DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.

Alba Berlanga Bustos

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Doctoral Thesis

Directed by Prof. Cristóbal Manuel Richart Jurado
and
Dra. Maria Teresa Auguet Quintillà

Department of Medicine and Surgery

UNIVERSITAT ROVIRA I VIRGILI

Tarragona 2015

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FEM CONSTAR que aquest treball titulat "**Deregulation of fatty acid metabolism and cannabinoid receptors in liver of morbidly obese women with non-alcoholic fatty liver disease**", que presenta Alba Berlanga Bustos per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament de Medicina i Cirurgia d'aquesta universitat i que compleix els requeriments per poder optar a menció internacional.

Tarragona, 3 de Setembre de 2015

El director de la tesi doctoral — La codirectora de la tesi doctoral

Prof. Cristóbal Manuel Richart Jurado — Dra. Maria Teresa Auguet Quintillà

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A mis padres y

a Ana

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"El que logra empezar un camino lo tiene ya medio hecho"

Séneca

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AGRADECIMIENTOS

Me gustaría hacer mención a todas aquellas personas que, de forma directa o indirecta, han permitido la elaboración de esta tesis doctoral.

En primer lugar, dar las gracias al Profesor Cristóbal Manuel Richart Jurado por haberme brindado la oportunidad de adentrarme en el mundo de la investigación formando parte del Grupo de Estudio de Enfermedades Metabólicas e Insulin Resistencia durante estos últimos cuatro años.

A la Dra. Maria Teresa Auguet Quintillà por su gran apoyo y positivismo, así como, por enseñarme que con dedicación y constancia siempre hay tiempo para todo.

A la Dra. Salomé Martinez por su colaboración en la valoración de los resultados histológicos.

Al Servicio de Cirugía General del Hospital Universitari Sant Joan de Reus por su crucial colaboración en la realización de este trabajo.

A todos los miembros que forman, y han formado, parte del grupo de investigación. A Ximena Terra, Isabel Quesada y Josep Maria Orellana por su inestimable dedicación durante los primeros años de la tesis. A Carmen Aguilar por su alegría, sabios consejos y cuidar de todos nosotros como si de una madre se tratase. A Pilar Budesca por no tardar ni un segundo en brindar su ayuda y levantar el teléfono ante cualquier duda administrativa. A Manoli por su simpatía y amabilidad. A Esther Guiu, junto a quien empecé esta aventura del doctorado, gracias por compartir codo con codo el trabajo de laboratorio y ser mi confidente en momentos difíciles. A Natàlia Enguix por su ayuda con el diseño gráfico. Y finalmente, a Gemma Aragonès y Sandra Armengol, por su gran apoyo y prácticos consejos durante el último periodo de la tesis, y quizás el más estresante. Gracias.

A todos los compañeros/as de la Unitat de Recerca del Hospital Universitari de Joan XXIII. En especial, a Ana, Javi, Vero y a mi “tronks”, por hacer amenas las horas de trabajo. También a Esther Rodriguez, que a pesar de su tardía incorporación, agradezco su alegría, ánimos y consejos.

AGRADECIMIENTOS

A mis amigas Núria, Eli, Lorena, Elena y las chicas de la Uni por los ánimos y el apoyo durante este largo recorrido, así como, por demostrar que la verdadera amistad es aquella que no entiende de distancias. Hacer especial mención a “Gema Gemita Gema”, quien de forma inesperada me hizo saber de la existencia de la beca predoctoral ofertada por la Universidad, y que posteriormente, me fue concedida.

A mi familia, por enseñarme los valores importantes a seguir en la vida, apoyarme en todas las decisiones que tomo, escucharme, entenderme y confiar en mí. Gracias por vuestra paciencia y comprensión. También me gustaría agradecer a “mi yayo y mi yaya”, quienes empezaron conmigo este camino y ahora están siempre conmigo en mi corazón.

Para finalizar, agradecer también a todos los pacientes que han participado en los estudios, así como, a los miembros del tribunal y evaluadores externos de la tesis.

Muchas gracias a todos.

AASLD: the American association for the Study of Liver Diseases

ACC1: acetyl-coenzyme A carboxylase 1

ACG: the American College of Gastroenterology

ADIPOR2: adiponectin receptor 2

AGA: the American Gastroenterological Association

ALP: alkaline phosphatase

ALT: alanine aminotransaminase

ApoB: apolipoprotein B

ARBs: angiotensin receptors blockers

AST: aspartate aminotransaminase

AT: adipose tissue

BMI: body mass index

CB1: cannabinoid receptor 1

CB2: cannabinoid receptor 2

CD36: hepatic fatty acid translocase

ChREBP: carbohydrate response element binding protein

CK18: caspase-cleaved cytokeratin-18

CPT1: carnitine palmitoyl-transferse 1

CPT2: carnitine palmitoyl-transferse 2

CRP: c-reactive protein

CT: computed tomography

CVD: cardiovascular disease

DAG: diacylglycerol

DNL: *de novo* lipogenesis

EC: endogenous cannabinoids

ECM: extracellular matrix

ER: endoplasmic reticulum

FA: fatty acids

FABP4: fatty acid binding protein 4

FAS: fatty acid synthase

FATP: fatty acid transporter protein

FFA: free fatty acids

FxR: farnesoid X receptor

GGT: γ-glutamyl transferase

GLP-1: glucagon-like peptide-1

GNG: gluconeogenesis

GWAS: genome-wide association studies

HbA1c: glycosylated hemoglobin

HCC: hepatocellular carcinoma

HDL: high density lipoprotein cholesterol

HGP: hepatic glucose production

HOMA2-IR: homeostatic model assessment method insulin resistance

HSC: hepatic stellate cells

HSL: hormone-sensitive lipase

IL1β: interleukin 1β

IL6: interleukin 6

IL8: interleukin 8

IR: insulin resistance

KC: kupper cells

LDL: low density lipoprotein cholesterol

LPL: lipoprotein lipase

LPS: lipopolysacharide

LxRα: liver X receptor alpha

MetS: metabolic syndrome

MR: nuclear magnetic resonance

mTORC1: mammalian target of rapamycin complex 1

MTP: microsomal triglyceride transfer protein

MUFA: monounsaturated fatty acids

NAFLD: non-alcoholic fatty liver disease

NAS: NAFLD activity score

NASH: non-alcoholic steatohepatitis

NEFA: non-sterified fatty acids

NFS: NAFLD fibrosis score

NL: normal liver

PKCε: protein kinase-Cε

PNPLA3: patatin-like phospholipase domain-containing protein 3

PPARα: peroxisome-proliferator-activated receptor α

PPARγ: peroxisome-proliferator-activated receptor γ

PPARδ: peroxisome-proliferator-activated receptor δ

PTX: Pentoxyphylline

PUFAs: polyunsaturated fatty acids

QM: chylomicrons

RAS: renin–angiotensin system

ROS: reactive oxygen species

SFA: saturated fatty acids

SREBP1c: sterol-regulatory-element-binding protein

SS: Simple steatosis

T2DM: type 2 diabetes mellitus

TCA: tricarboxylic acid cycle

TG: triglycerides

TGFβ1: transforming growth factor beta-1

TNFRI: tumour necrosis factor receptor I

TNFRII: tumour necrosis factor receptor II

TNFα: tumour necrosis factor alpha

TZD: thiazolidinedione

UDCA: Ursodeoxycholic acid

VLDL: Very low density lipoprotein

WAT: white adipose tissue

WC: waist circumference.

II. Introduction

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1. NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease (NAFLD) is becoming an increasingly important health issue due to the fact that it is the most common cause of chronic liver disease in the Western world, and its incidence is increasing rapidly[1]. NAFLD is a progressive inflammatory disease that encompasses a wide spectrum of liver damage, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Simple steatosis is defined by hepatic fat accumulation in form of triglycerides (TG), exceeding 5% of liver mass, in the absence of high alcohol intake. NASH includes the presence of simple steatosis, lobular inflammation, and hepatocellular injury (balloning) with or without fibrosis[2,3]. While simple steatosis is generally a benign, non-progressive clinical entity, NASH can progress to cirrhosis, which in rare cases gives rise to hepatocellular carcinoma (HCC)[4] (**Figure 1**).

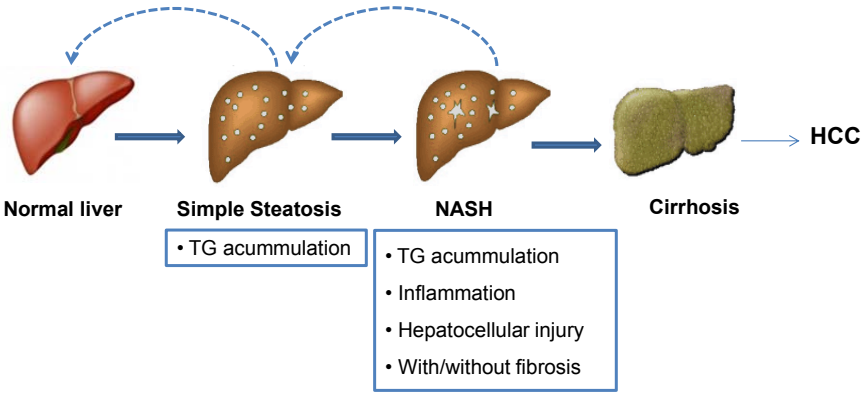


Figure 1. The disease spectrum of non-alcoholic fatty liver disease. The accumulation of triglycerides (TG) within lipid droplets in hepatocytes causes simple steatosis. Simple steatosis associated with lobular inflammation and hepatocellular injury (ballooning) with or without fibrosis is referred to as NASH. Both simple steatosis and NASH are considered reversible through weight-loss, changes in diet and increased physical activity. However, NASH can progress to irreversible fibrosis, leading to cirrhosis and a high risk of hepatocellular carcinoma (HCC) [5].

NAFLD has frequently been associated with components of the metabolic syndrome (MetS) such as obesity, dyslipidaemia, insulin resistance (IR) and type 2 diabetes mellitus (T2DM)[6,7], and thought to represent the hepatic manifestation of MetS. However, many people with NAFLD are not obese, and many people with NAFLD do not have T2DM. Increasing evidence indicates that the presence and severity of NAFLD not only further increases the risk of T2DM, but is also an independent risk factor of cardiovascular and chronic kidney disease; this suggests that NAFLD is a multisystem disease, affecting extra-hepatic organs and regulatory pathways. Although the primary liver pathology in NAFLD affects hepatic structure and function, causing morbidity and mortality from cirrhosis, liver failure and HCC, the majority of deaths among NAFLD patients are attributable to cardiovascular disease (CVD)[8,9].

Table 1 shows the risk factors associated with NAFLD. Although obesity, insulin resistance, T2DM, and dyslipidaemia are the most important risk factors, other endocrine conditions are also associated with NAFLD[2].

Table 1. Risk factors associated with NAFLD

Conditions with established associations	Conditions with emerging associations
Obesity	Polycystic ovary syndrome
Insulin resistance	Hypothyroidism
Type 2 diabetes mellitus	Obstructive sleep apnea
Dyslipidaemia	Hypopituitarism
Metabolic syndrome*	Hypogonadism
	Pancreato-duodenal resection

*Metabolic syndrome, as defined by the Adult Treatment Panel (ATP) III criteria, is defined by the presence of three or more of the following: (1) waist circumference greater than 102 cm in men or greater than 88 cm in women; (2) triglyceride level higher than 150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides; (3) highdensity lipoprotein (HDL) cholesterol level lower than 40 mg/dL (1.03 mmol/L) in men and less than 50 mg/dL (1.29 mmol/L) in women or on drug treatment for low HDL; (4) systolic blood pressure ≥130 mm Hg or diastolic pressure ≥85 mm Hg or treatment for hypertension; and (5) fasting plasma glucose level≥110mg/dL or drug treatment for elevated blood glucose[10].

1.1 NAFLD epidemiology

The prevalence of NAFLD worldwide varies between different countries and regions, which can be divided into three different groups: high, low and unknown prevalence groups[11]. However, the globally accepted worldwide prevalence of NAFLD based on a variety of assessment methods ranges from 6.3% to 33%, with a median of 20% in the general population, while the estimated prevalence of NASH is lower, ranging from 3 to 5%[12]. Moreover, NAFLD has been reported as more prevalent among obese subjects and in patients with type 2 diabetes, regardless of the degree of obesity. The prevalence increases to 57% in obese subjects, 70% in diabetic subjects and 90% in morbidly obese people[13].

The high prevalence group is mostly represented by "Western" countries where the population has an “urban” lifestyle. This group includes the United States and Europe, as well as the Middle- East, Latin America, Australia, Japan and China. The prevalence rates are constantly and rapidly increasing in these countries, due to the increase in obesity, IR, lipid impairments and T2DM related to incorrect dietary habits and sedentary life-styles. The prevalence of NAFLD In these populations ranges between 20% and 30%, and the prevalence of NASH from 3% to 16%.

The low prevalence group is mostly represented by Asian countries and developing countries. The majority of the population in these countries resides in rural areas where traditional diets and lifestyles are still present, and the reported prevalence of NAFLD is therefore about 10%. Nevertheless, in these same countries, people that reside in urban areas have a higher prevalence, similar to rates in Western countries. These data suggest that adoption of a sedentary lifestyle and the globalization of the Western diet are associated with an increase in the prevalence of NAFLD in developing countries.

The unknown prevalence group is mostly represented by "third-world" countries, and particularly those in Africa. As these are developing countries, with a high proportion of the population residing in rural areas, it

is reasonable to expect that the prevalence should be similar to the "low prevalence group"[11]. Indeed, a Nigerian study estimated the prevalence at around 9%[14].

Apart from obesity, IR, dyslipidaemia, MetS and diabetes, ***age***, ***gender***, and ***ethnicity*** are also risk factors associated with a differential prevalence for NAFLD. A number of studies have shown that the prevalence of NAFLD and NAFLD-related fibrosis increases with age[2,12], and reach the higher value in the age ranges between 40 and 65 years[11]. Although a smaller prevalence in children (2.6-9.6%) compared to adults was previously reported, it is rapidly increasing, particularly among obese children, ranging between 10-80%[15], in whom NAFLD has become the most common cause of chronic liver disease[16]. This is likely to expose patients to liver injury for a longer period of time, increasing the risk of progression and complications, and could change the natural history of this disease. This is currently represents a major public health problem, which requires the full attention of Institutions responsable for providing appropriate prevention strategies in the near future[11].

It is now a universally accepted fact that there is a higher prevalence in men than in women, perhaps related to men's tendency towards visceral obesity[11]. Most epidemiological studies have demonstrated that NAFLD is almost twice prevalent in males compared to females. For instance, the prevalence of NAFLD in the Dallas Heart Study was 42% in white men, compared with only 24% in white women and this difference was not attributable to differences in body weight or insulin sensitivity[17]. Studies have suggested that estrogen may reduce the risk of developing NAFLD in women[18].

The contribution of ethnicity to the prevalence of NAFLD has been extensively studied. Various USA-based studies have demonstrated that the rates of NAFLD are decreasing at different rates among East Asian Indians (higher rates), Hispanics, Asians, white Caucasians and African Americans (lower rates), with the latter apparently protected from developing NAFLD

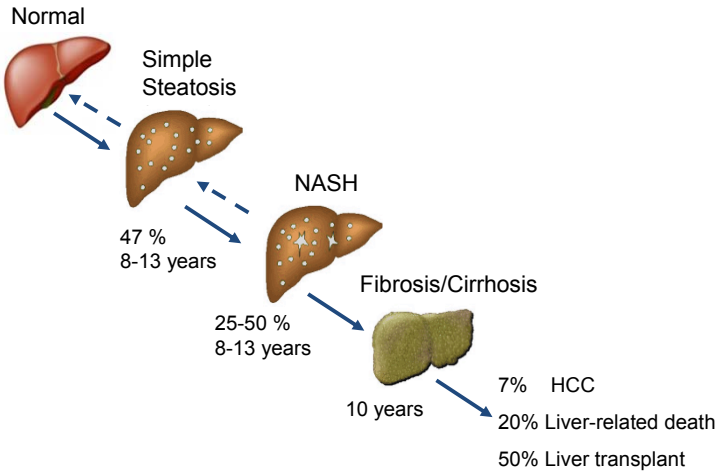
despite higher rates of obesity[11,19]. Recently, in accordance with these results, Williams *et al.* evaluated 328 individuals who underwent right upper quadrant ultrasound examinations, and found that Hispanics have the highest prevalence of NAFLD (58%) followed by Caucasians (44%) and African-Americans (35%)[20].

Smoking and **alcohol intake** are other risk factors associated with NAFLD. Modest alcohol consumption (one alcoholic beverage per day) had not been shown to increase the prevalence of NAFLD, and modest wine consumption would appear to reduce its incidence[21]. In a retrospective study, Hamabe *et al.* demonstrated over a 10-year period that smoking was an independent risk factor for NAFLD regardless of the presence of other metabolic risk factors[22]. Furthermore, the incidence of advanced hepatic fibrosis is high in smokers[23].

1.2 Natural history of NAFLD

Progression in NAFLD is difficult to assess, requiring years of follow-up and repeat biopsies that are prone to sampling errors[24]. The prognosis of subjects with NAFLD depends on the histological subtype of the disease. Subjects with simple steatosis (SS) have a relatively benign course and are at low risk of developing liver-related morbidity or mortality. Today, SS is widely accepted as a 'benign' adaption to lipid loading in the liver. By contrast, NASH is more frequently progressive and may lead to cirrhosis with complications of HCC, liver failure and liver-related death, or the need for liver transplantation[25]. Although only a small percentage of patients with NAFLD will eventually develop cirrhosis, and given the large number of patients with NAFLD, this represents a significant disease burden. Estimates suggest that approximately 5% of patients with NAFLD develop cirrhosis[26].

A long prospective study of the natural history of NAFLD using repeat biopsies, has reported that 47% of patients with SS progressed to NASH, and 25–50% of patients with NASH developed advanced fibrosis or cirrhosis in 8–13 years[27]. Patients with NAFLD-associated cirrhosis have been shown to have a very poor 10-year prognosis, with 50% needing a liver transplant, 20% dying from a liver-related cause and 7% developing HCC[28] (**Figure 2**).



Adapted from Proceedings of the Nutrition Society (2010), 69, 211–220[24]

Figure 2. Progression and stages of NAFLD

More recently, in a long-term follow-up evaluation of NAFLD patients, Rafiq *et al.* confirmed that NASH patients have increased liver related mortality compared to non-NASH patients[29]. This study also revealed that independent predictors of liver-related mortality not only include having histological NASH, but also T2DM and older age.

Both NASH and T2DM increase the risk of hepatocellular carcinoma. The underlying mechanisms by with NASH or T2DM increase the risk of developing HCC are not fully understood, but mechanisms involved in inflammation, metabolic stress and IR that are shared between NASH and T2DM may be also involved in HCC development[9]. Indeed, NASH is the third most common indication for liver transplantation in the

United States, and NASH has been predicted to be the most common underlying disease in patients requiring liver transplantation in 2020[30]. In addition to liver-related mortality, subjects with NASH are at increased risk of cardiac morbidity and mortality[25].

1.3 Diagnosis and staging of NAFLD

The American association for the Study of Liver Diseases (AASLD), the American College of Gastroenterology (ACG) and the American Gastroenterological Association (AGA) published new guidelines for the diagnosis and management of NAFLD[2]. They stipulated that the diagnosis of NAFLD requires **(a)** evidence of hepatic steatosis either by imaging or by histology, **(b)** the absence of significant alcohol consumption, and **(c)** the absence of secondary causes of hepatic steatosis. Common secondary causes of hepatic steatosis are summarized in **Table 2**.

Table 2. Common causes of Secondary Hepatic Steatosis

Macrovesicular steatosis
Excessive alcohol consumption
men: >21 drinks/week or 30g/day
women: >14 drinks/week or 20g/day
Hepatitis C
Wilson's disease
Lipodystrophy
Starvation
Parenteral nutrition
Abetalipoproteinemia
Medications (e.g corticosteroids)
Microvesicular steatosis
Reye's syndrome
Medications (e.g anti-retroviral medicines)
Acute fatty liver of pregnancy
HELLP syndrome
Inborn errors of metabolism

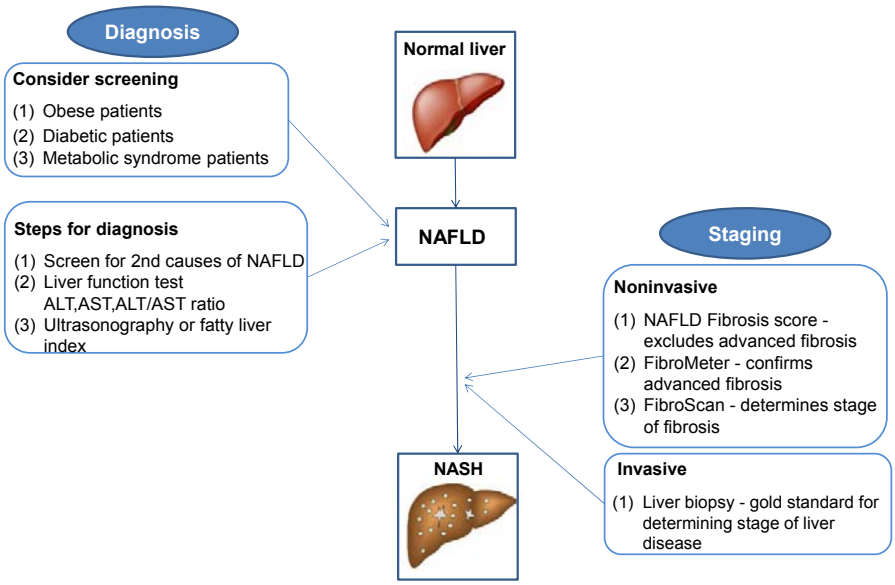
Gastroenterology 2012;142:1592-1609[2]

NAFLD should be suspected in individuals who are either obese, diabetic or have metabolic syndrome. However, it is frequently underdiagnosed, as it is mostly asymptomatic. Several non-invasive methods are used for diagnosis of NAFLD. In clinical practice, plasma liver aminotransferase levels and ultrasound are the most common diagnostic techniques[31]. Elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the absence of other liver diseases may support NAFLD, and an AST/ALT ratio less than 1 is also seen in NAFLD and supports NASH[32]. Both aminotransferase levels and ultrasonography are widely available, easy to perform and have a low cost; however, they have low sensitivity for fatty liver. Other imaging techniques, such as computed tomography (CT) and nuclear magnetic resonance (MR) can also detect liver steatosis. CT provides a semiquantitative method and can be used to diagnose moderate to severe hepatic steatosis, but has relatively low sensitivity and is not usually used for this purpose. MR remains the gold-standard technique, with a high sensitivity and specificity for steatosis (5.5 % intrahepatic TG content considered diagnostic for NAFLD), involves no radiation and allows quantification of liver steatosis, but it has limited availability and requires expensive hardware and software, making each test costly [31]. On the other hand, the Fatty Liver Index is an algorithm based on four markers: body mass index (BMI), waist circumference, triglyceride and γ-glutamyltransferase (GGT), which has been confirmed as accurately identifying NAFLD[32] (**Figure 3**).

It is important to note that serum aminotransferase levels and imaging tests do not reliably assess the presence of steatohepatitis and fibrosis in patients with NAFLD. Liver biopsy is currently the gold standard for characterizing liver histology (the degree of hepatocyte injury and level of fibrosis and inflammation) in patients with NAFLD. However, it is a procedure that is invasive and expensive, and involves some morbidity and very rare mortality risk. Nevertheless, liver biopsy should be considered in those patients with fatty liver who after imaging techniques, laboratory

abnormalities and/or non-invasive methods, are considered to be at increased risk of having NASH and advanced fibrosis [2].

Since liver biopsy still remains the gold standard, there is a significant interest in developing non-invasive biomarkers for identifying steatohepatitis in NAFLD. In this respect, circulating levels of caspase-cleaved cytokeratin-18 (CK18) fragments have been investigated as a promising biomarker for the presence of steatohepatitis in patients with NAFLD[33]. Moreover, recent breakthroughs have enabled non-invasive techniques to be used to diagnose the level of fibrosis/inflammation (**Figure 3**). The NAFLD fibrosis score (NFS) evaluates six variables: age, hyperglycemia, BMI, platelet count, albumin and AST/ALT ratio. This tool is widely used in practice to exclude advanced fibrosis. Another tool used in clinical practice is the FibroMeter, which uses age, weight, fasting glucose, AST, ALT, ferritin and platelet count to diagnose significant fibrosis and the percentage of hepatic fibrosis. This tool provides a more reliable diagnosis for significant fibrosis than the NFS; it can be used to confirm or rule out advanced fibrosis in NAFLD patients. FibroScan, also known as transient elastography, is another non-invasive method for assessing liver fibrosis. This method measures liver stiffness, and was originally designed for the hepatitis C population, but is being used in the NAFLD population to determine the stage of fibrosis. However, it has been shown to provide inacurate liver stiffness measurement in overweight and obese patients[32].



Adapted from Schwenger K et al. Clinical approaches to NAFLD. World J Gastroenterol 2014[32]

Figure 3. Diagnosis and staging of non-alcoholic fatty liver disease

1.4 Histopathology of NAFLD

Important pathological classifications of NAFLD/NASH were proposed by Matteoni, by Brunt and the NASH Clinical Research Network (CRN) Pathology Committee. In the 1999 classification by Matteoni *et al*. [34], the authors distinguished between NASH and non-NASH. They defined histological types 1 and 2 of NAFLD as "non-NASH", while types 3 and 4 were classified as NASH. When Matteoni's classification was published, Brunt *et al*.[35] proposed a semiquantitative grading and staging system for NASH. The authors graded steatosis, inflammation and ballooning degeneration, and the staging was based on the degree of fibrosis. This classification is only applicable to NASH, and not to the entire spectrum of NAFLD. In 2005, the pathology committee of the NASH CRN group therefore developed and validated a histological scoring system for use in NAFLD, based on Brunt's classification[3] (**Table 3**).

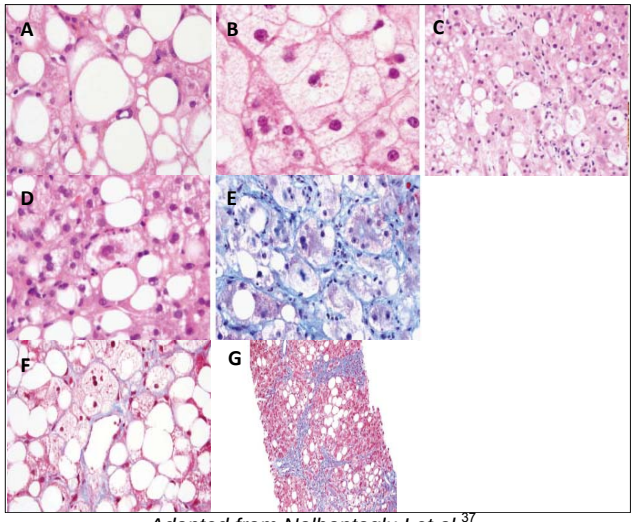
The NAFLD activity score (NAS) system is applicable to both adults and pediatric patients. The NAS represents the sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning degeneration (0-2). An NAS of 5 or more correlates with the diagnoses of NASH, scores of less than 3 correlates with "not NASH", while scores of 3 or 4 are uncertain for the diagnosis of NASH. Fibrosis is scored separately to give a fibrosis stage of between 0 and 4 (**Table 3**).

In short, the histological spectrum of NAFLD is characterized by steatosis, lobular inflammation, ballooning of hepatocytes, fibrosis, and other features that may or may not be present, such as Mallory–Denk bodies and portal inflammation. The NASH CRN grading and staging system of NASH is based on the use of haematoxylin and eosin and Masson's trichrome stain, so it can be used routinely by histopathologists[36]. **Figure 4** shows the histologic features, grading, and staging of NAFLD.

Table 3. Histological scoring system.

Steatosis grade	*Lobular inflammation*	*Hepatocyte balloning*
0: <5%	**0:** None	**0:** None
1: 5-33%	**1:** <2 foci	**1:** Mild
2: 34-66%	**2:** 2-4 foci	**2:** Moderate
3: >66%	**3:** >4 foci	
	NAFLD activity score (NAS)	**0-2** not NASH
		3-4 uncertain for NASH
		5-8 NASH
Fibrosis Staging		
Stage 1: 1A. Mild zone 3 perisinusoidal fibrosis; 2B. Moderate zone 3 perisinusoidal fibrosis; 1C. Portal fibrosis		
Stage 2: perisinusoidal and portal/periportal fibrosis		
Stage 3: bridging fibrosis		
Stage 4: cirrhosis		

From Kleiner et al.[3]



Adapted from Nalbantoglu I et al.[37]

Figure 4. Histologic features, grading, and staging of NAFLD. **A**: Mixed large and small droplet steatosis, single droplet, with nucleus pushed to one side, HE stain, 600×; **B**: Microvesicular steatosis, HE stain, 600 ×; **C**: Ballooned hepatocytes with flocculent cytoplasm, HE stain, 600 ×; **D**: Mallory-Denk body, HE stain, 600 ×; **E**: Mallory-Denk body in blue-green color and dense perisinusoidal fibrosis, Trichrome stain, 600 ×; **F**: Delicate perisinusoidal fibrosis, Trichrome stain, 600 ×; **G**: Bridging fibrosis, Trichrome stain, 200 ×.HE: haematoxylin and eosin

1.5 Management of NAFLD

The best approach for the management of NAFLD remains controversial due to the incomplete understanding of the natural history of the disease. While many cases of NAFLD can be diagnosed using plasma aminotransferase levels and imaging, a liver biopsy is still required to confirm the diagnosis and stage of the disease[31].

The most recent guidelines suggest management and treatment of patients with NAFLD, considering both the liver disease and the associated metabolic co-morbidities, such as obesity, dyslipidaemia, IR and T2DM[2]. At present, there are three approaches to focusing on management strategies in NAFLD: lifestyle modification, liver-directed pharmacotherapy, and managing the complications of cirrhosis[38]. All patients with NAFLD require advice about lifestyle modification, as well as treatment of any associated metabolic risk factors. However, patients with NASH and fibrosis require more aggressive lifestyle modification and if this fails liver-directed pharmacotherapy can be considered. For patients who have progressed to cirrhosis, surveillance for HCC is essential and some treatments have been shown to reduce the risk of HCC[38]. Interventions for the treatment of NAFLD are summarized in **Table 4**.

1.5.1. Lifestyle modification

Reduction in caloric intake and exercise: lifestyle modification, aimed at weight loss and increased physical activity, is still the mainstay of management of patients with NAFLD, irrespective of their underlying liver histology. A calorie-restricted diet (600 Kcal less than a person needs to remain at the same weight) should be recommended aiming to lose 0.5–1 kg per week until the target weight is achieved. Ideally, patients should be encouraged to lose >10% of their body weight and maintain the weight loss[38]. Previous studies have shown that >7%[39] and ≥9%[40] loss of body weight was associated with reduced steatosis, hepatocellular injury and hepatic inflammation. A higher level of physical activity is associated with

lower levels of steatosis[41]. Although the optimum exercise to treat NAFLD is not known, aerobic exercise has been shown to improves insulin sensivity[42]. Furthermore, moderate, high and resistance exercise have shown improvements in liver enzymes and reduction in liver fat, independent of weight loss, but the effects on histology remain unknown[38]. One approach is to recommend 30 min of moderate exercise five times weekly[43].

Weight loss therapies: a weight loss therapy using pharmacological agents can be considered for subjects who have not achieved their target weight by lifestyle intervention and have a BMI >30 kg/m^2. National Institute of Health and Care Excellence (NICE) guidelines[43] for obesity recommend Orlistat in these cases. Orlistat is an enteric lipase inhibitor that leads to malabsorption of dietary fat and can aid weight loss in subjects with obesity in conjunction with lifestyle modification. Zelber-Sagi *et al.* reported that Orlistat improved ALT and steatosis, but its effect on liver histology could not be evaluated[44]. However, Harrison *et al.* demonstrate that Orlistat did not improve body weight or liver histology[40].

The endocannabinoid CB1 receptors expressed in hepatocytes and hepatic myofibroblasts contribute to high fat storage, alcohol-induced steatosis, fibrogenesis and insulin resistance. In contrast to CB1, although the role of CB2 receptors in the development of fatty liver has not been extensively investigated, there is some evidence of a potentially anti-fibrotic effect of CB2 receptor activation. CB1 receptor antagonism and CB2 receptor agonism have therefore been identified as promising therapeutic strategies for the management of liver diseases[45]. Rimonabant (a CB1-antagonist) was initially approved for the management of overweight, liver steatosis and related cardio-metabolic risks, but it was withdrawn due to an alarming rate of adverse effects on mood, which were primarily psychiatric in nature due to its concentration in the brain[46].

Meanwhile, NICE guidelines suggest that bariatric surgery should be considered as a treatment for obesity in patients with BMI > 40 kg/m^2 or between 35 and 40 kg/m^2 with another significant disease that could be improved with weight loss, as well as a first-line option for the treatment of

obesity in adults with a BMI greater than 50 kg/m^2. Weight loss after bariatric surgery has beneficial effects on the components of the metabolic síndrome, including improving insulin sensitivity and the lipid profile, as well as reducing long-term mortality[47,48]. It also has specific effects on liver histology, including reduced steatosis, steatohepatitis and fibrosis[49]. However, it cannot be considered as a primary treatment for NASH[1].

1.5.2 Liver directed-pharmacotherapy

1.5.2.1 Targeting components of the metabolic syndrome

Insulin sensitizers: given the importance of IR in the pathogenesis of NAFLD, insulin sensitizers such as metformin, thiazolidinedione (TZDs) and incretin-based therapies have been extensively studied in the treatment of T2DM and the MetS in NAFLD[50].

Metformin is a biguanide insulin sensitizer, whose major action is mediated through activation of AMPK. Although metformin has no significant effect on liver histology and is not recommended as a specific treatment for liver disease in adults with NASH[2], it is recommended as the first-line pharmacological treatment for T2DM. This improves peripheral glucose intake, reduces hepatic gluconeogenesis and lipogenesis, and also increases beta oxidation of free fatty acids[51].

TZDs are agonists for the peroxisome-proliferator-activated receptor γ, a transcriptor factor that is very abundant in adipose tissue, and their actions in NAFLD are probably indirect and largely relate to their effects on adipose tissue. Since several studies have demonstrated that Pioglitazone improves insulin sensitivity as well as reducing hepatic steatosis and inflammation in subjects with NASH and T2DM, it has been considered a second-line treatment of T2DM in subjects with NASH[52,53]. However, there are also some concerns about its long-term safety due to its association with weight gain, increased risk of congestive cardiac failure, bladder cancer and reduced bone density[38].

Glucagon-like peptide-1 (GLP-1) is an incretin secreted by ileal L-cells dependent on the presence of food in the small intestine. It increases insulin sensitivity, inhibits gastric emptying and decreases food intake by increasing satiety in the brain. Analogues of GLP-1 should be considered as an alternative to insulin as a third-line treatment of T2DM in subjects with NASH. They reduce glycosylated hemoglobin (HbA1c) by 1% and patients frequently lose an average of 3 kg weight[38]. Furthermore, they have been associated with an improvement of liver enzymes as well as reduced steatosis[54]. However, recent evidence suggests that an GLP-1 agonist might induce pancreatitis[55].

Lipid lowering drugs: as mentioned above, patients with NAFLD and NASH are at an increased risk of CVD, and several studies have established CVD as their most common cause of death[56]. An effective treatment of dyslipidaemia is therefore vital in the management of NAFLD to reduce patients' cardiovascular risk profile. Statins, fibrates and omega-3 polyunsaturated fatty acids (PUFAs) are used to manage dyslipidaemia in patients with NAFLD and NASH[57]. Several studies have provided compelling evidence that lipid-lowering agents are safe and effective in patients with either NAFLD or NASH, and that some of these agents can induce a reduction in the extent of the hepatic steatosis[57].

Hypertension: angiotensin receptor blockers (ARBs) are widely used as antihypertensive agents with a well-characterized safety profile. Targeting the renin–angiotensin system (RAS) might be beneficial in all patients with NAFLD, as the RAS plays a role in liver fibrogenesis and blocking the RAS inhibits the proliferation of stellate cells, and reduces both inflammation and fibrosis[58]. Small-sample human studies have suggested that ARBs improve serum liver enzyme levels, insulin sensitivity and histological features of NAFLD[59]; however, further larger clinical trials are needed to corroborate these findings. A previous meta-analysis also showed a 20% reduction in the incidence of new onset diabetes with the use of ARBs[60].

1.5.2.2 Antioxidants and cytoprotective therapies

Vitamin E: vitamin E, a fat-soluble vitamin, has an anti-oxidant property and has been widely investigated for the treatment of NAFLD and NASH in both pediatric and adult populations. It has been shown to be associated with decreased aminotransferase levels and improvements in steatosis, inflammation, ballooning and resolution of steatohepatitis in adults with NASH[61]. Similar results were obtained in a pediatric population (the TONIC study)[62]. However, there are concerns about its long-term safety, since it has been shown to increase all-cause mortality, the risk of haemorrhagic stroke and prostate cancer at high doses[38]. Vitamine E is recommended as a first-line treatment for NAFLD patients with histologically confirmed NASH in the actual NAFLD AASLD guidelines, but it is not recommended for NASH patients with diabetes[2].

Ursodeoxycholic acid (UDCA): UDCA is a hydrophilic bile acid with cytoprotective and antioxidant properties. However, only limited data on the efficacy of UDCA in NAFLD is available[63]. In fact, two well-designed randomized controlled trials showed that UDA failed to show any significant improvement in the liver histology of patients with biopsy-proven NASH[64,65]. AASLD guidelines do not recommend UDCA for the treatment of patients with NAFLD or NASH[2].

