Nutrición Hospitalaria

Nutr Hosp. 2011;26(3):441-450
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Revisión

Molecular mechanisms of steatosis in nonalcoholic fatty liver disease

P. Pettinelli[1], A. M. Obregón[2] and L. A. Videla[3]

[1]Department of Food Science, Nutrition and Dietetics. Faculty of Pharmacy. University of Concepción. Concepción. Chile. [2]School of Nutrition and Dietetics. Faculty of Health Sciences. University of San Sebastián. Concepción. Chile. [3]Molecular and Clinical Pharmacology Program. Institute of Biomedical Sciences. Faculty of Medicine. University of Chile. Santiago. Chile.

Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most important cause of chronic liver disease and is considered the hepatic manifestation of the metabolic syndrome associated with diabetes mellitus type 2. The prevalence of NAFLD in the general population reaches 15-20%. It is also estimated that nonalcoholic steatohepatitis (NASH) affects 3% of the population. NAFLD refers to a wide spectrum of liver damage, which ranges from simple steatosis or intracellular triglyceride accumulation, to inflammation (NASH), fibrosis and cirrhosis. The mechanisms involved in the accumulation of triglycerides in the liver and subsequent hepatocellular damage are multifactorial and are not completely understood. However, metabolic changes such as insulin resistance (IR) are developed, being a common factor in the retention of fatty acids (FA) within the hepatocytes with oxidation and production of free radicals at the mitochondrial level, which are capable of causing lipid peroxidation, cytokine production, and necrosis. In addition, there are alterations in the hepatic bioavailability of long chain n-3 polyunsaturated fatty acids, conditions that alter the expression of a series of transcriptional factors involved in lipolytic and lipogenic processes in the liver. A greater knowledge of the etiopathogenic mechanisms of NAFLD is fundamental for the development of future effective therapeutic strategies. The pathophysiological fundamentals of liver steatosis are analyzed in this study.

(*Nutr Hosp.* 2011;26:441-450)

DOI:10.3305/nh.2011.26.3.5119

Key words: *Fatty liver. Obesity. PPAR-alfa. SREBP-1c. n-3 polyunsatured fatty acids.*

MECANISMOS MOLECULARES DE LA ESTEATOSIS EN LA PATOLOGÍA DE HÍGADO GRASO NO ALCOHÓLICO

Resumen

La enfermedad de Hígado graso no alcohólico (HGNA) es la causa más importante de enfermedad hepática crónica y es considerado la manifestación hepática del síndrome metabólico asociado a obesidad y diabetes mellitus tipo 2. La prevalencia de la enfermedad de HGNA en la población general alcanza el 15-20%, estimándose además que la esteatohepatitis no-alcohólica (EHNA) afecta al 3%. El HGNA se refiere a un amplio espectro de daño hepático, que va desde esteatosis simple o acumulación intracelular de triacilglicéridos (TAGs), a inflamación (EHNA), fibrosis y cirrosis. Los mecanismos implicados en la acumulación de TAGs a nivel hepático y subsecuente daño hepatocelular son de carácter multifactorial y no se conocen completamente. Sin embargo, es reconocido que existen alteraciones metabólicas, siendo la resistencia a la insulina (RI) un factor común que genera retención de ácidos grasos y TAGs dentro de los hepatocitos, con la producción de radicales libres a nivel mitocondrial capaces de inducir lipoperoxidación, producción de citoquinas y necrosis. Además, existen alteraciones en la biodisponibilidad hepática de ácidos grasos poli-insaturados de cadena larga de la serie n-3, condiciones que alterarían la expresión de una serie de factores de transcripción involucrados en el proceso de lipólisis y lipogénesis a nivel hepático. Un mayor conocimiento de los mecanismos etiopatogénicos de la enfermedad de HGNA es fundamental para el desarrollo de estrategias terapéuticas eficaces a futuro. Los fundamentos fisiopatológicos de la esteatosis son analizados a continuación.

(*Nutr Hosp.* 2011;26:441-450)

DOI:10.3305/nh.2011.26.3.5119

Palabras clave: *Hígado graso. Obesidad. PPAR-alfa. SREBP.1c. Ácidos grasos poli-insaturados n-3.*

Correspondence: Paulina Pettinelli.
University of Concepción.
Casilla 237, Concepción, Chile.
E-mail: ppettinelli@udec.cl

Recibido: 21-IV-2010.
1.ª Revisión: 5-XI-2010.
Aceptado: 4-III-2011.

Introduction

Non alcoholic fatty liver disease (NAFLD) has emerged as the most important cause of chronic liver disease related to the increase in incidence of obesity and diabetes mellitus type 2 in the population.[1] There are secondary, less common, causes of NAFLD including the use of different medicines, antiretroviral drugs, and nutritional causes such as rapid weight loss or total parenteral nutrition.[2] NAFLD refers to a wide spectrum of liver damage, which ranges from simple steatosis or intracellular triglyceride accumulation to inflammation (non alcoholic steatohepatitis, NASH) fibrosis and cirrhosis.[3] The characteristics of liver biopsies include steatosis, when it is more than 5% of the liver weight, mixed inflammatory cellular infiltration, degeneration and hepatocyte necrosis, nuclear glycogen, Mallory bodies and fibrosis.[4,5] Its clinical implications are mainly derived from its occurrence in the population and its potential to progress to cirrhosis and liver failure, steatosis being comparatively benign with a 0-4% risk of developing cirrhosis in a period of one to two decades, in contrast to the 5-8% of patients with NASH that could develop cirrhosis in a period of approximately 5 years.[4,6]

Liver biopsy is the gold standard for the diagnosis of NAFLD, considering that it is the only method that can distinguish between simple steatosis, NASH and the degree of fibrosis.[6,7] However, there are many techniques, such as ultrasound, computed tomography, or magnetic resonance, which can confirm the presence of liver steatosis with a high degree of precision. In addition, alcohol consumption is an important factor, which is restricted to less than 20 and 30 g of alcohol per day in women and man, respectively. Furthermore, it is also necessary to rule out other possible causes such as viral diseases, autoimmune responses, hereditary or metabolic factors, drugs, and toxins.[5,8]

NAFLD is considered the hepatic manifestation of metabolic syndrome, with a prevalence of 15-20% in the general population, whereas that of NASH affects 3% of the population.[4,9] In patients with diabetes mellitus type 2 the incidence steatosis is close to 50% and it goes up to 76 to 90% in the obese population. NASH is developed in almost 35% of the cases in obese subjects[10] and in all patients who exhibit morbid obesity and diabetes.[5] This means that many patients with NAFLD have multiple components of metabolic syndrome, IR being an important predicting factor of NAFLD and NASH.[11,12]

An important issue in the management strategy for these patients is the use of diets promoting a decrease in body weight and improve control over blood sugar, insulinemia, dyslipidemia, and cardiovascular risk. There are a variety of diets that have been recommended for the prevention and treatment of all of the components of metabolic syndrome,[13] however, their use in the treatment of NAFLD is still unknown.

