007; 13: 1579-84.
[28] Jepsen P, Vilstrup H, Mellemkjaer L, Thulstrup AM, Olsen JH, Baron J, *et al.* Prognosis of patients with a diagnosis of fatty liver - a registry-based cohort study. Hepatogastroenterology 2003; 50: 2101-4.
[29] Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? Diabetologia 2008; 51: 1947-53.
[30] Fracanzani AL, Burdick L, Raselli S, Pedotti P, Grigore L, Santorelli G, *et al.* Carotid artery intima-media thickness in nonalcoholic fatty liver disease. Am J Med 2008; 121: 72-8.
[31] Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, *et al.* Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology 2005; 42: 473-80.
[32] Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, *et al.* Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care 2007; 30: 2119-21.
[33] Targher G, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, *et al.* Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. Diabetologia 2008; 51: 444-50.
[34] Arner P. Not all fat is alike. Lancet 1998; 351: 1301-2.
[35] Bergman RN, Kim SP, Hsu IR, Catalano KJ, Chiu JD, Kabir M, *et al.* Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. Am J Med 2007; 120: S3-8; discussion S29-32.
[36] Kelley DE, McKolanis TM, Hegazi RA, Kuller LH, Kalhan SC. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. Am J Physiol Endocrinol Metab 2003; 285: E906-16.
[37] Nguyen-Duy TB, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. Am J Physiol Endocrinol Metab 2003; 284: E1065-71.
[38] Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2002; 283: E1135-43.
[39] Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, *et al.* Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002; 87: 3023-8.
[40] Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, *et al.* Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 2008; 168: 1609-16.
[41] Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, *et al.* Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab 2009; 10: 419-29.
[42] Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, *et al.* Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. Cell Metab 2009; 10: 178-88.
[43] Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, *et al.* GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. J Clin Invest 2009; 119: 1201-15.
[44] Adiels M, Taskinen MR, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, *et al.* Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia 2006; 49: 755-65.
[45] Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, *et al.* Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. Diabetes Care 2007; 30: 3093-8.
[46] Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted *via* lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005; 115: 1343-51.
[47] Musso G, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, *et al.* Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. Hepatology 2003; 37: 909-16.
[48] Musso G, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: possible pathogenetic role in NAFLD. Hepatology 2008; 46: 1167-77.
[49] Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. Diabetes Care 2006; 29: 1845-50.
[50] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003; 111: 1805-12.
[51] Kerner A, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, *et al.* Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. Arterioscl Thromb Vasc Biol 2005; 25: 193-7.
[52] Park SH, Kim BI, Yun JW, Kim JW, Park DI, Cho YK, *et al.* Insulin resistance and C-reactive protein as independent risk factors for non-alcoholic fatty liver disease in non-obese Asian men. J Gastroenterol Hepatol 2004; 19: 694-8.
[53] Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis 2007; 191: 235-40.
[54] Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, *et al.* High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. J Gastroenterol 2007; 42: 573-82.
[55] Lautamaki R, Borra R, Iozzo P, Komu M, Lehtimaki T, Salmi M, *et al.* Liver steatosis coexists with myocardial insulin resistance and coronary dysfunction in patients with type 2 diabetes. Am J Physiol Endocrinol Metab 2006; 291: E282-90.
[56] Perseghin G, Lattuada G, De Cobelli F, Esposito A, Belloni E, Ntali G, *et al.* Increased mediastinal fat and impaired left ventricular energy metabolism in young men with newly found fatty liver. Hepatology 2008; 47: 51-8.
[57] Bugianesi E. Nonalcoholic fatty liver disease (NAFLD) and cardiac lipotoxicity: another piece of the puzzle. Hepatology 2008; 47: 2-4.
[58] Goland S, Shimoni S, Zornitzki T, Knobler H, Azoulai O, Lutaty G, *et al.* Cardiac abnormalities as a new manifestation of nonalcoholic fatty liver disease: echocardiographic and tissue Doppler imaging assessment. J Clin Gastroenterol 2006; 40: 949-55.
[59] Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 2005; 42: 987-1000.
[60] Choudhury J, Sanyal AJ. Insulin resistance and the pathogenesis of nonalcoholic fatty liver disease. Clin Liver Dis 2004; 8: 575-94, ix.
[61] Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, *et al.* Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005; 48: 634-42.
[62] Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, *et al.* Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001; 50: 1844-50.
[63] Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002; 277: 25226-32.
[64] Tiikkainen M, Hakkinen AM, Korsheninnikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes 2004; 53: 2169-76.
[65] Kintscher U, Law RE. PPARgamma-mediated insulin sensitization: the importance of fat *versus* muscle. Am J Physiol Endocrinol Metab 2005; 288: E287-91.
[66] Hwang JH, Stein DT, Barzilai N, Cui MH, Tonelli J, Kishore P, *et al.* Increased intrahepatic triglyceride is associated with peripheral

insulin resistance: *in vivo* MR imaging and spectroscopy studies. Am J Physiol Endocrinol Metab 2007; 293: E1663-9.

[67] Bugianesi E, Marchesini G, Gentilcore E, Cua IH, Vanni E, Rizzetto M, *et al.* Fibrosis in genotype 3 chronic hepatitis C and nonalcoholic fatty liver disease: Role of insulin resistance and hepatic steatosis. Hepatology 2006; 44: 1648-55.

[68] Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, *et al.* High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001; 34: 738-44.

[69] Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, *et al.* Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science (New York, NY) 2004; 306: 457-61.

[70] Svegliati-Baroni G, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, *et al.* Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. Hepatology 1999; 29: 1743-51.

[71] Day CP, James OFW. Steatohepatitis: a tale of two "hits". Gastroenterology 1998; 114: 842-5.