1.5.2.3 Other drugs

The manipulation of microbiota through probiotics, prebiotics, and antibiotics is providing encouraging results for the treatment of obesity, diabetes, and NAFLD/NASH, but data in humans are scarce. It has been shown that probiotics may reduce NAFLD liver injury and may improve liver enzymes. Moreover, fructo-oligosaccharides are now becoming increasingly popular due to their prebiotic effects, but the existing data are difficult to reconcile, given the use of different strains, dosages and durations of treatment. Studies in an experimental model of non-alcoholic steatohepatitis indicate that anti- tumour necrosis factor alpha (TNFα) antibodies are

effective against liver necrosis, inflammation and fibrosis, but more studies on animal models are needed before testing these molecules on humans[45]. Furthermore, Pentoxyphylline (PTX) is a TNFα inhibitor which has been also evaluated in NASH for its anti-inflammatory properties. A meta-analysis of randomized, double-blinded, placebo-controlled trials showed that PTX could reduce aminotransferase activities and improve histological parameters in NAFLD patients[66]. These results need to be confirmed in larger studies, and the underlying mechanism responsible for the beneficial effects of PTX in NASH remains unidentified. Finally, elevated iron indices are described in NAFLD, and as such, iron reduction has been suggested as a potential therapy[45].

1.5.3 Managing the complications of cirrhosis and liver transplantation

Patients with NASH cirrhosis are at risk of the same complications of cirrhosis as with any other aetiology of liver disease, such as hepatocellular carcinoma. There is emerging evidence that metformin reduces the risk of cancer (including HCC) in patients with diabetes in a dose-dependent manner[67], and statins might reduce the incidence of HCC[68]. Moreover, in patients with end-stage liver disease due to NAFLD, liver transplantation is the only definitive treatment[69].

Table 4. Interventions for the treatment of NAFLD

Lifestyle intervention	**Weight loss**	Calorie restricted diet	
		Anti-obesity drugs:	Orlistat Rimonabant
		Bariatric surgery	
	Exercise	30 min moderate 5x/week	
Targeting components of the metabolic syndrome	**Insulin sensitizers**	Metformin	
		Thiazolidinedione (TZDs):	Pioglitazone
		Incretin based therapies:	GLP-1 analogues
	Dyslipidaemia	Statins	
		Fibrates	
		Omega-3 polyunsaturated fatty acids (PUFAs)	
	Hypertension	Angiotensin receptor blockers (ARBs)	
Antioxidants and cytoprotective therapies	**Vitamin E**		
	Ursodeoxycholic acid (UDCA)		
Others	**Probiotics/Prebiotics**		
	Anti-TNFα antibodies		
	Pentoxifylline (PTX)		
	Iron depletion		

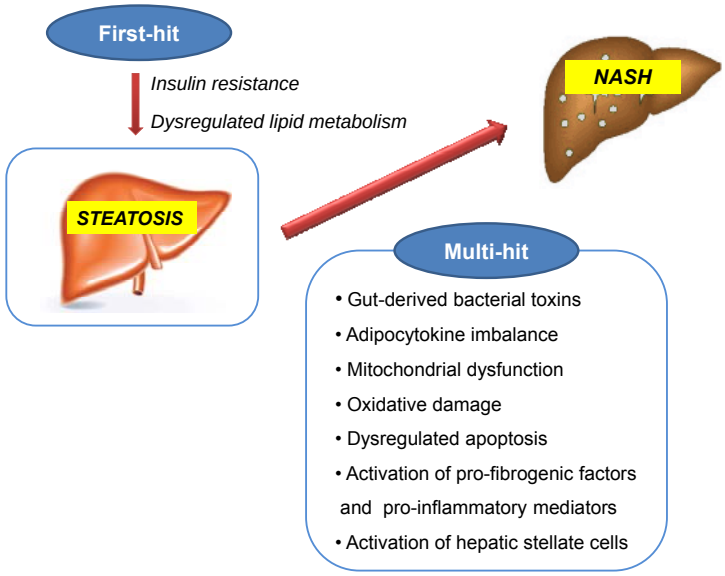
2. PATHOGENESIS OF NAFLD

2.1 The two-hit vs multi-hit hypothesis

The molecular mechanism underlying NAFLD progression has often been interpreted in terms of a "double-hit" process, which was proposed in 1998 by Day *et al*[70]. The primary insult or the "first hit" includes the accumulation of TG and free fatty acids (FFA) in hepatocytes. Insulin resistance and subsequent hyperinsulinemia, typically associated with weight gain or obesity, appears to be the key component of the "first hit" that results in hepatic steatosis. These changes have been postulated as resulting in increased sensitivity to the "second hit", which involves pro-inflammatory cytokines, mitochondrial dysfunction, oxidative stress and subsequent lipid peroxidation, leading to hepatocyte damage, inflammation and the development of steatohepatitis.
This paradigm suggests that in the "first hit", TG accumulation predisposes to further liver damage in the pathogenesis of NASH. However, it has recently been replaced by a more complex model in which hepatic TG accumulation seems to be a benign symptom of hepatic steatosis, while FFA and TG-derived metabolites may be the true lipotoxic agents that contribute to the development of NASH; and TG breakdown via metabolic lipases contributes to the pathogenesis and progression of NAFLD[71].

The classic "two-hit" hypothesis has been modified by the "multiple parallel hits" hypothesis to explain the development of NAFLD and the progression from simple steatosis to NASH[72]. In the "multi-hit" hypothesis, imbalanced lipid metabolism and insulin resistance are considered as the "first hit", leading to the development of hepatic steatosis. After the development of steatosis, the liver becomes more vulnerable to many hits that may act in parallel, including gut-derived bacterial toxins, adipokine/cytokine imbalance, mitochondrial dysfunction, oxidative damage, dysregulated hepatocyte apoptosis, release of pro-fibrogenic factors and

pro-inflammatory mediators from impaired organelles, and hepatic stellate cell and Kupffer cell activation. These factors may collectively stimulate inflammation, apoptosis, fibrosis and finally tumour development[73] (**Figure 5**). Iron accumulation and genetic factors are also involved in the development of NASH[74].



Adapted from Int. J. Mol. Sci. 2014, 15, 6184-6223[73]

Figure 5. The Multi-hit hypothesis of NAFLD pathogenesis. The "first hit", insulin resistance and lipid metabolism dysregulation, leads to the development of simple steatosis and hepatocytes become susceptible to "multi-hits".

3. THE LIVER: a key organ in metabolic homeostasis

The liver is the central organ responsible for both carbohydrate and lipid homeostasis. Insulin, an anabolic hormone released from pancreatic β-cells when dietary carbohydrates or amino acids are abundant, plays an essential role in this process.

3.1 Regulation of hepatic glucose metabolism

One of the principal functions of the liver is to contribute to maintaining blood glucose levels. In response to increased circulating levels of glucose after a meal, insulin is secreted by pancreatic β-cells. The result of insulin signalling in the liver is: **1)** reduced hepatic glucose production (HGP) via decreased gluconeogenesis (glucose biosynthesis) and glycogenolysis (glycogen breakdown), and **2)** conversion into glycogen of a large proportion of the glucose absorbed from the small intestine taken up by hepatocytes. This means that the liver is a pivotal location for glucose uptake and storage with decreased HGP. However, when nutrient availability is limited and circulating glucose levels fall, the liver becomes the main center for glucose production and secretion, via glycogenolysis and gluconeogenesis activation, which are largely governed by the combined actions of glucagon and glucocorticoids[75](**Figure 6**).

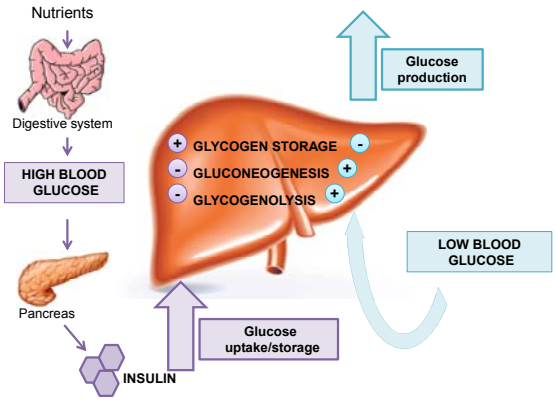


Figure 6. The liver's contribution to maintaining blood glucose levels. In response to elevated glucose levels, insulin is secreted from pancreatic β-cells. As a result of insulin signaling, the liver becomes a pivotal location for glucose uptake and storage, via decreasing gluconeogenesis and glycogenolysis with an increased glycogen storage. However, in response to low blood glucose levels, the liver becomes the main center for glucose production, via glycogenolysis or gluconeogenesis.

3.2 Regulation of hepatic lipid metabolism

Because the liver is not used as a storage depot for fat, the steady state concentration of hepatic triglycerides is low under physiological conditions. Nevertheless, there is considerable trafficking of both TG and fatty acids (FA) into and out of the liver in response to feeding and fasting[76] .

Under feeding conditions, dietary FA are absorbed from the small intestine, assembled into TG and incorporated into chylomicrons (QM). These are secreted into lymphatics and enter the plasma as triglyceride-rich QM, where they mainly deliver their FA to adipose tissue (for storage) and muscle (for energy) by the action of lipoprotein lipase (LPL) activity, an endothelium-associated enzyme which catalyses the hydrolysis of triglyceride-rich QM releasing FA. After the delipidation of chylomicrons by intravascular lipolysis, they are transform into QM remnant particles, which are taken up by the liver after further hydrolysis by intravascular hepatic lipase (HL)[77]. In addition, in the setting of excess carbohydrates, FA are also newly made within the liver through de novo lipogenesis (DNL). Under fasting conditions, FA are released from white adipose tissue and return to the liver by way of plasma non-sterified fatty acids (NEFA)[76].

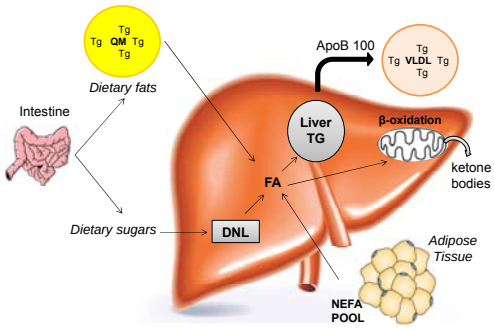


Figure 7. Hepatic lipid metabolism

In the liver, FA derived from either peripheral tissue, endogenous synthesis, or diet, can then be used for **1)** energy and ketone body production via mitochondrial β-oxidation; or **2)** sterified into TG that can be stored as lipid droplets within hepatocytes or packaged with apolipoprotein B (ApoB) 100 into very low density lipoproteins (VLDL) to be secreted into the circulation[78]. Alterations in any of these processes therefore influence both hepatic and whole-body energy metabolism and may contribute to disease development (**Figure 7**).

4. PATHOPHYSIOLOGICAL MECHANISMS LEADING TO NAFLD

The unequivocal histological hallmark of NAFLD in both adults and children is steatosis, defined as the histological manifestation of intracytoplasmic hepatic lipid accumulation in the form of triglycerides; the generally accepted dogma in NAFLD pathogenesis is that TG accumulation occurs when **hyperinsulinemia** and **insulin resistance**, commonly associated with obesity and T2DM, are present[74].

In NAFLD patients, the presence of IR is common at liver, adipose tissue and muscle level[79].

Insulin signalling

Under normal physiological conditions, insulin is released into the circulation from the beta cells in the islets of Langerhans in the pancreas in response to the ingestion of a meal. Upon binding to its receptor, insulin stimulates a well-described signaling cascade[80] involving the phosphorylation, docking and translocation of a series of signaling molecules, ultimately leading to alterations in specific endpoints of glucose and lipid metabolism. In muscle and adipose tissue insulin increases glucose uptake by the translocation of the glucose transporter GLUT4 from intracellular sites to plasma membrane, and in liver inhibits hepatic glucose production. Insulin also stimulates cell growth and differentiation, and promotes the storage of substrates in adipose tissue, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown[81]. **Figure 8** summarizes the main biological functions of insulin in glucose and lipid homeostasis.

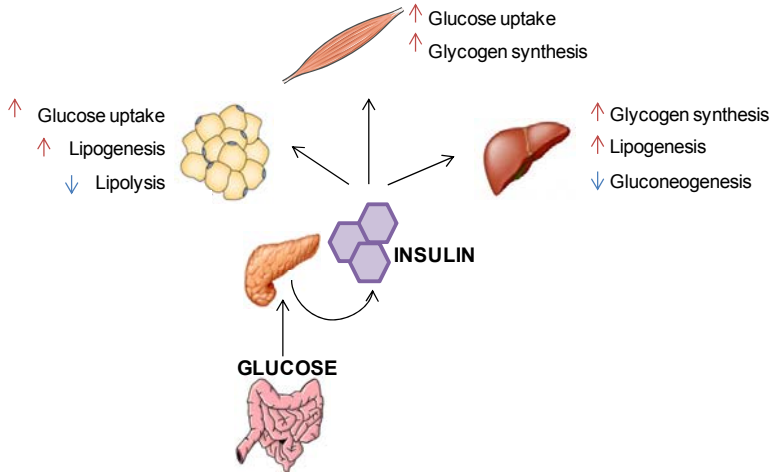


Figure 8. Biological functions of insulin in glucose and lipid homeostasis. Insulin is produced by the pancreatic beta cells, mainly in response to postprandial hyperglycemia. The main insulin target tissues are skeletal muscle, adipose tissue and liver. In skeletal muscle, insulin promotes glucose uptake and also stimulates glycogen synthesis. In adipose tissue, insulin stimulates glucose uptake and lipid synthesis, and additionally represses lipolysis. In liver, actions of insulin are the suppression of gluconeogenesis and the promotion of glycogen and lipid synthesis.

Under physiological conditions, insulin binds to the insulin receptor resulting in the phosphorylation of tyrosine residues on insulin receptor substrates (IRS), which leads the activation of phosphatidylinositol-3-OH kinase activation (PI3K). Activated PI3K phosphorylates membrane phospholipids, the major product being phosphatidylinositol-3,4,5-trisphosphate (PIP3), which in turn activates the enzyme PIP3-dependent kinase 1 (PDK-1), resulting in the phosphorylation and recruitment of Akt (also known as protein kinase B, PKB) to the cell membrane. There are three members of the PKB/Akt family identified as Akt1, Akt2, and Akt3. It is Akt2 that is important in insulin-mediated glucose homeostasis. Phosphorylated Akt is thought to suppress hepatic glucose production through two key mechanisms: first, by phosphorylation and nuclear exclusion of the forkhead box protein FoxO1, resulting in the decreased transcription of its pro-gluconeogenic targets genes, such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK); and second, by phosphorylation and inactivation of glycogen synthase kinase-3β (GSK-3), resulting in the dephosphorylation and activation of

glycogen synthase (GS). Additional targets of Akt include AKT Substrate of 160KDa (AS160). AS160 normally inhibits translocation of GLUT4 through its interaction with RabGTPase protein. However, phosphorylation of AS160 by Akt relieves the inhibitory effect on GLUT4, facilitating glucose uptake in adipocytes and muscle cells by allowing the translocation of GLUT4 to the plasma membrane. Akt also phosphorylates and inhibits the TSC1/2 complex (also known as tuberous sclerosis complex-1 and -2), resulting in the activation of the mammalian target of rapamycin (mTOR) pathway, which regulates protein synthesis[80]. On the other hand, Insulin also stimulates the mitogen-activated protein (MAP) kinase extracellular signal-regulated kinase (ERK). This pathway involves the tyrosine phosphorylation of IRS proteins and/or SHC, which in turn interact with the adapter protein Grb2, recruiting the Son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. Once activated, Ras operates as a molecular switch, stimulating a serine kinase cascade through the stepwise activation of Raf, MEK and ERK. Activated ERK can translocate into the nucleus, initiating a transcriptional programme that leads to cellular proliferation or differentiation[81] (**Figure 9**).

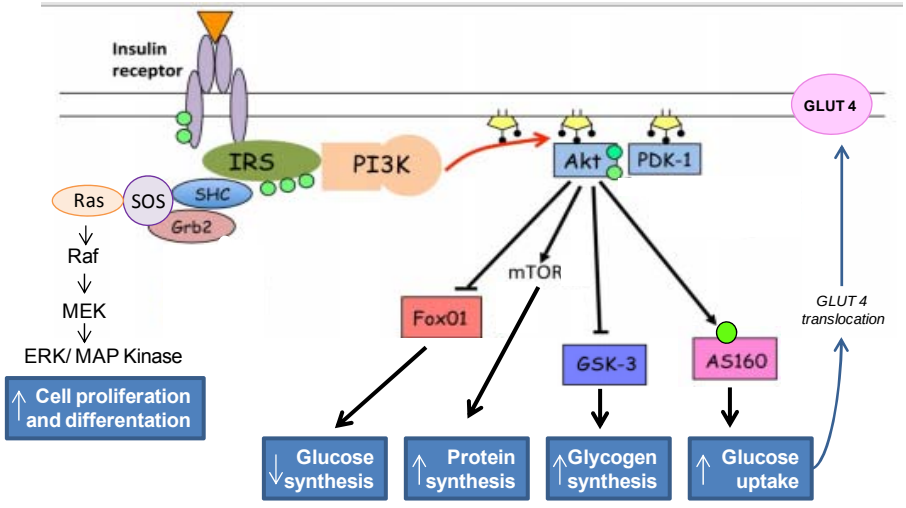


Figure 9. Insulin signaling pathway.

SHC, Src Homology 2 domain. Grb2, growth factor receptor-bound protein.

Insulin resistance

The insulin resistance state is defined as the pathological condition in which cells fail to respond to the normal actions of the hormone insulin. Insulin resistance occurs primarily in liver, adipose tissue and muscle, the main insulin target tissues.

Impaired insulin signalling in liver: under IR conditions, the ability of insulin to stimulate liver glycogen synthesis and suppress hepatic glucose production is diminished, resulting in increased plasma glucose concentrations[82] (**Figure 10**).

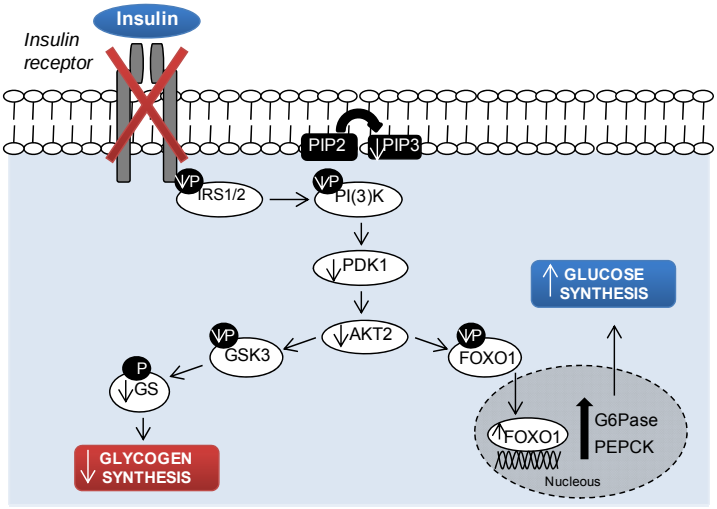


Figure 10. Hepatic insulin resistance Reduced phosphorylation of IRS and PI(3)K impairs Akt2 activity by reductions in PDK1 activity. Impaired Akt2 activity reduces the phosphorylation of GSK3. In its active (non-phosphorylated) form, GSK3 catalyzes phosphorylation and inactivation of glycogen synthase (GS), thus reducing glycogen synthesis. Impaired Akt2 activity also reduces insulin suppression of hepatic gluconeogenesis by promoting FOXO1 to the nucleus due to reduced phosphorylation, and subsequent increasing expression of gluconeogenic proteins, such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). IRS: insulin receptor substrate; PI(3)k: phosphatidylinositol-3-OH kinase; PDK1: 3-phosphoinositide dependent protein kinase 1; GSK3: glycogen synthase kinase-3β; FOXO1: forkhead box protein.

Impaired insulin signalling in adipose tissue: adipose tissue is the major site for storage of excess energy in form of triglycerides, and it contains multiple cell types, including adipocytes, preadipocytes, endothelial cells and immune cells. During positive energy balance, triglycerides are stored in the lipid droplets within the adipocytes, which are mobilized by lipolysis to release FFA into the circulation when energy is needed between meals or during physical activity. The resulting FFA are then transported to other tissues to be used as an energy source[73]. Under IR conditions, adipose tissue becomes resistant to the antilipolytic effect of the insulin. Insulin fails to inhibit the hormone-sensitive lipase, the enzyme which regulates the release of FFA from adipose tissue, resulting in an increased delivery of FFA to the liver[83,84].

Impaired insulin signalling in muscle: skeletal muscle insulin resistance typically accompanies insulin resistance at other sites. The independent effect of muscle insulin resistance on exacerbating NAFLD has been demonstrated in rodents[85,86] and translated to humans, in which selective muscle insulin resistance in healthy young lean individuals has been shown to predispose them to increased hepatic *de novo* lipogenesis, hepatic TG accumulation and atherogenic dyslipidaemia after eating high-carbohydrate meals. This is because ingested glucose is diverted away from muscle glycogen storage to the liver, where it is converted to TG driven by the compensatory hyperinsulinemia that is secondary to muscle insulin resistance[87]. However, there is evidence that these defects in muscle insulin signaling can be reversed by a single 45-minute bout of exercise, resulting in increased postprandial muscle glycogen synthesis following carbohydrate ingestion, and a 40% and 30% reduction in hepatic DNL and hepatic TG synthesis, respectively[88] (**Figure 11**).

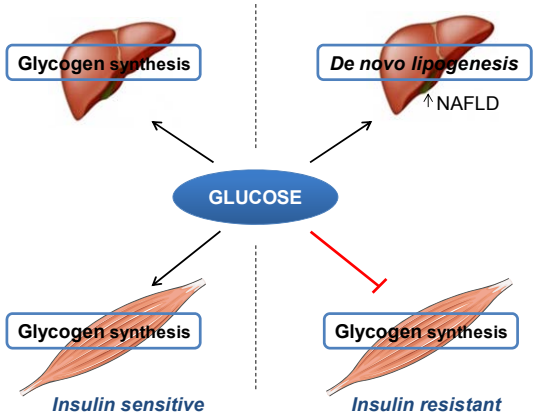


Figure 11. Skeletal muscle insulin resistance.
In insulin-sensitive subjects, insulin stimulates glycogen synthesis in both liver and muscle; however, in those with skeletal muscle insulin resistance, insulin fails to promote glycogen synthesis, diverting the substrate to the *de novo* lipogenesis. The increased lipid synthesis contributes to non-alcoholic fatty liver disease (NAFLD).

4.1 "First-hit" Imbalanced lipid metabolism and IR

As mentioned above, in patients with NAFLD, the presence of IR is common not only in muscle but also in liver and adipose tissue, resulting in increased insulin (hyperinsulinemia) and glucose (hyperglycemia) circulating levels.

High insulin levels should inhibit adipose tissue lipolysis, but this does not occur in these patients, due to the adipose tissue IR, thereby increasing FFA flux to the liver[79]. Moreover, as a consequence of muscle IR, impaired glucose uptake is observed in this tissue, contributing to elevated plasma glucose concentrations. Hepatic IR also contributes to hyperglycemia in NAFLD, due to the fact that insulin cannot suppress the hepatic gluconeogenesis, leading to excess glucose production. The pancreatic beta islet cells adapt to hyperglycemia by increasing insulin secretion, leading to hyperinsulinemia. Chronic elevated plasma glucose concentrations therefore promote an increase in hepatic DNL through two distinct mechanisms: **1)** directly, by increasing tricarboxylic acid cycle activity and the synthesis of Acetyl-CoA, which acts as a substrate of DNL, and **2)** indirectly, by activating the expression of the carbohydrate response element binding protein (ChREBP), which in turn promotes gene

transcription of lipogenic enzymes. Meanwhile, elevated insulin levels also stimulate DNL by activativating the sterol-regulatory-element-binding protein (SREBP1c), which in turn also promotes gene transcription of lipogenic enzymes[79] (**Figure 12**). All these metabolic alterations result in ectopic lipid accumulation in the form of triglycerides.

In patients with NAFLD, 26% of fatty acids in the liver have been demonstrated to derive from hepatic DNL, 59% from lipolysis of adipose tissue storage, and 15% from diet[89].

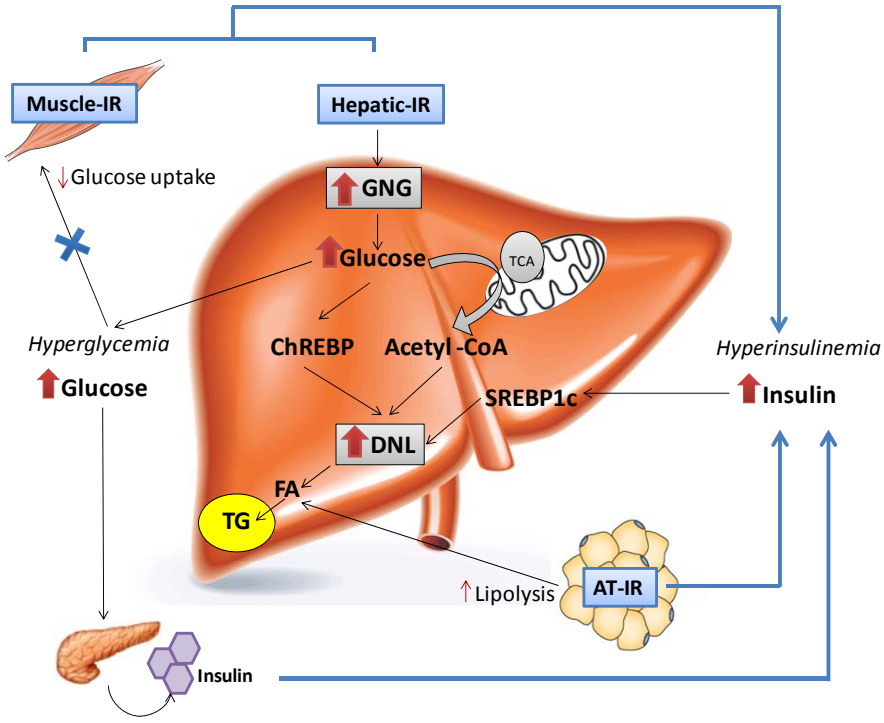


Figure 12. Systemic insulin resistance and hepatic triglyceride accumulation in NAFLD. Insulin resistance (IR) in adipose tissue (AT) results in an increased rate of lipolysis, with the consequent release of fatty acids (FA) to the liver. In skeletal muscle, glucose absorption decreases, while in the hepatocyte gluconeogenesis (GNG) and *de novo* lipogenesis (DNL) increase; resulting in hepatic triglyceride (TG) accumulation. ChREBP: carbohydrate response element binding protein; SREBP1c: sterol-regulatory-element-binding protein; TCA: tricarboxylic acid cycle.

When the biosynthesis of triglycerides exceeds the rate of secretion, TG excess accumulates into lipid droplets in the liver resulting in steatosis. Indeed, in NAFLD patients, TG accumulation in the cytoplasm of hepatocytes arises from an imbalance between lipid acquisition and removal: the influx of lipids, via increased fatty acid import or *de novo* fatty acid synthesis, exceeds the ability of hepatic lipid clearance by fatty acid oxidation or triglyceride export[78,90,91] (**Figure 13**). This highlights the fact that there is no a single pathway that is universally responsible for the development of steatosis, and the difficulty of understanding the pathogenesis of NAFLD.

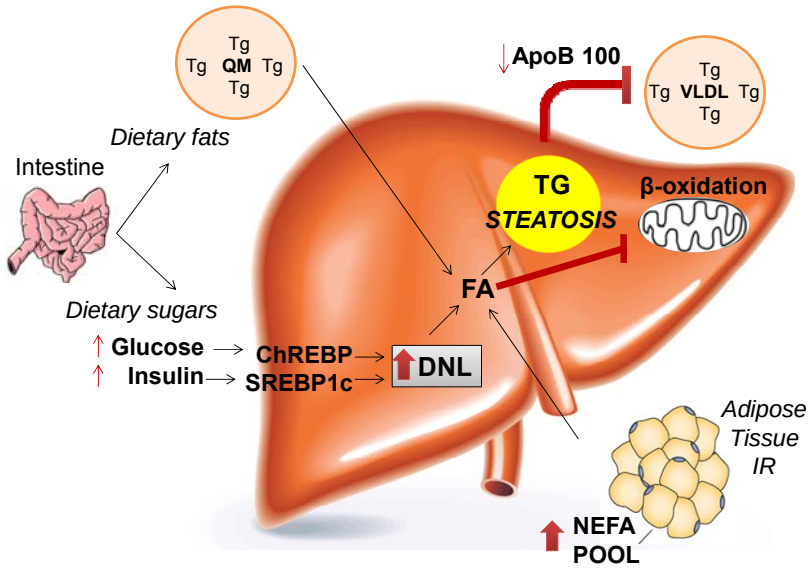


Figure 13. Imbalanced hepatic lipid metabolism in NAFLD. The hallmark of NAFLD is triglyceride accumulation in the cytoplasm of hepatocytes as a result of an imbalance between lipid input and output, **1.** An increase in fatty acids (FA) uptake derived from the circulation, due to the increased lipolysis from insulin resistance (IR) adipose tissue and/or from the diet in form of chylomicrons (QM); **2.** An increase in glucose and insulin levels in response to carbohydrate intake that promotes *de novo* lipogenesis (DNL); **3.** A decrease in FA mitochondrial β-oxidation; **4.** A decrease in triglyceride (TG) hepatic secretion by packaging with apolipoprotein B (ApoB) into very low density lipoproteins (VLDL). ChREBP: carbohydrate response element binding protein; SREBP1c: sterol-regulatory-element-binding protein

4.2 Progression of steatosis to NASH

As described previously, steatosis develops once excessive triglycerides have been accumulated in the liver. However, to develop NASH, multiple pathways or "multiple hits" are required to develop inflammation, cellular injury, and fibrosis[74]. 'Hits' that may contribute include direct hepatic lipotoxicity, oxidative stress, mitochondrial dysfunction, hepatocyte apoptosis, hepatic stellate cell and Kupffer cell activation, activation of pro-fibrogenic factors and pro-inflammatory mediators, hormones derived from adipose tissue (adipokines), endotoxins of intestinal origin, iron accumulation, and genetic factors. All these mechanisms are not mutually exclusive, but are more likely to act in a coordinated and cooperative manner[79]. **Figure 14** shows the molecular mechanisms leading to the formation of steatosis, as well as to the progression from steatosis to NASH.

4.2.1 Lipotoxicity

The current theory of lipotoxicity focuses on an increase in the flux of FFA within hepatocytes, which is a direct consequence of an increased dietary fats intake, *de novo* lipogenesis and adipose tissue lipolysis in the setting of insulin resistance and impairment of compensatory oxidative processes. This leads to the generation of toxic lipid FFA-derived metabolites, such as ceramides, diacylglycerols, lysophosphatidyl choline, and oxidized cholesterol metabolites, which act as reactive oxygen species (ROS), causing lipotoxic hepatocellular injury manifested as endoplasmic reticulum stress, inflammation, apoptosis, and necrosis[92]. Triglyceride accumulation may therefore actually be a protective response to prevent lipotoxicity from free fatty acid-derived metabolites.

4.2.2 Oxidative stress

Oxidative stress is a condition due to an altered balance between the production of reactive oxygen species (ROS) and the antioxidant

defenses capacity. FA catabolism in liver takes place mainly via mitochondrial β-oxidation. If there is an excessive overload of FFA, this process can lead to the generation of ROS in the mitochondrial chain which act upon the fatty acids of the cell membranes, causing lipid peroxidation. Once the mitochondria are exhausted or their function is impaired, FFA are then metabolized at other sites of hepatocyte, including the endoplasmic reticulum (ω-oxidation) and perixosomes (β-oxidation), also leading to the generation of ROS[93,94]. These cytotoxic ROS and lipid peroxidation products can diffuse into extracellular space, affecting Kupffer cells and hepatic stellate cells (HSC). This cellular oxidative stress from hepatocytes and the direct uptake of FFA or free cholesterol in Kupffer cells induces the activation of nuclear transcription factors such as NF-kβ, which regulates the synthesis of several pro-inflammatory cytokines, such as: **a)** tumour necrosis factor alpha (TNFα), which activates the caspase pathway and leads to hepatocyte apoptosis; **b)** transforming growth factor beta-1 (TGFβ1), which activates collagen synthesis due to HSC; **c)** the Fas ligand that cause 'fratricide deaths' between adjacent hepatocytes; and **d)** interleukin 8 (IL8), a powerful neutrophil chemotactic[95]. Furthermore, the end-products of lipid peroxidation, such as 4-hydroxynonenal and malondialdehyde, are also involved in the pathogenesis of liver damage. These molecules exert a chemotactic action on neutrophils, and may activate HSC and nonparenchymal cells of the liver and synthesis of the extracellular matrix, which increase the production and deposition of fibrous tissue[96].

4.2.3 Adipokines

Obesity, which is often associated with IR, is seen as a chronic systemic low grade of inflammation, in which adipose tissue has a central role[97,98]. Adipose tissue, and particularly white adipose tissue (WAT), has been recognized as an active endocrine organ that communicates with other tissues by secreting different soluble factors that are called adipokines (including chemokines, cytokines and hormones)[99]. Both an increased

release of free fatty acids from WAT and a dysregulated adipokine secretion, characterized by increased pro-inflammatory and decreased anti-inflammatory adipokine secretion, are observed in the obese state[73]. Many of these pro-inflammatory adipokines have been reported as promoting insulin resistance[100–102], leading to enhanced delivery of FFA to the liver, and thence to hepatic steatosis. Moreover, these molecules may cause direct damage to the liver or act indirectly by increasing oxidative stress, liver fibrosis, and tumor development by activating the oncogenic factor STAT3[72]. Clinical studies suggest that the expression of adipokines can vary in patients with NAFLD/NASH compared to healthy controls. In particular, serum levels of pro-inflammatory adipokines such as leptin, resistin, visfatin, TNFα and interleukin 6 (IL6) are significantly higher in NAFLD/NASH patients, while levels of insulin-sensitizing adipokines with anti-inflammatory properties, such as adiponectin, are significantly reduced[103]. Although adipose tissue secretes the majority of adipokines, they are also produced by other organs. In this regard, in human liver biopsies, hepatic adiponectin receptor mRNA increased in biopsy-proven NASH[104]. By contrast, Kaser S *et al.* found low mRNA expression of adiponectin and adiponectin receptor 2 (ADIPOR2) in the liver of patients with NASH compared to those with simple steatosis, and ADIPOR2 expression was inversely related to alanine aminotransferase and the fibrosis stage[105]. More recently, Moschen *et al.* demonstrated in a prospective study that rapid weight loss after bariatric surgery results in a significant improvement of both histological and biochemical liver parameters, accompanied by an increase of adiponectin serum levels, as well as hepatic mRNA adiponectin expression[106]. Hepatic TNFα and TNFα receptor I (TNFRI) expression was increased in patients with NASH compared with an obese group of similar age without NASH. In these patients, more advanced fibrosis was also accompanied by increased hepatic expression of TNFα[107]. As for IL6, Wieckowska *et al.* demonstrated a marked increase in hepatic IL6 expression in NASH patients compared to those with simple steatosis or a normal liver, and its expression positively correlated with the degree of inflammation, stage of fibrosis and IR[108]. In

another study, weight loss resulted in a dramatic decrease in IL6 expression in liver[109]. Recently, Chuan Shen *et al*. have demonstrated that hepatic resistin overexpression in NASH patients is associated with the severity of liver inflammation and fibrosis[110]. Finally, our research group has recently found that serum and hepatic visfatin to be higher in morbidly obese women with NAFLD than in those with normal liver histology[111].

4.2.4 Gut microbiota

A number of recent reviews have highlighted the importance of gut microbiota, which is now also considered a metabolic organ, in the pathogenesis of metabolic and inflammatory diseases such as obesity, T2DM and NAFLD[112–116]. Aron-Wisnewsky *et al.*[117] summarized the influence of gut microbiota in stimulating fat deposition and promoting NASH through 5 mechanisms: 1) it promotes obesity by improving the energy yield from food; 2) it regulates gut permeability, low-grade inflammation and immune balance; 3) it modulates dietary choline metabolism; 4) it regulates bile acid metabolism; 5) it increases endogenous ethanol production by bacteria. All these factors are molecular mechanisms by which microbiota can induce NAFLD and its progression.

Bacterial overgrowth and increased intestinal permeability, characterized by disruption of the intracellular tight junctions, have been observed in patients with NAFLD[118]. When the tight junction is impaired, intestinal permeability increases, leading to the delivery of gut-derived bacterial products such as endotoxin (lipopolysacharides, LPS) to the liver via the portal vein. Ruiz *et al.* demonstrated that plasma endotoxin levels were elevated in fatty liver patients, which were further increased in individuals with NASH, and were associated with a rise in TNFα gene expression in the hepatic tissue[119]. Moreover, Zhu *et al.* found an increased abundance of alcohol production in the microbiota of NASH patients, as well as elevated blood-ethanol concentrations, leading to increased oxidative stress and liver inflammation due to alcohol metabolism[120]. In addition, ethanol also contributes to

functional and morphological damage to the small bowel, increasing its permeability to endotoxins derived from the intestinal lumen[118].