Pathophysiology of NAFLD

The mechanisms associated with the accumulation of TAGs in the liver and the subsequent hepatocellular damage are multifactorial and not fully understood.[2] The first metabolic abnormality that leads to liver steatosis, involving a lipotoxic reaction with a component of oxidative stress, includes nutritional factors and changes in liver lipid metabolism, which is mainly the result of IR.[1,10,14,15]

Oxidative Stress and Insulin Resistance Associated with Obesity

Experimental studies indicate that the excess of FAs induces high levels of β-oxidation, with the production of reactive oxygen species (ROS) such as superoxide radical [$O_2^{\bullet-}$] and hydrogen peroxide [H_2O_2]) at a mitochondrial respiratory chain level, simultaneously with the induction of necrosis.[16] These results suggest that overeating can cause the excess of FAs in the liver, inducing high rates of β-oxidation and ROS production, which is in accordance with changes in the parameters related to oxidative stress observed in the liver of obese patients with steatosis[17-20] (fig. 1). In fact, relative to the control values, the liver of obese patients presents (i) a decrease in the antioxidant potential (a decreased superoxide dismutase activity and glutathione content), (ii) an increase in pro-oxidant activity (an increased lipid peroxidation, hydroperoxide content, and protein oxidation), and (iii) Kupffer cell activation (enhanced rates of $O_2^{\cdot-}$ production and lipid peroxidation). These parameters are associated with decreased plasma antioxidant capacity and increased levels of serum F_2-isoprotanes, the products of arachidonic acid peroxidation.[1,3]

The imbalance of redox status observed in the liver of obese patients represents a nutritional oxidative stress phenomenon, which is caused by excessive and prolonged consumption of metabolic fuels (carbohydrates and lipids) and/or inadequate supply of dietary antioxidants.[21] In conditions of liver oxidative stress, obese patients exhibit two important alterations associated with this redox imbalance, namely, (i) development of IR,[22] shown by the increase in the HOMA index, which exceeds its normal value by 100%,[1] and (ii) substantial decreased of liver content of long-chain polyunsaturated fatty acids n-3 (LCPUFA n-3)[20] (fig. 1).

Under most conditions, FAs are the main liver fuel, however, in pathologies such as obesity, the large influx of carbohydrates and lipids cause significant changes in the intermediary metabolism of the liver.[23] Under these conditions, hyperinsulinemia and hyperglycemia promote the synthesis of FAs from glucose and inhibit β-oxidation of FA, and the excess of FAs is redirected to the formation of TAGs[2,23] (fig. 2).

IR causes an increase in peripheral lipolysis, leading to enhancement in free fatty acids (FFA) levels in the

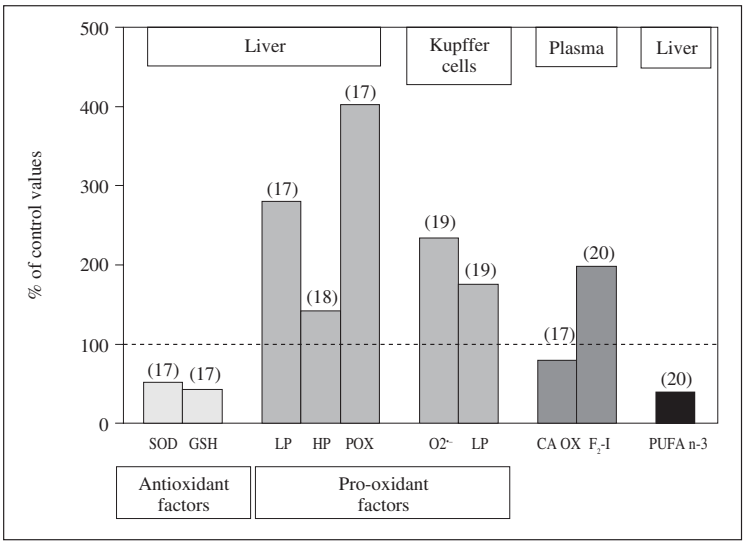

Fig. 1.—Parameters associated with oxidative stress in obese patients with non alcoholic fatty liver disease, expressed as a percentage of the control values. The numbers in parentheses correspond to the specific references cited. Abbreviations: SOD, superoxide dismutase; GSH, reduced glutathione; LP, lipid peroxidation, measured as production of malondialdehyde; HP, hydroperoxide products of the LP; POX, oxidized proteins; $O_2^{\bullet-}$, superoxide radical; CAOX, plasma antioxidant capacity; F_2-I, F_2-isoprostane products of arachidonic acid peroxidation; PUFA n-3, polyunsaturated fatty acid n-3 (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]).

liver, which is potentially toxic for the organ. Although IR causes an alteration in the insulin glucoregulatory pathway, the insulin lipogenic effects are maintained[24], and the hepatocytes protect themselves by transforming, catabolizing, and exporting the excess FFA.[24,25] These changes appear to play a key role in the appearance of fatty liver by IR, promoting the mobilization of peripheral FA towards the liver.[26]

The retention of FA and TAGs within the hepatocytes that depends on IR and hyperinsulinemia leads to the production of free radicals at a mitochondrial level, capable of inducing lypoperoxidation, cytokine pro-

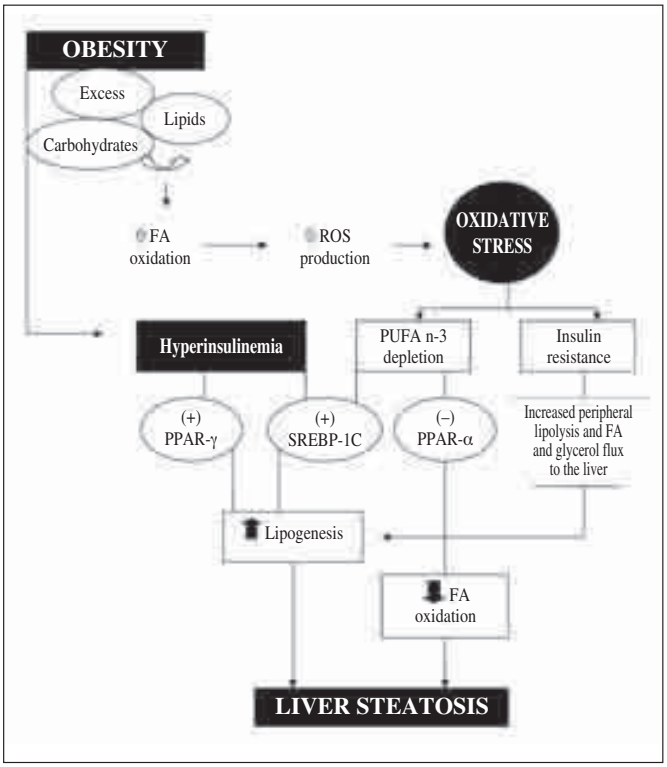

Fig. 2.—Induction of oxidative stress and its relationship with insulin resistance (IR) and steatosis in non alcoholic fatty liver associated with obesity. Abbreviations: FA, fatty acids; ROS, reactive oxygen species; PUFA n-3, polyunsaturated fatty acids n-3; PPAR-α (γ), peroxisome proliferator-activated receptor-alfa (gamma); SREBP-1c, sterol regulatory element binding protein 1-c.