[72] Day CP. From fat to inflammation. Gastroenterology 2006; 130: 207-10.

[73] Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, *et al.* Relative contribution of iron burden, HFE mutations and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology 2004; 39: 179-87.

[74] Fassio E, Alvarez E, Dominguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology 2004; 40: 820-6.

[75] Biddinger SB, Kahn CR. From mice to men: insights into the insulin resistance syndromes. Annu Rev Physiol 2006; 68: 123-58.

[76] Considine RV, Nyce MR, Allen LE, Morales LM, Triester S, Serrano J, *et al.* Protein kinase C is increased in the liver of humans and rats with non-insulin-dependent diabetes mellitus: an alteration not due to hyperglycemia. J Clin Invest 1995; 95: 2938-44.

[77] Taniguchi CM, Ueki K, Kahn R. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. J Clin Invest 2005; 115: 718-27.

[78] Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. Cell Metabol 2008; 7: 95-6.

[79] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, *et al.* Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 2007; 45: 1366-74.

[80] DeFronzo RA. Dysfunctional fat cells, lipotoxicity and type 2 diabetes. Int J Clin Pract Suppl 2004; 143: 9-21.

[81] Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis 2008; 28: 360-9.

[82] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114: 1752-61.

[83] Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004; 99: 1497-502.

[84] Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. *In situ* detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. J Hepatol 2002; 37: 56-62.

[85] Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, *et al.* Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001; 120: 1183-92.

[86] Perez-Carreras M, Del Hoyo P, Martin MA, Rubio JC, Martin A, Castellano G, *et al.* Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology 2003; 38: 999-1007.

[87] Hannun YA, Obeid LM. The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J Biol Chem 2002; 277: 25847-50.

[88] Mari M, Fernandez-Checa JC. Sphingolipid signalling and liver diseases. Liver Int 2007; 27: 440-50.

[89] Li Z, Berk M, McIntyre TM, Gores GJ, Feldstein AE. The lysosomal-mitochondrial axis in free fatty acid-induced hepatic lipotoxicity. Hepatology 2008; 47: 1495-503.

[90] Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, *et al.* Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression *via* a lysosomal pathway. Hepatology 2004; 40: 185-94.

[91] Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: Present and future. Hepatology 2007; 46: 582-9.

[92] Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol 2008; 8: 923-34.

[93] Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, *et al.* Feeding our immune system: impact on metabolism. Clin Dev Immunol 2008; 2008: 639803.

[94] Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, *et al.* IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 2005; 11: 191-8.

[95] DiAngelo JR, Bland ML, Bambina S, Cherry S, Birnbaum MJ. The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. Proc Natl Acad Sci USA 2009; [Oct 27 Epub ahead of print].

[96] Rajala MW, Scherer PE. Minireview: the adipocyte--at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 2003; 144: 3765-73.

[97] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; 117: 175-84.

[98] Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003; 38: 681-7.

[99] Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, *et al.* Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-87.

[100] DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc 2008; 83: 460-9.

[101] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-72.

[102] Bergheim I, Weber S, Vos M, Kramer S, Volynets V, Kaserouni S, *et al.* Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008; 48: 983-92.

[103] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-25.

[104] Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. Hepatology 2009; 50: 1094-104.

[105] Fiorentino L, Vivanti A, Cavalera M, Marzano V, Ronci M, Fabrizi M, *et al.* Increased tumor necrosis factor alpha-converting enzyme activity induces insulin resistance and hepatosteatosis in mice. Hepatology 2010; 51: 103-10.

[106] Ota T, Takamura T, Kurita S, Matsuzawa N, Kita Y, Uno M, *et al.* Insulin resistance accelerates a dietary rat model of nonalcoholic steatohepatitis. Gastroenterology 2007; 132: 282-93.

[107] Gabbay E, Avraham Y, Ilan Y, Israeli E, Berry EM. Endocannabinoids and liver disease--review. Liver Int 2005; 25: 921-6.

[108] Teixeira-Clerc F, Julien B, Grenard P, Tran Van Nhieu J, Deveaux V, Li L, *et al.* CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. Nat Med 2006; 12: 671-6.

[109] Struben VM, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. Am J Med 2000; 108: 9-13.

[110] Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. Am J Gastroenterol 2001; 96: 2957-61.

[111] Bathum L, Petersen HC, Rosholm JU, Hyltoft Petersen P, Vaupel J, Christensen K. Evidence for a substantial genetic influence on biochemical liver function tests: results from a population-based Danish twin study. Clin Chem 2001; 47: 81-7.

[112] Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, *et al.* Heritability of nonalcoholic fatty liver disease. Gastroenterology 2009; 136: 1585-92.

[113] Browning JD, Kumar KS, Saboorian MH, Thiele DL. Ethnic differences in the prevalence of cryptogenic cirrhosis. Am J Gastroenterol 2004; 99: 292-8.

[114] Musso G, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. Hepatology 2008; 47: 1167-77.

[115] Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, *et al.* Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol 2004; 40: 781-6.

[116] Miele L, Beale G, Patman G, Nobili V, Leathart J, Grieco A, *et al.* The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. Gastroenterology 2008; 135: 282-91 e1.

[117] Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut 2008; 57: 649-54.

[118] Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes 2005; 54: 603-8.

[119] Valtuena S, Pellegrini N, Ardigo D, Del Rio D, Numeroso F, Scazzina F, *et al.* Dietary glycemic index and liver steatosis. Am J Clin Nutr 2006; 84: 136-42; quiz 268-9.

[120] Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, *et al.* Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. J Hepatol 2007; 47: 711-7.

[121] Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. Am J Clin Nutr 2004; 79: 1164S-8S.

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