4.2.5 Iron accumulation

Some studies have shown that increased hepatic iron concentration in patients with NAFLD is associated with IR and may therefore indirectly contribute to disease progression[121,122]. Ferritin levels reflect total body iron stores, and as such raised levels indicate iron overload. Studies suggest that ferritin can act as a cytokine to induce the release of further tissue cytokines and activate Kupffer and stellate cells, thereby exacerbating fibrosis[123]. However, despite the observation of correlations between serum ferritin levels and the presence of NASH[124–126], the importance of raised ferritin in NASH and the role of iron as a source of oxidative stress remain unresolved[127].

4.2.6 Genetic factors

Genome-wide association studies (GWAS) have suggested that patatin-like phospholipase domain-containing protein 3 (PNPLA3), also known as adiponutrin, is a genetic factor responsible for a higher prevalence of NAFLD and contributes to its development and progression[128–130]. PNPLA3 encodes a protein that is highly expressed in the liver and is involved in the metabolism of triglycerides. A single nucleotide polymorphism (rs738409) of PNPLA3 has been associated with the hepatic fat content, the progression of fibrosis[131–133] and with an increased risk of HCC in patients with NAFLD/NASH[134].

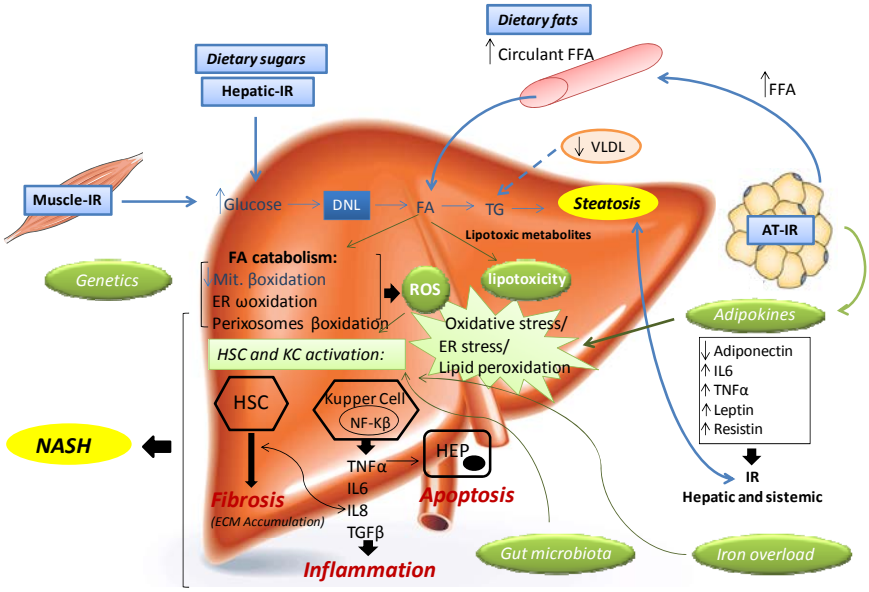


Figure 14. Molecular mechanisms involved in NAFLD development and progression. The mechanisms involved in steatosis formation via increased hepatic triglyceride content are shown in blue, and include insulin resistance and obesity, increased sugar and fats intake, increased *de novo* lipogenesis, increased free fatty acid flux from adipose tissue to the liver, impaired VLDV secretion, and decreased β-oxidation due to mitochondrial damage (especially in the presence of NASH). The multiple hits involved in the progression from steatosis to NASH are in green, and include lipotoxicity, hepatocellular oxidative stress secondary to ROS formation during β- and ω-oxidation of fatty acids, role of adipokines, role of iron accumulation, role of microbiota, and genetic factors.
AT: adipose tissue; DNL: *de novo* lipogenesis; ECM: extracellular matrix; ER: endoplasmic reticulum; FA: fatty acids; FFA: free fatty acids; HEP: hepatocyte; HSC: hepatic stellate cells; IL: interleukin; IR: insulin resistance; KC: Kupffer cells; Mit: mitochondrial; NASH: non-alcoholic steatohepatitis; ROS: reactive oxygen species; TG: triglycerides; TGFβ: transforming growth factor beta-1; TNFα: tumour necrosis factor alpha; VLDL: very low density lipoprotein

5. DISSOCIATION BETWEEN IR AND NAFLD

In the previous sections, we have seen that NAFLD is strongly associated with insulin resistance, not only at the level of the liver, but also at muscle and adipose tissue level. However, whether NAFLD is a consequence or a cause of IR is a matter of debate.

5.1 NAFLD as a consequence of insulin resistance

Insulin exerts its action by binding to the extracellular portion of the insulin receptor, and triggers a cascade of intracellular signaling events involving three critical nodes which are sequential phosphorylation and activation of insulin receptor substrates, PI(3)K and Akt[80]. In normal liver, intact insulin signaling inhibits glucose production and promotes fatty acid synthesis[81]. Multiple animal models support a direct causal relationship between insulin resistance, hyperinsulinemia, and hepatic steatosis[135,136]. Evidence that insulin resistance causes steatosis in humans comes from patients with Akt2 mutations[137], who showed strong resistance to the glucoregulatory actions of insulin when suffering from this pathological condition, but presumably retained sensitivity to the lipogenic effects of the hormone. In fact, hyperinsulinemia activates the transcriptional factor SREBP1c, promoting lipogenic enzyme gene expression in spite of insulin resistance[138]. These data support the theory that insulin sensitivity contributes to hepatosteatosis, but how does insulin resistance contribute to a vicious cycle that exacerbates this condition? To resolve this paradox, Brown and Goldstein formulated the concept of “selective insulin resistance”[139]. This proposes that, although the insulin-mediated suppression of hepatic gluconeogenesis is blunted due to disruption of some aspects of insulin signaling in the liver, other insulin signaling mechanisms remain intact and continue to drive hepatic lipogenesis. Li S, *et al.* suggested that the mammalian target of rapamycin complex 1 (mTORC1) is such a bifurcation point of the insulin signaling that lies downstream of Akt[140]. The inhibition of mTORC1 in rats blocked the insulin-

induced upregulation of lipogenic genes expression, but it did not affect the insulin-mediated suppression of gluconeogenic genes expression. These results establish mTORC1 as an essential component in the insulin-regulated pathway for hepatic lipogenesis but not gluconeogenesis, and may help to resolve the paradox of selective insulin resistance in livers of diabetic rodents[140]. However, an alternative explanation for the concomitant elevation of gluconeogenesis and lipogenesis in human type 2 diabetes or models of IR is that lipogenic and gluconeogenic enzymes are both target genes of ChREBP induced by hyperglycemia (See section 6.2.3).

5.2 NAFLD as a cause of insulin resistance

The coincident occurrence of hepatic steatosis and IR has led to the hypothesis that excess triglyceride in liver causes insulin resistance. However, this hypothesis is contradicted by observations in mice with defects in diverse pathways that cause hepatic steatosis without the development of IR[135,141].

Samuel, V.T *et al.* demonstrated the induction of NAFLD in rats, which after just 3 days of being fed a high-fat diet, developed hepatic steatosis and IR, with no changes in body weight, adiposity or IR in skeletal muscle[142]. Interestingly, an increase in the FFA-derived metabolite diacylglycerol (DAG) was observed in the liver of these animals. The relationship between hepatic accumulation of DAG and IR could be attributable to the activation of protein kinase-Cε (PKCε), highly expressed in the liver. These changes were associated with reductions in the phosphorylation of the insulin receptor and the Akt2 activity. In this model, the activity of insulin to induce glycogen synthesis and inhibit gluconeogenesis is therefore diminished due to DAG-mediated activation of PKCε (**Figure 15**). PKCε is a novel isoform of the PKC family with a much higher affinity for DAG than the conventional PKC isoforms[143]. The crucial role of PKCε in mediating lipid-induced hepatic IR has been convincingly demonstrated in a study in which antisense oligonucleotides were used in

rats to knockdown the hepatic expression of PKCε[144]. The authors were able to show that knocking down PKCε expression in the liver protected rats from lipid-induced hepatic IR, despite increases in hepatic lipid content. In addition, they found that PKCε activation caused hepatic IR by directly binding to and inhibiting insulin receptor kinase activity. These results were replicated in PKCε knockout mice, which were protected from IR induced by high-fat feeding[145]. Moreover, the interaction between DAG, PKCε activation and hepatic RI has been demonstrated in numerous other rodent models of NAFLD-associated hepatic IR[146].

This model for lipid-induced hepatic IR has also been translated to humans. Kumashiro *et al.* evaluated the possible mechanisms involved in hepatic IR in a group of patients with morbid obesity and NAFLD. In these patients, hepatic DAG content and PKCε activation were the strongest predictors of IR in liver[147]. However, they found no association between insulin sensitivity and other factors involved in the development of hepatic IR, such as ceramides, endoplasmic reticulum (ER) stress markers or inflammatory cytokines. These results were replicated in another study, in which the content of DAG in liver was also the best predictor of hepatic IR in obese humans, whereas no association with the content of ceramides or markers of inflammation was found[148]. In fact, it has been suggested that liver inflammation is a consequence rather than a cause of insulin resistance[149].

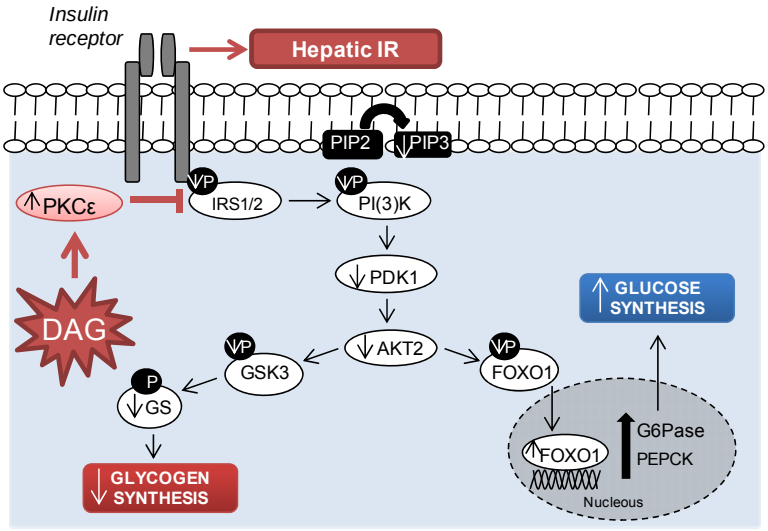


Figure 15. Mechanism of DAG-PKCε-mediated hepatic insulin resistance[71]**.** Membrane-near intracellular DAG lead to activation of PKCε, which in turn inhibits the insulin receptor kinase. This then leads to decreased insulin-stimulated tyrosine phosphorylation of IRS-1 and -2, PI(3)K activation and downstream insulin signaling. The net result is a decrease in hepatic glycogen synthesis, resulting from decreased activation of glycogen synthase (GS), and increased hepatic gluconeogenesis through reduced inactivation of forkhead box protein O (FOXO1). DAG: diacylglycerol; PKCε: protein kinase-Cε; IRS: insulin receptor substrate; PI(3)k: phosphatidylinositol-3-OH kinase; PDK1: 3-phosphoinositide dependent protein kinase 1; GSK3: glycogen synthase kinase-3β; G6Pase: glucose-6-phosphatase;PEPCK: phosphoenolpyruvate carboxykinase.

The model described, which explains the relationship between DAG, PKCε and hepatic IR, focuses mainly on the inability of insulin to alter hepatic glucose metabolism. However, the ability of insulin to activate lipogenesis appears to be intact in most NAFLD models. It is well established that it is possible to experimentally induce insulin resistance without NAFLD, or induce NAFLD without insulin resistance under certain conditions[82]. ChREBP and SREBP1c have recently been independently implicated in dissociating NAFLD and IR in mice and humans[150,151]. To explain this paradox, Cantley *et al.* examined the subcellular localization of DAG in liver cells, and observed that the knockdown of the gene encoding CGI -58, an activator of adipose tissue triglyceride lipase (ATGL) involved in the hydrolysis of TG, promotes DAG accumulation in lipid droplets,

protecting against DAG accumulation in the cell membrane and the subsequent translocation of PKCε[152]. These results suggest that compartmentalization of DAG in hepatocytes could be an important factor in the pathogenesis of hepatic IR. Indeed, the compartmentalization of DAG and TG in neutral compartments, where it cannot activate PKCε, may explain why hepatic DAG content may not always correlate with hepatic insulin resistance in some animal models. **Figure 16** summarizes the metabolic pathways leading to hepatic DAG accumulation.

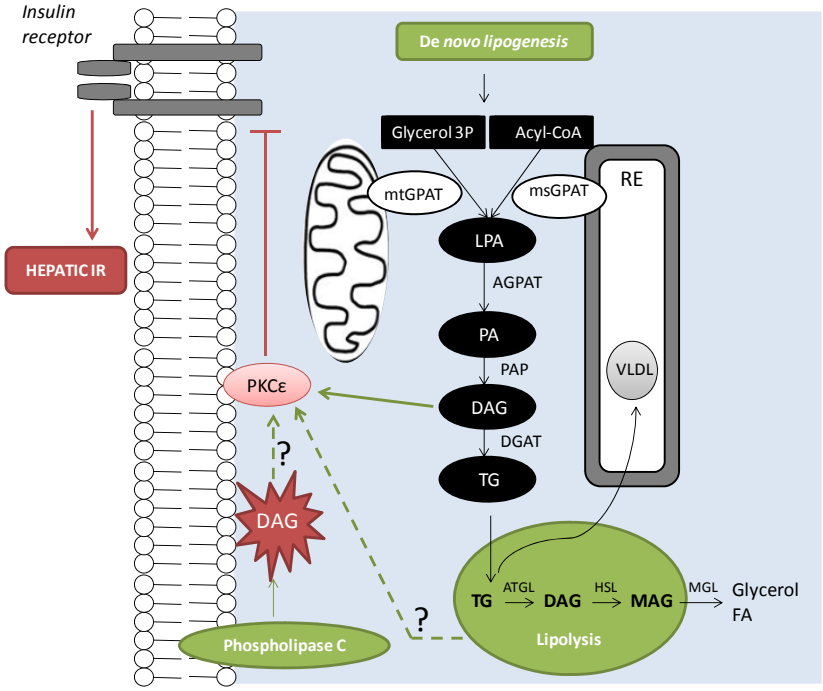


Figure 16. Metabolic pathways leading to hepatic DAG accumulation[71]. The glycerol 3-phosphate (Glycerol 3P) pathway represents the *de novo* lipogenesis route in the synthesis of triglycerides (TG) and phospholipids. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the acylation of glycerol 3P with acyl-coenzyme A (Acyl-CoA) to generate lysophosphatidic acid (LPA), which is thought to be the rate-controlling step in TG synthesis. The enzymes acyl-glycerol-phosphate acyl transferase (AGPAT), phosphatidic acid phosphatase (PAP) and diacylglycerol acyltransferase (DGAT) then catalyze the formation of phosphatidic acid (PA), diacylglycerol

(DAG) and TG, respectively. LPA and PA require translocation through the cytosol for TG synthesis at the endoplasmic reticulum (ER) if they are not synthesized in the ER. In the liver, TG are either deposited in intracellular vacuoles or exported in particles of very low density lipoproteins (VLDL). In the lipid droplets, the conversion from TG to diacylglycerol (DAG) is mediated by adipose triglyceride lipase (ATGL) during lipolysis, and then DAG can be hydrolyzed to monoacylglycerol (MAG) by hormone-sensitive lipase (HSL) and subsequently to glycerol by monoglyceride lipase (MGL); which can be used as a substrate for gluconeogenesis. These reactions also release fatty acids (FA). Phospholipase C can release DAG from membrane lipids. DAG activates protein kinase-Cε (PKCε) membrane translocation, which in turn, inhibits the insulin receptor kinase. However, whether DAG derived from phospholipase C pathway and lipid droplets can lead to PKCε activation and hepatic insulin resistance remains to be determined.

6. MECHANISMS OF HEPATIC FAT ACCUMULATION IN NAFLD

Steatohepatitis develops in only a fraction of patients with NAFLD, while the majority only present simple steatosis. Lipid accumulation thus appears to be a prerequisite for the development of NASH, and the early and crucial step in NAFLD manifestation. It is therefore important to understand the events that lead to initial lipid accumulation in the liver**.** **Figure 15** shows the transcriptional regulation of mechanisms leading to hepatic lipid accumulation.

6.1 Hepatic fatty acid uptake and transport

Free fatty acids in the plasma can be taken up by the liver, and are important sources for hepatic TG synthesis. Plasma FFA is normally generated by white adipocytes via lipolysis, which is induced by beta adrenergic receptor agonists such as catecholamine under fasting conditions[83]. This process involves the regulation of protein kinase A (PKA)-dependent phosphorylation and activation of hormone-sensitive lipase (HSL), a key rate-limiting enzyme in the lipolysis, to promote this pathway. This pathway is reversed by insulin under feeding conditions, limiting the liberation of FFA and instead inducing *de novo* lipogenesis in this tissue. However, the IR state goes along with increased adipocyte lipolysis, leading to abundant FFA in the plasma pool regardless of the nutritional status[153]. Studies in humans have shown that uptake of exogenous FFA is the single largest source of FA in stored hepatic TG, and that this contribution is further increased with fasting and NAFLD[89,154]. Moreover, increased visceral fat content is correlated with hepatic TG content[155,156] and has been implicated in NAFLD etiology. Meanwhile, clearance of the chylomicron-remnant TG also contributes to the hepatic FA pool.

The rate of FA uptake from plasma into cells depends on the FA concentration in the plasma and the hepatocellular capacity for FA uptake,

which also depends on the number and activity of transporter proteins in the sinusoidal plasma membrane of the hepatocyte. The main plasma membrane transporters for FFA are FA transporter protein (FATP), caveolins, FA translocase (FAT/CD36), and FA-binding protein (FABP)[78]. Six **FATP** isoforms have been identified in mammalian cells, which contain a common motif for FA uptake and fatty acyl-CoA synthetase function[157]. Of these isoforms, FATP2 and FATP5 are highly expressed in the liver, and are utilized as major FATPs for the normal physiological context. Hepatic FA uptake is decreased in mice lacking FATP2 or FATP5 in the liver[158,159]. In humans, a promoter polymorphism in the liver-specific FATP5 is associated with features of the metabolic syndrome and steatosis[160]. **Caveolins** consist of three protein family members termed caveolins 1, 2, and 3, and are found in the membrane structures called caveolae, which are important for protein trafficking and the formation of lipid droplets. Caveolin-1 knockout mice exhibited lower TG accumulation in the liver[161]. Some authors suggest that there is an involvement of caveolin-1 in abnormal lipogenesis and mitochondrial function typical of steatotic hepatocytes in NAFLD[162]. **FAT/CD36** is a transmembrane protein that accelerates FA uptake via facilitated diffusion[163]. Its hepatic expression is normally weak, but its expression is enhanced in rodents with fatty liver[164]. Moreover, some authors have demonstrated that FAT/CD36 mRNA levels increase concomitantly with hepatic TG content in different animal models of liver steatosis[165,166]. Further studies have shown that FAT/CD36 is a common target gene of the liver X receptor, pregnane X receptor, and peroxisome-proliferator-activated receptor γ in promoting hepatic steatosis in a murine model[167]. However, little is known about the significance of FAT/CD36 in human liver diseases. In morbidly obese patients with NAFLD, Greco *et al.* showed that hepatic FAT/CD36 mRNA levels were positively related to liver fat content[168] and Bechmann *et al*. found a significant correlation between hepatic FAT/CD36 mRNA and apoptosis in patients with NASH[169]. Other authors have reported that hepatic FAT/CD36 upregulation is significantly associated with IR, hyperinsulinemia, and increased steatosis in patients with NASH[170]. **FABPs** are cytosolic lipid binding proteins that facilitate the

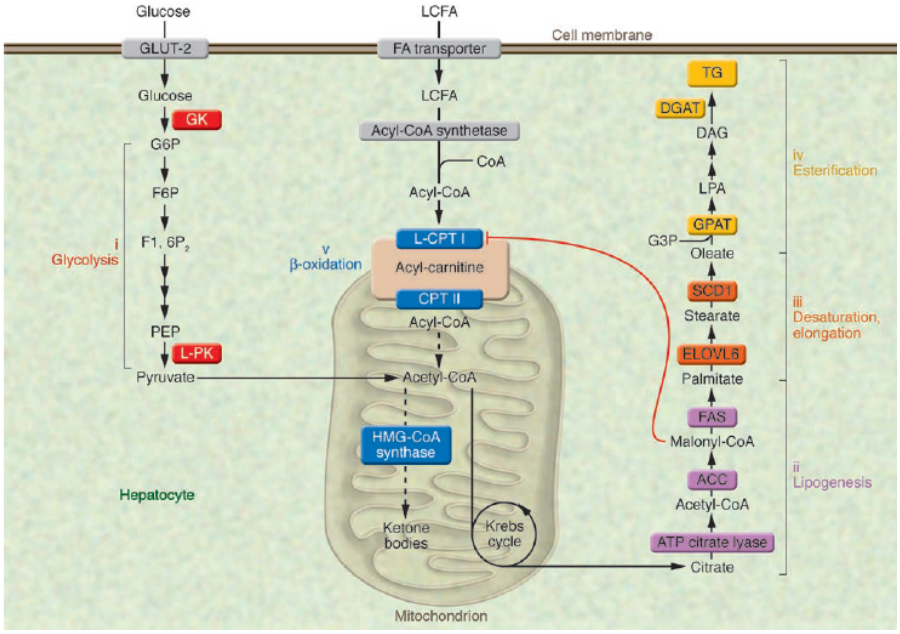
intracellular transport of FFA[163]. The functions of FABPs include enhancement of FFA solubility and transport to specific enzymes and cellular compartments (to the mitochondria and peroxisomes for oxidation; to the endoplasmic reticulum for reesterification; into lipid droplets for storage; or to the nucleus for gene expression regulation)[171]. Few studies have assessed the involvement of hepatic FABP4 expression in NAFLD. For instance, Greco *et al*. has described FABP4 as being upregulated in subjects with high liver fat content[168]. Moreover, the expression of FABP4 and FABP5 in the liver has been correlated with hepatic fatty infiltration in NAFLD patients[172].

Peroxisome-proliferator-activated receptor γ (**PPARγ**) is the master transcriptional regulator in the control of genes involved in lipogenic pathways of adipocytes, promoting the uptake of fatty acid and adipocyte differentiation (adipogenesis). PPARγ also confers sensitization to insulin through the transcriptional activation of adiponectin gene in adipocytes, up-regulating its expression[173]. The net effect of this process is to increase triglyceride storage in adipose tissue, reducing delivery of fatty acids to the liver. Despite PPARγ is present in the liver to a lesser degree than in adipose tissue, increased PPARγ expression is a feature of steatotic liver. Several studies attribute a casual role to PPARγ in liver triglyceride accumulation by mechanisms involving activation of lipogenic genes and *de novo* lipogenesis[173–177], suggesting a pro-steatotic role of hepatic PPARγ. In contrast to these studies, which show a deleterious effect of PPARγ on NAFLD, other studies have shown PPARγ to have anti-inflammatory and anti-fibrotic effects in stellate cells, macrophages, and endothelial cells (Review in Tailleaux *et al.*[177]). Westerbacka and Paulina Pettinelli *et al*. have described that PPARγ as being over-expressed in the fatty liver of obese human subjects[172,178].

6.2 De novo synthesis of fatty acids

As described above, the non-esterified fatty acids that are incorporated into triglycerides within the liver may be derived from the plasma or may be newly synthesized from glucose by *de novo* lipogenesis. Dysregulation of DNL is observed in patients with obesity, metabolic syndrome or NAFLD.

DNL is an integrated pathway that consist of glycolysis (conversion of glucose to acetyl-CoA), biosynthesis of saturated fatty acid followed by desaturation, and the formation of TG. Key rate limiting enzymes in the process include glucokinase (GK) and L-pyruvate kinase (L-PK) in the glycolysis, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) in the fatty acid synthesis, long chain fatty acid elongase 6 (ELOVL6) and stearoyl-CoA desaturase 1 (SCD1) in the formation of monounsaturated fatty acids, and glycerol-3-phosphate acyltransferase (GPAT), lipins, and acyl-CoA:diacylglycerol acyltransferase (DGAT) in the formation of TG[163]. During DNL, glucose is converted to acetyl-CoA, and it is then converted to malonyl-CoA by ACC1. FAS catalyzes the formation of palmitic acid from malonyl-CoA and acetyl-CoA. Palmitic acid is then elongated and desaturated by ELOVL6 and SCD1 to generate monounsaturated fatty acids, which are the major fatty acid constituents of triglycerides. GPAT then catalyzes the esterification of glycerol-3-phosphate from glycolysis with the newly synthesized fatty acid to generate lysophosphatidic acids. Lysophosphatidic acids are substrates for GPAT to catalyze the formation of phosphatidic acids. Phosphatidic acids are then processed to diacylglycerols by lipin 1, followed by the formation of triglycerides through DGAT[76] (**Figure 17**).



Postic C et al. J Clin Invest. 2008[90].

Figure 17. Metabolic pathways leading to the synthesis of TGs in liver. The synthesis of TGs in liver is nutritionally regulated. The ingestion of a LF/HC diet causes a marked induction of enzymes involved in key metabolic pathways, including (i) glucokinase (GK) and L-PK for glycolysis; (ii) ATP citrate lyase, ACC, and FAS for lipogenesis; (iii) ELOVL6 and SCD1 for fatty acid elongation and desaturation steps; and finally (iv) GPAT and DGAT for TG synthesis. Under these nutritional conditions, elevation in malonyl-CoA concentrations, the product of the lipogenic enzyme ACC, inhibits L–CPT I, the rate-limiting enzyme of β-oxidation (v), which regulates the transfer of long-chain acyl-CoAs from the cytosol into the mitochondria, thus resulting in a shift from an oxidative (production of ketone bodies) to an esterification pathway (TG synthesis). F6P, fructose 6-phosphate; F1, 6P2, fructose 1,6 diphosphate; G3P, glycerol 3-phosphate; G6P, glucose 6-phosphatase; PEP, phosphoenol pyruvate; LCFA, long-chain fatty acids; CPT II, carnitine palmitoyltransferase II.

The rate of DNL is regulated primarily at the transcriptional level (**Figure 18**). Several nuclear transcription factors such as liver X receptor alpha (LxRα), SREBP1c, ChREBP, and farnesoid X receptor (FxR) are involved. Chronic hyperinsulinemia and hyperglycemia, as found in NAFLD, promote DNL through the upregulation of lipogenic transcription factors. In humans, NAFLD has been associated with increased hepatic expression of several genes involved in *de novo* lipogenesis[179,180]. Moreover, a recent study has confirmed that increased *de novo* lipogenesis is a distinct characteristic of subjects with NAFLD[181].

6.2.1 LxRs

LxRs are ligand-activated transcription factors that belong to the nuclear receptor superfamily. There are two LxR isoforms termed α and β. LxRα is mainly expressed in the liver, adipose tissue, and intestine, whereas LxRβ is ubiquitously expressed. In addition to modulating cholesterol metabolism, LxRs have been characterized as major regulators of hepatic FA biosynthesis. A major function of LxRα in the liver is the stimulation of *de novo* lipogenesis, through the direct transcriptional activation of lipogenic enzymes, such as ACC and FAS, but also indirectly via the insulin-mediated transcriptional activation of SREBP1c[182,183] (**Figure 18**). Several authors have described an enhanced expression of LxRα and SREBP1c in NAFLD[180,184–187]. More recently, Sang Bong Ahn *et al.* have demonstrated that LxRα expression is positively correlated with the degree of hepatic fat deposition, as well as with hepatic inflammation and fibrosis in NAFLD patients; suggesting that it could be an attractive target for the treatment and regulation of hepatic inflammation and fibrosis[188].

6.2.2 SREBP1c

SREBPs are a family of membrane-bound transcription factors that are synthesized as precursors embedded in the endoplasmatic reticulum. A proteolytic cleavage then allows the accumulation of mature SREBP in the nucleus. There are different SREBP isoforms: SREBP1c and SREBP2 that are expressed in the liver, and SREBP1a expressed only at very low levels in the liver of adult mice, rats, and humans. SREBP1c is the predominant isoform in the liver and preferentially affects the transcription of genes that regulate DNL. However, SREBP2 regulates genes involved in cholesterol biosynthesis and metabolism. SREBP1a isoform, despite its very low levels in the liver, transactivates both lipogenic and cholesterol genes[78,189]. To date, the main regulation demonstrated for SREBP1c is at the transcriptional level. SREBP1c transcription is induced by two quite disparate stimuli: insulin, released in response to carbohydrate intake and

leading to a parallel increase in both the membrane-bound precursor and the mature nuclear form, and by LxRα. In response to feeding, SREBP1c promotes the expression of lipogenic genes, including ACC and FAS, the two rate-limiting enzymes in *de novo* FA synthesis, thereby stimulating DNL[190–192] (**Figure 18**).

Various authors have described an enhanced expression of SREBP1c and LxRα in NAFLD[180,184–187]. However, Nagaya *et al.* demonstrated the downregulation of the hepatic SREBP1c-mediated lipogenic pathway in advanced NASH patients; SREBP1c mRNA levels were inversely correlated with the fibrosis stage[193]. These discrepancies might be explained by differences in the cohort of patients studied. For example, Higuchi *et al.* included normal weight patients with NAFLD[184] and Lima-Cabello *et al.* included patients with NAFLD and with steatosis related to chronic hepatitis C virus infection in mildly overweight men and women[185]. Moreover, Higuchi *et al.* did not evaluate either histological findings nor protein levels or intracellular localization of SREBP1c[184].

6.2.3 ChREBP

ChREBP is a glucose-responsive transcription factor that relocates to the nucleus in response to increased glucose concentrations. It was first identified as a regulator for hepatic glycolysis by activating transcription of the L-PK gene, but in recent years, ChREBP has also been reported as playing an important role in glucose-mediated lipogenesis within the liver[194]. Interestingly, ChREBP has also been identified as a direct target of LXRs, an important regulator of the lipogenic pathway through the transcriptional control of SREBP1c and lipogenic enzymes[195]. Although oxysterols are known ligands of LXRs, glucose has been shown to activate LXRs and to induce their target genes, including ChREBP[196] (**Figure 18**).

Postprandial hyperglycemia raises the hepatic concentrations of phosphorylated intermediates, causing activation of ChREBP, which binds to the promoter of its target genes. ChREBP target genes include not only

enzymes of glycolysis and lipogenesis that predispose to hepatic steatosis, but also glucose 6-phosphatase (G6Pase), which catalyzes the final reaction in glucose production, and glucokinase regulatory protein (GCKR), which inhibits hepatic glucose uptake[197,198]. Transcriptional induction of G6PC and GCKR manifests as hepatic glucose intolerance or IR[199].

Study results of the role and impact of ChREBP in glucose and lipid metabolism have been contradictory, with global ChREBP deficiency resulting in IR in one murin model[200] *vs*. improved hepatic steatosis and other related metabolic alterations, including IR[201–203]. Benhamed *et al.* hypothesized that these opposite phenotypes in these murine models may be due to the fact that ChREBP controls both glycolysis and lipogenesis, and that the beneficial effect of ChREBP deficiency may only be apparent in the context of lipid overload[151]. The authors showed that, on a standard diet, mice overexpressing ChREBP remained insulin sensitive, despite increased lipogenesis resulting in hepatic steatosis. However, mice that overexpress ChREBP, on a high-fat diet, showed normal insulin levels and improved insulin signaling and glucose tolerance compared to the controls, despite having greater hepatic steatosis. This effect seems to be mediated by the fact that ChREBP modifies the monounsaturated FA/saturated FA (MUFA/SFA) balance in favor of MUFA, by stimulating SCD1 activity. Taken together, these results show that increasing ChREBP, by buffering detrimental FA and favoring lipid partitioning, can dissociate hepatic steatosis from IR, with beneficial effects on both glucose and lipid metabolism. Interestingly, ChREBP expression in liver biopsies from patients with NASH was higher when steatosis was greater than 50% and lower in the presence of severe IR[151], supporting this conclusion.

6.2.4 FxR

The FxR is a member of the nuclear hormone receptor superfamily and a receptor for bile acids. It is mainly expressed in the liver, intestine, kidneys, and the adrenal glands, with less expression in adipose tissue and the heart[204]. FxR has emerged as a master regulator of both lipid and

glucose homeostasis in the liver, as well as of inflammatory processes at hepatic and extrahepatic sites. Moreover, it has been demonstrated in mouse models that FxR needs to be activated in order to reduce the expression of SREBP1c[205] .

FxR knockout mice exhibited severe fatty liver accompanied by elevated circulating levels of FFA, elevated glucose levels and impaired IR[206]. The activation of the nuclear receptor FxR also improved hyperglycemia and hyperlipidemia in diabetic mice[207]. These results suggest a key role for FxR in lipid and glucose metabolism. In addition, a number of synthetic FxR agonists have been used in the treatment of different hepatic and metabolic disorders, resulting in a lower inflammatory and fibrogenic process[208]. For instance, treatment of obese *db/db* (diabetic) mice with a dual agonist for FxR and TGR5 (Takeda G-protein couples receptor 5; a bile acid receptor) improved the histological features of NASH, and increased the number of intrahepatic monocytes with an anti-inflammatory phenotype[209]. Moreover, Obeticholic acid (OCA) is an specific FxR agonist that does not activate other nuclear hormone receptors, and beneficially controls liver inflammation in an NF-Kβ-dependent manner[210,211]. The first controlled clinical trial demonstrating the efficacy and safety of an OCA FxR receptor agonist in patients with T2DM and NAFLD has been published recently[212]. In the light of the positive results on insulin sensitivity and markers of liver fibrosis, the National Institute of Health initiated a Phase IIb clinical study with this agent in patients with NASH (the FLINT study), comparing placebo and 25mg OCA dialy for 72 weeks (http://www.clinicaltrials.gov; NCT01265498). Analysis showed that treatment with OCA resulted in a highly statistically significant improvement in the primary histological end point, defined as a decrease in the NAFLD Activity Score of at least two points with no worsening of fibrosis, compared to the placebo. Another hepatic protective mechanism of FxR activation has been shown to be maintenance of gut integrity against gut-derived endotoxins through the induction of antibacterial factors such as angiogenin, inducible nitric oxide (NO) synthase, and interleukin-18 (IL18)[213].

Regarding the role of FxR in lipid metabolism, the majority of literature seems to point to the fact that FxR activation is beneficial in situations of excess, such as obesity and diabetes. FxR activation seems to reduce TG levels by: **1)** reducing FA synthesis in the liver, through the reduction of SREBP1c and LxR expression; **2)** inducing the expression of PPARα, which promotes FFA catabolism via β-oxidation; **3)** increasing TG clearance; and **4)** increasing adipose tissue storage and altering adipokine patterns[204]. Patients with NAFLD have lower protein and mRNA FxR levels, which has been attributed to higher TG synthesis and induced expression of SREBP1c and LxRα[214].

6.2.5 Lipogenic enzymes

FAS and ACC, the two rate-limiting enzymes in fatty acid biosynthesis, are currently considered an attractive target for regulating the human diseases of obesity, diabetes, cancer, and cardiovascular complications[78]. Two isoforms of ACC exist, with each one differing in its physiological function. ACC1 is primarily cytosolic and produces malonyl-CoA that is largely used as a substrate by FAS for DNL. In contrast, ACC2 is localized on the mitochondria and produces a local pool of malonyl-CoA. Malonyl-CoA resulting from *de novo* lipogenesis activation inhibits carnitine palmitoyl-transferse 1 (CPT1)[215]. Dorn *et al.* found that FAS mRNA expression was significantly higher in steatotic human livers samples than in normal liver tissue[216]. In accordance with Dorn *et al.*, several authors have not only described an enhanced hepatic expression of FAS in NAFLD, but also of ACC1[184–186]. Moreover, in evidence supporting increased FA synthesis in NAFLD, Morgan *et al.* found that ACC1 and FAS mRNA expression were significantly higher in high-fat mice[217].

6.3 Fatty acid oxidation

The steady state of hepatic triglycerides is also controlled by consumption of fatty acids by oxidation. FA can be catabolized through 3 distinct pathways of oxidation: α, ω, and β. ω-oxidation occurs exclusively in microsomes and α- and β-oxidation can occur in both peroxisomes and mitochondria. Mitochondrial β-oxidation is the primary catabolic pathway in the liver for most FA, but perixosomal β-oxidation is largely responsible for the initial oxidation of very long-chain FA (>20)[215].

Fatty acid β-oxidation in mitochondria is a process shortening the FA into acetyl-CoA, which can subsequently be converted into keton bodies or can be incorporated into the tricarboxylic acid (TCA) cycle for the full oxidation[163]. To initiate the process, FA are activated by acyl-CoA-synthetase to acyl-CoA in the cytosol to enable fatty acids to cross membranes. While short- and medium-chain FA pass the mitochondrial membrane without activation, activated long-chain fatty acids need to be transported across the membrane in a carnitine-dependent manner[218]. Fatty acyl-CoAs are converted to fatty acyl-carnitines by CPT1 in the outer mitochondrial membrane for the translocation into the intermembrane space. Fatty acyl-carnitines are then transported across the inner mitochondrial membrane by carnitine acylcarnitine translocase. Carnitine palmitoyltransferase 2 (CPT2), which is expressed on the inner mitochondrial membrane, converts fatty acyl-carnitines back to acyl-CoAs for fatty acid β-oxidation inside the mitochondrial matrix[163] (**Figure 17**).