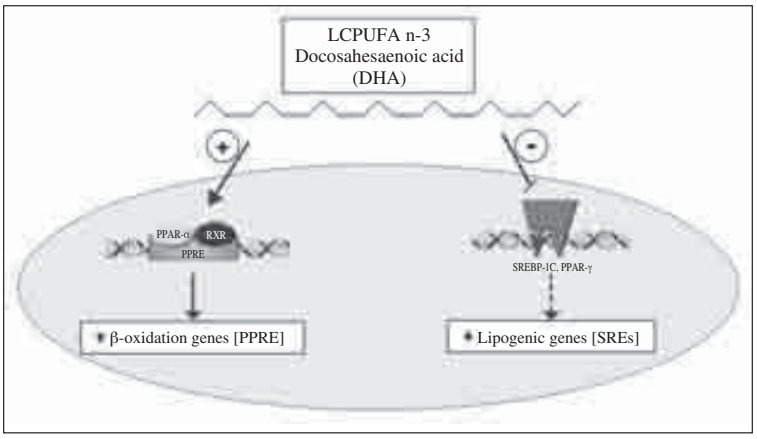

Fig. 3.—Nuclear mechanism of regulation of gene expression by long chain polyunsaturated fatty acids n-3 (LCPUFA-n3). Abbreviations: PPAR-α (γ), peroxisome proliferator-activated receptor -α (g); PPRE, PPAR response element; SREBP-1c, sterol regulatory element binding protein; SREs, response elements to sterols.

duction and hepatocyte necrosis,[16] which may trigger NAFLD progression to the more severe state of NASH.[1,27]

Therefore, from a temporary point of view, fatty liver develops when *de novo* synthesis exceed the oxidation and re-secretion of TAGs.[15] The sources of FAs that potentially may contribute to fatty liver are (i) peripheral lipids stored in the adipose tissue that reach the liver via plasma FFA, considering that in patients with NAFLD lipolysis in adipose tissue is not totally suppressed,[14] (ii) increased *de novo* lipogenesis (DNL),[28] (iii) alteration in the synthesis or secretion of lipoproteins,[29] and/or; (iv) reduction in FA oxidation.[10,14,15]

The effects of hyperglycaemia and hyperinsulinemia on hepatic glycolysis and lipogenesis are mediated by the activation of different transcription factors such as sterol regulatory element binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor γ (PPAR-γ), which transcriptionally activate the expression of a machinery of genes necessary for lipogenesis.[3,30,31] In addition, high concentrations of glucose in the liver, independently of insulin levels, activate carbohydrate responsive element-binding protein (ChREBP) upregulating the transcription of both pyruvate kinase and lipogenic genes.[10,12,32]

Alterations in the hepatic bioavailability of long-chain polyunsaturated fatty acids n-3 (LCPUFA n-3), as mediators of the control of metabolic distribution of FAs and glucose in the liver

LCPUFA n-3, mainly EPA (C20:5n3, eicosapentaenoic acid) and DHA (C22:6n3, docosahexaenoic acid) exert their effects by regulating the metabolism of the lipids in the liver through modifications in the gene transcription.[30,33] This is carried out by means of (i) inhibition of the expression and processing of SREBP-1c (fig. 3) though a reduced trans-activation of the liver receptor X-α (LXR-α), an agonist of SREBP-1c,[34-36] with the consequent inhibition of the transcription of the lipogenic and glycolitic genes, and (ii) activation of the transcription of genes involved in FA oxidation acting as a ligand of the peroxisome proliferators-activated receptor a (PPAR-α), which directly influences the distribution of the metabolic fuel (fig. 3)[3]. Several studies have shown that the liver of patients with NAFLD shows altered LCPUFA n-3 content, reaching 50% decrease in EPA and DHA levels both in steatosis and steatohepatitis,[37-40] which can cause down-regulation in signal transduction associated with PPAR-α and a decrease in membrane fluidity and in the number of GLUT4 receptors in insulin-dependent tissues, critical factors of IR.[41] Additionally, one of the factors involved with the depletion of these LCPUFA n-3 is a diminution in the activity by delta-5 and delta-6 desaturases, responsible for the biosynthesis of highly unsaturated FAs.[42]

In an animal model, LCPUFA n-3 supplementation was associated with an improvement of liver steatosis and insulin sensitivity, as well as a decrease in the concentration of fasting FFA and TAGs levels.[43] In obese patients with NAFLD, LCPUFA n-3 supplementation for 12 months decreased the degree of steatosis measured by ultrasonography and ECO-Doppler, in comparison with non supplemented patients; however, the LCPUFA n3 levels in liver phospholipids and the presence or absence of IR were not evaluated.[44] Recently, it has been shown that the administration of LCPUFA n3 in obese patients with NAFLD improve IR, as well as reduce the content of hepatic lipids and the serum levels of alanine aminotransferase and TNF-α.[45]

Transcription Factors in NAFLD

The regulation of lipogenesis and hepatic lipoxidation is under the strict control of a network of nuclear

receptors, which regulate the expression of enzymes that participate in the lipid metabolism in a coordinated manner.[15] While in healthy subjects in a postprandial state, DNL increases by 20-30%, in subjects with NAFLD this process is increased in fasting conditions, without an elevation in postprandial states.[28] These observations support the concept of a sustained increase of DNL in the liver of patients with NAFLD.

Regarding hepatic lipogenesis, it has been shown that the transcription factors SREBP-1c, PPAR-γ, and ChREBP play an essential role in the regulation of genes involved with the synthesis of lipids, glycolysis and lipogenesis, respectively.[46-48] In addition, factor XBP-1 has recently been identified as a possible factor involved with the accumulation of TAGs in the liver of animal models.[49] The participation of these factors as mechanisms involved in liver steatosis in obesity will be analyzed in the following sections.