In the postprandial state, β-oxidation in the liver is suppressed. This occurs in part due to the antilipolytic effect of insulin on white adipose tissue, which reduces the flux of non-esterified fatty acids to the liver, and in part due to the direct control by glucose and insulin over the rate of fatty acid entry into the mitochondria[219]. As described previously, insulin facilitates DNL through the upregulation and activation of SREBP1c and induction of ACC. Malonyl-CoA produced by ACC activity inhibits the activity

of CPT1 (**Figure 17**), and thereby decreases the rate of β-oxidation by reducing fatty acid entry to mitochondria. Under fasting conditions, glucagon promotes fatty acid oxidation. Glucagon signaling activates AMP-activated protein kinase (AMPK), which in turn inactivates ACC1 and ACC2 by phosphorylation, resulting in the blockade of the synthesis of malonyl-CoA[220]. Kohjima *et al.* showed that CPT1 expression in humans is reduced by 50% in NAFLD compared with levels in the normal liver[221].

6.3.1 PPARα

peroxisome-proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor belonging to the NR1C nuclear receptor superfamily. Besides PPARα, the PPAR subfamily contains two other isotypes encoded by PPARδ (NR1C2) and PPARγ (NR1C3) genes[222]. PPARs form heterodimers with the retinoid X receptor (RXR), and activate transcription by binding to a specific DNA element, termed the perixosome proliferator response element (PPRE), in the regulatory region of genes encoding proteins that are involved in lipid metabolism and energy balance[223]. Binding agonists within the ligand-binding site of PPARs cause a conformational change, resulting in the transcriptional activation of their target genes. PPARα ligands are FA derivates formed during lipolysis, *de novo* lipogenesis or FA catabolism[222]. Activated PPARα promotes the expression of genes involved in perixosomal and mitochondrial β-oxidation, FA transport and hepatic glucose metabolism, with the latter being rodent-specific[224]. In addition, PPARα negatively regulates pro-inflammatory and acute phase response signaling pathways, as seen in rodent models of systemic inflammation, atherosclerosis and NASH[222,225].

Studies in NAFLD patients have yielded mixed results in terms of alterations in rates of fatty acid oxidation. Impaired adenosisne triphosphate (ATP) production has been described in patients with NAFLD and insulin resistance[226,227], while others have reported evidence for increased rates of fatty acid oxidation in NAFLD[228,229]. Interestingly, Francque S *et al.* have found that liver PPARα gene expression negatively correlates with NASH

severity, visceral adiposity and insulin resistance and positively with adiponectin. The histological improvement is associated with an increase in expression of PPARα and its target genes[230]. PPARα agonists may therefore potentially be useful in the management of NAFLD. Animal models have revealed that the administration of PPARα agonists not only prevents the development of steatohepatitis, but also reverses hepatic fibrosis by decreasing the expression of fibrotic markers and reducing the number of hepatic stellate cells[231,232].

Like PPARα, some studies have demonstrated a role in FA oxidation and inflammation for peroxisome-proliferator-activated receptor δ (PPARδ). Nagasawa *et al*. demonstrated that the administration of the PPARδ agonist GW501516 to mice with steatohepatitis decreased fat storage in liver mainly by activating the genes involved in FA oxidation, as well as reducing inflammatory gene expression[233]. Moreover, activation of PPARδ not only improved fatty acid oxidation in the liver of obese diabetic mice, but also suppressed *de novo* lipogenesis and glucose production[234]. Interestingly, animal models have also shown that PPARδ agonists were also able to improve hepatic inflammation by suppression of pro-inflammatory cytokines synthesis[235] and provided protection from liver fibrosis[236]. A dual PPARα/δ agonist was recently tested in animal models of NASH/NAFLD, resulting in improvement of steatosis, inflammation and fibrosis[237]. This agonist (GFT505) has already been tested in humans and initial studies in IR patients have shown that GFT505 improves hepatic and peripheral I sensitivity, liver function tests and systemic inflammation[238,239]

6.4 Triglyceride secretion

The liver secretes triglycerides in the form of VLDL particles for delivery to peripheral tissues, including skeletal and cardiac muscle, and adipose tissue. VLDL consists of hydrophobic core lipids containing TG and cholesterol esters, which are covered by hydrophilic phospholipids and ApoB 100[76]. ApoB 100 is a liver-specific ApoB that is critical in VLDL assembly, while apoB 48 in the intestine is associated with chylomicron formation. The VLDL assembly process initially occurs in the rough of ER during the translation and translocation of the ApoB 100 across the ER membrane, resulting in the formation of a partially lipidated ApoB 100, termed nascent VLDL. Nascent VLDL particles are then transported from ER to the Golgi for maduration, where they are lipidated by the activity of microsomal triglyceride transfer protein (MTP), and subsequently released from the liver via exocytosis[240]. The VLDL secretion rate depends not only on the availability of hepatic triglycerides, but also on the overall capacity for VLDL assembly. When the triglyceride availability is reduced, lipid-free apoB 100 is degraded by both proteasomal and non-proteasomal pathways[241]. Insulin is critical in the regulation of VLDL assembly. In response to postprandial insulin release, hepatic VLDL production is suppressed to limit plasma triglyceride excursion, by degradation of apoB 100 and inhibition of MTP transcription[241,242].

Impaired VLDL assembly and secretion result in excessive lipid accumulation in the liver. Indeed, hepatic steatosis has been reported in subjects carrying mutations in apoB 100 (hypobetalipoproteinemia) and in MTP (abetalipoproteinemia)[243]. Moreover, ApoB 100 secretion is not increased in NAFLD, suggesting that ApoB 100 production limits the liver's capacity to export hepatic triglycerides[244,245]. In this respect, prolonged exposure of the liver to non-esterified fatty acids, such as that occurring in the obesity and insulin resistance state, would promote excessive ER stress and other oxidative stress in the liver, leading to the degradation of ApoB 100, thereby decreasing triglyceride secretion and worsening steatosis[76,246].

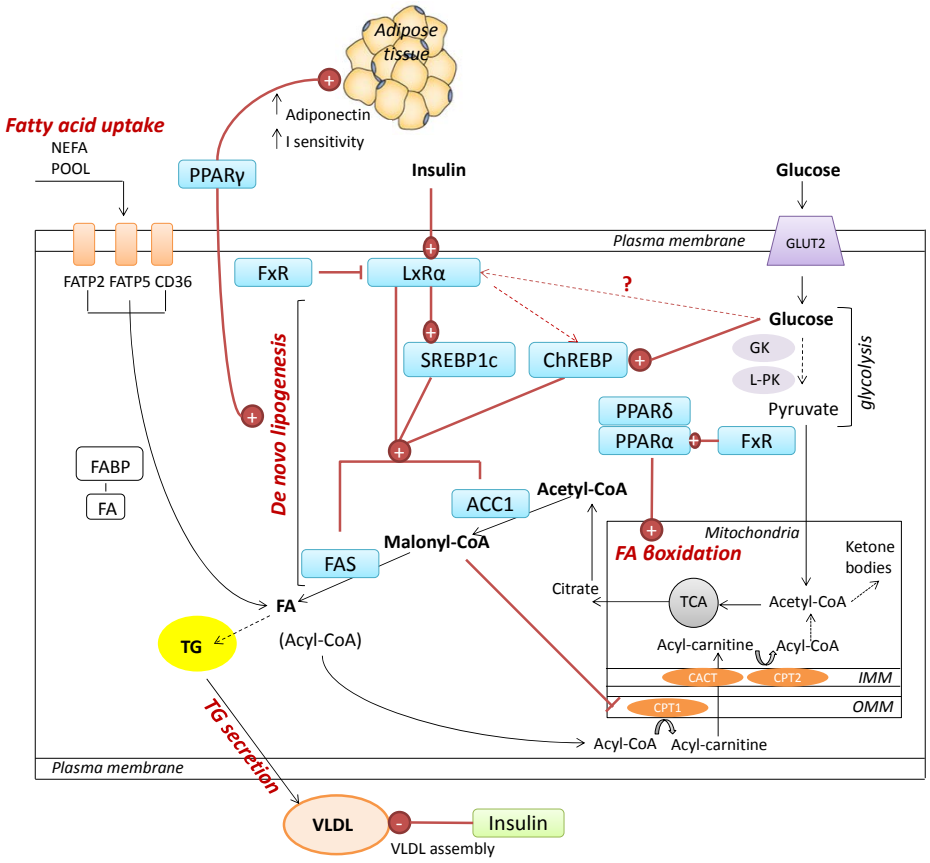


Figure 18. Transcriptional regulation of mechanisms leading to TG accumulation in liver Fatty acid uptake: fatty acid transport protein (FATP) 2, FATP5 and fatty acid translocase (CD36) mediate transport of non-esterified fatty acids (NEFA) across the plasma membrane. Once taken up into cytosol, fatty acids (FA) are activated to form acyl-CoA. Fatty acid binding proteins (FABP) facilitate intracellular transport of FA. *De novo* lipogenesis: the conversion of glucose into FA through *de novo* lipogenesis is nutritionally regulated by glucose and insulin signalling pathways. Insulin activates the transcription factor sterol-regulatory-element-binding protein (SREBP1c), which is under the control of liver X receptor alpha (LxRα), while glucose activates the transcription factor carbohydrate response element binding protein (ChREBP), thereby stimulating the transcriptional activation of lipogenic enzymes, such as acetyl-CoA carboxylase 1 (ACC1) and fatty acis synthase (FAS), the two rate-limiting enzymes in the biosynthesis of FA. Interestingly, ChREBP has also been identified as a

direct target of LxRs, and glucose has been shown to activate LxRs and to induce their target genes, including ChREBP. Peroxisome-proliferator-activated receptor γ (PPARγ) plays a role in promoting FA uptake into adipocytes, adipocyte differentiation, as well as in increasing insulin sensitivity through the transcriptional activation of adiponectin gene in adipocytes, resulting in a reduced delivery of FA to the liver. Meanwhile, several studies attribute a casual role of PPARγ in steatosis devolpment by activation of *de novo* lipogenesis. Fatty acid oxidation: acyl-CoAs are transported into mitochondria across the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) by the activities of carnitine palmitoyl-transferase (CPT) 1, CPT2 and carnitine acylcarnitine translocase (CACT). Within mitochondria, acyl-CoAs are oxidized to form acetyl-CoA. Malonyl-CoA resulting from *de novo* lipogenesis inhibits the activity of CPT1, and thereby decreases the rate of β-oxidation by reducing fatty acid entry to the mitochondria. Activated peroxisome-proliferator-activated receptor α (PPARα) promotes the expression of genes involved in mitochondrial β-oxidation. Hepatic farnesoid x receptor (FxR) activation inhibits FA synthesis by suppressing LxRα activation, and induces the expression of PPARα, which promotes mitochondrial FA β-oxidation. Some animal models have demonstrated a role for peroxisome-proliferator-activated receptor δ (PPARδ) in decreased fat storage in the liver mainly by activating the genes involved in FA oxidation. Triglyceride secretion: triglycerides (TG) are packaged together with apolipoprotein B (ApoB) 100 into very low density lipoprotein (VLDL) in the endoplasmic reticulum by the activity of microsomal triglyceride transfer protein (MTP) and released from the liver. Insulin inhibits VLDL assembly, degrading ApoB 100 and inhibiting the transcription of MTP.

7. THE ROLE OF ENDOCANNABINOID SYSTEM IN NAFLD

The endocannabinoid system mainly consists of cannabinoid receptors type 1 (CB1) and type 2 (CB2), their endogenous ligands, known as endocannabinoids (EC), and the enzymes dedicated to EC biosynthesis and degradation[247]. It has been reported that the EC system plays an important role in NAFLD by modulating lipid metabolism[248]. In particular, CB1 and CB2 receptors in hepatocytes are increasingly being recognized as key mediators of fatty liver by regulating the expression of key enzymes of lipid synthesis and transport[249,250]. As said at the beginning of the introduction section, modulation of cannabinoid receptors is currently emerging as a potential novel therapeutic approach for management of NAFLD[251].

EC mimic the pharmacological actions of the psychoactive principle of marijuana, Δ9-tetrahydrocannabinol (Δ9-THC). They are endogenous lipid-signaling molecules, and are implicated in different physiological and pathological functions (regulation of food intake, immunomodulation, inflammation, analgesia, cancer, addictive behavior, epilepsy and others). The two most extensively studied endocannabinoids are arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG)[252]. Anandamide exhibits a higher affinity for CB1 than CB2 receptors, and 2-AG binds to CB1 and CB2 with similar affinities[253,254]. Both anandamide and 2-AG are synthesized from membrane phospholipid precursors, via parallel pathways involving phospholipase D for anandamide and diacylglycerol lipase for 2-AG[255]. It occurs “on demand” and they are released from cells immediately after production to activate the cannabinoid receptor to elicit a biological response, after which they are inactivated through reuptake[247]. The catabolism of 2-AG is catalysed by monoacylglycerol lipase (MAGL) and that of anadamide by fatty acid amide hydrolase (FAAH)[255].

The cannabinoid receptors are membrane G-protein coupled receptors, first discovered as molecular targets of Δ9-THC. These receptors are involved in the physiological modulation various functions of the central nervous system (CNS) and various peripheral tissues after activation by their endogenous agonists. CB1 receptors are mainly expressed in CNS, but they are also located in liver and other peripheral tissues (gastrointestinal tract, pancreas, skeletal muscle, adipose tissue, heart and reproductive system). Meanwhile, CB2 receptors are primarily expressed in cells of the hematopoietic and immune system. However, the presence of CB2 have been also described in the brain and in peripheral tissues such as pancreas and liver[253,254]. Under physiological conditions, the EC system is silent, since CB1 and CB2 receptors are faintly expressed. By contrast, induction of CB receptors and/or increased levels of EC are common features of liver injuries of diverse origins. In particular, CB1 receptors are upregulated in hepatocytes, hepatic myofibroblasts and endothelial cells, whereas CB2 receptors are induced in Kupffer cells and hepatic myofibroblasts, but are not expressed by hepatocytes. During liver pathology, EC levels are increased with varying patterns depending on the nature of the liver insult, with anandamide produced mainly by Kupffer cells, and 2-AG by hepatic stellate cells and hepatocytes[253]. **Table 5** provides a comparison between the two types of receptors and their role in fatty liver disease.

Table 5. Comparison between CB1 and CB2 receptors

	CB1	CB2
Distribution	Throughout the body, highest density in the CNS	Cells of the immune system
Expression in normal liver	Faint	Faint/absent
Expression in liver pathology	Hepatocytes, endothelial cells, hepatic myofibroblast	Kupffer cells, hepatic myofibroblasts
Main roles	Mood, appetite, emesis control, memory, spatial coordination muscle tone and analgesia	Immune-modulatory, anti-inflammatory, pain, bone loss
Role in fatty liver and MetS	liver steatosis, food intake/body weight, insulin resistance, lipogenesis, splanchnic, vasodilation	Inflammation, fibrogenesis
Role in liver fibrosis	Profibrogenic	Antifibrogenic

Saudi J Gastroenterol 2013;19:144-51[247]

Several items of experimental evidence and clinical trials support the role of both CB1 and CB2 receptors in fatty liver disease

7.1 Role of CB1 receptor in NAFLD

CB1-mediated EC tone is enhanced in experimental diet-induced or genetic models of NAFLD, and is characterized by the upregulation of adipose tissue and hepatocyte CB1 receptors, and by increased liver synthesis of anandamide. The pathogenic role of CB1 receptors in NAFLD is supported by the resistance to steatosis of obese mice with a global or hepatocyte-specific CB1 deletion, and of rodents administered CB1 antagonists[256–258]. Other studies further indicate that the steatogenic

properties of CB1 arise from altered hepatic lipid metabolism, consisting of a combination of hepatocyte activation of SREBP1c-mediated lipogenesis, the reduction of FA oxidation via inhibition of AMP kinase, and reduced release of TG-rich VLDL[256,257,259,260]. In addition, the adipose tissue may largely contribute to the steatogenic process via the CB1-induced release of FFA by adipocytes[261,262] (**Figure 19**).

A potential impact of CB1 receptors on the inflammatory response associated with NASH has been suggested by experiments in obese rats showing that the CB1 antagonist Rimonabant reduces liver inflammation[258]. In addition, fat CB1 receptors reduce the production of adiponectin, an adipokine which reduces hepatic inflammation[257,258]. Chanda *et al*. demonstrated a novel mechanism of action of activated CB1 receptor signaling to induce hepatic gluconeogenesis in primary rat and human hepatocytes; this suggests a role for CB1 receptors activation in regulating the hepatic glucose metabolism[263]. With regard to fibrosis, hepatic CB1 receptors have shown profibrogenic effects in NASH animal models. In these models, antagonism or blockade of CB1 receptors reduces fibrogenesis compared to control animals[264,265]. Finally, activation of CB1 receptors has been also associated with liver regeneration, as well as with cardiovascular alterations associated with advanced cirrhosis[266,267].

In humans, enhanced EC tone has been reported in obese patients prone to developing fatty liver disease and metabolic syndrome. In several studies, obese individuals displayed higher serum levels of EC than lean individuals. There was a strong association between high plasma EC levels and visceral obesity, high TG, low high-density lipoprotein (HDL) cholesterol, and IR in obese as well as T2DM patients[268,269]. More recently, a positive correlation between liver fat content and the arterial and hepatic venous concentrations of 2-AG has been reported, indicating that the human fatty liver takes up 2-AG and overproduces TG containing saturated fatty acids, which might reflect increased *de novo* lipogenesis[248]. Clinical trials with Rimonabant in overweight and obese populations have shown

clear benefits for weight reduction, abdominal obesity, liver steatosis and the improvement of other cardiometaboilc syndrome parameters, including improved insulin sensitivity, elevated plasma adiponectin and HDL cholesterol, and reduced plasma TG and low-density lipoprotein (LDL) cholesterol levels[270–276]. However, the marketing approval for Rimonabant has been withdrawn by the European Regulatory Authorities due to the fact that this drug increases the incidence of psychiatric disorders: depression, anxiety, irritability, and aggression[277,278]. For this reason, in recent years, research in this area has focused on the development of compounds - either antagonists or inverse agonists - which do not cross the blood-brain barrier, and which block peripheral CB1 receptors[279,280].

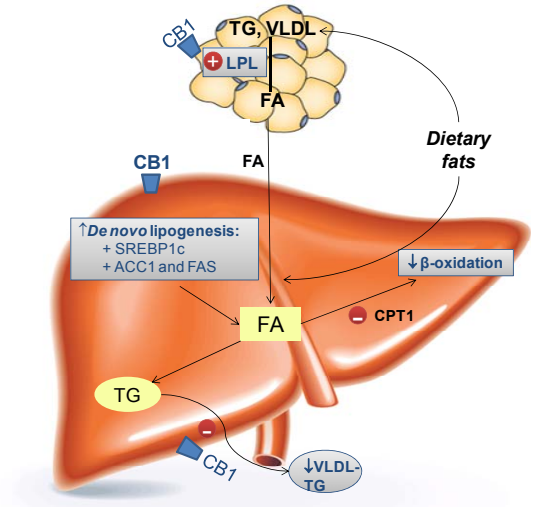


Figure 19. Mechanisms of CB1 involved in hepatic lipid accumulation.
The activation of CB1 receptors in adipose tissue promotes lipoprotein lipase (LPL) activity, which results in an increased release of fatty acids (FA) into the liver. The activation of hepatic CB1 receptors contributes to liver fat accumulation by: 1. Increased *de novo* lipogenesis, inducing the expression of transcription factor SREBP1c and its target key enzymes such as ACC1 and FAS; 2. Decreased fatty acid β-oxidation; 3. Reduced secretion of triglycerides (TG) in the form of very low density lipoprotein (VLDL) particles.

7.2 Role of CB2 receptor in NAFLD

Unlike CB1 receptors, the role of CB2 receptors in the development of fatty liver is still under-investigated. The potential role of CB2 in the pathogenesis of fatty liver has been supported by the finding that, in wild-type (WT) mice fed with a high-fat diet for 6 weeks, the administration

of JWH-133 (CB2 agonist) enhanced liver TG accumulation, IR and potentiated fat inflammation[281]. In contrast, genetic and pharmacological inactivation of CB2 receptors decreased adipose tissue macrophage infiltration, protected mice from both age-related and diet-induced IR[282]. In human studies, CB2 receptors are expressed in all liver samples from patients with steatosis and steatohepatitis[283]. Taken together, these results suggest that CB2 receptors have a potential role in liver steatogenesis and fat inflammatory response associated with insulin resistance.

The role of CB2 receptors in fibrogenesis has not been well-characterized. However, there is some evidence of a potential anti-fibrogenic role of CB2 activation. Selective activation of hepatic CB2 receptors significantly reduced hepatic collagen content in rats with pre-existing cirrhosis and enhanced regenerative response to acute liver injury[284]. Furthermore, CB2–/– mice had enhanced response to fibrogenic stimuli and delayed liver regeneration in response to carbon tetrachloride CCl_4-induced injury[285]. However, treatment with a CB2 agonist, JWH-133, in CCl_4-treated WT mice reduced the injury and accelerated liver regeneration[285]. Moreover, in liver biopsy specimens from patients with active cirrhosis of various etiologies, CB2 receptors were highly up-regulated in cirrhotic liver, and predominantly in hepatic fibrogenic cells. By contrast, they were not detected in normal human liver. Their activation triggered potent antifibrogenic effects – namely growth inhibition and apoptosis[286].

All these data support the hypothesis that an enhanced CB1 tone promotes liver fibrogenesis and cardiovascular alterations associated with cirrhosis, and contributes to the pathogenesis of NAFLD. However, upregulated CB2 signaling displays hepatoprotective effects, reducing liver inflammation, and improving liver fibrogenesis. Consequently, antagonism of CB1 and agonism of CB2 receptors have been identified as promising therapeutic strategies for the management of liver diseases[78]

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III. Hypothesis and Objectives

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The work carried out in this doctoral thesis has focused on one of the main research lines of the GEMMAIR (Grup d'Estudi de Malalties Metabòliques i Insulin Resistència) Research Group. GEMMAIR has a track record of over 25 years' experience in the study of liver and metabolic chronic diseases, and more than 15 years in the study of pathophysiology of obesity and its associated metabolic diseases.

Due to the fact that:

- NAFLD is an universal disorder which is now considered the most common liver disease in the western world
- NAFLD not only affects the adult population, but also children
- NAFLD can progress slowly from simple steatosis to non-alcoholic steatohepatitis, and subsequently to hepatic fibrosis, cirrhosis and hepatocellular carcinoma
- NASH-related cirrhosis and hepatocellular carcinoma will be the major health care problem and the leading indication of liver transplantation in the near future
- Most patients with NAFLD remain asymptomatic until they develop cirrhosis
- There is no specific test that can predict the progression of simple steatosis to non-alcoholic steatohepatitis
- Hepatic biopsy, an invasive diagnostic test, is still the gold standard for determining the staging and grading of NAFLD
- There are currently no effective therapies for NAFLD apart from lifestyle modification, aimed at weight loss

the study of pathophysiological mechanisms involved in the development and progression of NAFLD is of particular interest in order to develop therapeutic and preventive therapies.

The majority of the literature seems to point to the fact that lipid accumulation in the cytoplasm of hepatocytes seems to be the hallmark of NAFLD, and is the early and crucial step in NAFLD development. For this reason, an improved understanding of the underlying mechanisms leading to the initial lipid accumulation in the liver could be of great interest for controlling the progression of NAFLD. It has also been reported that the EC system, mediated mainly by CB1 and CB2 cannabinoid receptors, plays an important role in NAFLD pathogenesis by modulating lipid metabolism.

We therefore hypothesized that in patients with NAFLD, the expression of genes and transcription factors involved in the regulation of hepatic fatty acid metabolism, as well as the expression of cannabinoid receptors could be altered; and this alteration may be related to the onset of liver damage. As a result, the main objectives of this thesis/study were to investigate the fatty acid metabolism in liver of morbidly obese (MO) women with NAFLD, and to study the association of cannabinoid receptors with the disease. To that end, six specific objectives were proposed:

Study 1- Hepatic lipid metabolism

1.1 To evaluate the hepatic mRNA expression of some key genes involved in the *de novo* synthesis of fatty acids (LxRα, SREBP1c, ACC1, FAS), fatty acid uptake and transport (PPARγ, CD36, FABP4), fatty acid oxidation (PPARα) and, finally, inflammation-related genes (IL6, TNFα, CRP, PPARδ) of MO women according to their liver pathology (normal liver, NL; simple steatosis, SS; steatohepatitis, NASH).

1.2 To assess the relationship between the expression of the genes studied and the presence of hepatic fat accumulation, classifying the SS group into different histological degrees: mild, moderate or severe SS.

1.3 To analyse the protein expression of the genes differentially expressed between each group of study by western blot analysis.

1.4 To asses the relationship between the genes differentially expressed in each group of study and glucose metabolism parameters.

Study 2- Cannabinoid receptors

2.1 To evaluate the gene expression profiles of cannabinoid receptors (CB1 and CB2) in the liver of MO women according to their liver pathology.

2.2 To asses the relationship between CB1 and CB2 gene expression and genes related to *de novo* synthesis of fatty acids (ChREBP, SREBP1c, LxRα, FxR, ACC1, and FAS), fatty acid oxidation (PPARα), fatty acid uptake and transport (PPARγ, CD36, and FABP4), inflammation (IL6, TNFα, CRP, PPARδ), and adipokines (adiponectin and resistin) expression in the liver of MO women.

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IV. Results

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IV. Results

1. Altered Fatty Acid Metabolism-Related Gene Expression in Liver from Morbidly Obese Women with Non-Alcoholic Fatty Liver Disease

Int. J. Mol. Sci. 2014, *15*, 22173-22187; doi:10.3390/ijms151222173

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OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

Altered Fatty Acid Metabolism-Related Gene Expression in Liver from Morbidly Obese Women with Non-Alcoholic Fatty Liver Disease

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External Editor: Johannes Haybaeck

Received: 2 October 2014; in revised form: 25 November 2014 / Accepted: 25 November 2014 / Published: 2 December 2014

Abstract: Lipid accumulation in the human liver seems to be a crucial mechanism in the pathogenesis and the progression of non-alcoholic fatty liver disease (NAFLD). We aimed to evaluate gene expression of different fatty acid (FA) metabolism-related genes in

morbidly obese (MO) women with NAFLD. Liver expression of key genes related to *de novo* FA synthesis (LXRα, SREBP1c, ACC1, FAS), FA uptake and transport (PPARγ, CD36, FABP4), FA oxidation (PPARα), and inflammation (IL6, TNFα, CRP, PPARδ) were assessed by RT-qPCR in 127 MO women with normal liver histology (NL, n = 13), simple steatosis (SS, n = 47) and non-alcoholic steatohepatitis (NASH, n = 67). Liver FAS mRNA expression was significantly higher in MO NAFLD women with both SS and NASH compared to those with NL (p = 0.003, p = 0.010, respectively). Hepatic IL6 and TNFα mRNA expression was higher in NASH than in SS subjects (p = 0.033, p = 0.050, respectively). Interestingly, LXRα, ACC1 and FAS expression had an inverse relation with the grade of steatosis. These results were confirmed by western blot analysis. In conclusion, our results indicate that lipogenesis seems to be downregulated in advanced stages of SS, suggesting that, in this type of extreme obesity, the deregulation of the lipogenic pathway might be associated with the severity of steatosis.

Keywords: insulin resistance; morbid obesity; fatty acid metabolism; non-alcoholic fatty liver disease; simple steatosis; non-alcoholic steatohepatitis

1. Introduction

Non-alcoholic fatty liver disease is characterized by an accumulation of triglycerides (TG) in hepatocytes and has frequently been associated with obesity, type 2 diabetes mellitus, hyperlipidemia, and insulin resistance (IR) [1]. NAFLD is an increasingly recognized condition associated with increased cardiovascular and liver-related mortality [2,3]. The pathogenesis of NAFLD has been interpreted by the "double-hit" hypothesis, comprising lipid accumulation as the primary insult or "first hit" in the liver [4,5], followed by a "second hit" in which proinflammatory mediators induce inflammation, hepatocellular injury and fibrosis [6]. Recently, however, some studies have shown that while hepatic TG accumulation seems to be a benign symptom of hepatic steatosis, fatty acid (FA) metabolites contribute to the progression of NAFLD to NASH. IR promotes the recruitment of free FAs from the serum pool as well as intrahepatic fatty acid accumulation, which induces apoptosis and the formation of reactive oxygen species (ROS). FAs themselves also promote hepatic insulin resistance via Toll-like receptor 4 (TLR4) activation that increases the release of inflammatory biomediators such as IL6, IL1β, and the TNFα receptor [7], indicating a vicious cycle of lipid accumulation, and IR as a crucial mechanism in the pathogenesis of NASH, among other mechanisms. In this regard, some authors have suggested a "multiple parallel hits hypothesis" to explain the pathophysiology of NAFLD [8,9].

Lipid accumulation in the human liver seems to be a crucial mechanism in NAFLD pathophysiology, so its regulatory mechanisms need to be understood in order to control the progression of NAFLD. It is known that hyperinsulinemia promotes *de novo* synthesis of fatty acids from glucose and increases free FA flux to the liver due to peripheral IR through the sterol regulatory element-binding protein-1c (SREBP1c) and inhibits fatty acid oxidation through the nuclear receptor peroxisome proliferators-activated receptor-α (PPARα). Then, insulin signalling and nuclear receptors

(including PPARs and LXRα) regulate both hepatic fatty acid and glucose metabolism. Both pathways are closely interrelated and share common regulatory elements and indistinguishably contribute to NAFLD [10]. Regarding that, some authors have described overexpression of genes involved in FA partitioning and binding, lipolysis and inflammation in the human fatty liver [11–15]. In addition, more recently, Ahn *et al.* found that LXRα expression correlated with the degree of hepatic fat deposition, as well as with hepatic inflammation and fibrosis in NAFLD patients [16].

In a previous study we demonstrated that lipogenesis and FA oxidation were downregulated in subcutaneous adipose tissue (SAT) samples from morbidly obese women, suggesting that SAT works to limit any further development of fat mass [17]. Based on that data, we wished to further investigate the fatty acid metabolism in the liver of MO women with NAFLD by evaluating the expression of some key genes involved in *de novo* synthesis of fatty acids (LXRα, SREBP1c, ACC1, FAS), fatty acid uptake and transport (PPARγ, CD36, FABP4), fatty acid oxidation (PPARα) and, finally, inflammation related genes (IL6, TNFα, CRP, PPARδ). Furthermore, as the lipid accumulation in the cytoplasm of hepatocytes seems to be the hallmark of NAFLD, we assessed the relationship between the expression of these genes and the presence of hepatic fat accumulation in this cohort.

2. Results

2.1. Baseline Characteristics of Subjects

The cohort of morbidly obese women was classified according to the liver pathology into normal liver (NL), simple steatosis (SS) and non-alcoholic steatohepatitis (NASH) (Table 1). Age and anthropometrical measurements were not significantly different between the three morbidly obese groups. However, insulin and HbA1c levels were significantly higher in both SS and NASH groups than in the NL group. Glucose levels were significantly higher in SS and tended to be higher in NASH ($p = 0.05$), compared with the NL group. Also, IL6 levels were significantly higher in NASH than in the NL group. Our results indicated that ALT and ALP activity was higher in both SS and NASH groups than in obese women with normal liver histology. Furthermore, AST levels tended to be higher in SS ($p = 0.05$) and were significantly higher in NASH, compared with the NL group.

Table 1. Anthropometric and metabolic variables of the study cohort classified according to the liver pathology.

Variables	NL (*n* = 13)	SS (*n* = 47)		NASH (*n* = 67)		
	Mean ± SEM	Mean ± SEM	*p*-Value 1	Mean ± SEM	*p*-Value 1	*p*-Value 2
Age (years)	44.5 ± 3.2	47.7 ± 1.5	n.s	47.1 ± 1.3	n.s	n.s
Weight (kg)	122.9 ± 4.3	121.6 ± 2.4	n.s	119.4 ± 1.8	n.s	n.s
WC (cm)	131.5 ± 6.2	128.7 ± 1.6	n.s	130.8 ± 1.7	n.s	n.s
BMI (kg/m^2)	49.1 ± 1.9	48.3 ± 1.1	n.s	46.5 ± 0.5	n.s	n.s
Glucose (mg/dL)	100.8 ± 6.8	128.1 ± 6.2	0.026	128.8 ± 6.0	0.05	n.s
Insulin (mUI/L)	13.7 ± 2.6	20.4 ± 1.7	0.048	23.4 ± 3.1	0.04	n.s
HbA1c (%)	5.1 ± 0.3	6 ± 0.3	0.031	6.3 ± 0.2	0.028	n.s
HOMA2-IR	2.1 ± 0.5	2.7 ± 0.2	n.s	2.9 ± 0.5	n.s	n.s

Table 1. *Cont.*

Variables	NL (*n* = 13)	SS (*n* = 47)		NASH (*n* = 67)		
	Mean ± SEM	Mean ± SEM	*p*-Value 1	Mean ± SEM	*p*-Value 1	*p*-Value 2
HDL-C (mg/dL)	43.3 ± 2.6	39.4 ± 1.8	n.s	39.6 ± 1	n.s	n.s
LDL-C (mg/dL)	96 ± 6.9	99.7 ± 4.3	n.s	100.7 ± 3.7	n.s	n.s
Triglycerides (mg/dL)	142.7 ± 13	197.2 ± 15	n.s	156.6 ± 7.9	n.s	n.s
AST (U/L)	25.6 ± 4	45.6 ± 5.3	0.05	43.2 ± 3.5	0.042	n.s
ALT (U/L)	24.6 ± 2.4	44.3 ± 4.6	<0.001	43.2 ± 3.3	<0.001	n.s
GGT (U/L)	23.3 ± 6.9	28.6 ± 3.2	n.s	36.2 ± 5.2	n.s	n.s
ALP (U/L)	57.8 ± 3.2	68.3 ± 2.4	0.028	71.4 ± 2.6	0.032	n.s
Adipo/Cytokine Circulating Levels						
HMW adiponectin (μg/mL)	3.8 ± 1.7	3.3 ± 0.7	n.s	3 ± 0.4	n.s	n.s
IL6 (pg/mL)	2.1 ± 0.3	2.7 ± 0.5	n.s	3.3 ± 0.5	0.031	n.s
TNFRI (ng/mL)	2.8 ± 0.3	3.1 ± 0.2	n.s	3 ± 0.2	n.s	n.s
TNFRII ng/mL	4.2 ± 0.7	5.2 ± 0.5	n.s	5.7 ± 0.4	n.s	n.s
CRP (mg/dL)	2.2 ± 2.1	1.4 ± 0.3	n.s	3 ± 0.8	n.s	0.046
FABP4 (ng/mL)	56.8 ± 16.9	62.6 ± 4.6	n.s	56.4 ± 5.1	n.s	n.s

NL, morbidly obese subjects with normal liver; SS, morbidly obese subjects with simple steatosis; NASH, morbidly obese subjects with steatohepatitis; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C reactive protein; FABP4, fatty acid binding protein 4; GGT, gamma-glutamyltransferase; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment 2-insulin resistance; IL6, interleukin 6; LDL-C, low density lipoprotein cholesterol; TNFRI and II, tumour necrosis factor receptor I and II; WC, waist circumference. ANOVA test was used to compare the gene expression in the different groups. *p*-Value 1 indicates significant differences respect NL group ($p < 0.05$); *p*-Value 2 indicates significant differences respect SS group ($p < 0.05$). n.s indicates no significant differences. Data are expressed as mean ± SEM.

2.2. Evaluation of the Expression of Genes Related to Lipid Metabolism and Inflammation in Liver and Their Protein Expression

We analysed liver expression, in our cohort of morbidly obese women, of some key genes related to the *de novo* synthesis of fatty acids (LXRα, SREBP1c, ACC1, FAS), fatty acid (FA) uptake and transport (PPARγ, CD36, FABP4), FA oxidation (PPARα), and related to inflammation (IL6, TNFα, CRP, PPARδ).

We first classified the whole cohort into NL, SS, and NASH (Table 2). The results indicate that among the key genes related to the *novo* fatty acid synthesis, only FAS mRNA expression was significantly higher in MO NAFLD women with both SS and NASH compared to those with normal liver histology. Regarding inflammation, IL6 hepatic mRNA expression was significantly higher in NASH than in the SS group. Hepatic TNFα mRNA expression tended to be higher in NASH compared with the SS group ($p = 0.05$). No more significant differences were found regarding the other studied fatty acid metabolism-related genes (Table 2).

Table 2. Hepatic expression of genes related to de *novo* fatty acid synthesis, fatty acid uptake and transport, fatty acid oxidation, and inflammation in morbidly obese women according to the liver pathology.

Gene Expression	NL (*n* = 13)	SS (*n* = 47)		NASH (*n* = 67)		
	Mean ± SEM	Mean ± SEM	*p*-Value 1	Mean ± SEM	*p*-Value 1	*p*-Value 2
***De novo* lipogenesis**						
LXRα	9.5 ± 2.6	10.4 ± 1.4	n.s	8.5 ± 1.1	n.s	n.s
SREBP1c	8.1 ± 1.3	10.2 ± 1	n.s	8.7 ± 0.9	n.s	n.s
ACC1	4.4 ± 1.0	6.7 ± 1.7	n.s	7.6 ± 2.1	n.s	n.s
FAS	5.9 ± 1.1	13.9 ± 2.3	0.003	16.8 ± 2.8	0.001	n.s
Fatty acid uptake and transport						
PPARγ	2.9 ± 0.5	4.6 ± 0.7	n.s	4.8 ± 1.1	n.s	n.s
CD36	6.1 ± 0.9	6.3 ± 0.7	n.s	5.8 ± 0.7	n.s	n.s
FABP4	1.1 ± 0.4	3.3 ± 0.8	n.s	3.5 ± 1.3	n.s	n.s
Fatty acid oxidation						
PPARα	26.1 ± 4.5	26.6 ± 3.6	n.s	21.2 ± 3	n.s	n.s
Inflammation						
IL6	1.1 ± 0.7	0.5 ± 0.1	n.s	1.5 ± 0.4	n.s	0.033
TNFα	0.8 ± 0.6	0.4 ± 0.1	n.s	1 ± 0.2	n.s	0.050
CRP	117.4 ± 19.9	167.4 ± 30	n.s	165.4 ± 26.4	n.s	n.s
PPARδ	3.6 ± 0.8	4.9 ± 0.7	n.s	3.7 ± 0.5	n.s	n.s

NL, morbidly obese subjects with normal liver; SS, morbidly obese subjects with simple steatosis; NASH, morbidly obese subjects with steatohepatitis. ANOVA test was used to compare the gene expression in the different groups. *p*-Value 1 indicates significant differences respect NL group ($p < 0.05$); *p*-Value 2 indicates significant differences respect SS group ($p < 0.05$). n.s indicates no significant differences. Data are expressed as mean ± SEM.

Then, in order to add to the current knowledge about the role of lipid metabolism alterations in simple steatosis, we assessed the relationship between the expression of the studied genes and the presence of hepatic fat accumulation, classifying the SS group into different grades: mild, moderate or severe SS (Table 3).

Table 3. Hepatic expression of genes related to *de novo* fatty acid synthesis, fatty acid uptake and transport, fatty acid oxidation, and inflammation in morbidly obese women diagnosed with different degrees of simple steatosis (SS).

Gene Expression	MILD SS (*n* = 18)	MODERATE SS (*n* = 16)		SEVERE SS (*n* = 13)		
	Mean ± SEM	Mean ± SEM	*p*-Value 1	Mean ± SEM	*p*-Value 1	*p*-Value 2
***De novo* lipogenesis**						
LXRα	12.5 ± 3.1	11 ± 1.8	n.s	4.8 ± 2.2	0.05	0.05
SREBP1c	10.9 ± 2.3	11.8 ± 1.6	n.s	8.7 ± 2.0	n.s	n.s
ACC1	6.6 ± 1.9	6.4 ± 1.3	n.s	2.4 ± 0.4	0.042	0.008
FAS	15.2 ± 4.4	16.3 ± 3.8	n.s	7.4 ± 1.6	n.s	0.047

Table 3. *Cont.*

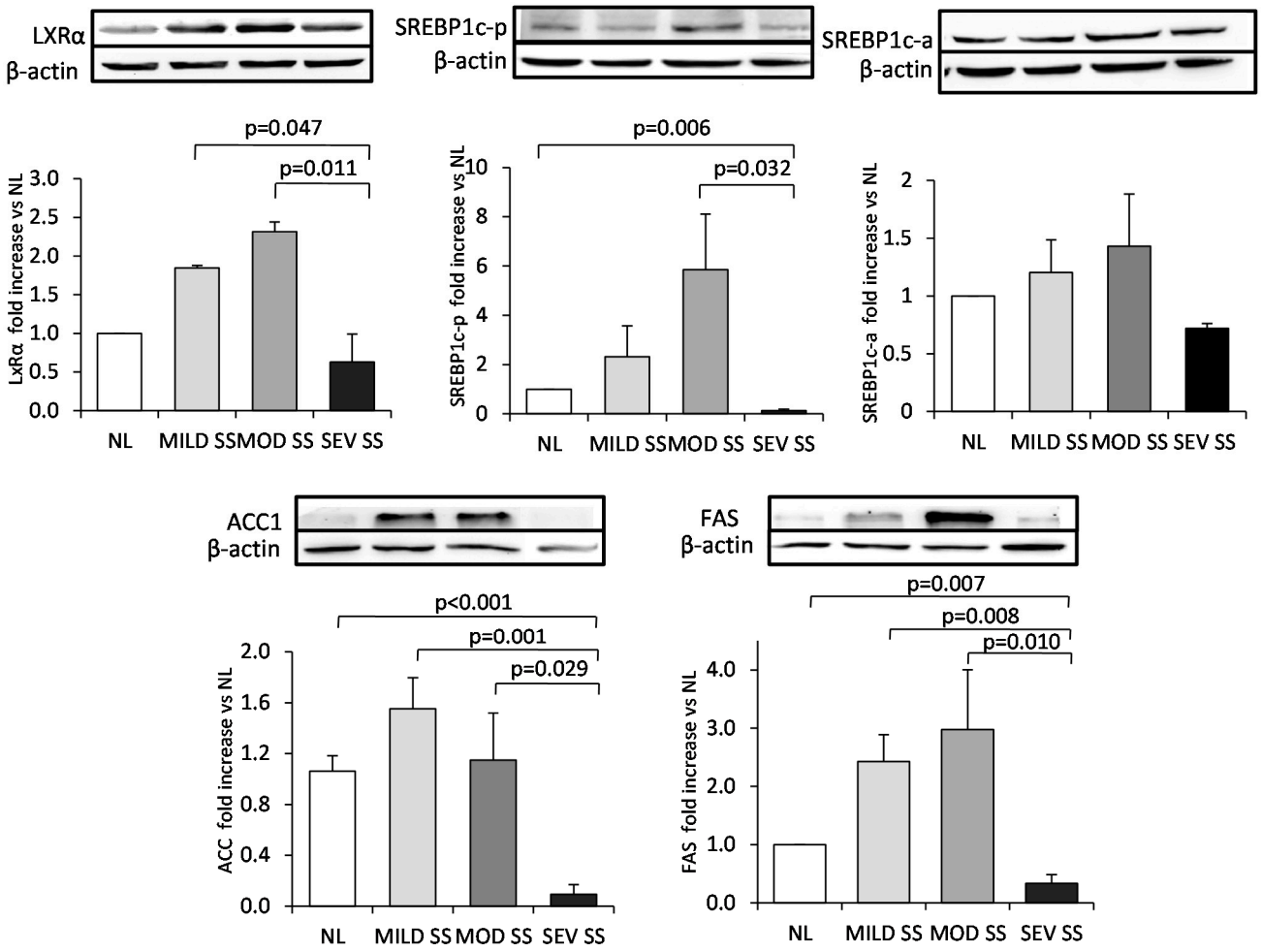
Gene Expression	MILD SS (*n* = 18) Mean ± SEM	MODERATE SS (*n* = 16) Mean ± SEM	*p*-Value 1	SEVERE SS (*n* = 13) Mean ± SEM	*p*-Value 1	*p*-Value 2
Fatty acid uptake and transport						
PPARγ	6.1 ± 1.6	4.7 ± 1.2	n.s	2.9 ± 0.9	n.s	n.s
CD36	5.4 ± 1.4	8.4 ± 0.9	n.s	5.3 ± 1.2	n.s	n.s
FABP4	1.7 ± 0.6	4.7 ± 1.4	n.s	4.9 ± 2.3	n.s	n.s
Fatty acid oxidation						
PPARα	31.5 ± 7.9	24.5 ± 4.9	n.s	23.1 ± 6.2	n.s	n.s
Inflammation						
IL6	0.6 ± 0.2	0.5 ± 0.1	n.s	0.4 ± 0.2	n.s	n.s
TNFα	0.3 ± 0.1	0.4 ± 0.1	n.s	0.5 ± 0.2	n.s	n.s
CRP	129.9 ± 63.7	132.7 ± 34.5	n.s	139 ± 64.1	n.s	n.s
PPARδ	5.7 ± 1.2	5.6 ± 1	n.s	4.3 ± 2.2	n.s	n.s

NL, morbidly obese subjects with normal liver; MILD SS, morbidly obese subjects with mild simple steatosis; MODERATE SS, morbidly obese subjects with moderate simple steatosis; SEVERE SS, morbidly obese subjects with severe simple steatosis. ANOVA test was used to compare the gene expression in the different groups. *p*-Value 1 indicates significant differences respect MILD SS group ($p < 0.05$); *p*-Value 2 indicates significant differences respect MODERATE SS group ($p < 0.05$). n.s indicates no significant differences. Data are expressed as mean ± SEM.

Regarding *de novo* synthesis of fatty acids, liver ACC1 mRNA expression was down regulated in severe SS compared to both mild and moderate SS groups. In addition, LXRα mRNA expression tended to be lower in severe SS compared to both mild and moderate SS groups ($p = 0.05$). Hepatic FAS mRNA expression levels were significantly lower in severe SS compared to the moderate SS group. Regarding SREBP1c, despite we did not find any significant difference, its mRNA expression seem to be lower in severe SS compared to both mild and moderate SS groups (Table 3).

In order to confirm these results regarding lipogenic gene expression, we also analyzed the protein expression of the key genes related to the *de novo* fatty acid synthesis by western blot analysis. There were similar results with respect to its protein expression and those obtained in the gene expression analysis. LXRα, SREBP1c precursor form, ACC1 and FAS protein expression was significantly lower in morbidly obese women with severe SS compared to those with moderate SS ($p < 0.05$). We also determined the activated SREBP1c form by Western blot analysis. Our results show that, although we did not find any significant difference between groups, activated SREBP1c protein has a similar profile of both ACC1 and FAS mRNA and protein expression, two well known target genes of SREBP1c (Figure 1).

Figure 1. Liver expression of lipogenic transcription factors and enzymes in morbidly obese patients diagnosed with different degree of simple steatosis. Representative Western blot analysis showing LXRα, SREBP1c-precursor form (SREBP1c-p), SREBP1c-active form (SREBP1c-a), ACC1, FAS and β-actin protein expression and bar graphs showing the quantification of bands normalized by values of β-actin bands (n = 28: 6 NL, 8 MILD SS, 8 MOD SS, 6 SEV SS). Results are shown as mean ± SD. $p < 0.05$ are considered statistically significant. NL, morbidly obese subjects with normal liver; MILD SS, morbidly obese subjects with mild simple steatosis; MOD SS, morbidly obese subjects with moderate simple steatosis; SEV SS, morbidly obese subjects with severe simple steatosis.



We also studied the genes related to FA oxidation, FA uptake and transport, and related to inflammation in the SS cohort classified into different grades of simple steatosis. In this case, we did not find any significant difference on its mRNA expression levels (Table 3).

2.3. Correlations between the Expression of Genes Related to Lipid Metabolism and Inflammation with Glucose Metabolism Parameters

Our results showed that liver FAS expression correlated positively with glucose circulating levels in the morbidly obese cohort (Table 4).

Regarding FA uptake and transport, hepatic CD36 expression correlates positively with insulin circulating levels, and also with HOMA2-IR in the morbidly obese group. In addition, we found a positive correlation between FABP4 expression and glucose, insulin, HbA1c and HOMA2-IR (Table 4). Interestingly, when we classify the morbidly obese cohort into NL, SS and NASH, we observed that both CD36 and FABP4 correlations with glucose metabolism parameters became stronger in NASH (CD36: Insulin $r = 0.550$, $p = 0.010$; HOMA2-IR $r = 0.546$, $p = 0.010$) (FABP4: Glucose $r = 0.801$, $p < 0.001$; Insulin $r = 0.833$, $p < 0.001$; HbA1c $r = 0.893$, $p < 0.001$; HOMA2-IR $r = 0.838$, $p < 0.001$).

Finally, liver IL6 expression correlated positively with insulin circulating levels and with HOMA2-IR in the morbidly obese group (Table 4).

Table 4. Significant correlations between the expression of genes related to lipid metabolism and inflammation with glucose metabolism parameters in the morbidly obese cohort.

Variables	FABP4		FAS		CD36		IL6	
	r	*p*-Value	*r*	*p*-Value	*r*	*p*-Value	*r*	*p*-Value
Glucose (mg/dL)	0.465	0.001	0.185	0.035	−0.055	0.697	0.014	0.925
Insulin (mu/L)	0.710	<0.001	0.008	0.933	0.357	0.013	0.371	0.011
Homa2-IR	0.714	<0.001	0.079	0.514	0.354	0.014	0.369	0.012
Hba1c (%)	0.742	<0.001	0.118	0.24	0.155	0.325	0.185	0.252

HbA1c, glycosylated haemoglobin; HOMA2-IR, homeostatic model assessment 2-insulin resistance.

3. Discussion

The novelty of this study lies in the fact that it establishes a clear relationships between NAFLD and genes related to *de novo* synthesis of fatty acids, FA uptake and transport, FA oxidation, and related to inflammation, in an extensive cohort of women with morbid obesity (BMI > 40 kg/m^2). Moreover, this design provides a comparison of gene expression and protein levels between different degrees of simple steatosis according to the percentage of liver fat deposition (mild, moderate or severe SS).

The present study demonstrates that FAS, well known as an important lipogenic enzyme, is overexpressed in the liver of MO NAFLD patients with both simple steatosis and non-alcoholic steatohepatitis, in agreement with other authors [13,14,18]. Other studies have shown enhanced expression of LXRα and SREBP1c in NAFLD [13–15,18,19]. However, we were unable to find any other significant difference in other related genes to the *de novo* lipogenesis pathway. These discrepancies might be explained by differences in the cohort of the patients studied. Higuchi *et al.* [13] included normal weight patients with NAFLD and Lima-Cabello *et al.* [14] included patients with NAFLD and with steatosis related to chronic hepatitis C virus infection in mildly overweight men and women. Finally, Nakamuta *et al.*, who included a cohort of obese patients, observed that the expression of LXRα and ACC1 was upregulated in NAFLD and it was more noticeable in non-obese than in obese patients [19].

In order to add to the current knowledge about the role of lipid metabolism alterations in simple steatosis, we evaluated the expression of these genes in morbidly obese patients with different histopathology types of SS according to the hepatic fat deposition. The most outstanding finding of the present study is that the liver expression of key genes related to *de novo* fatty acid synthesis (LXRα, ACC1 and FAS) have an inverse relationship with the grade of steatosis, that is to say, it diminishes

when the grade of steatosis increases. This novel finding was confirmed evaluating the protein expression, obtaining similar results with respect to LXRα, ACC1, FAS levels, and also with respect to SREBP1c. Our findings indicate that, in this type of extreme obesity, the hepatic lipogenic pathway seems to be downregulated in advanced stages of simple steatosis. The explanation for these results are complex, however experimental studies have shown that in mice with total insulin resistance in liver, insulin fails to stimulate the synthesis of fatty acids and triglycerides [20,21]. In this context, the liver of an extremely obese patient with severe steatosis might behave as if there were total insulin resistance, which could be responsible for the downregulation of the lipogenic pathway shown in the liver of these patients. However, in the present study we did not perform hyperinsulinemic euglycemic clamp to measure hepatic insulin sensitivity in order to confirm this hypothesis.

We also found that IL6 and TNFα, two important proinflammatory adipocytokines hugely expressed in the adipose tissue of obese human subjects and patients with IR [22,23], were overexpressed in the liver of NAFLD MO women with NASH compared to those with simple steatosis. These results are in agreement with the literature, which supports that they correlate with histological severity in obese patients. For instance, Crespo *et al.* reported increased hepatic expression of TNFα in patients with NASH compared to SS patients [24]. Moreover, Wieckowska *et al.* demonstrated markedly increased IL6 expression in the liver of NAFLD patients with NASH compared to those with SS or normal liver [25].

Insulin resistance is known to be an intrinsic defect in NAFLD that is closely associated with steatosis, inflammation and disease progression in NASH. Moreover, IR has been described as the main factor associated with NASH development, as well as genetic and environmental factors [12,26,27]. In this regard, we found correlations between some important genes related to the hepatic uptake and transport of fatty acids (CD36 and FABP4) and the presence of IR and insulin circulating levels in our cohort of MO NAFLD women, becoming stronger in those with NASH. These findings are in agreement with Miquilena-Colina *et al.* who observed a significant correlation between hepatic CD36 expression and plasma insulin levels and insulin resistance (HOMA-IR) in patients with NASH [28]. Furthermore, we observed the same correlation regarding IL6 gene expression. These results indicate that hepatic fatty acid accumulation, as well as inflammation, might be contributing to NAFLD progression in relation with insulin resistance.

In addition, we found a positive correlation between glucose circulating levels and liver FAS expression in the MO cohort. This finding supports the reported observation that glucose binds and activates LXRs transcription factors and induces their target genes, including SREBP1c, ACC1 and FAS [29,30].

Our cohort of severely obese women has made it possible to establish clear relationships between NAFLD and fatty acid metabolism-related genes without the interference of such confounding factors as gender or age. These results cannot be extrapolated to other obesity groups, normal-weight or over-weight women or men. Further studies, including these cohorts, would be useful in order to validate our findings. Another limitation is that we did not assess the hepatic insulin sensitivity by hyperinsulinemic euglycemic clamp.

4. Materials and Methods

4.1. Subjects

The study was approved by the institutional review board (Comitè d'Ètica d'Investigació Clínic, Hospital Sant Joan de Reus, 09-06-25/6proj2). All participants gave written informed consent for participation in medical research. We included 127 morbidly obese women (BMI > 40 kg/m^2) of Western European descent. Liver biopsies were obtained during planned laparoscopic bariatric surgery. All biopsies were performed for clinical indications.

The diagnosis of NAFLD was made using the following criteria: (1) liver pathology; (2) an intake of less than 10 gr. of ethanol/day; and (3) appropriate exclusion of other liver diseases.

The weight of all subjects was stable with no fluctuation in body weight greater than 2% for at least 3 months prior to surgery. The exclusion criteria were: (1) concurrent use of medications known to produce hepatic steatosis; (2) patients using lipid-lowering medications including PPARα or -γ agonists; (3) diabetic women who were receiving insulin or on medication likely to influence endogenous insulin levels; (4) menopausal and post-menopausal women and subjects receiving contraceptive treatment; (5) patients who had an acute illness, current evidence of acute or chronic inflammatory or infectious diseases or end-stage malignant diseases.

4.2. Liver Pathology

Liver samples were stained with hematoxylin and eosin, and Manson's trichrome stains and scored by two experienced hepatopathologists using the methods described before [31,32]. Simple steatosis (SS) was graded as follows: grade 1 or mild SS: more than 5% and less than 33% of hepatocytes affected; grade 2 or moderate SS: 33% to 66% of hepatocytes affected; or grade 3 or severe SS: more than 66% of hepatocytes affected. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus.

According to their liver pathology [30,31], patients were sub-classified into the following groups: (1) normal liver (NL) histology (n = 13); (2) simple steatosis (SS) (micro/macrovesicular steatosis without inflammation or fibrosis, n = 47); (3) non-alcoholic steatohepatitis (NASH) (Brunt grade 1–3, n = 67).

4.3. Biochemical Analyses

A complete anthropometrical, biochemical, and physical examination was carried out on each patient. Body height and weight were measured with the patient standing in light clothes and shoeless. Body mass index was calculated as body weight divided by height squared (kg/m^2). The subjects' waist circumference was measured with a soft tape midway between the lowest rib and the iliac crest. Laboratory studies included glucose, insulin, glycated haemoglobin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and transaminases, all of which were analysed using a conventional automated analyser. Insulin resistance was estimated using homeostasis model assessment of IR (HOMA2-IR) [33].

We determined the circulating levels of several molecules related to inflammation including adipokines (HMW adiponectin), acute phase proteins (CRP) and proinflammatory cytokines (IL6, TNFRI and TNFRII). Circulating levels of HMW adiponectin (EMD Millipore, St. Charles, MO, USA), CRP (Dade Behring, Marburg, Germany), IL6 (Quantikine, R&D Systems, Minneapolis, MN, USA), FABP4 (Biovendor, Modrice, Czech Republic), TNFRI and TNFRII (AssayPro, St. Charles, MO, USA) were measured in duplicate using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's instructions.

4.4. RNA Isolation and Real-Time PCR

Liver samples were conserved in RNAlater (Sigma, Barcelona, Spain) for 24 h at 4 °C and then stored at −80 °C. Total RNA from liver was isolated according to the manufacturers' protocols RNeasy mini kit (Qiagen, Barcelona, Spain). RNA was digested with DNase I (RNase-Free DNase set; Qiagen). First-strand cDNA was synthesized using an equal amount of total RNA with the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Madrid, Spain). Real-time quantitative PCR was carried out in a final volume of 20 μL, which contained 10 ng of reverse-transcribed cDNA, 10 μL of 2× TaqMan Fast Universal PCR Master Mix (Applied Biosystems) and 1 μL TaqMan Assay predesigned by Applied Biosystems for the detection of LXRα, SREBP1c, ACC1, FAS, PPARγ, CD36, FABP4, PPARα, IL6, TNFα, CRP, PPARδ, and 18S ribosomal RNA, which was used as the housekeeping gene. The mRNA expression for each gene and sample was calculated using the recommended $2^{-\Delta\Delta Ct}$ method. All reactions were carried out in duplicate in 96-well plates using the 7900HT Fast Real-Time PCR systems (Applied Biosystems).

4.5. Western Blot Analysis

Protein levels were evaluated in a subgroup of 28 subjects (6 MO women with NL, 8 with mild SS, 8 with moderate SS and 6 with severe SS), for whom enough tissue was available. Liver samples were homogenized in a medium containing 50 mM HEPES, 150 mM NaCl, 1 mM DTT, 0.1% SDS and 1% protease inhibitor cocktail (Thermo Scientific, Madrid, Spain). Protein concentrations were determined by using a BCA assay Kit (Thermo Scientific). For Western blot analysis, equal amounts of protein (50 μg) were separated by SDS/PAGE (7% acrylamide) and transferred onto nylon membranes. Non-specific binding was blocked by preincubation of the membranes with 5% (*w*/*v*) non-fat milk powder in 0.1% PBS-Tween for 1 h. Specific protein expression was detected by incubating with goat anti-LXRα (Santa Cruz Biotechnology, Inc., Heidelberg, Germany), rabbit anti-SREBP1c (Thermo Scientific), rabbit anti-ACC1 (Cell Signaling Technology, Inc., Barcelona, Spain) and rabbit anti-FAS (Cell Signaling Technology) antibodies overnight at 4 °C, followed by an incubation with anti-mouse IgG (GE Healthcare, Freiburg, Germany), anti-goat IgG (Santa Cruz Biotechnology, Inc.) or anti-rabbit IgG (GE Healthcare) antibodies for 2 h at room temperature and developed with SuperSignal West Pico Chemiluminescent or SuperSignal Femto Maximum Sensitivity Substrate (Thermo Scientific). The density of specific bands was determined by densitometry and quantified by the Phoretix 1D software from TotalLab. The expression pattern of all proteins was normalized by β-actin (Sigma) liver expression.

4.6. Statistical Analysis

All the values reported were analyzed using the SPSS/PC+ for windows statistical package (version 19.0; SPSS, Chicago, IL, USA). One-way ANOVA with post-hoc Tukey test was used to compare continuous variables between groups. The strength of association between variables was calculated using Pearson's method for parametric variables and Spearman's ρ-correlation test for non-parametric contrasts. *p*-Values <0.05 were considered to be statistically significant.

5. Conclusions

In conclusion, although it was not possible to determine the causality that leads to the downregulation of *de novo* fatty acid synthesis in advanced stages of simple steatosis, our results suggest that, in NAFLD patients with this type of extreme obesity, the deregulation of the lipogenic pathway might be associated with the severity of simple steatosis. Prospective studies are needed in order to better understand the alteration of fatty acid metabolism-related pathways in morbidly obese subjects with NAFLD.

Acknowledgments

This study was supported by the Ministry of Science and Innovation of the government of Spain (grant number SAF 2008-02278, to Cristóbal Richart), the Fondo de Investigación Sanitaria (grant number PS09/01778 to Teresa Auguet and PI13/00468, to Teresa Auguet), by funds from Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR 2009 SGR 959 to Cristóbal Richart), Grup de Recerca en Medicina Aplicada URV (2010 PFR-URV-B2-14 to Cristóbal Richart) and by the Fundación Biociencia.

Author Contributions

Teresa Auguet and Alba Berlanga participated in the design of the study, in the analysis and interpretation of data and were involved in drafting the manuscript. Esther Guiu-Jurado and Carmen Aguilar carried out the experimental work. Gemma Aragonès reviewed/edited the manuscript. Salomé Martinez and Joan Josep Sirvent are the pathologists who scored liver samples. José Antonio Porras, Fátima Sabench and Mercé Hernandez made substantial contributions to the conception and design of the study, and to the acquisition of data. Daniel Del Castillo and Cristóbal Richart revised the draft and gave final approval for publication. The authors have all seen the final version.

Abbreviations

ACC1, acetyl-coenzyme A carboxylase 1; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; ALP, alkaline phosphatase; BMI, body mass index; CD36, hepatic fatty acid translocase; CRP, c-reactive protein; FA, fatty acid; FABP4, fatty acid binding protein 4; FAS, fatty acid synthase; 18S, 18S ribosomal RNA; GGT, γ-glutamyl transferase; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment

method insulin resistance; IL6, interleukin 6; IL1β, interleukin 1β; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; LXRα, liver X receptor; MOD SS, moderate simple steatosis; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NL, normal liver; PPARα, peroxisome-proliferator-activated receptor α; PPARδ, peroxisome-proliferator-activated receptor δ; PPARγ, peroxisome-proliferator-activated receptor *γ*; SEV SS, severe simple steatosis; SREBP1c, sterol-regulatory-element-binding protein; SS, simple steatosis; TG, triglycerides; TLR4, Toll-like receptor 4; TNFα, tumour necrosis factor; TNFRI, tumour necrosis factor receptor I; TNFRII, tumour necrosis factor receptor II; WC, waist circumference.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Marchesini, G.; Bugianesi, E.; Forlani, G.; Cerrelli, F.; Lenzi, M.; Manini, R.; Natale, S.; Vanni, E.; Villanova, N.; Melchionda, N.; *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* **2003**, *37*, 917–923.
2. Angulo, P. Obesity and nonalcoholic fatty liver disease. *Nutr. Rev.* **2007**, *65*, 57–63.
3. Nakamuta, M.; Kohjima, M.; Morizono, S.; Kotoh, K.; Yoshimoto, T.; Miyagi, I.; Enjoji, M. Evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **2005**, *16*, 631–635.
4. Haider, D.G.; Schindler, K.; Schaller, G.; Prager, G.; Wolzt, M.; Ludvik, B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 1578–1581.
5. Farrell, G.C.; Larter, C.Z. Nonalcoholic fatty liver disease: From steatosis to cirrhosis. *Hepatology* **2006**, *43*, S99–S112.
6. Tacke, F.; Luedde, T.; Trautwein, C. Inflammatory pathways in liver homeostasis and liver injury. *Clin. Rev. Allergy Immunol.* **2009**, *36*, 4–12.
7. Jialal, I.; Kaur, H.; Devaraj, S. Toll-like receptor status in obesity and metabolic syndrome: A translational perspective. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 39–48.
8. Tilg, H.; Moschen, A.R. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. *Hepatology* **2010**, *52*, 1836–1846.
9. Berlanga, A.; Guiu-Jurado, E.; Porras, J.A.; Auguet, T. Molecular pathways in non-alcoholic fatty liver disease. *Clin. Exp. Gastroenterol.* **2014**, *7*, 221–239.
10. Bechmann, L.P.; Hannivoort, R.A.; Gerken, G.; Hotamisligil, G.S.; Trauner, M.; Canbay, A. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J. Hepatol.* **2012**, *56*, 952–964.
11. Lemoine, M.; Barbu, V.; Girard, P.M.; Kim, M.; Bastard, J.P.; 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.

12. Westerbacka, J.; Kolak, M.; Kiviluoto, T.; Arkkila, P.; Sirén, J.; Hamsten, A.; Fisher, R.M.; 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.
13. Higuchi, N.; Kato, M.; Shundo, Y.; Tajiri, H.; Tanaka, M.; Yamashita, N.; Kohjima, M.; Kotoh, K.; Nakamuta, M.; Takayanagi, R.; *et al.* 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.
14. Lima-Cabello, E.; Garcia-Mediavilla, M.V.; Miquilena-Colina, M.E.; Vargas-Castrillon, J.; Lozano-Rodriguez, T.; Fernandez-Bermejo, M.; Olcoz, J.L.; Gonzalez-Gallego, J.; Garcia-Monzon, C.; Sanchez-Campos, S. Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. *Clin. Sci.* **2011**, *120*, 239–250.
15. Kohjima, M.; Higuchi, N.; Kato, M.; Kotoh, K.; Yoshimoto, T.; Fujino, T.; Yada, M.; Yada, R.; Harada, N.; Enjoji, M.; *et al.* SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **2008**, *21*, 507–511.
16. Ahn, S.B.; Jang, K.; Jun, D.W.; Lee, B.H.; Shin, K.J. Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease. *Dig. Dis. Sci.* **2014**, *59*, 2975–2982.
17. Auguet, T.; Guiu-Jurado, E.; Berlanga, A.; Terra, X.; Martinez, S.; Porras, J.A.; Ceausu, A.; Sabench, F.; Hernandez, M.; Aguilar, C.; *et al.* Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women. *Obesity (Silver Spring)* **2014**, *22*, 2032–2038.
18. Kohjima, M.; Enjoji, M.; Higuchi, N.; Kato, M.; Kotoh, K.; Yoshimoto, T.; Fujino, T.; Yada, M.; Yada, R.; Harada, N.; *et al.* Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **2007**, *20*, 351–358.
19. Nakamuta, M.; Fujino, T.; Yada, R.; Yada, M.; Yasutake, K.; Yoshimoto, T.; Harada, N.; Higuchi, N.; Kato, M.; Kohjima, M.; *et al.* Impact of cholesterol metabolism and the LXRalpha-SREBP-1c pathway on nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **2009**, *23*, 603–608.
20. Brown, M.S.; Goldstein, J.L. Selective *vs.* total insulin resistance: A pathogenic paradox. *Cell Metab.* **2008**, *7*, 95–96.
21. Biddinger, S.B.; Hernandez-Ono, A.; Rask-Madsen, C.; Haas, J.T.; Alemán, J.O.; Suzuki, R.; Scapa, E.F.; Agarwal, C.; Carey, M.C.; Stephanopoulos, G.; *et al.* Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab.* **2008**, *7*, 125–134.
22. Hotamisligil, G.S.; Shargill, N.S.; Spiegelman, B.M. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* **1993**, *259*, 87–91.
23. Kern, P.A.; Saghizadeh, M.; Ong, J.M.; Bosch, R.J.; Deem, R.; Simsolo, R.B. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J. Clin. Investig.* **1995**, *95*, 2111–2119.

24. Crespo, J.; Cayón, A.; Fernández-Gil, P.; Hernández-Guerra, M.; Mayorga, M.; Domínguez-Díez, A.; Fernández-Escalante, J.C.; Pons-Romero, F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* **2001**, *34*, 1158–1163.
25. Wieckowska, A.; Papouchado, B.G.; Li, Z.; Lopez, R.; Zein, N.N.; Feldstein, A.E. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am. J. Gastroenterol.* **2008**, *103*, 1372–1379.
26. Petta, S.; Muratore, C.; Craxì, A. Non-alcoholic fatty liver disease pathogenesis: The present and the future. *Dig. Liver Dis.* **2009**, *41*, 615–625.
27. Utzschneider, K.M.; Kahn, S.E. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 4753–4761.
28. Miquilena-Colina, M.E.; Lima-Cabello, E.; Sánchez-Campos, S.; García-Mediavilla, M.V.; Fernández-Bermejo, M.; Lozano-Rodríguez, T.; Vargas-Castrillón, J.; Buqué, X.; Ochoa, B.; Aspichueta, P.; *et al.* Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut* **2011**, *60*, 1394–1402.
29. Denechaud, P.-D.; Dentin, R.; Girard, J.; Postic, C. Role of ChREBP in hepatic steatosis and insulin resistance. *FEBS Lett.* **2008**, *582*, 68–73.
30. Mitro, N.; Mak, P.A.; Vargas, L.; Godio, C.; Hampton, E.; Molteni, V.; Kreusch, A.; Saez, E. The nuclear receptor LXR is a glucose sensor. *Nature* **2007**, *445*, 219–223.
31. Kleiner, D.E.; Brunt, E.M.; van Natta, M.; Behling, C.; Contos, M.J.; Cummings, O.W.; Ferrell, L.D.; Liu, Y.C.; Torbenson, M.S.; Unalp-Arida, A.; *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **2005**, *41*, 1313–1321.
32. Brunt, E.M.; Janney, C.G.; di Bisceglie, A.M.; Neuschwander-Tetri, B.A.; Bacon, B.R. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.* **1999**, *94*, 2467–2474.
33. Terra, X.; Auguet, T.; Broch, M.; Sabench, F.; Hernandez, M.; Pastor, R.M.; Quesada, I.M.; Luna, A.; Aguilar, C.; del Castillo, D.; *et al.* Retinol binding protein-4 circulating levels were higher in nonalcoholic fatty liver disease *vs.* histologically normal liver from morbidly obese women. *Obesity (Silver Spring)* **2012**, *21*, 170–177.

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Biomed Res Int. 2014;2014:502542. doi: 10.1155/2014/502542.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 502542, 7 pages
http://dx.doi.org/10.1155/2014/502542

Research Article

Endocannabinoid Receptors Gene Expression in Morbidly Obese Women with Nonalcoholic Fatty Liver Disease

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Received 20 February 2014; Accepted 28 March 2014; Published 23 April 2014

Academic Editor: Luca Miele

Background. Recent reports suggest a role for the endocannabinoid system in the pathology of nonalcoholic fatty liver disease (NAFLD). The aim of this study was to investigate the relationship between liver expression of cannabinoid (CB) receptor subtypes, CB1 and CB2, in morbidly obese (MO) women with different histological stages of NAFLD. *Methods*. We analysed hepatic CB1 and CB2 mRNA expression, and the expression of genes involved in lipid metabolism in 72 MO women, subclassified by liver histology into MO with normal liver (NL, $n = 16$), simple steatosis (SS, $n = 28$), and nonalcoholic steatohepatitis (NASH, $n = 28$) by enzyme-linked immunosorbent assay and RT-PCR. *Results*. We found that CB1 mRNA expression was significantly higher in NASH compared with SS and correlated negatively with PPARα. Regarding CB2, CB2 mRNA expression correlated positively with ACC1, PPARγ, IL6, TNFα, resistin, and adiponectin. *Conclusions*. The increased expression of CB1 in NASH and the negative correlation with PPARα suggest a deleterious role of CB1 in NAFLD. Regarding CB2, its positive correlation with the anti-inflammatory molecule adiponectin and, paradoxically, with inflammatory genes suggests that this receptor has a dual role. Taken together, our results suggest that endocannabinoid receptors might be involved in the pathogenesis of NAFLD, a finding which justifies further study.

1. Introduction

Obesity, as part of the metabolic syndrome, is one of the major risk factors in the development of fatty liver [1, 2]. Nonalcoholic fatty liver disease (NAFLD) has become the most common liver disorder in developed countries, affecting over one-third of the population [3, 4]. NAFLD has frequently been associated with obesity, type 2 diabetes mellitus, hyperlipidemia, and insulin resistance [5]. The spectrum of the disease ranges from simple steatosis to steatohepatitis, a condition that associates steatosis, liver inflammation, hepatocellular injury, and activation of fibrogenic pathways with a 10–20% risk of developing cirrhosis within 10 to 20 years [6]. The transition from steatosis (SS) to nonalcoholic steatohepatitis (NASH) is not completely understood and appears multifactorial. Recent studies have revealed a role of lipotoxic fatty acid metabolites originating from the adipose tissue or from *de novo* lipogenesis in the development of hepatocellular injury [7]. Increasing evidence suggests that a fatty liver is more vulnerable to factors that lead to inflammation and fibrosis [8, 9]. Different studies confirm that *de novo* lipogenesis might be upregulated in NAFLD [10–12]. Recent reports have shown that endogenous cannabinoids (EC) are related to fatty liver metabolism [13, 14] although the molecular mechanism by which EC modulates the metabolism within hepatocytes is still not clear.

EC are lipid mediators that produce similar effects to those of marijuana by acting on membrane-bound receptors and regulating appetite behaviour [15]. Cannabinoid receptors are mainly localized in the brain, but are also present in small amounts in liver and other peripheral tissue (CB1) and in immune and haematopoietic cells (CB2) [16, 17]. EC may also regulate peripheral energy metabolism, as demonstrated by their CB1-mediated effect on lipoprotein lipase activity in adipocytes [18] and their ability to stimulate lipogenesis in hepatocytes [13, 14]. In agreement with that data, other studies have shown that CB1 receptor antagonists represent an important therapeutic target, owing to beneficial effects on lipid metabolism and in light of its antifibrogenic properties. Unfortunately, the brain-penetrant CB1 antagonist rimonabant was withdrawn because of an alarming adverse effect on mood. However, the efficacy of peripherally-restricted CB1 antagonists with limited brain penetration has now been validated in preclinical models of NAFLD [19].

Taken together, these findings indicate that CB1 receptors mediate metabolic steatogenesis in the liver by central and peripheral effects. Regarding CB2, results of recent studies have suggested that this receptor could be a promising anti-inflammatory and antifibrogenic target [19, 20], although clinical development of its agonists is still awaited.

In order to investigate the associations of CB1 and CB2 with NAFLD we aimed to (1) find out the gene expression profiles of CB1 and CB2 in liver of morbidly obese women with or without NAFLD, (2) assess the relationship between its gene expressions and the presence of hepatic fat and inflammation, and (3) study the relationship between liver CB1 and CB2 mRNA expression and liver mRNA expression of key genes involved in lipid metabolism: genes involved in *de novo* synthesis of fatty acids (ChREBP, SREBP1c, LxRα, FxR, ACC1, and FAS), fatty acid oxidation (PPARα), uptake and transport (PPARγ, CD36, and FABP4), and inflammatory related genes (PPARδ, IL6, TNFα, and CRP).