SREBP (Sterol Regulatory Element Binding Protein)

The protein SREBP is represented by SREBP-1a, SREBP-1c and SREBP-2, key factors in the control of various genes involved in the homeostatic regulation of cholesterol and lipid metabolism. SREBP-1a, SREBP-1c are encoded in a single gene through the use of alternative transcription start sites, differing in the terminal amino sequence, whereas SREBP-2 is encoded in a different gene.[30,50,51] SREBP-1a is a potent activator of all SREBP-responsive genes that the synthesis of cholesterol, fatty acids, and TAGs. SREBP-2 activates genes involved in cholesterol synthesis, while SREBP-1c increases the transcription of genes involved in FA synthesis (fig. 3)[30].

The distribution pattern of the SREBPs also differs, being SREBP-1c the major (90%) isoform that controls FA synthesis in the liver.[51-53] The SREBPs are synthesized as inactive precursors attached to the membrane of the endoplasmic reticulum (ER), where they are associated to two membrane proteins, SCAP (activating protein anchored to the SREBP) and Insig-1 (Insulin-induced gene 1) or Insig-2 (Insulin-induced gene 2), the latter being selectively associated to SREBP-1c.[54] In order to be activated, they are transported through vesicles (COP II) to the Golgi apparatus where, through proteolytic processing by SP1 and SP2, they are freed in order to fulfill their function at a nuclear level. In this way, they reach the nucleus and join the sterol response elements (SREs) of multiple genes, activating their transcription.[30,34,52,54]

SREBP-1c is regulated in response to a series of nutritional and hormonal stimulus both by transcriptional and post-transcriptional mechanisms. Multiple lines of research suggest that insulin has a stimulating effect on FA synthesis through an increase in SREBP-1c, even under IR conditions,[34,53] whose underlying mechanisms have been recently reviewed.[51,54]

Activation of the pro-lipogenic factor SREBP-1c in obesity

Over a decade ago, it was shown that the genetically obese, insulin resistant, and hyperinsulinemic mouse (*ob*/*ob*) had elevated hepatic levels of SREBP-1c,[52] together with an increased expression of synthase fatty acid (FAS), acetyl-CoA carboxylase 1 (ACC-1) and PPAR-γ.[48] In relation to transgenic models, knockout SREBP-1c *ob*/*ob* mice presented a significant reduction in the hepatic expression of lipogenic genes preventing liver steatosis,[55] and in mice with overexpressed SREBP-1c, an increase in the content of TAGs in the liver as well as an increase in the enzyme mRNA levels involved with FA synthesis are observed, without an increase in the enzymes that controls cholesterol synthesis.[30]

Among the genes that are regulated by SREBP-1c constituting the main regulators of DNL are ACC-1 (located in lipogenic tissues such as adipose tissue and liver), ACC-2 (located in non-lipogenic tissues such as heart, muscle, and skeletal, and to a lesser extent in the liver), and FAS. In the liver, the FAS gene can be transcriptionally activated by a stimulating factor and by LXR ligands, as it has LXR response elements, therefore maintaining the FA synthesis.[56]

The malonil-CoA formed by ACC-1 is used by FAS to synthesize palmitic acid (C16:0), and through successive elongation and desaturation steps it is possible to form oleic acid (C18:1).[30,32,57] One study showed that obese patients with NAFLD presented a significant increase of C18:1 (final product of DNL) in hepatic phospholipids when compared to eutrophic subjects without NAFLD,[37] which suggests that under the condition of IR, the DNL is increased**.** The malonil-CoA made by the ACC-2 in lipid consuming tissues allosterically inhibits carnitine-palmitoyl transferase-I (CPT-I).[57]

HIV patients present lipodystrophy, NAFLD, and steatohepatitis related to of the adverse effects of antiretroviral therapy. One study determined that patients with HIV and IR had higher hepatic expression of both transcription factors compared with non-obese NAFLD patients.[58] Studies in humans have shown that there are many differences between obese and non-obese patients with NAFLD concerning the expression of hepatic genes related to lipid metabolism, regeneration, apoptosis, and detoxification.[59] When the expression of LXR-α, ChREBP, SREBP-1c, ACC-1, and FAS is studied in non-obese patients with NAFLD and control subjects, increases in the expression LXR-α, SREBP-1c, ACC-1 and FAS are shown, as well as a decrease in the expression of ChREBP in the group of eutrophic patients with NAFLD in comparison with controls. These findings suggest that SREBP-1c may be the dominating factor involved in the regulation of the lipid metabolism, activating the expression of lipogenic genes.[60]

Recently, it was shown that the liver of obese patients had higher level of expression of the pro-

lipogenic factor SREBP-1c in comparison with control subjects,[39] with concomitant 107% increase in the expression of FAS, results that are concurrent with the increase in the DNL process observed in obesity.[28,61,62] The previously mentioned decrease of the LCPUFA n3 may constitute a mechanism contributing to the increase in the hepatic DNL in obese patients with NAFLD, favoring the proteolytic release of SREBP-1c attached to the ER membrane and its nuclear abundance, and/or altering the composition of membrane phospholipids, which is associated with IR.[39]

Peroxisome proliferator-activated receptor or PPARs

PPARs are proteins of approximately 56 kD that belong to the super family of steroid nuclear receptors. There are three known isoforms of PPAR, namely, α, β, and γ, which are similarly encoded by individual genes.[24] PPARs act by modulating different cellular functions, including adipocyte differentiation, glucose metabolism, FA oxidation, and inhibition of the expression of inflammatory genes.[24,63] They present different tissue distribution patterns. For example, PPAR-α is mainly expressed in those tissues involved with the metabolism of FA such as liver, heart, and brown adipose tissue. PPAR-β function has been less characterized. It may have a role in the metabolism of FA with a ubiquitous expression pattern, with greater comparative levels in skeletal and cardiac muscles, as well as nervous tissue. PPAR-γ has a restricted expression pattern, with a prominent expression in the white and brown adipose tissues, with lower levels in spleen, intestine, liver, and lymph nodes.[35,63-65] Binding of ligands to PPAR induces a conformational change and the receptor dimerizes forming a heterodimer with 9-cis retinoic acid receptor (RXR), which causes its activation. The activated receptor interacts with specific sequences in the DNA or peroxisome proliferator response elements (PPRE), present in the target gene promoter. The initial PPAR activation process involves degradation of a co-repressor complex and recruitment of co-activating complexes, leading to up-regulation of the expression of the target genes.[24,31,65,66]

Several studies have identified a series of ligands for PPARs such as unsaturated fats, oxidized low density lipoproteins (LDL-ox), VLDL, derived metabolites of linoleic acid, and pharmaceuticals such as fibrates and thiazolidinediones (TZD).[65]

Increase of the expression of the pro-lipogenic factor PPAR-γ in obesity

The *Pparg* gene, located in the chromosome 3p25, with a highly conserved structure in rats and humans, produces 2 proteins, namely, PPAR-γ1 and PPAR-γ2 has 30 additional amino acids in its extreme amino-terminal, allowing a greater capability of stimulating transcription.[66] PPAR-γ plays a fundamental part in the control of genes involved in lipogenic pathways of adipocytes, promoting the uptake of FA and the differentiation of the adipocyte, with the consequent increase in the content of TAGs in the adipocytes and reduction in the delivery of FA to the liver.[31,35] It also confers sensitization to insulin through the transcriptional activation of the adiponectin gene in adipocytes, up-regulating its expression. PPAR-γ is the molecular target of TZDs, which increase sensibility to insulin by increasing the plasmatic levels of adiponectin in humans. Because of this action, TZDs are used for their anti-diabetic effects on the liver, skeletal muscle, and adipose tissue.[67,68]

In animal models of obesity, IR, and diabetes mellitus, increased hepatic PPAR-γ mRNA and protein levels have been found as potential mechanisms of steatosis (fig. 2).[69,70] In some infections, such as the hepatitis B and C viruses, multiple observations suggest that liver steatosis is a common histological characteristic in which an increase in the expression and/or activity of PPAR-γ could contribute to the regulation of lipid synthesis, causing the development of liver steatosis.[71-73] Recent findings indicate that hepatic PPAR-γ mRNA levels are significantly increased in obese NAFLD patients with either steatosis or NASH, over lean controls,[74] in agreement with data assessing the PPAR-γ2 isoform.[75]

Similar to SREBP-1c factor, PPAR-γ is regulated by insulin in normal conditions,[76] which probably persists under the condition of IR. Furthermore, a factor that may be related to the expression of PPAR-γ is the presence of polymorphisms in the *Pparg* gene, a condition that causes susceptibility of developing NAFLD through the adiponectin pathway.[77]

PPAR-α

PPAR-α is expressed in high concentrations in liver tissue in rats and humans.[35,65] It plays a crucial role in controlling the oxidation of FA by modulating the expression of genes that encode enzymes involved in mitochondrial, peroxisomal, and microsomal FA oxidation. It also regulates the expression of proteins involved in FA binding and esterification and export of FA in VLDL.[64,78]

There are numerous proteins induced by PPAR-α, among them, Acyl-CoA synthetase (ACS), Acyl-CoA oxidase (AOX), and the CPT system, which have PPREs in their promoting region.[36,79] The CPT system consists of multiple components, namely, (i) CPT-1, an integral transmembrane protein located in the mitochondrial external membrane that catalyzes the transfer of acyl-CoA to carnitine to generate acyl-carnitine, being the limiting enzyme in mitochondrial FA β-oxidation; in addition to having a PPRE region, CPT-1 has

a regulating sequence that responds to LCPUFA;[80] (ii) carnitine-acylcarnitine translocase (CACT), a transporter located in the mitochondrial internal membrane, and (iii) CPT-2, a membrane protein of the mitochondrial internal membrane facing the matrix that reverts the reaction catalyzed by the CPT-1.[35,81] Three tissue specific CPT-1 isoforms have been reported, L-CPT-1 that is mainly expressed in the liver, M-CPT-1 expressed in skeletal muscle and other tissues, and B-CPT-1 located in brain, whereas CPT-2 exists as a unique protein.[5,25,80] Factors such as FAs, insulin, and thyroid hormone regulate L-CPT-1.[80]

Inhibition of the lipoxidative factor PPAR-α in obesity

In hepatocytes, FAs are oxidized to acetyl-CoA by mitochondrial and peroxisomal β-oxidation. Under normal circumstances, peroxisomal β-oxidation is a less important pathway in FA oxidation compared with mitochondrial β-oxidation, however, in conditions of high fat diets and other metabolic alterations, PPAR-α is activated.[25,64] PPAR-α activation causes an increase in FA uptake at a mitochondrial and peroxisomal level.[15] Several animal and cellular models indicate that the activation of PPAR-α prevents the infiltration of TAGs in the liver under conditions of increased inflow. Animal models of hepatic steatosis have shown a lower expression of this lipoxidative factor and enzymes with PPRE.[64,82] knockout animals for PPAR-α present minimal hepatic steatosis under normal conditions, which is drastically enhanced when they are subjected to fasting. In knockout animals for both AOX and PPAR-α, a marked alteration in the FA oxidation is observed after 48-72 hours, higher than that in only PPARα -/- rats, which caused a slight increase in the expression of PPAR-α. These data suggest that PPAR-α plays a critical role in FA oxidation, determining the degree of hepatic steatosis (fig. 2).[83] One of the difficulties with some of the animal models of obesity and IR to study the expression of PPAR-α is the genetic background, considering the involvement of other genetic factors and/or interactions that may contribute to PPAR-α function in the metabolism of FA in these models.[84]

Studies in humans have evaluated the expression of PPAR-α in patients with hepatitis C, a pathology that coexists with steatosis. In these studies, the expression of hepatic mRNA and protein levels of CPT-1 are decreased in patients with untreated hepatitis C in comparison with control patients, suggesting that the hepatitis C virus could alter PPAR-α expression and activation, playing a role in the development of steatosis.[85,86]

Recently, a significant decrease in the levels of PPAR-α mRNA with a reduction in the expression of CPT-1a have been reported in NAFLD obese patients with steatosis and steatohepatitis, associated with depletion of LCPUFA n-3.[39] Considering that PPAR-α is activated by a direct interaction of LCPUFA n-3 to its ligand binding domain,[87] hepatic PPAR-α function could be compromised by a deficient activation due to decreased binding of LCPUFA n-3[39] (fig. 3). Similarly, an inhibition in the expression of hepatic PPAR-α, without significant alterations in the expression of hepatic SREBP-1c has been reported in patients with NAFLD.[88] The discrepancy observed in the hepatic expression of SREBP-1c in these studies could be caused by the fact that the patients did not exhibit the same nutritional status, as those with SREBP-1c up-regulation were morbidly obese,[39] while those with normal SREBP-1c levels were not.[88] A relevant finding recently published by our research group is the significant increase in the hepatic SREBP-1c/PPAR-α ratio in obese patients with NAFLD in comparison with the control group.[39] This implies a metabolic imbalance between the DNL and the oxidation of FAs in favor of the lipogenic process, a central and determining factor of steatosis in the liver of obese patients. In addition, the SREBP-1c/PPAR-α relationship is associated with plasma insulin levels and the HOMA-IR index, pro-lipogenic factors that determine the increase in peripheral lipolysis and the onset of non-esterificated FA flux to the liver.[39]