2. Patients and Methods

2.1. Subjects. The institutional review board approved the study. All participants gave written informed consent for participation in medical research. This study included 72 morbidly obese (MO) women (body mass index, BMI > 40 Kg/m^2) of Western European descent.

We analysed 72 liver samples from MO women. Liver biopsies were obtained during planned bariatric surgery. All biopsies were carried out under clinical indications.

NAFLD was diagnosed by the following criteria: (1) liver pathology, (2) an intake of less than 10 gr of ethanol/day, and (3) appropriate exclusion of other liver diseases. Liver samples were scored by two experienced hepatopathologists using the methods described before [21, 22].

According to their liver pathology, patients were subclassified into the following groups: (1) MO with normal liver (NL) histology ($n = 16$), (2) MO with simple steatosis (SS) (micro/macrovesicular steatosis without inflammation or fibrosis, $n = 28$), and (3) MO with nonalcoholic steatohepatitis (NASH) (Brunt grade 1–3, $n = 28$).

Subjects' weight was stable, with no fluctuation greater than 2% of body weight for at least 3 months prior to surgery. The exclusion criteria were (1) concurrent use of medication known to produce hepatic steatosis, (2) patients using antidiabetics or lipid-lowering medications, including PPARα or -γ agonists, (3) diabetic women that were receiving insulin or on medication likely to influence endogenous insulin levels, (4) menopausal and postmenopausal women and subjects receiving contraceptive treatment, and (5) patients who had an acute illness, current evidence of acute or chronic inflammatory or infectious diseases, or end-stage malignant diseases.

2.2. Anthropometrical and Biochemical Analysis. A complete anthropometrical examination and a biochemical analysis were carried out on each patient. Height and weight were measured with the patient standing in light clothes and shoeless. BMI was calculated as body weight divided by height squared (kg/m^2). Laboratory studies included glucose, insulin, glycated haemoglobin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and transaminases, all of which were analysed using a conventional automated analyser. Insulin resistance (IR) was estimated using homeostasis model assessment of IR (HOMA2-IR) [23]. Serum levels of adiponectin (Linco Research, Inc., St. Charles, USA), resistin (Biovendor, Modrice, Czech Republic), interleukin 6 (IL6) (Quantikine, R&D Systems, Minneapolis, USA), tumour necrosis factor receptor 2 (TNFRII) (AssayPro, St. Charles, USA), and C-reactive protein (CRP) (Dade Behring, Marburg, Germany) were measured in duplicate using ELISA, following the manufacturer's instructions.

2.3. RNA Isolation and Real Time PCR. Liver samples were conserved in RNAlater (Sigma, Barcelona, Spain) for 24 hours at 4°C and then stored at −80°C. Total RNA from liver tissue was isolated using the RNeasy mini kit (Qiagen, Barcelona, Spain), according to the manufacturers' protocols. RNA was digested with DNase I (RNase-Free DNase set; Qiagen). First-strand cDNA was synthesized using an equal amount of total RNA with High Capacity RNA-to-cDNA Kit (Applied Biosystems, Madrid, Spain). The Real-time quantitative PCR was carried out in a final volume of 20 μL, which contained 10 ng of reverse-transcribed cDNA, 10 μL of 2X TaqMan Fast Universal PCR Master Mix (Applied Biosystems), and 1 μL TaqMan Assay predesigned by Applied Biosystems for the detection of CB1, CB2, ChREBP, SREBP1c, FxR, LxRα, ACC1, FAS, PPARα, PPARγ, CD36, FABP4, PPARδ, IL6, TNFα, CRP, adiponectin, and resistin gene and for GAPDH, which was used as the housekeeping gene. The mRNA expression for each gene and sample was calculated using the recommended $2^{-\Delta\Delta Ct}$ method. All reactions were carried out in duplicate in 96-well plates using the 7900HT Fast Real-Time PCR systems (Applied Biosystems).

2.4. Statistical Analysis. All the values reported are expressed as mean ± SD (standard deviation) and were analysed using SPSS/PC+ for windows statistical package (version

20.0; SPSS, Chicago, IL). Differences between groups were calculated using the Student's t-test or one-way ANOVA analysis. The strength of association between variables was calculated using Pearson's method for parametric variables and Spearman's ρ-correlation test for nonparametric contrasts. A multiple linear regression analysis was carried out. The predictors for stepwise linear regression analysis were based on correlation analysis and selected from the variables known to be associated with the dependent variable. P values <0.05 were considered to be statistically significant.

3. Results

3.1. Baseline Characteristics and General Laboratory Data of the Subjects in the Study. Morbidly obese women were classified according to the presence of NAFLD (Table 1). Age, anthropometrical measurements, glucose, HbA1c, insulin, HDL and LDL-Cholesterol, and triglycerides were not significantly different among morbidly obese women in the NL, SS, or NASH groups. However, our results indicated that AST and ALT activity were higher in both the SS and the NASH groups than in obese women with normal liver histology (SS: AST $P = 0.05$, ALT $P < 0.001$; NASH: AST $P = 0.042$, ALT $P < 0.001$). ALP activity was higher in the NASH group than in NL ($P = 0.032$). Moreover, the levels of adipocytokines determined were not significantly different between the morbidly obese with NL, SS, or NASH (Table 1).

3.2. Evaluation of CB1 and CB2 mRNA Expression in Liver. We analysed hepatic CB1 and CB2 mRNA expression in the MO cohort in relation to the presence of NAFLD. When we subclassified that cohort into NL, SS, and NASH, we observed that CB1 mRNA expression was significantly higher in NASH compared with SS (SS: 0.09 ± 0.07; NASH: 0.14 ± 0.03; $P < 0.010$) (Figure 1(a)). However, CB2 gene expression was similar among the three groups (Figure 1(b)).

Additionally, we studied the relationship between the grade of steatohepatitis and CB1 and CB2 gene expression. When we subclassified the MO cohort into those with NASH Brunt 1 and with NASH Brunt 2/3, we found that both CB1 and CB2 mRNA expression were similar in both groups (data not shown).

3.3. Correlations between the Gene Expression of CB1 and CB2 and Genes Related to Fatty Acid Synthesis (ChREBP, SREBP1c, LxRα, FxR, ACC1, and FAS), Fatty Acid Oxidation (PPARα), Uptake and Transport (PPARγ, CD36, and FABP4), Inflammation (PPARδ, IL6, TNFα, and CRP), and Adipokines (Adiponectin and Resistin) in Liver from MO Cohort. We found a negative correlation between CB1 and PPARα gene expression. We also found that CB2 mRNA expression correlated positively with ACC1 and PPARγ mRNA expression (Table 2).

Regarding inflammation and adipokines, we did not find any correlation between CB1 gene expression and inflammatory genes expression, nor with adipokines expression. However, we found positive correlations between CB2 and IL6, TNFα, resistin, and adiponectin geneexpression (Table 2).

In addition, we performed a stepwise multiple linear regression analysis, which included age, BMI, triglycerides, PPARα, and the presence of NASH as independent variables, and CB1 expression as a dependent variable. The results indicated that NASH and PPARα (inverse) were the only variables associated with CB1 expression ($R^2 = 0.255$, $P = 0.002$; $R^2 = 0.129$, $P = 0.014$, resp.).

4. Discussion

The present study demonstrates that in morbidly obese women with NAFLD, liver CB1 gene expression is significantly higher at the histological stage of NASH compared to SS. This finding might agree with experimental studies in obese rats, which showed that rimonabant, a selective CB1 receptor antagonist used as an adjunctive treatment of obesity, reduces liver inflammation [24, 25]. The underlying mechanism has not yet been delineated but in hepatocytes CB1 receptors might contribute to the acute phase response via activation of ChREBP, a liver-specific transcription factor that upregulates acute phase response genes [26]. In our case, we were not able to demonstrate a positive relationship between CB1 and ChREBP mRNA expression, nor with mRNA expression of inflammatory genes.

Studies in cultured hepatocytes and in animal models have observed steatogenic properties of CB1 as a result of hepatic lipogenesis activation, reduction of fatty acid oxidation, and decreased release of TG-rich VLDL, combined to CB1-dependent release of free fatty acids from the adipose tissue [24, 25, 27]. Our cohort did not demonstrate any relationship between the hepatic CB1 gene expression and simple steatosis. However, we did find a negative correlation between CB1 and PPARα gene expression. PPARα plays a pivotal role in fatty acid (FA) catabolism by upregulating the expression of numerous genes involved in mitochondrial FA oxidation, peroxisomal FA oxidation, and other aspects of FA metabolism [28]. Furthermore, PPARα is related to adipoR2. Activation of adipoR2 can increase PPARα levels and activate PPARα pathways, leading to increased fatty acid oxidation and a reduction in oxidative stress [29, 30]. Recently in experimental studies the adipoR agonist (AdipoRon) bound to both adipoR1 and AdipoR2 *in vitro*. AdipoRon showed very similar effects to adiponectin in muscle and liver, such as an activation of AMK and PPARα pathways, and ameliorated IR and glucose intolerance in mice fed a high-fat diet, which was completely obliterated in adipoR1 and AdipoR2 double-knockout mice [31]. In conclusion, the higher expression of CB1 in NASH and the negative correlation with PPARα suggest a deleterious role of CB1 in NAFLD.

Regarding hepatic CB2 expression, we did not find any differences between MO with NL, SS or NASH. It is important to note that the role of CB2 in liver diseases is controversial. Some authors have reported that CB2 receptors display protective properties during liver injury. These effects largely depend on anti-inflammatory and antifibrogenic signals generated by CB2-expressing hepatic immune cells and/or

Table 1: Characteristics of study cohort classified according to the liver pathology.

		Morbidly Obese (n = 72)	
	NL (n = 16)	SS (n = 28)	NASH (n = 28)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	44.0 ± 3.2	47.4 ± 1.5	45.9 ± 1.4
Weight (kg)	121.0 ± 12.1	121.8 ± 16.3	123.8 ± 14.3
WC (cm)	129.5 ± 6.4	132.1 ± 10.7	135.1 ± 9.9
BMI (kg/m^2)	48.6 ± 2.6	48.1 ± 7.8	47.5 ± 5.4
Glucose (mg/dL)	101.8 ± 18.9	125.4 ± 38.2	119.0 ± 30.0
Insulin (mUI/L)	11.4 ± 3.1	21.2 ± 11.5	23.1 ± 28.1
HbA1c (%)	4.3 ± 0.3	5.9 ± 1.7	5.8 ± 1.7
HOMA2-IR	1.5 ± 0.5	2.9 ± 1.4	3.0 ± 3.4
HDL-C (mg/dL)	43.6 ± 7.6	39.3 ± 9.5	41.5 ± 9.0
LDL-C (mg/dL)	102.0 ± 31.7	104.7 ± 24.6	102.5 ± 32.6
Triglycerides (mg/dL)	144.4 ± 51.9	178.4 ± 68.5	183.1 ± 86.6
AST (U/L)	25.8 ± 8.5	43.7 ± 34.4*	44.9 ± 29.3*
ALT (U/L)	25.4 ± 10.5	45.6 ± 31.6*	46.3 ± 28.3*
GGT (U/L)	18.3 ± 13.8	30.6 ± 22.2	36.1 ± 31.7
ALP (U/L)	58.2 ± 12.6	66.3 ± 16.0	71.9 ± 15.2*
Adipocytokine levels			
Adiponectin (μg/mL)	10.1 ± 2.0	6.8 ± 3.5	6.7 ± 2.4
IL6 (pg/mL)	2.1 ± 0.6	2.9 ± 2.5	3.3 ± 2.0
Resistin (ng/mL)	5.1 ± 2.1	4.6 ± 2.0	4.5 ± 1.6
TNFRII (ng/mL)	4.2 ± 1.4	5.5 ± 2.5	5.6 ± 1.7
CRP (mg/dL)	1.0 ± 0.8	0.9 ± 0.7	1.1 ± 0.7

NL: morbidly obese subjects with normal liver; SS: morbidly obese subjects with simple steatosis; NASH: morbidly obese subjects with steatohepatitis; ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index; CRP: C-reactive protein; GGT: gamma-glutamyltransferase; HbA1c: glycosylated haemoglobin; HDL-C: high density lipoprotein; HOMA2-IR: homeostatic model assessment 2-insulin resistance; IL6: interleukin 6; LDL-C: low density lipoprotein; TNFRII: tumour necrosis factor receptor II; WC: waist circumference. *indicates significant differences respect NL group ($P < 0.05$).

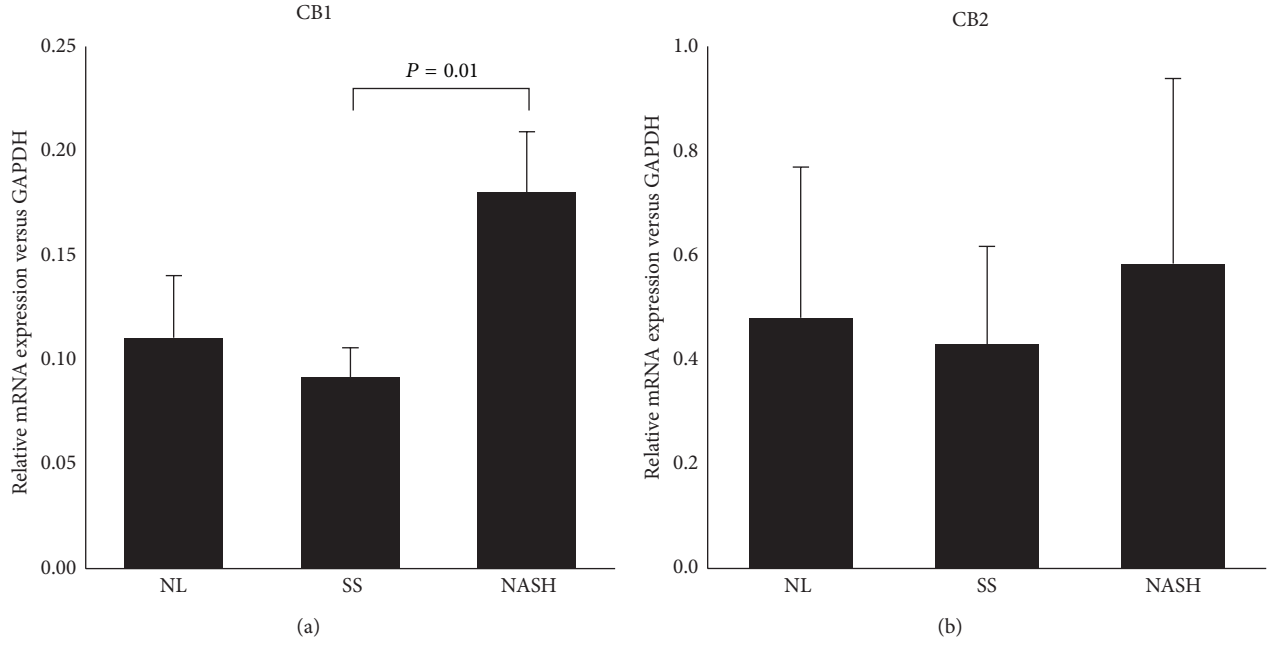

Figure 1: Liver CB1 and CB2 mRNA expression in morbidly obese women classified according to the liver pathology. NL: with normal liver histology; SS: simple steatosis; NASH: nonalcoholic steatohepatitis. Results are shown as mean ± SD. $P < 0.05$ is considered statistically significant.

Table 2: Correlations between the expression of CB1 and CB2 and genes related to fatty acid synthesis, to fatty acid oxidation, uptake and transport, inflammation, and adipokines in liver from morbidly obese women.

	CB1		CB2	
	r	P value	r	P value
De novo synthesis of Fatty acids				
ChREBP	0.185	0.198	0.072	0.603
SREBP1c	0.074	0.590	0.031	0.816
LxRα	0.052	0.709	0.126	0.346
FxR	0.079	0.571	0.095	0.482
ACC1	0.207	0.126	0.257	**0.047**
FAS	0.164	0.232	0.182	0.167
Fatty acid oxidation				
PPARα	−0.354	**0.037**	0.155	0.347
Fatty acid uptake and transport				
PPARγ	0.161	0.240	0.600	<**0.001**
CD36	−0.028	0.846	0.157	0.257
FABP4	0.105	0.460	0.040	0.776
Inflammation				
IL6	0.116	0.431	0.288	**0.040**
TNFα	−0.018	0.902	0.293	**0.033**
CRP	−0.210	0.140	−0.002	0.988
PPARδ	−0.077	0.576	0.126	0.342
Adipokines				
Adiponectin	0.031	0.846	0.625	<**0.001**
Resistin	−0.058	0.701	0.506	<**0.001**

$P < 0.05$ are considered statistically significant.

hepatic myofibroblasts, with paracrine impact on hepatocytes, which do not express CB2 [19, 32]. The endogenous or exogenous activation of CB2 receptors has been described as a protective pathway in several models of acute liver injury in which CB2 receptors undergo early induction in nonparenchymal cells [16, 20, 33, 34].

We found that CB2 liver expression correlated positively with hepatic ACC1 gene expression, a gene related to fatty acid synthesis. In fatty acid synthesis process, ACC1 converts acetyl-CoA, an essential substrate of fatty acids, to malonyl-CoA. Fatty acids as well as acyl-CoA and acetyl-CoA have been identified as potential causes of lipotoxicity [35]. Furthermore, we found that CB2 liver expression correlated positively with PPARγ gene expression. In NAFLD, PPARγ is upregulated in liver tissue and liver-specific *PPARγ* knockout mice are protected from diet-induced steatosis [36, 37]. However, these isolated correlations do not clarify the role of CB2 in lipogenic and uptake and transport pathways.

Other studies provide additional evidence that supports the potential involvement of CB2 receptors in inflammatory liver diseases [38]. However, in our study, we found a positive correlation between liver CB2 and adiponectin gene expression, which is a molecule with an anti-inflammatory role. Taken together, all of this suggests that CB2 seems to be a molecule with a dual action.

Our cohort of morbidly obese women has made it possible to establish clear relationships between NASH and CB1 liver expression without the interference of such confounding factors as gender or age. However, the results of our study cannot be extrapolated to other obesity groups, to men, or to normal-weight subjects.

The main results of our study demonstrate that liver CB1 mRNA expression is induced in nonalcoholic steatohepatitis and correlates negatively with hepatic PPARα expression, which suggests a deleterious role of CB1 in NAFLD. Furthermore, liver CB2 mRNA expression is related to the expression of key genes involved in hepatic lipid metabolism. With regard to inflammation, CB2 seems to act as a dual molecule because it correlates positively with the anti-inflammatory molecule adiponectin and, paradoxically, also with inflammatory genes. Our results suggest that endocannabinoid receptors might be involved in the physiopathological processes of NAFLD and justify the need for further study.

List of Abbreviations

ACC1: Acetyl-coenzyme A carboxylase 1
ALT: Alanine aminotransaminase
AST: Aspartate aminotransaminase
ALP: Alkaline phosphatase
BMI: Body mass index
CB1: Cannabinoid receptor 1
CB2: Cannabinoid receptor 2
CD36: Fatty acid translocase
ChREBP: Carbohydrate response element binding protein

CRP: C-reactive protein
EC: Endogenous cannabinoids
FABP4: Fatty acid binding protein 4
FAS: Fatty acid synthase
FxR: Farnesoid X receptor
GGT: γ-glutamyl transferase
HbA1c: Glycosylated hemoglobin
HDL-C: High-density lipoprotein
HOMA2-IR: Homeostatic model assessment method insulin resistance
IL6: Interleukin 6
IR: Insulin resistance
LDL-C: Low-density lipoprotein
LxRα: Liver X receptor
NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis
NL: Normal liver
PPARα: Peroxisome-proliferator-activated receptor α
PPARδ: Peroxisome-proliferator-activated receptor δ
PPARγ: Peroxisome-proliferator-activated receptor γ
SS: Simple steatosis
SREBP1c: Sterol-regulatory-element-binding protein
TNFα: Tumour necrosis factor
VLDL: Very low-density lipoprotein
WC: Waist circumference.

Conflict of Interests

The authors declare that they have no conflict of interests to disclose.

Authors' Contribution

Teresa Auguet and Alba Berlanga contributed equally to this work.

Acknowledgments

This study was supported by the Ministerio de Ciencia e Innovación of the government of Spain (Grant no. SAF 2008-02278, to Cristóbal Richart), the Fondo de Investigación Sanitaria (Grant no. PS09/01778, to Teresa Auguet), funds from Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR 2009 SGR 959 to Cristóbal Richart), Grup de Recerca en Medicina Aplicada URV (2010PFR-URV-B2-14 to Cristóbal Richart), and the Fundación Biociencia.

References

[1] S. A. Harrison and A. M. Diehl, "Fat and the liver—a molecular overview," *Seminars in Gastrointestinal Disease*, vol. 13, no. 1, pp. 3–16, 2002.

[2] J. M. Clark, F. L. Brancati, and A. M. Diehl, "Nonalcoholic fatty liver disease," *Gastroenterology*, vol. 122, no. 6, pp. 1649–1657, 2002.

[3] P. Angulo, "Medical progress: nonalcoholic fatty liver disease," *The New England Journal of Medicine*, vol. 346, no. 16, pp. 1221–1231, 2002.

[4] G. Vernon, A. Baranova, and Z. M. Younossi, "Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults," *Alimentary Pharmacology and Therapeutics*, vol. 34, no. 3, pp. 274–285, 2011.

[5] G. Marchesini, E. Bugianesi, G. Forlani et al., "Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome," *Hepatology*, vol. 37, no. 4, pp. 917–923, 2003.

[6] J. P. Ong and Z. M. Younossi, "Epidemiology and natural history of NAFLD and NASH," *Clinics in Liver Disease*, vol. 11, no. 1, pp. 1–16, 2007.

[7] H. Tilg and A. R. Moschen, "Evolution of inflammation in non-alcoholic fatty liver disease: the multiple parallel hits hypothesis," *Hepatology*, vol. 52, no. 5, pp. 1836–1846, 2010.

[8] C. P. Day, "Clinical spectrum and therapy of non-alcoholic steatohepatitis," *Digestive Disease*, vol. 30, supplement 1, pp. 69–73, 2012.

[9] C. Postic and J. Girard, "Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice," *Journal of Clinical Investigation*, vol. 118, no. 3, pp. 829–838, 2008.

[10] E. Lima-Cabello, M. V. García-Mediavilla, M. E. Miquilena-Colina et al., "Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C," *Clinical Science*, vol. 120, no. 6, pp. 239–250, 2011.

[11] M. Nakamuta, T. Fujino, R. Yada et al., "Impact of cholesterol metabolism and the LXRα-SREBP-1c pathway on nonalcoholic fatty liver disease," *International Journal of Molecular Medicine*, vol. 23, no. 5, pp. 603–608, 2009.

[12] M. Kohjima, M. Enjoji, N. Higuchi et al., "Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease," *International Journal of Molecular Medicine*, vol. 20, no. 3, pp. 351–358, 2007.

[13] D. Osei-Hyiaman, M. DePetrillo, P. Pacher et al., "Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity," *Journal of Clinical Investigation*, vol. 115, no. 5, pp. 1298–1305, 2005.

[14] W.-I. Jeong, D. Osei-Hyiaman, O. Park et al., "Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver," *Cell Metabolism*, vol. 7, no. 3, pp. 227–235, 2008.

[15] V. di Marzo, F. Piscitelli, and R. Mechoulam, "Cannabinoids and endocannabinoids in metabolic disorders with focus on diabetes," *Handbook of Experimental Pharmacology*, vol. 2011, pp. 75–104, 2011.

[16] P. Pacher and R. Mechoulam, "Is lipid signaling through cannabinoid 2 receptors part of a protective system?" *Progress in Lipid Research*, vol. 50, no. 2, pp. 193–211, 2011.

[17] P. Pacher, S. Bátkai, and G. Kunos, "The endocannabinoid system as an emerging target of pharmacotherapy," *Pharmacological Reviews*, vol. 58, no. 3, pp. 389–462, 2006.

[18] D. Cota, G. Marsicano, M. Tschöp et al., "The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis," *Journal of Clinical Investigation*, vol. 112, no. 3, pp. 423–431, 2003.

[19] A. Mallat, F. Teixeira-Clerc, and S. Lotersztajn, "Cannabinoid signaling and liver therapeutics," *Journal of Hepatology*, vol. 59, no. 4, pp. 891–896, 2013.

[20] N. Mendez-Sanchez, D. Zamora-Valdes, R. Pichardo-Bahena et al., "Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease," *Liver International*, vol. 27, no. 2, pp. 215–219, 2007.

[21] D. E. Kleiner, E. M. Brunt, M. van Natta et al., "Design and validation of a histological scoring system for nonalcoholic fatty liver disease," *Hepatology*, vol. 41, no. 6, pp. 1313–1321, 2005.

[22] E. M. Brunt, C. G. Janney, A. M. di Bisceglie, B. A. Neuschwander-Tetri, and B. R. Bacon, "Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions," *American Journal of Gastroenterology*, vol. 94, no. 9, pp. 2467–2474, 1999.

[23] X. Terra, T. Auguet, M. Broch et al., "Retinol binding protein-4 circulating levels were higher in nonalcoholic fatty liver disease vs. histologically normal liver from morbidly obese women," *Obesity*, vol. 21, no. 1, pp. 170–177, 2013.

[24] J. Tam, V. K. Vemuri, J. Liu et al., "Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity," *Journal of Clinical Investigation*, vol. 120, no. 10, pp. 2953–2966, 2010.

[25] M. Gary-Bobo, G. Elachouri, J. F. Gallas et al., "Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese zucker fa/fa rats," *Hepatology*, vol. 46, no. 1, pp. 122–129, 2007.

[26] T. Jourdan, L. Djaouti, L. Demizieux, J. Gresti, B. Vergès, and P. Degrace, "CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice," *Diabetes*, vol. 59, no. 4, pp. 926–934, 2010.

[27] D. Osei-Hyiaman, J. Liu, L. Zhou et al., "Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice," *Journal of Clinical Investigation*, vol. 118, no. 9, pp. 3160–3169, 2008.

[28] S. Mandard, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor α target genes," *Cellular and Molecular Life Sciences*, vol. 61, no. 4, pp. 393–416, 2004.

[29] M. Iwabu, T. Yamauchi, M. Okada-Iwabu et al., "Adiponectin and AdipoR1 regulate PGC-1α and mitochondria by Ca^{2+} and AMPK/SIRT1," *Nature*, vol. 464, no. 7293, pp. 1313–1319, 2010.

[30] S. Kersten, B. Desvergne, and W. Wahli, "Roles of PPARS in health and disease," *Nature*, vol. 405, no. 6785, pp. 421–424, 2000.

[31] M. Okada-Iwabu, T. Yamauchi, M. Iwabu et al., "A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity," *Nature*, vol. 503, pp. 493–499, 2013.

[32] A. Louvet, F. Teixeira-Clerc, M.-N. Chobert et al., "Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice," *Hepatology*, vol. 54, no. 4, pp. 1217–1226, 2011.

[33] S. Lotersztajn, F. Teixeira-Clerc, B. Julien et al., "CB2 receptors as new therapeutic targets for liver diseases," *British Journal of Pharmacology*, vol. 153, no. 2, pp. 286–289, 2008.

[34] J. Agudo, M. Martin, C. Roca et al., "Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age," *Diabetologia*, vol. 53, no. 12, pp. 2629–2640, 2010.

[35] S. H. Myoung, Y. P. Sun, K. Shinzawa et al., "Lysophosphatidylcholine as a death effector in the lipoapoptosis of hepatocytes," *Journal of Lipid Research*, vol. 49, no. 1, pp. 84–97, 2008.

[36] H.-Y. Jiang, S. A. Wek, B. C. McGrath et al., "Phosphorylation of the α subunit of eukaryotic initiation factor 2 is required for activation of NF-κB in response to diverse cellular stresses," *Molecular and Cellular Biology*, vol. 23, no. 16, pp. 5651–5663, 2003.

[37] J. Seo, E. S. Fortuno III, M. S. Jae et al., "Atf4 regulates obesity, glucose homeostasis, and energy expenditure," *Diabetes*, vol. 58, no. 11, pp. 2565–2573, 2009.

[38] V. Deveaux, T. Cadoudal, Y. Ichigotani et al., "Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis," *PLoS ONE*, vol. 4, no. 6, Article ID e5844, 2009.

V. Summary Results

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.
Alba Berlanga Bustos
Dipòsit Legal: T 1705-2015

In the first study of this doctoral thesis, we have analysed the liver expression of some key genes related to the *de novo* synthesis of fatty acids (LxRα, SREBP1c, ACC1, FAS), fatty acid uptake and transport (PPARγ, CD36, FABP4), fatty acid oxidation (PPARα), and related to inflammation (IL6, TNFα, CRP, PPARδ) in an extensive cohort of 127 morbidly obese (MO) women with a body mass index greater than 40 kg/m^2. We first classified the cohort according to their liver histology into normal liver, SS or NASH. We found that, in liver of both MO women with SS and MO women with NASH, the main lipogenic enzyme fatty acid synthase (FAS) is over expressed ($p<0.05$). For inflammation, we found that IL6 gene expression is significantly higher in NASH patients compared to those with SS ($p<0.05$). TNFα, another important inflammatory molecule, tended to be higher in NASH ($p=0.05$). We did not find any more significant differences for the other fatty acid metabolism-related genes studied. We then assessed the relationship of the hepatic expression of fatty acid metabolism and inflammation-related genes with the presence of hepatic lipid accumulation. In order to do so, we classified the steatotic patients according to mild, moderate or severe degree of SS. Our results showed that the liver expression of key genes related to *de novo* fatty acid synthesis (ACC1, FAS and LxRα) had an inverse relationship with the degree of steatosis; their mRNA expression diminished when the degree of simple steatosis increased: liver ACC1 mRNA expression was down-regulated in severe SS compared to both mild and moderate SS groups ($p<0.05$); hepatic FAS mRNA expression levels were significantly lower in severe SS compared to the moderate SS group ($p<0.05$); LxRα mRNA expression tended to be lower in severe SS compared to both mild and moderate SS groups ($p=0.05$). For SREBP1c, although we did not find any significant difference, its mRNA expression seemed to be lower in severe SS MO women compared to those with mild or moderate SS. In order to confirm these results, we also analysed LxRα, SREBP1c (precursor and active form), ACC1, and FAS protein expression according to the degree of simple steatosis. Interestingly, these results were similar for its protein expression and those obtained in the gene expression analysis. For FA oxidation, FA

uptake and transport, and inflammation–related genes, we found no significant difference in its mRNA expression levels according to the grade of simple steatosis. Finally, we also assessed the correlations of hepatic expression of fatty acid metabolism and inflammation -related genes with glucose metabolism parameters in our cohort of morbidly obese women. Liver FAS expression correlated positively with glucose circulating levels; CD36 and IL6 with insulin circulating levels, and also with HOMA2-IR; and FABP4 with glucose, insulin, HbA1c and HOMA2-IR. Interestingly, both CD36 and FABP4 correlations with glucose metabolism parameters became stronger in NASH.

Meanwhile, in order to add to the current knowledge about the role of the endocannabinoid system in physiopathology of non-alcoholic fatty liver disease, in the second study of this thesis, we analysed the gene expression of cannabinoid receptor 1 (CB1) and 2 (CB2) in the liver of 72 MO women according to their liver histology. We observed that CB1 was over expressed in liver of MO women with NASH ($p<0.05$). Unlike CB1, CB2 gene expression in the liver of MO women was similar for those with normal liver, simple steatosis or NASH histology. We also assessed the correlations of CB1 and CB2 hepatic gene expression with *de novo* synthesis of FA, FA oxidation, FA uptake and transport, and inflammation–related genes, as well as with resistin and adiponectin adipokines in the cohort of MO women. We found that CB1 hepatic gene expression correlated negatively with PPARα gene expression levels, while CB2 correlated positively with ACC1, PPARγ, IL6, TNFα, adiponectin and resistin gene expression levels. Finally, we performed a stepwise multiple linear regression analysis, which included age, BMI, triglycerides, PPARα, and the presence of NASH as independent variables, while CB1 hepatic gene expression was included as dependent variable. The results indicated that NASH and PPARα (inverse) were the only variables associated with CB1 gene expression in the liver of the MO women cohort.

VI. General Discussion

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.
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As mentioned in the introduction to this thesis, NAFLD encompasses a histological spectrum from simple steatosis to non-alcoholic steatohepatitis, with the latter being more frequently progressive. Although the disease is asymptomatic most of the time, in a subset of patients it can lead to progressive liver disease such as cirrhosis, liver failure and hepatocellular carcinoma, which has led to NAFLD becoming a serious public health concern. NAFLD is now the most common cause of chronic liver disease in Western countries, and its prevalence worldwide has risen as more cultures have adopted a western lifestyle and diet[1,19]. It is now predicted to be the most common indication of liver transplantation in the near future[30]. Metabolic risk factors such as obesity, IR, dyslipidaemia, metabolic syndrome and diabetes, which are also well known risk factors for cardiovascular disease, have been associated to NAFLD[11]. In fact, the majority of deaths among NAFLD patients are attributable to CVD[8,9]. While simple steatosis does not affect mortality, patients with NASH are at increased risk of cardiovascular death as well as liver-related mortality[27,287,288]. It is therefore especially important distinguish NASH patients from those with simple steatosis, not only because of the risk of progression to cirrhosis and HCC, but also of risk of cardiovascular death. However, although clinical scoring methods, laboratory tests, and imaging techniques are being developed, liver biopsy - an invasive procedure - is still the gold standard for reliably distinguishing NASH from simple steatosis[289]. Moreover, management of NAFLD patients largely depends on the stage of the disease, with emphasis on the importance of careful risk stratification[38]. Although a number of pharmacological therapies have been evaluated in NASH, and agents such as vitamin E and thiazolidinediones have shown some promise, there are currently no effective therapies for NAFLD apart from lifestyle modification, aimed at weight loss and increased physical activity. As a result, a better understanding of the underlying mechanisms in NAFLD pathogenesis could be of great interest for controlling the progression of NAFLD, and it might help in the development of non-invasive diagnostic and optimal therapeutic interventions. In this context, as lipid accumulation in the human liver seems to be the early and

crucial step in NAFLD development[91], we wished to further investigate the hepatic fatty acid metabolism of morbidly obese women with NAFLD and normal liver histology, by evaluating the expression of key genes involved in the molecular mechanisms that cause the initial hepatic lipid accumulation.

It has been reported that, in NAFLD, the mechanisms leading to excessive hepatic lipid accumulation arise from an imbalance between lipid acquisition and removal, with an increased delivery of non-esterified fatty acids from peripheral expanded adipose tissue to the liver, and/or an increased intake of dietary fats and sugars, enhanced *de novo* lipid synthesis via the lipogenic pathway, and decreased hepatic clearance capacity by means of fatty acid oxidation or triglyceride export[78,90,91]. We therefore decided to investigate the expression levels of hepatic genes that play significant roles in the metabolism of fatty acids in an extensive cohort of women with morbid obesity (BMI > 40 Kg/m^2). Their roles include the *de novo* synthesis (LxRα, SREBP1c, ACC1, and FAS), uptake and transport (PPARγ, CD36, and FABP4) and oxidation (PPARα) of fatty acids. We first classified the cohort according to their liver histology into normal liver, simple steatosis or NASH. Donnelly *et al.* have shown that in NAFLD patients, although several pathways are involved in hepatic lipid accumulation, elevated peripheral fatty acids and *de novo* lipogenesis predominantly contribute to the accumulation of hepatic fat. A recent study has confirmed that increased *de novo* lipogenesis is a distinct characteristic of subjects with NAFLD[181]. In this context, and in agreement with other authors[184–186,216], we found that FAS, the enzyme that catalyzes the last step in FA biosynthesis and which is considered a major determinant of the maximal hepatic capacity to generate FA by *de novo* lipogenesis pathway[216], is over-expressed in the liver of both morbidly obese women simple steatosis and morbidly obese women with NASH. These authors[184–186], in accordance with other studies[180,187], have also shown enhanced expression of LxRα and SREBP1c, the major transcriptional factors in lipogenic gene expression[183,189] in NAFLD patients. However, apart from FAS, we were unable to find any other significant difference in other genes

related to the *de novo* lipogenesis pathway. These discrepancies might be explained by differences in the cohort of patients studied. It is important to note that we studied a homogenous cohort of MO women, while the authors of these studies included normal weight[184], mildly over weight[185] or obese[187] patients with NAFLD, as well as men and women in the same cohort of patients, or patients with steatosis related to other etiologies, such as chronic hepatitis C virus infection[185]. In addition, Nakamuta *et al.* found that the hepatic expression of LxRα was significantly higher in non-obese NAFLD than in obese patients[187].