The inverse correlation established between the hepatic SREBP-1c/PPAR-α ratio and the content of LCPUFA n3 in the control group and obese patients with NAFLD is of particular interest[39], as it supports the fundamental role of LCPUFA n-3 depletion as a mechanism that directs FAs away from oxidation towards triglyceride storage (fig. 3).[89]

Besides the key regulating action of the hepatic metabolism of lipids, the activation of PPAR-α plays an important role in the control of inflammatory processes, an action that is carried out by the interference in the activation of the transcriptional factors nuclear factor-κB (NF-κB) and activating protein 1 (AP-1).[24] Recently, obese patients with steatohepatitis showed (i) inverse and significant correlations between NF-κB or AP-1 with levels of PPAR-α mRNA, and (ii) increases of 7.8 times and 15.1 times in the NF-κB/PPAR-α and AP-1/PPAR-α ratios over control values, respectively.[90] These findings suggest that, in addition to the pro-lipogenic role that hepatic PPAR-α down-regulation implies, it may represent an important factor in the progression of steatosis to the steatohepatitis associated with obesity.

Conclusions

In healthy human beings, saturated FAs are the main metabolic fuel of the liver under most circumstances, whereas the contribution of DNL to the hepatic content of TAGs is rather low (1.6-4.7%).[61,62] However, under the conditions of insulin resistance, as occurs in hyperinsulemic obese patients with NAFLD, fasting DNL is substantially increased, a response that probably involves the induction of enzymes that participate in

the hepatic DNL process.[61,62] Although liver DNL is mainly controlled by SREBP-1c signaling, other factors such as LXR-α and/or activation of ChREBP could also play an important role in the induction of SREBP-1c;[60,91-93] however, additional studies are required to verify this assertion in morbid obesity in humans.

Based on relevant data obtained in humans, rather then in animal models that often present characteristics not observed in man,[94] it is concluded that the expression of the transcriptional factors that control lipid metabolism is markedly altered in the liver of obese NAFLD patients with steatosis. This alteration in the liver signaling pathways is associated with several metabolic alterations that take place within the context involving overnutrition, and the appearance of oxidative stress and IR.

Acknowledgements

The authors would like to express their gratitude for the funding given by the National Fund of Scientific and Technological Development (*Fondo Nacional de Desarrollo Científico y Tecnológico* - FONDECYT Grants 1060105 and 1090020), CONICYT and UCO 0202 Doctoral Scholarships.

References

1. Videla LA, Rodrigo R, Araya J, Poniachik J. Insulin resistance and oxidative stress interdependency in non-alcoholic fatty liver disease. *Trends Mol Med* 2006; 12: 555-558.
2. Adams LA, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diab Med* 2005*;* 22: 1129-1133.
3. Videla LA. 2008. Oxidative stress and insulin resistance as interdependent pathogenic mechanisms in non-alcoholic fatty liver disease associated with obesity. In: S. Alvarez, P. Evelson (Eds.), Free Radical Pathophysiology. pp. 369-385. Transworld Research Network, Kerala, India.
4. Angulo P. Nonalcoholic fatty liver disease. *N Eng J Med* 2002*;* 346: 1221-1231.
5. Reddy JK, Rao MS. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol* 2006; 290: 852-858.
6. Adams LA, Angulo P. Treatment of non-alcoholic fatty liver disease. *Postgrad Med J* 2006; 82: 315-322.
7. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; 123: 745-750.
8. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; 43: 99-112.
9. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; 48: 792-798.
10. Anderson N, Borlak J. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacol Rev* 2008; 60: 311-357.
11. Boza C, Riquelme A, Ibañez L, Duarte I, Norero E, Viviani P, Soza A, Fernandez JI, Raddatz A, Guzman S, Arrese M. Predictors of nonalcoholic steatohepatitis (NASH) in obese patients undergoing gastric bypass. *Obesity Surgery* 2005; 15: 1148-1153.
12. Marra F, Gastaldelli A, Svegliati-Baroni G, Tell G,Tiribelli C. Molecular basis and mechanisms of progression of non-alcoholic steatohepatitis. *Trends Mol Med* 2008; 14: 72-81.
13. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr* 2007; 86: 285-300.
14. Méndez-Sánchez N, Arrese M, Zamora-Valdés D, Uribe M. Current concepts in the pathogenesis of nonalcoholic fatty liver Disease. *Liver Int* 2007; 27: 423-433.
15. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res* 2009; 48: 1-26.
16. Aronis A, Madar Z, Tirosh O. Mechanism underlying oxidative stress-mediated lipotoxicity: exposure of J774.2 macrophages to triacylglycerols facilitates mitochondrial reactive oxygen species production and cellular necrosis. *Free Radic Biol Med* 2005; 38: 1221-1230.
17. Videla LA, Rodrigo R, Orellana M, Fernández V, Tapia G, Quiñones L, Varela N, Contreras J, Lazarte R, Csendes A, Rojas J, Maluenda F, Burdiles P, Diaz JC, Smok G, Thielemann L, Poniachik J. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin Sci* 2004; 106: 261-268.
18. Oliveira CP, Faintuch J, Rascovski A, Furuya CK Jr, Bastos MS; Matsuda M, Della Nina BI, Yahnosi K, Abdala DS, Vezozzo DC, Alves VA, Zilberstein B, Garrido AB Jr, Halpern A, Carrilho FJ, Gama-Rodrigues JJ. Lipid peroxidation in bariatric candidates with nonalcoholic fatty liver disease. *Obes Surg* 2005; 15: 502-505.
19. Malaguarnera L, Di Rosa M, Zambito AM, dell, Ombra N, Nicoletti F, Malaguarnera M. Chotitriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. *Gut* 2006; 55: 1313-1320.
20. Elizondo A, Araya J, Rodrigo R, Signorini C, Sgherri C, Comporti M, et al. Effects of weight loss on liver and erythrocyte polyunsaturated fatty acid pattern and oxidative stress status in obese patients with non-alcoholic fatty liver disease. *Biol Res* 2008; 41: 59-68.
21. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. *J Nutr* 2005; 135: 969-972.
22. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; 440: 944-948.
23. Videla LA. Oxidative stress signaling underlying liver disease and hepatoprotective mechanisms. *World J Hepatol* 2009; 1: 73-79.
24. George J, Liddle C. Nonalcoholic fatty liver disease: pathogenesis and potential for nuclear receptors as therapeutic targets. *Mol Pharm* 2008; 5: 49-59.
25. Seo YS, Kim JH, Jo NY, Choi KM, Baik SH, Park JJ, Kim JS, Byun KS, Bak YT, Lee CH, Kim A, Yeon JE. PPAR agonists treatment is effective in a nonalcoholic fatty liver disease animal model by modulating fatty-acid metabolic enzymes. *J Gastroenterol Hepatol* 2008; 23: 102-109.
26. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* 2008; 134: 424-431.
27. Maher JJ, Leon P, Ryan JC. Beyond insulin resistance: Innate immunity in nonalcoholic steatohepatitis. *Hepatology* 2008; 48: 670-678.
28. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Bold MD, Parks EJ. Sources of fatt fatty liver disease. *J Clin Invest* 2005; 115: 1343-1351.
29. Fujita K, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, Endo H, Takahashi H, Inamori M, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nakajima A. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology* 2009; 50: 772-780.
30. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; 109: 1125- 1131.