In order to add to current knowledge of the role of lipid metabolism alterations, we then evaluated the hepatic expression of fatty acid metabolism-related genes according to the hepatic fat deposition, classifying the steatotic patients in different histopathology types of simple steatosis (SS): mild, moderate or severe. The most interesting finding was that liver expression of key genes related to *de novo* lipogenesis (LxRα, ACC1 and FAS) has an inverse relationship with the degree of SS, i.e it diminishes when the degree of steatosis increases. This novel finding was confirmed by evaluating the protein expression. Interpreting these findings is extremely complicated. Under normal conditions, dietary glucose stimulates insulin secretion from the pancreas, which travels directly to the liver via the portal vein and elicits two key actions at the gene transcription level. In order to keep blood glucose low, insulin first stimulates the phosphorylation of the transcription factor FOXO1, preventing it from entering the nucleus[290], which results in the downregulation of genes required for gluconeogenesis. Insulin then also activates the transcription factor SREBP1c, which enhances the transcription of genes required for fatty acid and triglyceride biosynthesis, and particularly ACC and FAS[189,291]. In the liver, intact insulin signaling is therefore required for lipogenesis activation and for gluconeogenesis suppression. Paradoxically, in liver of animal models of insulin-resistant type 2 diabetes[139] as well as in humans with mutations in the insulin receptor[137], insulin fails to suppress gluconeogenesis but continues to activate lipogenesis, leading to a clear

relationship between insulin resistance and hepatic steatosis. To resolve this paradox, Brown and Goldstein[139] introduced the concept of "selective insulin resistance", according to which although the effect of insulin in both gluconeogenic and lipogenic pathways requires the insulin receptor, at some distal point, the FOXO1 pathway becomes insensitive to insulin, whereas the SREBP1c pathway remains sensitive. Li S, *et al.*[140] suggested that mTORC1 is the responsible branch point and an essential component in the insulin-regulated pathway for hepatic lipogenesis, but not for gluconeogenesis. Surprisingly, our results show a down-regulated lipogenesis in the liver of morbidly obese women exhibiting a severe simple steatosis. In this context, Biddinger *et al.* showed that in mice with total insulin resistance in liver, insulin fails to stimulate both gluconeogenesis and synthesis of fatty acids and triglycerides[292]. We could thus assume that the liver of an extremely obese patient with severe steatosis might behave as if there were total insulin resistance, which could be responsible for the downregulation of the lipogenic pathway in the liver of these patients. However, because we did not use any hyperinsulinemic euglycemic clamp technique to measure hepatic insulin sensitivity, we are unable to confirm this hypothesis.

Obesity, which is commonly observed in NAFLD, is associated with a chronic low-grade inflammatory state characterized by abnormal cytokine production, increased synthesis of acute-phase reactants and activation of inflammatory signaling pathways[91,293]. Adipose tissue, and especially white adipose tissue, has been recognized as a crucial site in this state, due to the fact that many of the interactions between metabolism and the immune system are mediated by a complex network of soluble mediators derived mainly from adipocytes, known as adipokines [294]. In the obese state, a dysregulated adipokine secretion is observed, characterized by increased pro-inflammatory and decreased anti-inflammatory adipokine secretion[73]. According to the "portal theory", in obese individuals, the increasing pro-inflammatory citokines released from visceral fat, as well as increasing amounts of free fatty acids, reach the liver via the portal system,

promoting the development of hepatic insulin resistance and liver steatosis [295]. In fact, serum levels of pro-inflammatory adipokines such as leptin, resistin, visfatin, TNFα and IL6 has been reported are significantly higher in NAFLD/NASH patients, while levels of anti-inflammatory cytokines such as adiponectin are significantly reduced[103]. Moreover, it is important to recognize that although the majority of adipokines are secreted by adipose tissue, they are also produced by other organs, such as the liver[91]. Because various studies have reported adipokines and pro-inflammatory cytokines as having a key role in the pathogenesis of NAFLD, as well as in obesity-related insulin resistance[91,103,293], we also decided to investigate the expression levels of hepatic genes that play a significant role in inflammation. When we classified our cohort of morbidly obese women according to their liver histology, we found that IL6 gene expression is over-expressed in the liver of NASH patients compared to those with simple steatosis, while the gene expression of TNFα also tends to be higher in NASH. These results are consistent with the literature, which supports the theory that these two pro-inflammatory cytokines are correlated with histological NAFLD severity in obese patients. For instance, Crespo *et al.* reported increased hepatic TNFα and TNFRI expression in the liver of obese patients with NASH compared to those without NASH[107]. For IL6, Wieckowska *et al.* demonstrated a marked increase in hepatic IL6 expression in NASH patients compared to those with hepatic steatosis or normal liver histology, and its expression positively correlated with the degree of inflammation, stage of fibrosis and systemic insulin resistance[108].

The generally accepted dogma in NAFLD pathogenesis is that hepatic lipid accumulation occurs when hyperinsulinemia and IR is present[74]. Hyperinsulinemia is widely considered a consequence of IR where β-cells, as a compensatory response to the insulin resistant state, produce and secrete increased levels of insulin, resulting in elevated insulin basal levels. However, there is increasing data to suggest that hyperinsulinemia is often both a result and a driver of insulin resistance[296], and that hyperinsulinemia is an independent risk factor for the development

of perturbed glucose tolerance, type 2 diabetes mellitus and NAFLD[297–299]. In this regard, in a recent study, Steneberg *et al.* presented *in vivo* and *ex vivo* data providing evidence that increased insulin circulating levels trigger hepatic steatosis development and IR, by stimulating hepatic expression of the FA transporter CD36[300]. In accordance with this theory, we found that not only hepatic gene expression of CD36, but also the FA transporter FABP4, correlate positively with circulating insulin levels and the presence of insulin resistance (HOMA2-IR) in our cohort of morbidly obese women, becoming stronger in those with NASH. Moreover, in agreement with our results, Miquelina-Colina *et al.* observed significant correlations between CD36 expression and insulin resistance, and plasma insulin levels in patients with NASH[170]. In addition, we found that the expression of the pro-inflammatory IL6 cytokine in the liver of MO women also correlates with insulin circulating levels and HOMA2-IR. Taken together, these results indicate that hepatic fatty acid accumulation, as well as inflammation, might be contributing to NAFLD progression in relation to the presence of insulin resistance. Finally, we also found that the glucose circulating levels of morbidly obese women correlate positively with liver FAS expression, supporting the observation that glucose binds and activates LXRs transcription factors, there by inducing their target genes, including SREBP1c, ACC1 and FAS[196,301].

The endocannabinoid system, mediated mainly by CB1 and CB2 cannabinoid receptors, has recently been reported as playing an important role in NAFLD by modulating lipid metabolism[248]. Although traditionally associated with the central nervous system, increasing energy intake by stimulating the appetite[261], cannabinoid receptors in hepatocytes are increasingly being recognized as key mediators of fatty liver by regulating the expression of key enzymes of lipid synthesis and transport[249,250], and associated insulin resistance[258,263] caused by high-fat diet[256], viral hepatitis[302] or ethanol intake[303]. Modulation of cannabinoid receptors, and specially antagonism of CB1 receptors and CB2 receptor agonism, is thus emerging as a potential novel therapeutic approach for the management of

NAFLD[247,251,253]. Consequently, in order to further investigate the role of the endocannabinoid system in the physiopathology of NAFLD and its relationship with lipid metabolism, for this doctoral thesis, we also studied the gene expression profiles of both CB1 and CB2 in the liver of morbidly obese women with NAFLD and normal liver histology, as well as the correlations between them and the hepatic gene expression of the *de novo* synthesis of FA, FA oxidation, FA uptake and transport, and inflammation–related genes, and resistin and adiponectin adipokines. Our results show that CB1 hepatic gene expression is significantly higher at the histological stage of NASH, suggesting that CB1 might have a role in the progression of NAFLD. M. Gary-Bobo *et al.* found that Rimonabant, a selective CB1 receptor antagonist, decreased the high level of local hepatic TNFα, currently associated with steatohepatitis, in obese rats[258]. However, we were unable to find significant correlations between CB1 gene expression and the pro-inflammatory cytokine TNFα, which has been shown to be a key mediator of the progression of liver diseases to more serious forms[304,305]. On the other hand, studies in cultured hepatocytes and in animal models have observed steatogenic properties of CB1 as a result of hepatic lipogenesis activation, reduction of fatty acid oxidation, and decreased release of TG-rich VLDL, combining to produce a CB1-dependent release of free fatty acids from the adipose tissue[256,257,259–262]. Although our cohort of MO women did not demonstrate a relationship between the hepatic CB1 gene expression and *de novo* synthesis of fatty acids, we did find that CB1 hepatic gene expression correlates negatively with PPARα in the liver of these women. It is well known that PPARα plays a pivotal role in FA catabolism by upregulating the expression of numerous genes involved in FA oxidation, as well as other aspects of FA metabolism[306]. In addition, it has been demonstrated that PPARα gene expression negatively correlates with NASH severity in NAFLD patients[230]. The induced liver gene expression of CB1 in NASH and the negative correlation with PPARα therefore suggest that CB1 might be playing a deleterious role in the physiopathological process of NAFLD.

Unlike to CB1, our results show that CB2 gene expression in liver of morbidly obese women is similar among those with normal liver, simple steatosis and NASH histology. It is important to note that the role of CB2 in liver disease is controversial. Some authors have reported that CB2 receptors have a potential role in liver steatogenesis and fat inflammatory response associated with IR[281,282], while others have reported that CB2 receptors exert hepatoprotective effects, reduce liver inflammation, and display antifibrogenic propierties (review in Mallat *et al.*[253] and Khalid *et al.*[247]). As for the steatogenic role of CB2, we found that it correlates positively with ACC1 gene expression, a rate-limiting enzyme during the fatty acid biosynthesis process by *de novo* lipogenesis in liver, and with PPARγ gene expression, which has been found to be increased in steatotic livers, and several studies attribute a causal role to PPARγ in steatosis development by mechanisms involving the activation of lipogenic genes and *de novo* lipogenesis[174,177,307]. Accordingly, targeted deletion of PPARγ in hepatocytes and in macrophages protected mice against diet-induced hepatic steatosis[175], suggesting a pro-steatotic role for PPARγ. Moreover, Paulina Pettinelli *et al.* showed that PPARγ is up-regulated in the liver of obese patients with simple steatosis and NASH, and significantly correlates with SREBP1c mRNA levels, acting as an additional reinforcing lipogenic mechanism in the development of hepatic steatosis[178]. However, these isolated correlations do not fully clarify the role of CB2 in liver steatogenesis. With regard to inflammation, we found that CB2 hepatic gene expression correlates positively with the anti-inflammatory molecule adiponectin and paradoxically, also with the pro-inflammatory cytokines TNFα, IL6 and resistin, suggesting that CB2 is a molecule with a dual action in NAFLD.

To summarize, the major finding in this doctoral thesis is that in our cohort of morbidly obese women who were diagnosed with isolated simple steatosis, *de novo* fatty acid synthesis appears to be down-regulated in advanced histological stages of simple steatosis. We were unable to determine the casuality that leads to a down-regulated lipogenesis in a

severe steatotic liver, but these results suggest that the deregulation of the lipogenic pathway may be associated with the severity of simple steatosis. Regarding the role of the endocannabinoid system, our results suggest a deleterious role of CB1 in NAFLD, while the role of CB2 is not fully clarified; which justifies the need for further study of cannabinoid receptors and its relationship with the physiopathological processes of non-alcoholic fatty liver disease. Finally, I would like to emphasize that our cohort of MO women made it possible to establish relationships between NAFLD and fatty acid metabolism-related genes and the CB1 receptor, with no interference by confounding factors such as gender or age. However, these results cannot be extrapolated to other obesity groups, normal-weight or male gender subjects. Further studies including these cohorts would be useful in order to validate our results.

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.
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VII. Conclusions

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1. The main lipogenic enzyme fatty acid synthase (FAS) is over-expressed in the liver of morbidly obese NAFLD women, and its hepatic gene expression is positively correlated with glucose circulating levels.

2. Gene and protein expression of key genes related to *de novo* fatty acid synthesis has an inverse relationship with the histological degree of simple steatosis in the liver of morbidly obese NAFLD women with simple steatosis. They diminish when the degree of steatosis increases.

3. The inflammatory molecules IL6 and TNFα are over-expressed in the liver of morbidly obese NAFLD women with NASH compared to those with simple steatosis.

4. Gene expression of the inflammatory molecule IL6, as well as the fatty acid transporters CD36 and FABP4 in liver of morbidly obese women is positively correlated with glucose metabolism parameters.

5. Cannabinoid receptor 1 (CB1) mRNA expression is significantly higher in the liver of morbidly obese NASH women compared to those with simple steatosis, and is negatively correlated with hepatic PPARα gene expression, suggesting that it might have a deleterious role in NAFLD.

6. Cannabinoid receptor 2 (CB2) mRNA expression in the liver of morbidly obese women is similar in those with normal, simple steatosis and NASH histology.

7. CB2 mRNA expression in liver of morbidly obese NAFLD women correlates positively with both ACC1 and PPARγ hepatic gene expression. However, these isolated correlations do not fully clarify the role of CB2 in liver steatogenesis.

8. CB2 seems to act as a dual molecule in NAFLD due to the fact that its hepatic mRNA expression is positively correlated with the hepatic gene expression of the anti-inflammatory molecule adiponectin and paradoxically, also with inflammatory molecules such as IL6, TNFα and resistin.

VIII. References

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1. Masuoka HC, Chalasani N. Nonalcoholic fatty liver disease: An emerging threat to obese and diabetic individuals. *Ann N Y Acad Sci*. 2013;1281(1):106-122.

2. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*. 2012;142(7):1592-1609.

3. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;41(6):1313-1321.

4. Hashimoto E, Tokushige K, Ludwig J. Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges. *Hepatol Res*. 2015:1-9.

5. Cohen J, Horton J, Hobbs H. Human fatty liver disease: old questions and new insights. *Science (80-)*. 2011;332(6037):1519-1523.

6. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37(4):917-923.

7. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab*. 2007;92(9):3490-3497.

8. Brea A, Puzo J. Non-alcoholic fatty liver disease and cardiovascular risk. *Int J Cardiol*. 2013;167(4):1109-1117.

9. Byrne CD, Targher G. NAFLD: A multisystem disease. *J Hepatol*. 2015;62(1):S47-S64.

10. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation*. 2005;112(17):2735-2752.

11. Masarone M, Federico A, Abenavoli L, Loguercio C, Persico M. Non alcoholic fatty liver: epidemiology and natural history. *Rev Recent Clin Trials*. 2014;9(3):126-133.

12. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther*. 2011;34(3):274-285.

13. Gaggini M, Morelli M, Buzzigoli E, DeFronzo R a., Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients*. 2013;5(5):1544-1560.

14. Onyekwere CA, Ogbera AO, Balogun BO. Non-alcoholic fatty liver disease and the metabolic syndrome in an urban hospital serving an African community. *Ann Hepatol*. 10(2):119-124.

15. Papandreou D, Rousso I, Mavromichalis I. Update on non-alcoholic fatty liver disease in children. *Clin Nutr*. 2007;26(4):409-415. doi:10.1016/j.clnu.2007.02.002.

16. Aggarwal A, Puri K, Thangada S, Zein N, Alkhouri N. Nonalcoholic fatty liver disease in children: recent practice guidelines, where do they take us? *Curr Pediatr Rev*. 2014;10(2):151-161.

17. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004;40(6):1387-1395.

18. Gutierrez-Grobe Y, Ponciano-Rodríguez G, Ramos MH, Uribe M, Méndez-Sánchez N. Prevalence of non alcoholic fatty liver disease in premenopausal, posmenopausal and polycystic ovary syndrome women. The role of estrogens. *Ann Hepatol*. 2010;9(4):402-409.

19. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol*. 2013;10(11):686-690.

20. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. *Gastroenterology*. 2011;140(1):124-131.

21. Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased prevalence of suspected nonalcoholic fatty liver disease. *Hepatology*. 2008;47(6):1947-1954.

22. Hamabe A, Uto H, Imamura Y, et al. Impact of cigarette smoking on onset of nonalcoholic fatty liver disease over a 10-year period. *J Gastroenterol*. 2011;46(6):769-778.

23. Zein CO, Unalp A, Colvin R, Liu Y-C, McCullough AJ. Smoking and severity of hepatic fibrosis in nonalcoholic fatty liver disease. *J Hepatol*. 2011;54(4):753-759.

24. Moore JB. Symposium 1: Overnutrition: consequences and solutions Non-alcoholic fatty liver disease: the hepatic consequence of obesity and the metabolic syndrome. *Proc Nutr Soc*. 2010;69(2):211-220.

25. Smith BW, Adams LA. Non-alcoholic fatty liver disease. *Crit Rev Clin Lab Sci*. 2011;48(3):97-113.

26. Adams L a., Lymp JF, St. Sauver J, et al. The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology*. 2005;129(1):113-121.

27. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006;44(4):865-873.

28. Ratziu V, Bugianesi E, Dixon J, et al. Histological progression of non-alcoholic fatty liver disease: a critical reassessment based on liver sampling variability. *Aliment Pharmacol Ther*. 2007;26(6):821-830.

29. Rafiq N, Bai C, Fang Y, et al. Long-Term Follow-Up of Patients With Nonalcoholic Fatty Liver. *Clin Gastroenterol Hepatol*. 2009;7(2):234-238.

30. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology*. 2011;141(4):1249-1253.

31. Lomonaco R, Sunny NE, Bril F, Cusi K. Nonalcoholic fatty liver disease: Current issues and novel treatment approaches. *Drugs*. 2013;73(1):1-14.

32. Schwenger KJP, Allard JP. Clinical approaches to non-alcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(7):1712-1723.

33. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med*. 2011;43(8):617-649.

34. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology*. 1999;116(6):1413-1419.

35. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri B a., Bacon BR. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. *Am J Gastroenterol*. 1999;94(9):2467-2474.

36. Levene AP, Goldin RD. The epidemiology, pathogenesis and histopathology of fatty liver disease. *Histopathology*. 2012;61(2):141-152.

37. Nalbantoglu Ilk, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(27):9026-9037.

38. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. *Frontline Gastroenterol*. 2014:1-10.

39. Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology*. 2010;51(1):121-129.

40. Harrison S a., Fecht W, Brunt EM, Neuschwander-Tetri B a. Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. *Hepatology*. 2009;49(1):80-86.

41. Perseghin G, Lattuada G, De Cobelli F, et al. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care*. 2007;30(3):683-688.

42. Kirwan JP, Solomon TPJ, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab*. 2009;297(1):E151-E156.

43. (UK) C for PHE at N, (UK) NCC for PC. Obesity: The Prevention, Identification, Assessment and Management of Overweight and Obesity in Adults and Children.

44. Zelber-Sagi S, Kessler A, Brazowsky E, et al. A Double-Blind Randomized Placebo-Controlled Trial of Orlistat for the Treatment of Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2006;4(5):639-644.

45. Federico A. Focus on emerging drugs for the treatment of patients with non-alcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(45):16841.

46. Wierzbicki AS, Pendleton S, McMahon Z, et al. Rimonabant improves cholesterol, insulin resistance and markers of non-alcoholic fatty liver in morbidly obese patients: a retrospective cohort study. *Int J Clin Pract*. 2011;65(6):713-715.

47. Sjöström L, Lindroos A-K, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med*. 2004;351(26):2683-2693.

48. Sjöström L, Narbro K, Sjöström CD, et al. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med*. 2007;357(8):741-752.

49. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2008;6(12):1396-1402.

50. Chang E, Park C-Y, Park SW. Role of thiazolidinediones, insulin sensitizers, in non-alcoholic fatty liver disease. *J Diabetes Investig*. 2013;4(6):517-524.

51. Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. AMPK activation: a therapeutic target for type 2 diabetes? *Diabetes Metab Syndr Obes*. 2014;7:241-253.

52. Boettcher E, Csako G, Pucino F, Wesley R, Loomba R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2012;35(1):66-75.

53. Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med*. 2006;355(22):2297-2307.

54. Armstrong MJ, Houlihan DD, Rowe IA, et al. Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. *Aliment Pharmacol Ther*. 2013;37(2):234-242.

55. Nauck MA. A critical analysis of the clinical use of incretin-based therapies: The benefits by far outweigh the potential risks. *Diabetes Care*. 2013;36(7):2126-2132.

56. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med*. 2010;363(14):1341-1350.

57. Nseir W, Mograbi J, Ghali M. Lipid-lowering agents in nonalcoholic fatty liver disease and steatohepatitis: human studies. *Dig Dis Sci*. 2012;57(7):1773-1781.

58. Bataller R, Sancho-Bru P, Ginès P, et al. Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II. *Gastroenterology*. 2003;125(1):117-125.

59. Georgescu EF, Ionescu R, Niculescu M, Mogoanta L, Vancica L. Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. *World J Gastroenterol*. 2009;15(8):942-954.

60. Al-Mallah M, Khawaja O, Sinno M, Alzohaili O, Samra ABA. Do angiotensin converting enzyme inhibitors or angiotensin receptor blockers prevent diabetes mellitus? A meta-analysis. *Cardiol J*. 2010;17(5):448-456.

61. Sanyal AJ, Chalasani N, Kowdley K V, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. 2010;362(18):1675-1685.

62. Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA*. 2011;305(16):1659-1668.

63. Xiang Z, Chen Y, Ma K, et al. The role of ursodeoxycholic acid in non-alcoholic steatohepatitis: a systematic review. *BMC Gastroenterol*. 2013;13:140.

64. Leuschner UFH, Lindenthal B, Herrmann G, et al. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology*. 2010;52(2):472-479.

65. Lindor KD, Kowdley K V, Heathcote EJ, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology*. 2004;39(3):770-778.

66. Zeng T, Zhang C-L, Zhao X-L, Xie K-Q. Pentoxifylline for the treatment of nonalcoholic fatty liver disease: a meta-analysis of randomized double-blind, placebo-controlled studies. *Eur J Gastroenterol Hepatol*. 2014;26(6):646-653.

67. Bo S, Benso A, Durazzo M, Ghigo E. Does use of metformin protect against cancer in Type 2 diabetes mellitus? *J Endocrinol Invest*. 2012;35(2):231-235.

68. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. *Gastroenterology*. 2013;144(2):323-332.

69. Newsome PN, Allison ME, Andrews PA, et al. Guidelines for liver transplantation for patients with non-alcoholic steatohepatitis. *Gut*. 2012;61(4):484-500.

70. Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology*. 1998;114(4):842-845.

71. Berlanga A, Guiu-Jurado E, Porras JA, Aragonès G, Auguet T. Papel de las lipasas metabólicas y la lipotoxicidad en el desarrollo de esteatosis hepática y esteatohepatitis no alcohólica. *Clínica e Investig en Arterioscler*. 2015;(xx).

72. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. *Hepatology*. 2010;52(5):1836-1846.

73. Jung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci*. 2014;15(4):6184-6223.

74. Noureddin M, Mato JM, Lu SC. Nonalcoholic fatty liver disease: Update on pathogenesis, diagnosis, treatment and the role of S-adenosylmethionine. *Exp Biol Med*. 2015:1-12.

75. Le Lay J, Kaestner KH. The Fox genes in the liver: from organogenesis to functional integration. *Physiol Rev*. 2010;90(1):1-22.

76. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol*. 2013;48(4):434-441.

77. Tony Y. Wang1, 2, Min Liu3, Piero Portincasa4 and DQ-HW 1Department. New insights into the molecular mechanism of intestinal fatty acid absorption Tony. *Changes*. 2012;29(6):997-1003.

78. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. *Clin Exp Gastroenterol*. 2014;7(1):221-239.

79. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes: Common Pathophysiologic Mechanisms. *Curr Diab Rep*. 2015;15(6):1-13.

80. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*. 2006;7(2):85-96.

81. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*. 2001;414(6865):799-806.

82. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*. 2014;510(7503):84-91.

83. Arner P. Human fat cell lipolysis: Biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab*. 2005;19(4):471-482.

84. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. *Atherosclerosis*. 2015;239(1):192-202.

85. Kim JK, Zisman A, Fillmore JJ, et al. Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. *J Clin Invest*. 2001;108(1):153-160.

86. Wang HY, Ducommun S, Quan C, et al. AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. *Biochem J*. 2013;449(2):479-489.

87. Petersen KF, Dufour S, Savage DB, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci U S A*. 2007;104(31):12587-12594.

88. Rabøl R, Petersen KF, Dufour S, Flannery C, Shulman GI. Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. *Proc Natl Acad Sci U S A*. 2011;108(33):13705-13709.

89. 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(5):1343-1351.

90. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest*. 2008;118(3):829-838.

91. Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends Endocrinol Metab*. 2008;19(10):371-379.

92. Neuschwander-Tetri B a. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: The central role of nontriglyceride fatty acid metabolites. *Hepatology*. 2010;52(2):774-788.

93. Fromenty B, Robin MA, Igoudjil A, Mansouri A, Pessayre D. The ins and outs of mitochondrial dysfunction in NASH. *Diabetes Metab*. 2004;30(2):121-138.

94. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab*. 2011;14(6):804-810.

95. Martín-domínguez V, González-casas R, Mendoza-jiménez-ridruejo J, García- L, Moreno-otero R. Pathogenesis , diagnosis and treatment of non-alcoholic fatty liver disease. 2013;105:409-420.

96. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014.

97. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans*. 2005;33(Pt 5):1078-1081.

98. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112(12):1821-1830.

99. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89(6):2548-2556.

100. Asrih M, Jornayvaz FR. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. *J Endocrinol*. 2013;218(3):R25-R36.

101. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem*. 2003;278(46):45777-45784.

102. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259(5091):87-91.

103. Abenavoli L, Peta V. Role of adipokines and cytokines in non-alcoholic fatty liver disease. *Rev Recent Clin Trials*. 2014;9(3):134-140.

104. Nannipieri M, Cecchetti F, Anselmino M, et al. Pattern of expression of adiponectin receptors in human liver and its relation to nonalcoholic steatohepatitis. *Obes Surg*. 2009;19(4):467-474.

105. Kaser S, Moschen A, Cayon A, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut*. 2005;54(1):117-121.

106. Moschen AR, Molnar C, Wolf AM, et al. Effects of weight loss induced by bariatric surgery on hepatic adipocytokine expression. *J Hepatol*. 2009;51(4):765-777.

107. Crespo J, Cayón A, Fernández-Gil P, et al. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology*. 2001;34(6):1158-1163.

108. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2008;103(6):1372-1379.

109. Moschen AR, Molnar C, Geiger S, et al. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor alpha expression. *Gut*. 2010;59(9):1259-1264.

110. Shen C, Zhao C-Y, Wang W, et al. The relationship between hepatic resistin overexpression and inflammation in patients with nonalcoholic steatohepatitis. *BMC Gastroenterol*. 2014;14:39.

111. Auguet T, Terra X, Porras JA, et al. Plasma visfatin levels and gene expression in morbidly obese women with associated fatty liver disease. *Clin Biochem*. 2013;46(3):202-208.

112. Ferolla S, Armiliato G, Couto C, Ferrari T. The Role of Intestinal Bacteria Overgrowth in Obesity-Related Nonalcoholic Fatty Liver Disease. *Nutrients*. 2014;6(12):5583-5599.

113. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology*. 2014;146(6):1513-1524.

114. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol*. 2014;30(3):332-338.

115. Dai X, Wang B. Role of Gut Barrier Function in the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Gastroenterol Res Pract*. 2015;2015:1-6.

116. Harris K, Kassis A, Major G, Chou CJ. Is the gut microbiota a new factor contributing to obesity and its metabolic disorders? *J Obes*. 2012;2012:879151.

117. Aron-Wisnewsky J, Gaborit B, Dutour a., Clement K. Gut microbiota and non-alcoholic fatty liver disease: New insights. *Clin Microbiol Infect*. 2013;19(4):338-348.

118. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology*. 2009;49(6):1877-1887.

119. Ruiz AG, Casafont F, Crespo J, et al. Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha gene expression in obese patients: evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. *Obes Surg*. 2007;17(10):1374-1380.

120. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology*. 2013;57(2):601-609.

121. Machado M, Cortez-Pinto H. Nash, insulin resistance and iron. *Liver Int*. 2006;26(10):1159-1162.

122. Mendler MH, Turlin B, Moirand R, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology*. 1999;117(5):1155-1163.

123. Ruddell RG, Hoang-Le D, Barwood JM, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. *Hepatology*. 2009;49(3):887-900.

124. Morrison ED, Brandhagen DJ, Phatak PD, et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Ann Intern Med*. 2003;138(8):627-633.

125. Fargion S, Mattioli M, Fracanzani AL, et al. Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2001;96(8):2448-2455.

126. Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology*. 2004;39(1):179-187.

127. Peverill W, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci*. 2014;15(5):8591-8638.

128. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2008;40(12):1461-1465.

129. Kitamoto T, Kitamoto A, Yoneda M, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. *Hum Genet*. 2013;132(7):783-792.

130. Lin Y-C, Chang P-F, Chang M-H, Ni Y-H. Genetic variants in GCKR and PNPLA3 confer susceptibility to nonalcoholic fatty liver disease in obese individuals. *Am J Clin Nutr*. 2014;99(4):869-874.

131. Hotta K, Yoneda M, Hyogo H, et al. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. *BMC Med Genet*. 2010;11:172.

132. Kawaguchi T, Sumida Y, Umemura A, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One*. 2012;7(6):e38322.

133. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2011;53(6):1883-1894.

134. Liu Y-L, Patman GL, Leathart JBS, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol*. 2014;61(1):75-81.

135. Hebbard L, George J. Animal models of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol*. 2011;8(1):35-44.

136. Ota T, Takamura T, Kurita S, et al. Insulin resistance accelerates a dietary rat model of nonalcoholic steatohepatitis. *Gastroenterology*. 2007;132(1):282-293.

137. Semple RK, Sleigh A, Murgatroyd PR, et al. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *J Clin Invest*. 2009;119(2):315-322.

138. Tamura S, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J Clin Invest*. 2005;115(5):1139-1142.

139. Brown MS, Goldstein JL. Selective versus Total Insulin Resistance: A Pathogenic Paradox. *Cell Metab*. 2008;7(2):95-96.

140. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci U S A*. 2010;107(8):3441-3446.

141. Brown JM, Betters JL, Lord C, et al. CGI-58 knockdown in mice causes hepatic steatosis but prevents diet-induced obesity and glucose intolerance. *J Lipid Res*. 2010;51(11):3306-3315.

142. Samuel VT, Liu Z-X, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem*. 2004;279(31):32345-32353.

143. Dries DR, Gallegos LL, Newton AC. A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. *J Biol Chem*. 2007;282(2):826-830.

144. Samuel VT, Liu Z-X, Wang A, et al. Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J Clin Invest*. 2007;117(3):739-745.

145. Frangioudakis G, Burchfield JG, Narasimhan S, et al. Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: regulation of lipogenesis by protein kinase C delta. *Diabetologia*. 2009;52(12):2616-2620.

146. Jornayvaz FR, Shulman GI. Diacylglycerol activation of protein kinase Cε and hepatic insulin resistance. *Cell Metab*. 2012;15(5):574-584.

147. Kumashiro N, Erion DM, Zhang D, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc Natl Acad Sci*. 2011;108(39):16381-16385.

148. Magkos F, Su X, Bradley D, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology*. 2012;142(7):1444-1446.e2.

149. Funke A, Schreurs M, Aparicio-Vergara M, et al. Cholesterol-induced hepatic inflammation does not contribute to the development of insulin resistance in male LDL receptor knockout mice. *Atherosclerosis*. 2014;232(2):390-396.

150. Ruiz R, Jideonwo V, Ahn M, et al. Sterol regulatory element-binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver. *J Biol Chem*. 2014;289(9):5510-5517.

151. Benhamed F, Denechaud P-D, Lemoine M, et al. The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. *J Clin Invest*. 2012;122(6):2176-2194.

152. Cantley JL, Yoshimura T, Camporez JPG, et al. CGI-58 knockdown sequesters diacylglycerols in lipid droplets/ER-preventing diacylglycerol-mediated hepatic insulin resistance. *Proc Natl Acad Sci U S A*. 2013;110(5):1869-1874.

153. Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care*. 2007;10(2):142-148.

154. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab*. 2006;91(4):1446-1452.

155. Eguchi Y, Eguchi T, Mizuta T, et al. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. *J Gastroenterol*. 2006;41(5):462-469.

156. Fenkci S, Rota S, Sabir N, Akdag B. Ultrasonographic and biochemical evaluation of visceral obesity in obese women with non-alcoholic fatty liver disease. *Eur J Med Res*. 2007;12(2):68-73.

157. Doege H, Stahl A. Protein-mediated fatty acid uptake: novel insights from in vivo models. *Physiology (Bethesda)*. 2006;21:259-268.

158. Doege H, Baillie RA, Ortegon AM, et al. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. *Gastroenterology*. 2006;130(4):1245-1258.

159. Falcon A, Doege H, Fluitt A, et al. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *Am J Physiol Endocrinol Metab*. 2010;299(3):E384-E393.

160. Auinger A, Valenti L, Pfeuffer M, et al. A promoter polymorphism in the liver-specific fatty acid transport protein 5 is associated with features of the metabolic syndrome and steatosis. *Horm Metab Res*. 2010;42(12):854-859.

161. Fernández M a, Albor C, Ingelmo-Torres M, et al. Caveolin-1 is essential for liver regeneration. *Science*. 2006;313(5793):1628-1632.

162. Mastrodonato M, Calamita G, Rossi R, et al. Altered distribution of caveolin-1 in early liver steatosis. *Eur J Clin Invest*. 2011;41(6):642-651.

163. Koo S-H. Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. *Clin Mol Hepatol*. 2013;19(3):210-215.

164. Inoue M, Ohtake T, Motomura W, et al. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem Biophys Res Commun*. 2005;336(1):215-222.

165. Buqué X, Martínez MJ, Cano A, et al. A subset of dysregulated metabolic and survival genes is associated with severity of hepatic steatosis in obese Zucker rats. *J Lipid Res*. 2010;51(3):500-513.

166. Degrace P, Moindrot B, Mohamed I, et al. Upregulation of liver VLDL receptor and FAT/CD36 expression in LDLR-/- apoB100/100 mice fed trans-10,cis-12 conjugated linoleic acid. *J Lipid Res*. 2006;47(12):2647-2655.

167. Zhou J, Febbraio M, Wada T, et al. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology*. 2008;134(2):556-567.

168. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(5):G1281-G1287.

169. Bechmann LP, Gieseler RK, Sowa J-P, et al. Apoptosis is associated with CD36/fatty acid translocase upregulation in non-alcoholic steatohepatitis. *Liver Int*. 2010;30(6):850-859.

170. Miquilena-Colina ME, Lima-Cabello E, Sánchez-Campos S, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut*. 2011;60(10):1394-1402.

171. Zimmerman AW, Veerkamp JH. New insights into the structure and function of fatty acid-binding proteins. *Cell Mol Life Sci*. 2002;59(7):1096-1116.

172. Westerbacka J, Kolak M, Kiviluoto T, et al. 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(11):2759-2765.

173. Wang Y, Lam KSL, Yau M, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J*. 2008;409(3):623-633.

174. Gavrilova O, Haluzik M, Matsusue K, et al. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem*. 2003;278(36):34268-34276.

175. Morán-Salvador E, López-Parra M, García-Alonso V, et al. Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. *FASEB J*. 2011;25(8):2538-2550.

176. García-Ruiz I, Rodríguez-Juan C, Díaz-Sanjuán T, Martínez MA, Muñoz-Yagüe T, Solís-Herruzo JA. Effects of rosiglitazone on the liver histology and mitochondrial function in ob/ob mice. *Hepatology*. 2007;46(2):414-423.

177. Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: Potential therapeutic targets. *Biochim Biophys Acta*. 2012;1821(5):809-818.

178. 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*. 2011;96(5):1424-1430.

179. Mitsuyoshi H, Yasui K, Harano Y, et al. Analysis of hepatic genes involved in the metabolism of fatty acids and iron in nonalcoholic fatty liver disease. *Hepatol Res*. 2009;39(4):366-373.

180. Kohjima M, Higuchi N, Kato M, et al. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int J Mol Med*. 2008;21(4):507-511.

181. Lambert JE, Ramos-Roman M a., Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology*. 2014;146(3):726-735.

182. Baranowski M. Biological role of liver X receptors. *J Physiol Pharmacol*. 2008;59 Suppl 7:31-55.

183. Faulds MH, Zhao C, Dahlman-Wright K. Molecular biology and functional genomics of liver X receptors (LXR) in relationship to metabolic diseases. *Curr Opin Pharmacol*. 2010;10(6):692-697.

184. Higuchi N, Kato M, Shundo Y, et al. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol Res*. 2008;38(11):1122-1129.

185. Lima-Cabello E, Garcia-Mediavilla M V, Miquilena-Colina ME, et al. Enhanced expression of pro-inflammatory mediators and liver X-

receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. *Clin Sci (Lond)*. 2011;120(6):239-250.

186. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. 2007;20(3):351-358.

187. Nakamuta M, Fujino T, Yada R, et al. Impact of cholesterol metabolism and the LXRalpha-SREBP-1c pathway on nonalcoholic fatty liver disease. *Int J Mol Med*. 2009;23(5):603-608.

188. Ahn SB, Jang K, Jun DW, Lee BH, Shin KJ. Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease. *Dig Dis Sci*. 2014;59(12):2975-2982.

189. 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(9):1125-1131.

190. Schultz JR, Tu H, Luk A, et al. Role of LXRs in control of lipogenesis. *Genes Dev*. 2000;14(22):2831-2838.

191. Osborne TF. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem*. 2000;275(42):32379-32382.

192. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res*. 2001;40(6):439-452.

193. Nagaya T, Tanaka N, Suzuki T, et al. Down-regulation of SREBP-1c is associated with the development of burned-out NASH. *J Hepatol*. 2010;53(4):724-731.

194. Uyeda K, Repa JJ. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab*. 2006;4(2):107-110.

195. Cha J-Y, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem*. 2007;282(1):743-751.

196. Mitro N, Mak PA, Vargas L, et al. The nuclear receptor LXR is a glucose sensor. *Nature*. 2006;445(7124):219-223.

197. Arden C, Petrie JL, Tudhope SJ, et al. Elevated glucose represses liver glucokinase and induces its regulatory protein to safeguard hepatic phosphate homeostasis. *Diabetes*. 2011;60(12):3110-3120.

198. Ma L, Robinson LN, Towle HC. ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J Biol Chem*. 2006;281(39):28721-28730.