31. Kallwitz ER, McLachlan A, Cotler SJ. Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. *World J Gastroenterol* 2008; 14: 22-28.
32. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004; 114:147-152.
33. Clarke S.D. Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. *Am J Physiol Gastrointest Liver Physiol* 2001; 281: 865-869.
34. Howell G. 3rd, Deng X, Yellaturu C, Park EA, Wilcox HG, Raghow R, Elam MB. N-3 polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXR alpha. *Biochim Biophys Acta* 2009; 1791: 1190-1196.
35. Dubuquoy L, Dharancy S, Nutten S, Pettersson S. Auwerx, J., Desreumaux, P. Role of peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. *Lancet* 2002; 360: 1410-1418.
36. Duplus E, Forest C. Is there a single mechanism for fatty acid regulation of gene transcription?. *Biochem Pharmacol* 2002; 64: 893-901.
37. Araya J, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, Poniachik J. Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond.)* 2004; 106: 635-643.
38. Elizondo A, Araya J, Rodrigo R, Poniachik J, Csendes A, Maluenda F, Díaz JC, Signorini C, Sgherri C, Comporti M, Videla LA. Polyunsaturated fatty acid pattern in liver and erythrocyte phospholipids from obese patients. *Obesity (Silver Spring)* 2007; 15: 24-31.
39. Pettinelli P, Araya J, del Pozo T, Ayara J, Rodrigo R, Araya AV, Smok G, Csendes A, Gutierrez L, Rojas J, Korn O, Maluenda F, Díz JC, Rencoret G, Braghetto I, Castillo J, Poniachik J, Videla LA. Enhancement in liver SREBP-1c/PPAR-a ratio and steatosis in obese patients: correlations with insulin resistance and n-3 polyunsaturated fatty acid depletion. *Biochim Biophys Acta Mol Bases Dis* 2009; 1792: 1080-1086.
40. Allard JP, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, Jaladi P, Kandasamy T, Prayitno N, Sherman M, Guindi M, Ma DWL, Heatcote JE. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 2008; 48: 300-307.
41. Delarue J, LeFoll C, Corporeau C, Lucas D. N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? *Reprod Nutr Dev* 2004; 44: 289-299.
42. Araya J, Rodrigo R, Pettinelli P, Araya AV, Poniachik J, Videla LA. Decreased Liver Fatty Acid Delta-6 and Delta-5 Desaturase Activity in Obese Patients. *Obesity* 2010; 18: 1460-1463.
43. Alwayn IP, Gura K, Nosé V, Zausche B, Javid P, Garza J, Verbesey J, Voss S, Ollero M, Andersson C, Bistrian B, Folkman J, Puder M. Omega-3 fatty acid supplementation prevents hepatic steatosis in a murine model of nonalcoholic fatty liver disease. *Pediatr Res* 2005; 57: 445-452.
44. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, Svegliati- Baroni G, Sofi F, Milani S, Abbate R, Surrenti C, Casini A. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. *Aliment Pharmacol Ther* 2006; 23: 1143-1151.
45. Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, Papa G, Rabuazzo AM, Purrello F. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. *Dig Liver Dis* 2008; 40: 194-199.
46. Denechaud PD, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. *FEBS Lett* 2008; 582: 68-73.
47. Iizuka K, Horikawa Y. ChREBP: a glucose-activated transcription factor involved in the development of metabolic syndrome. *Endocr J* 2008; 55: 617-624.
48. Schadinger SE, Bucher NL, Schreiber BM, Farmer SR. PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am J Physiol Endocrinol Metab* 2005; 288: 1195-1205.
49. Glimcher LH, Lee AH. From sugar to fat: How the transcription factor XBP1 regulates hepatic lipogenesis. *Ann NY Acad Sci* 2009; 1173 (Suppl. 1): 2-9.
50. Ahmed MH, Byrne CD. Modulation of sterol regulatory element binding proteins (SREBPs) as potential treatments for non-alcoholic fatty liver disease (NAFLD). *Drug Discov Today* 2007; 12: 740-747.
51. Yellaturu CR, Deng X, Cagen LM, Wilcox HG, Mansbach CM. 2nd., Siddiqi SA, Park EA, Raghow R, Elam MB. Insulin enhances post-translational processing of nascent SREBP-1c by promoting its phosphorylation and association with COPII vesicles. *J Biol Chem* 2009; 284: 7518-7532.
52. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* 1999; 274: 30028-30032.
53. Osborne TF. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem* 2000; 275: 32379-32382.
54. Yellaturu CR, Deng X, Park EA, Raghow R, Elam MB. Insulin enhances the biogenesis of nuclear sterol regulatory element-binding protein (SREBP)-1c by posttranscriptional down-regulation of Insig-2A and its dissociation from SREBP cleavage-activating protein (SCAP).SREBP-1c complex. *J Biol Chem* 2009; 284: 31726-31734.
55. Yahagi N, Shimano H, Hasty AH, Matsuzaka T, Ide T, Yoshikawa T, Amemiya- Kudo M, Tomita S, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. *J Biol Chem* 2002; 31: 19353-19357.
56. Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, Tran J, Tippin TK, Wang X, Lusis AJ, Hsueh WA, Law RE, Collins JL, Willson TM, Tontonoz P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci USA* 2002; 99: 7604-7609.
57. Cheng D, Chu CH, Chen L, Feder JN, Mintier GA, Wu Y, Cook JW, Harpel MR, Locke GA, An Y, Tamura JK. Expression, purification, and characterization of human and rat acetyl coenzyme A carboxylase (ACC) isozymes. *Protein Expr Purif* 2007; 51: 11-21.
58. Lemoine M, Barbu V, Girard PM, Kim M, Bastard JP, Wendum D, Paye F, Housset C, Capeau J, Serfaty L. Altered hepatic expression of SREBP-1 and PPARgamma is associated with liver injury in insulin-resistant lipodystrophic HIV-infected patients. *AIDS* 2006; 20: 387-395.
59. Younossi ZM, Gorreta F, Ong JP, Schlauch K, Del Giacco L, Elariny H, Van Meter A, Younoszai A, Goodman Z, Baranova A, Christensen A, Grant G, Chandhoke V. Hepatic gene expression in patients with obesity-related non-alcoholic steatohepatitis. *Liver Int* 2005; 25: 760-771.
60. Higuchi N, Kato M, Shundo Y, Tajiri H, Tanaka M, Yamashita N, Kohjima M, Kotoh K, Nakamuta M, Takayanagi R, Enjoji M. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol Res* 2008; 38: 1122-1129.
61. Schwarz JM, Linfoot P, Dare D, Aghajanian K. Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. *Am J Clin Nutr* 2003; 77: 43-50.
62. Timlin MT, Parks EJ. Temporal pattern of de novo lipogenesis in the postprandial state in health men. *Am J Clin Nutr* 2005; 81: 35-42.
63. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med* 2004; 10: 355-361.
64. Yeon JE, Choi KM, Baik SH, Kim KO, Lim HJ, Park KH, Kim JY, Park JJ, Kim JS, Bak YT, Byun KS, Lee CH. Reduced

expression of peroxisome proliferatoractivated receptor-alpha may have an important role in the development of non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2004; 19: 799-804.
65. Bensinger SJ, Tontonoz P. Integration of metabolism and inflammation by lipid activated nuclear receptors. *Nature* 2008; 454: 470-477.
66. Vidal-Puig AJ, Considine RV, Jimenez-Liñan M, Werman A, Pories WJ, Caro JF, Flier JS. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 1997; 99: 2416-2422.
67. Matsuzawa Y. The metabolic syndrome and adipocytokines. *FEBS Lett* 2006; 580: 2917-2921.
68. Nishida M, Funahashi T, Shimomura I. Pathophysiological significance of adiponectin. *Med Mol Morphol* 2007; 40: 55-67.
69. Cheung O, Sanyal AJ. Abnormalities of lipid metabolism in nonalcoholic fatty liver disease. *Sem Liver Dis* 2008; 28: 351-359.
70. Rahimian R, Masih-Khan E, Lo M, van Breemen C, McManus BM, Dubé GP. Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. *Mol Cell Biochem* 2001; 224: 29-37.
71. Kim KH, Hong SP, Kim K, Park MJ, Kim KJ, Cheong J. HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARgamma. *Biochem Biophys Res Commun* 2007; 355: 883-888.
72. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, Kim HH, Yang US, Yu DY, Cheong J. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. *Gastroenterology* 2007; 132: 1955-1967.
73. Kim K, Kim KH, Ha E, Park JY, Sakamoto N, Cheong J. Hepatitis C virus NS5A protein increases hepatic lipid accumulation via induction of activation and expression of PPARgamma. *FEBS Lett* 2009; 583: 2720-2726.
74. Pettinelli P, Videla LA. Up-Regulation of PPAR-{gamma} mRNA Expression in the Liver of Obese Patients: an Additional Reinforcing Lipogenic Mechanism to SREBP-1c Induction. J Clin Endocrinol Metab. [Epub ahead of print].
75. Westerbacka J, Kolak M, Kiviluoto T, Arkkila P, Sirén J, Hamsten A, Fisher RM, Yki-Järvinen H. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* 2007; 56: 2759-2765.
76. Werman A, Hollenberg A, Solanes G, Bjorbaek C, Vidal- Puig AJ, Flier JS. Ligand-independent activation domain in the N terminus of peroxisome proliferator activated receptor gamma (PPARgamma): differential activity of PPARgamma1 and -2 isoforms and influence of insulin. *J Biol Chem* 1997; 272: 20230-20235.
77. Hui Y, Yu-Yuan L, Yu-Qiang N, Wei-Hong S, Yan-Lei D, Xiao-Bo L, Yong-Jian Z. Effect of peroxisome proliferator-activated receptors-gamma and co-activator-1alpha genetic polymorphisms on plasma adiponectin levels and susceptibility of non-alcoholic fatty liver disease in Chinese people. *Liver Int* 2008; 28: 385-392.
78. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J. Reddy JK, Spiegelman BM, Staels B, Wahli W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol Rev* 2006; 58: 726-741.
79. Mascaró C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J Biol Chem* 1998; 273: 8560-8563.
80. Louet JF, Chatelain F, Decaux JF, Park EA, Kohl C, Pineau T, Girard J, Pegorier JP. Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor alpha (PPARalpha)- independent pathway. *Biochem J* 2001; 354: 189-197.
81. Rufer AC, Thoma R, Hennig M. Structural insight into function and regulation of carnitine palmitoyltransferase. *Cell Mol Life Sci* 2009; 66: 2489-2501.
82. Fan CY, Pan J, Usuda N, Yeldandi AV, Rao MS, Reddy JK. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. *J Biol Chem* 1998; 273: 15639-15645.
83. Hashimoto T, Cook WS, Qi C, Yeldandi AV, Reddy JK, Rao MS. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. *J Biol Chem* 2000; 275: 28918-28928.
84. Macdonald GA, Prins JB. Peroxisomal fatty acid metabolism, peroxisomal proliferator-activated receptors and non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2004; 19: 1335-1337.
85. Cheng Y, Dharancy S, Malapel M, Desreumaux P. Hepatitis C virus infection down-regulates the expression of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl acyl-CoA transferase 1A. *World J Gastroenterol* 2005; 11: 7591-7596.
86. Dharancy S, Lemoine M, Mathurin P, Serfaty L, Dubuquoy L. Peroxisome Proliferator-Activated Receptors in HCV-Related Infection. *PPAR Res* 2009;2009:357204.
87. Murakami K, Ide T, Suzuki M, Mochizuki T, Kadowaki T. Evidence for direct binding of fatty acids and eicosanoids to human peroxisome proliferatorsactivated receptor alpha. *Biochem Biophys Res Commun* 1999; 260: 609-613.
88. Kohjima M, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Takayanagi R, Nakamuta M Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med* 2007; 20: 351-358.
89. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J Nutr* 2001; 131: 1129-1132.
90. Videla LA. Liver NF-κB and AP-1 activation and PPAR-α expression are negatively correlated in obese patients: pro-inflammaroty implications. *Clin Nutr* 2010; 29: 687-688.
91. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr* 2005; 25: 317-340.
92. Sato R. SREBPs: protein interaction and SREBPs. *FEBS J* 2009; 276: 622-627.
93. Ferré P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res* 2007; 68: 72-82.
94. Romestaing C, Piquet MA, Bedu E, Rouleau V, Dautresme M, Hourmand-Ollivier I, Filippi C, Duchamp C, Sibille B. Long term highly saturated fat diet does not induce NASH in Wistar rats. *Nutr Metab (Lond)* 2007; 21: 4-4.