199. De la Iglesia N, Mukhtar M, Seoane J, Guinovart JJ, Agius L. The role of the regulatory protein of glucokinase in the glucose sensory mechanism of the hepatocyte. *J Biol Chem*. 2000;275(14):10597-10603.

200. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A*. 2004;101(19):7281-7286.

201. Iizuka K, Miller B, Uyeda K. Deficiency of carbohydrate-activated transcription factor ChREBP prevents obesity and improves plasma glucose control in leptin-deficient (ob/ob) mice. *Am J Physiol Endocrinol Metab*. 2006;291(2):E358-E364.

202. Dentin R, Benhamed F, Hainault I, et al. Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes*. 2006;55(8):2159-2170.

203. Iizuka K, Takeda J, Horikawa Y. Hepatic overexpression of dominant negative Mlx improves metabolic profile in diabetes-prone C57BL/6J mice. *Biochem Biophys Res Commun*. 2009;379(2):499-504.

204. Teodoro JS, Rolo AP, Palmeira CM. Hepatic FXR: key regulator of whole-body energy metabolism. *Trends Endocrinol Metab*. 2011;22(11):458-466.

205. Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest*. 2004;113(10):1408-1418.

206. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. *J Clin Invest*. 2006;116(4):1102-1109.

207. Zhang Y, Lee FY, Barrera G, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A*. 2006;103(4):1006-1011.

208. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov*. 2008;7(8):678-693.

209. McMahan RH, Wang XX, Cheng LL, et al. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem*. 2013;288(17):11761-11770.

210. Wang Y-D, Chen W-D, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology*. 2008;48(5):1632-1643.

211. Adorini L, Pruzanski M, Shapiro D. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today*. 2012;17(17-18):988-997.

212. Mudaliar S, Henry RR, Sanyal AJ, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology*. 2013;145(3):574-582.e1.

213. Inagaki T, Moschetta A, Lee Y-K, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A*. 2006;103(10):3920-3925.

214. Yang Z-X, Shen W, Sun H. Effects of nuclear receptor FXR on the regulation of liver lipid metabolism in patients with non-alcoholic fatty liver disease. *Hepatol Int*. 2010;4(4):741-748.

215. Mashek DG. Hepatic fatty acid trafficking: multiple forks in the road. *Adv Nutr*. 2013;4(6):697-710. doi:10.3945/an.113.004648.

216. Dorn C, Riener M-O, Kirovski G, et al. Expression of fatty acid synthase in nonalcoholic fatty liver disease. *Int J Clin Exp Pathol*. 2010;3(5):505-514.

217. Morgan K, Uyuni A, Nandgiri G, et al. Altered expression of transcription factors and genes regulating lipogenesis in liver and adipose tissue of mice with high fat diet-induced obesity and nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2008;20(9):843-854.

218. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem*. 1997;244(1):1-14.

219. Sidossis LS, Stuart CA, Shulman GI, Lopaschuk GD, Wolfe RR. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. *J Clin Invest*. 1996;98(10):2244-2250.

220. Foster DW. Malonyl-CoA: the regulator of fatty acid synthesis and oxidation. *J Clin Invest*. 2012;122(6):1958-1959.

221. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. 2007;20(3):351-358.

222. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol*. 2015;62(3):720-733.

223. Yessoufou A, Wahli W. Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels. *Swiss Med Wkly*. 2010;140:w13071.

224. Xu J, Xiao G, Trujillo C, et al. Peroxisome proliferator-activated receptor alpha (PPARalpha) influences substrate utilization for hepatic glucose production. *J Biol Chem*. 2002;277(52):50237-50244.

225. Gervois P, Kleemann R, Pilon A, et al. Global suppression of IL-6-induced acute phase response gene expression after chronic in vivo treatment with the peroxisome proliferator-activated receptor-alpha activator fenofibrate. *J Biol Chem*. 2004;279(16):16154-16160.

226. Schmid AI, Szendroedi J, Chmelik M, Krssák M, Moser E, Roden M. Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. *Diabetes Care*. 2011;34(2):448-453.

227. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA*. 1999;282(17):1659-1664.

228. Satapati S, Sunny NE, Kucejova B, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. *J Lipid Res*. 2012;53(6):1080-1092.

229. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*. 2001;120(5):1183-1192.

230. Francque S, Verrijken A, Caron S, et al. PPARα gene expression correlates with severity and histological treatment response in patients with Non-alcoholic Steatohepatitis. *J Hepatol*. 2015.

231. Rao MS, Papreddy K, Musunuri S, Okonkwo A. Prevention/reversal of choline deficiency-induced steatohepatitis by a peroxisome proliferator-activated receptor alpha ligand in rats. *In Vivo*. 16(2):145-152.

232. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology*. 2004;39(5):1286-1296.

233. Nagasawa T, Inada Y, Nakano S, et al. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. *Eur J Pharmacol*. 2006;536(1-2):182-191.

234. Qin X, Xie X, Fan Y, et al. Peroxisome proliferator-activated receptor-delta induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice. *Hepatology*. 2008;48(2):432-441.

235. Liu S, Hatano B, Zhao M, et al. Role of peroxisome proliferator-activated receptor {delta}/{beta} in hepatic metabolic regulation. *J Biol Chem*. 2011;286(2):1237-1247.

236. Iwaisako K, Haimerl M, Paik Y-H, et al. Protection from liver fibrosis by a peroxisome proliferator-activated receptor δ agonist. *Proc Natl Acad Sci U S A*. 2012;109(21):E1369-E1376.

237. Staels B, Rubenstrunk A, Noel B, et al. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology*. 2013;58(6):1941-1952.

238. Cariou B, Hanf R, Lambert-Porcheron S, et al. Dual peroxisome proliferator-activated receptor α/δ agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. *Diabetes Care*. 2013;36(10):2923-2930.

239. Cariou B, Zaïr Y, Staels B, Bruckert E. Effects of the new dual PPAR α/δ agonist GFT505 on lipid and glucose homeostasis in abdominally obese patients with combined dyslipidemia or impaired glucose metabolism. *Diabetes Care*. 2011;34(9):2008-2014.

240. Tiwari S, Siddiqi S a. Intracellular trafficking and secretion of VLDL. *Arterioscler Thromb Vasc Biol*. 2012;32(5):1079-1086.

241. Ginsberg HN, Fisher E a. The ever-expanding role of degradation in the regulation of apolipoprotein B metabolism. *J Lipid Res*. 2009;50 Suppl:S162-S166.

242. Kamagate A, Dong HH. FoxO1 integrates insulin signaling to VLDL production. *Cell Cycle*. 2008;7(20):3162-3170.

243. Di Filippo M, Moulin P, Roy P, et al. Homozygous MTTP and APOB mutations may lead to hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia. *J Hepatol*. 2014;61(4):891-902.

244. 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(2):424-431.

245. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology*. 2002;35(4):898-904.

246. Ota T, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *J Clin Invest*. 2008;118(1):316-332.

247. Alswat KA. The role of endocannabinoids system in fatty liver disease and therapeutic potentials. *Saudi J Gastroenterol*. 19(4):144-151.

248. Westerbacka J, Kotronen A, Fielding BA, et al. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. *Gastroenterology*. 2010;139(6):1961-1971.e1.

249. Purohit V, Rapaka R, Shurtleff D. Role of cannabinoids in the development of fatty liver (steatosis). *AAPS J*. 2010;12(2):233-237.

250. De Gottardi A, Spahr L, Ravier-Dall'Antonia F, Hadengue A. Cannabinoid receptor 1 and 2 agonists increase lipid accumulation in hepatocytes. *Liver Int*. 2010;30(10):1482-1489.

251. Mallat A, Lotersztajn S. Endocannabinoids and their role in fatty liver disease. *Dig Dis*. 2010;28(1):261-266.

252. Guindon J, Hohmann AG. The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets*. 2009;8(6):403-421.

253. Mallat A, Teixeira-Clerc F, Lotersztajn S. Cannabinoid signaling and liver therapeutics. *J Hepatol*. 2013;59(4):891-896.

254. Valenzuela C, Castillo V, Ronco AM, Aguirre C, Hirsch S, Llanos M. [A role for the endocannabinoid system in hepatic steatosis]. *Rev Med Chil*. 2014;142(3):353-360.

255. Mallat a., Teixeira-Clerc F, Deveaux V, Manin S, Lotersztajn S. The endocannabinoid system as a key mediator during liver diseases: New insights and therapeutic openings. *Br J Pharmacol*. 2011;163(7):1432-1440.

256. Osei-Hyiaman D, Liu J, Zhou L, et al. Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest*. 2008;118(9):3160-3169.

257. Tam J, Vemuri VK, Liu J, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest*. 2010;120(8):2953-2966.

258. Gary-Bobo M, Elachouri G, Gallas JF, et al. Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats. *Hepatology*. 2007;46(1):122-129.

259. Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest*. 2005;115(5):1298-1305.

260. Jourdan T, Djaouti L, Demizieux L, Gresti J, Vergès B, Degrace P. CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes*. 2010;59(4):926-934.

261. Cota D, Marsicano G, Tschöp M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest*. 2003;112(3):423-431.

262. Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G, Kunos G. Endocannabinoids in liver disease. *Hepatology*. 2011;53(1):346-355.

263. Chanda D, Kim DK, Li T, et al. Cannabinoid Receptor Type 1 (CB1R) signaling regulates hepatic gluconeogenesis via induction of endoplasmic reticulum-bound transcription factor cAMP-responsive element-binding protein H (CREBH) in primary hepatocytes. *J Biol Chem*. 2011;286(32):27971-27979.

264. Giannone FA, Baldassarre M, Domenicali M, et al. Reversal of liver fibrosis by the antagonism of endocannabinoid CB1 receptor in a rat model of CCl(4)-induced advanced cirrhosis. *Lab Invest*. 2012;92(3):384-395.

265. DeLeve LD, Wang X, Kanel GC, Atkinson RD, McCuskey RS. Prevention of hepatic fibrosis in a murine model of metabolic syndrome with nonalcoholic steatohepatitis. *Am J Pathol*. 2008;173(4):993-1001.

266. Moezi L, Gaskari SA, Lee SS. Endocannabinoids and liver disease. V. endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. *Am J Physiol Gastrointest Liver Physiol*. 2008;295(4):G649-G653.

267. Mukhopadhyay B, Cinar R, Yin S, et al. Hyperactivation of anandamide synthesis and regulation of cell-cycle progression via cannabinoid type 1 (CB1) receptors in the regenerating liver. *Proc Natl Acad Sci U S A*. 2011;108(15):6323-6328.

268. Di Marzo V, Côté M, Matias I, et al. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. *Diabetologia*. 2009;52(2):213-217.

269. Côté M, Matias I, Lemieux I, et al. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)*. 2007;31(4):692-699.

270. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA*. 2006;295(7):761-775.

271. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet (London, England)*. 365(9468):1389-1397.

272. Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet (London, England)*. 2006;368(9548):1660-1672.

273. Després J-P, Golay A, Sjöström L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med*. 2005;353(20):2121-2134.

274. Nissen SE, Nicholls SJ, Wolski K, et al. Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA*. 2008;299(13):1547-1560.

275. Hollander PA, Amod A, Litwak LE, Chaudhari U. Effect of rimonabant on glycemic control in insulin-treated type 2 diabetes: the ARPEGGIO trial. *Diabetes Care*. 2010;33(3):605-607.

276. Després J-P, Ross R, Boka G, Alméras N, Lemieux I. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol*. 2009;29(3):416-423.

277. Sam AH, Salem V, Ghatei M a. Rimonabant: From RIO to Ban. *J Obes*. 2011;2011.

278. Christopoulou FD, Kiortsis DN. An overview of the metabolic effects of rimonabant in randomized controlled trials: potential for other cannabinoid 1 receptor blockers in obesity. *J Clin Pharm Ther*. 2011;36(1):10-18.

279. Silvestri C, Paris D, Martella A, et al. Two non-psychoactive cannabinoids reduce intracellular lipid levels and inhibit hepatosteatosis. *J Hepatol*. 2015;62(6):1382-1390.

280. Tam J, Cinar R, Liu J, et al. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab*. 2012;16(2):167-179.

281. Deveaux V, Cadoudal T, Ichigotani Y, et al. Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS One*. 2009;4(6):e5844.

282. Agudo J, Martin M, Roca C, et al. Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia*. 2010;53(12):2629-2640.

283. Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, et al. Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int*. 2007;27(2):215-219.

284. Muñoz-Luque J, Ros J, Fernández-Varo G, et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther*. 2008;324(2):475-483.

285. Teixeira-Clerc F, Belot M-P, Manin S, et al. Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology*. 2010;52(3):1046-1059.

286. Julien B, Grenard P, Teixeira-Clerc F, et al. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology*. 2005;128(3):742-755.

287. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol*. 2013;10(6):330-344.

288. Söderberg C, Stål P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology*. 2010;51(2):595-602.

289. Fielding CM, Angulo P. Hepatic steatosis and steatohepatitis: Are they really two distinct entities? *Curr Hepatol reports*. 2014;13(2):151-158.

290. Matsumoto M, Han S. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin …*. 2006;116(9).

291. Brown MS, Goldstein JL. The SREBP Pathway: Regulation Review of Cholesterol Metabolism by Proteolysisof a Membrane-Bound Transcription Factor. 1997;89(1):1-10.

292. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, et al. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab*. 2008;7(2):125-134.

293. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860-867.

294. Tilg H. Adipocytokines in nonalcoholic fatty liver disease: key players regulating steatosis, inflammation and fibrosis. *Curr Pharm Des*. 2010;16(17):1893-1895.

295. Item F, Konrad D. Visceral fat and metabolic inflammation: the portal theory revisited. *Obes Rev*. 2012;13 Suppl 2:30-39.

296. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care*. 2008;31 Suppl 2:S262-S268.

297. Rhee E-J, Lee W-Y, Cho Y-K, Kim B-I, Sung K-C. Hyperinsulinemia and the development of nonalcoholic Fatty liver disease in nondiabetic adults. *Am J Med*. 2011;124(1):69-76.

298. Dankner R, Chetrit A, Shanik MH, Raz I, Roth J. Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: a preliminary report. *Diabetes Care*. 2009;32(8):1464-1466.

299. Dankner R, Chetrit A, Shanik MH, Raz I, Roth J. Basal state hyperinsulinemia in healthy normoglycemic adults heralds dysglycemia after more than two decades of follow up. *Diabetes Metab Res Rev*. 2012;28(7):618-624.

300. Steneberg P, Sykaras AG, Backlund F, Straseviciene J, Soderstrom I, Edlund H. Hyperinsulinemia enhances hepatic expression of the fatty acid transporter Cd36 and provokes hepatosteatosis and hepatic insulin resistance. *J Biol Chem*. June 2015.

301. Denechaud P-D, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. *FEBS Lett*. 2008;582(1):68-73.

302. Van der Poorten D, Shahidi M, Tay E, et al. Hepatitis C virus induces the cannabinoid receptor 1. *PLoS One*. 2010;5(9).

303. Jeong W, Osei-Hyiaman D, Park O, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metab*. 2008;7(3):227-235.

304. Tomita K, Tamiya G, Ando S, et al. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut*. 2006;55(3):415-424.

305. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med*. 2000;343(20):1467-1476.

306. Mandard S, Müller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci*. 2004;61(4):393-416.

307. Yu S, Matsusue K, Kashireddy P, et al. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *J Biol Chem*. 2003;278(1):498-505.

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.
Alba Berlanga Bustos
Dipòsit Legal: T 1705-2015

IX. Annex

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM AND CANNABINOID RECEPTORS IN LIVER OF MORBIDLY OBESE WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE.
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Publications

Interleukin-17A Gene Expression in Morbidly Obese Women

Fernando Zapata-Gonzalez, Teresa Auguet, Gemma Aragonès, Esther Guiu-Jurado, Alba Berlanga, Salomé Martinez, Andreu Martí, Fátima Sabench, Mercé Hernandez, Carmen Aguilar, Joan Josep Sirvent, Rosa Jorba, Daniel Del Castillo and Cristóbal Richart. *Int. J. Mol. Sci.* 2015, *16*, 17469-17481

Role of metabolic lipases and lipotoxicity in the development of non-alcoholic steatosis and non-alcoholic steatohepatitis

Berlanga A*, Guiu-Jurado E*, Porras JA, Aragonès G, Auguet T. *Clin Investig Arterioscler 2015.* *These authors contributed equally

Altered fatty acid metabolism-related gene expression in liver from morbidly obese women with non-alcoholic fatty liver disease

Auguet T*****, Berlanga A*****, Guiu-Jurado E, Martinez S, Porras JA, Aragonès G, Sabench F, Hernandez M, Aguilar C, Sirvent JJ, Del Castillo C, Richart C. *Int J Mol Sci. 2014;15(12):22173-22187* *These authors contributed equally

Molecular pathways in non-alcoholic fatty liver disease.

Berlanga A*, Guiu-Jurado E*, Porras JA, Auguet T. *Clin Exp Gastroenterol*. 2014;7(1):221-239.*These authors contributed equally

Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women.

Auguet T, Guiu-Jurado E, Berlanga A, Terra X, Martinez S, Porras JA, Ceausu A, Sabench F, Hernandez M, Aguilar C, Sirvent JJ, Castillo DD, Richart C. *Obesity (Silver Spring). 2014;22(9):2032-2038.*

Endocannabinoid receptors gene expression in morbidly obese women with nonalcoholic fatty liver disease.

Auguet T*, Berlanga A*, Guiu-Jurado E, Terra X, Martinez S, Aguilar C, Filiu E, Alibalic A, Sabench F, Hernández M, Del Castillo D, Richart C. *Biomed Res Int. 2014;2014:502542.**These authors contributed equally to this work.

Clinical and adipocytokine changes after bariatric surgery in morbidly obese women.

Auguet T, Terra X, Hernández M, Sabench F, Porras JA, Orellana-Gavaldà JM, Llutart J, Guiu-Jurado E, Berlanga A, Martinez S, Aguilar C, Castillo DD, Richart C. *Obesity (Silver Spring)*. 2014;22(1):188-194.

Adipocytokine levels in women with anorexia nervosa. Relationship with weight restoration and disease duration.

Terra X, Auguet T, Agüera Z, Quesada IM, Orellana-Gavaldà JM, Aguilar C, Jiménez-Murcia S, Berlanga A, Guiu-Jurado E, Menchón JM, Fernández-Aranda F, Richart C. *Int J Eat Disord*. 2013;46(8):855-861.

Long-term changes in leptin, chemerin and ghrelin levels following different bariatric surgery procedures: Roux-en-Y gastric bypass and sleeve gastrectomy.

Terra X, Auguet T, Guiu-Jurado E, Berlanga A, Orellana-Gavaldà JM, Hernández M, Sabench F, Porras JA, Llutart J, Martinez S, Aguilar C, Del Castillo D, Richart C. *Obes Surg*. 2013;23(11):1790-1798

Congres attendance

miR33a/b AND miR122 hepatic expression in obese patients with non-alcoholic fatty liver disease
Berlanga A; Guiu-Jurado E; Auguet T; Aragonés G; Martinez S; Aguilar C; Sabench F; Armengol S; Alibalic A; del Castillo D; Richart C.
Type of participation: Poster
22nd European Congress on Obesity (ECO). Prague (Czech Republic) **2015**

Downregulation of de novo lipogenesis and fatty acid oxidation in subcutaneous adipose tissue of moderate obese women
Guiu-Jurado E; Berlanga A; Auguet T; Aragonés G, Sabench F; Martí A; Aguilar C; Armengol S; del Castillo D; Richart C
Type of participation: Poster
22nd European Congress on Obesity (ECO). Prague (Czech Republic) **2015**

Estudio de la expression génica de la fracción estromal vascular y de los adipocito maduros en pacientes obesos mórbidos
E. Guiu-Jurado, T. Auguet, F. Sabench, A. Berlanga, E. Raga, G. Aragonès, M. Hernández, C. Aguilar, C. Richart, D. Del Castillo
Type of participation: Poster.
XVII Congreso de la Sociedad Española de Cirugía de la Obesidad y de las Enfermedades Metabólica Y de laSección de Obesidad de la AEC. Vitoria (SPAIN) **2015**

Expresión génica de la interleuquina-17A en mujeres obesas mórbidas: Relación con la IL-6
T. Auguet, G. Aragonès, A. Muñoz, E. Guiu-Jurado, F. Sabench, A. Berlanga, M. Hernández, C. Aguilar, D. Del Castillo, C. Richart
Type of participation: Poster.

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Maternal metformin treatment during obese pregnancy reduces offspring fatty liver and hepatic inflammation
Hugh Thomas, Alba Berlanga, Christopher D Byrne, Felino R Cagampang
Type of participation: Conference
Experimental Biology 2015. Boston (United States) **2015**

Metformin treatment in obese pregnant mice protects adult offspring from increased adiposity and elevated fasting blood glucose
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Type of participation: Conference
Physiology 2014. London (United Kingdom) **2014**

Liver expression of transcription factors and lipogenic enzymes in morbidly obese women with non-alcoholic fatty liver disease.
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XXXVI Congreso de la Sociedad Española de Bioquímica y Biología Molecular (SEBBM). Madrid (SPAIN) **2013**

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XXXVI Congreso de la Sociedad Española de Bioquímica y Biología Molecular (SEBBM). Madrid (SPAIN) **2013**

Cambios en los niveles de Quemerina, Leptina y Grelina después de diferentes procedimientos de cirugía bariátrica: Bypass Gastroyeyunal en Y De Roux vs Gastrectomía Vertical
T.Auguet; F.Sabench; X.Terra; J.A Porras, M.Hernández; J.M Orellana; C.Aguilar; A.Berlanga; C.Richart Jurado; D.Del Castillo

Type of participation: Poster

1er Congreso médico-quirúrgico de la obesidad**.** Madrid (ESPAÑA) **2013**

Longitudinal changes in adipo/cytokine levels after bariatric surgery: preoperative concentrations as predictors of weight reduction and insulin sensitivity recovery.

Ximena Terra, Josep Maria Orellana-Gavaldà, Teresa Auguet, Esther Guiu, Alba Berlanga, Fàtima Sabench, Carmen Aguilar, Mercè Hernández, Daniel del Castillo and Cristobal Richart

Type of participation: Poster

22nd IUBMB- 37th FEBS Congress. From Single Molecules to Systems Biology. Sevilla (SPAIN) **2012**

The Role of Liver X Receptor Alpha in non-alcoholic liver disease.

Josep Maria Orellana-Gavaldà, Ximena Terra, Teresa Auguet, Alba Berlanga, Esther Guiu, Fàtima Sabench, Carmen Aguilar, Salomé Martínez, Mercè Hernández, Daniel del Castillo and Cristobal Richart

Type of participation: Poster

22nd IUBMB- 37th FEBS Congress. From Single Molecules to Systems Biology. Sevilla (SPAIN) **2012**

Clinical and Experimental Gastroenterology

Dovepress
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Open Access Full Text Article

REVIEW

Molecular pathways in non-alcoholic fatty liver disease

This article was published in the following Dove Press journal:
Clinical and Experimental Gastroenterology
5 July 2014
Number of times this article has been viewed

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological change characterized by the accumulation of triglycerides in hepatocytes and has frequently been associated with obesity, type 2 diabetes mellitus, hyperlipidemia, and insulin resistance. It is an increasingly recognized condition that has become the most common liver disorder in developed countries, affecting over one-third of the population and is associated with increased cardiovascular- and liver-related mortality. NAFLD is a spectrum of disorders, beginning as simple steatosis. In about 15% of all NAFLD cases, simple steatosis can evolve into non-alcoholic steatohepatitis, a medley of inflammation, hepatocellular injury, and fibrosis, often resulting in cirrhosis and even hepatocellular cancer. However, the molecular mechanism underlying NAFLD progression is not completely understood. Its pathogenesis has often been interpreted by the "double-hit" hypothesis. The primary insult or the "first hit" includes lipid accumulation in the liver, followed by a "second hit" in which proinflammatory mediators induce inflammation, hepatocellular injury, and fibrosis. Nowadays, a more complex model suggests that fatty acids (FAs) and their metabolites may be the true lipotoxic agents that contribute to NAFLD progression; a multiple parallel hits hypothesis has also been suggested. In NAFLD patients, insulin resistance leads to hepatic steatosis via multiple mechanisms. Despite the excess hepatic accumulation of FAs in NAFLD, it has been described that not only de novo FA synthesis is increased, but FAs are also taken up from the serum. Furthermore, a decrease in mitochondrial FA oxidation and secretion of very-low-density lipoproteins has been reported. This review discusses the molecular mechanisms that underlie the pathophysiological changes of hepatic lipid metabolism that contribute to NAFLD.

Keywords: non-alcoholic fatty liver disease, molecular pathways, insulin resistance, fatty acid metabolism

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major public health issue due to its high prevalence worldwide, and ranges widely from 11% to 46%,[1–3] and has potentially serious sequelae.[4] The prevalence increases to 58% in overweight individuals and can be as high as 98% in non-diabetic obese individuals.[5]

NAFLD is an inclusive term that takes in a spectrum of liver pathologies from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH). NASH involves hepatocellular injury and inflammation of the liver.[6]

Whereas SS is characterized by a relatively favorable clinical course, NASH much more frequently progresses to cirrhosis and hepatocellular carcinoma.[7,8] NAFLD should be suspected in individuals who are either obese, diabetic, or have metabolic syndrome.[9] Moreover, NAFLD is considered a hepatic manifestation of metabolic syndrome and

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a risk factor for type 2 diabetes mellitus, dyslipidemia, and hypertension.[10,11] The majority of patients with NAFLD are asymptomatic and the disease may be detected via routine blood tests showing elevated liver enzymes or when an ultrasound is performed for various reasons and detects liver steatosis. Secondary causes of hepatic steatosis or elevated liver enzymes, such as excess alcohol consumption, medications, toxins, lipodystrophy, autoimmune and inflammatory diseases, nutrition (malnutrition, total parenteral nutrition, severe weight loss, and refeeding syndrome), viral hepatitis, and metabolic liver disease should be excluded by reviewing the patient's history and proper investigation.[9,12]

Although it is still not possible to diagnose NAFLD based solely on blood work, elevated transaminases can be used as a first step.[13] An aspartate aminotransferase–alanine aminotransferase ratio <1 is also seen in NAFLD[14] and supports NASH. However, it is important to note that patients with normal transaminases and liver steatosis on imaging may also have NASH.[15] Ultrasonography is a noninvasive tool that is used in the detection of liver steatosis. Other imaging techniques such as computed tomography and nuclear magnetic resonance imaging can also detect liver steatosis, but neither of these more expensive techniques provide more information than ultrasonography,[16,17] except for fat quantification.[18] Diagnosis for NASH is confirmed when a liver biopsy shows the presence of perilobular inflammation, or the presence of hepatocyte ballooning, Mallory's hyaline, and acidophil bodies with or without fibrosis. Noninvasive tests such as Fatty Liver Index, NAFLD fibrosis score, FibroMeter, and Fibroscan[19] may suggest the presence of NASH by detecting fibrosis. Research is ongoing to assess surrogate markers for NASH such as CK18, but this remains experimental.[20,21]

Regarding the management of NAFLD, weight management through improvements in diet and increased physical activity can help to improve liver histology as well as delay disease progression.[22–24] Lifestyle interventions may not be effective in certain cases, and thus other approaches must be considered. Pharmacological treatment has been studied in this population, specifically insulin-sensitizing agents (metformin and thiazolidinediones [TZDs]); however, there are conflicting results. Clinical studies could not demonstrate the effectiveness of metformin in the treatment of NAFLD.[25] On the other hand, TZDs that are peroxisomal proliferator-activated receptor γ (PPARγ) agonists promote hepatic fatty acid (FA) oxidation and decrease hepatic lipogenesis.[26,27] In NAFLD patients, TZDs have been shown to decrease hepatic fat and decrease cellular injury. However, discontinuing TZD therapy resulted in NASH recurrence and long-term use of TZDs can result in medical complications such as edema, congestive heart failure, osteoporosis, and weight gain.[28,29] The use of statins in NAFLD patients with dyslipidemia can improve liver function tests,[30] as well as steatosis.[31] Furthermore, statins seem to be safe in NAFLD/NASH patients with dyslipidemia.[32] However, there is a lack of evidence for the use of statins in the treatment of NASH patients without dyslipidemia.[33] Further research is necessary to document the effect of other strategies, such as bariatric surgery, antioxidants, and fish oil in NAFLD.

Because there are currently no effective therapies for NAFLD apart from weight loss, ongoing research efforts are focused on understanding the underlying pathobiology of hepatic steatosis with the intention of identifying novel therapeutic targets. In this sense, this review analyses some of the molecular mechanisms that underlie the pathophysiological changes of hepatic lipid metabolism in NAFLD: the contribution of lipid metabolism, the influence of inflammation, and the role of lipotoxicity and cannabinoid receptors in NAFLD.

Contribution of lipid metabolism to NAFLD

The liver plays a major role in lipid metabolism, importing free FAs (FFAs) and manufacturing, storing, and exporting lipids; derangements in any of these processes can lead to the development of NAFLD.[34] FAs are involved in many important cellular events, such as synthesis of cellular membranes, energy storage, and intracellular signaling pathways. However, chronically elevated FFAs can disturb diverse metabolic pathways and induce insulin resistance (IR) in many organs. Hepatic fat accumulation has been strongly associated with IR.[35,36] IR in the peripheral adipose tissue enhances lipolysis and increases the delivery of adipose-derived FFAs to the liver. In particular, obesity increases tumor necrosis factor α (TNFα) production in adipocytes, facilitates adipocyte IR, and increases lipolysis rate.[37] Thus, the circulating pool of FFAs is increased in obese individuals and accounts for the majority of liver lipids in NAFLD.[38]

Under physiological conditions, triglyceride (TG) synthesis is stimulated to dispose of the excess of FFAs. The TGs can then be stored as lipid droplets within hepatocytes or secreted into the blood as very-low-density lipoprotein (VLDL).[39] Rodent studies have shown that the mechanisms leading to the excessive accumulation of hepatic TGs are associated with an increased supply of FFAs from peripheral adipose tissue to the liver and an enhanced de novo lipid synthesis via the lipogenic pathway. Conversely, liver disposal

via β-oxidation and VLDL export are moderately affected.[40] At the cellular level, defects in the insulin signaling pathways contribute to the increase of FFA flux in the liver, which in turn activates a series of signaling cascades and leads to the phosphorylation of several substrates.[41] Despite TGs being the main lipids stored in the liver of patients with NAFLD, large epidemiological studies suggest that they might exert protective functions. TG synthesis seems to be an adaptive, beneficial response in situations where hepatocytes are exposed to potentially toxic TG metabolites.[42–44] FFAs and cholesterol, especially when accumulated in the mitochondria, are considered the "aggressive" lipids leading to TNFα-mediated liver damage and reactive oxygen species (ROS) formation.[45,46] These lipids could also be present in a non-steatotic liver and act as early "inflammatory" hits, leading to the whole spectrum of NAFLD pathologies. The concept of lipotoxicity and involved lipid species has been introduced: abundant FAs cause lipotoxicity via the induction of ROS release, which causes inflammation, apoptosis, and thus, the progression to NASH and fibrogenesis.[46–48]

In summary, TG accumulation in the cytoplasm of hepatocytes, as the hallmark of NAFLD, arises from an imbalance between lipid acquisition (FA uptake and de novo lipogenesis) and removal (mitochondrial FA oxidation and export as a component of VLDL particles) and accompanies multiple pathophysiological mechanisms in NASH (Figure 1). In order to control the progression of NAFLD, it is important to understand the regulatory mechanisms of lipid accumulation in the human liver.

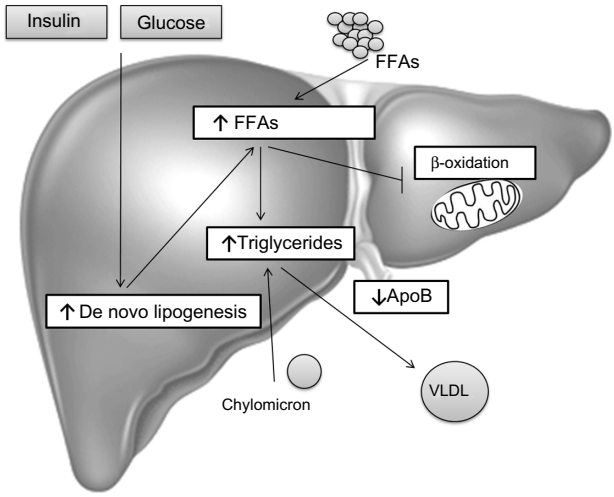

Figure 1 Hepatic steatosis.
Notes: The hallmark of NAFLD is triglyceride accumulation in the cytoplasm of hepatocytes as a result of an imbalance between lipid input and output: 1) an increase in FFAs uptake derived from the circulation due to increased lipolysis from adipose tissue and/or from the diet in the form of chylomicrons; 2) an increase in glucose and insulin levels in response to carbohydrate intake that promotes de novo lipogenesis; 3) a decrease in FA mitochondrial oxidation; 4) a decrease in triglyceride hepatic secretion by packaging with ApoB into VLDLs. In NAFLD patients, enhanced acquisition of FAs through uptake and de novo lipogenesis are not compensated by FA oxidation or production of VLDL particles.
Abbreviations: ApoB, apolipoprotein B; FFAs, free fatty acids; FA, fatty acid; NAFLD, non-alcoholic fatty liver disease; VLDL, very-low-density lipoprotein.

Hepatic FA uptake

One of the sources for hepatic FAs is FFA recruitment from the blood stream. FFAs are derived from lipolysis in adipocytes, which usually occurs in the fasting state, promoted by catecholamines, natriuretic peptides, and glucagon, and are usually repressed by insulin.[49] However, the IR state (obesity, metabolic syndrome) goes along with increased adipocyte lipolysis, leading to abundant FFAs in the plasma pool independently from the nutritional status.[50] FFAs are then taken up by the hepatocytes in a facilitated fashion rather than by passive processes.[51] FA uptake into the liver contributes to the steady balance of hepatic TGs, as well as the pathogenesis of NAFLD. The rate of FA uptake from plasma into cells depends on the FA concentration in the plasma and the hepatocellular capacity for FA uptake, which also depends on the number and activity of transporter proteins on the sinusoidal plasma membrane of the hepatocyte. The main plasma membrane transporters for FFAs are FA transporter protein (FATP), caveolins, FA translocase (FAT)/CD36, and FA-binding protein (FABP).[52–56]

FATP

Six FATP isoforms have been identified in mammalian cells, which contain a common motif for FA uptake and fatty acyl-CoA synthetase function.[57] Of these isoforms, FATP2 and FATP5 are highly expressed in the liver, and are utilized as major FATPs for the normal physiological context. In mouse hepatocytes, adenovirus-mediated knockdown of FATP2 or genetic deletion of FATP5 significantly decreases the rates of FA uptake.[58] Indeed, FATP5 knockout mice have shown resistance to diet-induced obesity and hepatic TG accumulation.[58] In humans, a promoter polymorphism in the liver-specific FATP5 is associated with features of the metabolic syndrome and steatosis.[59]

Caveolins

Caveolins consist of three protein family members termed caveolins 1, 2, and 3. They are found in the membrane structures called caveolae, which are important for protein trafficking and the formation of lipid droplets. Caveolin 1 knockout mice exhibited lower TG accumulation in the liver and showed resistance to diet-induced obesity, showing the importance of this protein in TG synthesis.[60] Some authors

suggest there is an involvement of caveolin 1 in abnormal lipogenesis and mitochondrial function typical of steatotic hepatocytes in NAFLD.[61]

FAT/CD36

It is well-known that FFAs are taken up into cells by passive diffusion and by protein-mediated mechanisms involving a number of FA transporters, of which FAT/CD36 is the best characterized. FAT/CD36 is expressed in a wide variety of cells including macrophages, adipocytes, myocytes, enterocytes, and hepatocytes. This transmembrane protein plays an important role in facilitating the uptake and intracellular trafficking of FFAs, as well as esterification into TGs in heart and skeletal muscle cells; this function is largely dependent on its translocation from intracellular depots to the plasma membrane. Insulin, muscular contractions, and the transcription factor Forkhead box protein O1 (FoxO1) induce FAT/CD36 translocation and enhance FFA uptake.[62]

Hepatic FAT/CD36 expression is normally weak, but its expression is enhanced in rodents with fatty liver.[63] Moreover, some authors have demonstrated that FAT/CD36 mRNA levels increase concomitantly with hepatic TG content in different animal models of liver steatosis.[64,65] Further studies have shown that FAT/CD36 is a common target gene of liver X receptor (LXR), pregnane X receptor, and PPARγ in promoting hepatic steatosis in a murine model.[66] However, little is known about the significance of FAT/CD36 in human liver diseases. In morbidly obese patients with NAFLD, Greco et al showed that hepatic FAT/CD36 mRNA levels were positively related to liver fat content[67] and Bechmann et al found a significant correlation between hepatic FAT/CD36 mRNA and apoptosis in patients with NASH.[68] Other authors have described that hepatic FAT/CD36 upregulation is significantly associated with IR, hyperinsulinemia, and increased steatosis in patients with NASH.[62]

FABPs

The FABPs are a group of molecules that coordinate inflammatory and metabolic responses in cells.[69] These proteins are a family of 14- to 15-kDa proteins that bind with high affinity to hydrophobic ligands such as saturated and unsaturated long-chain FAs (LCFAs).[70] Two isoforms of FABPs, aP2 (FABP4) and mal1 (FABP5) are the isoforms coexpressed in adipocytes and macrophages.[71] The expression of these FABP isoforms is controlled transcriptionally during adipocyte differentiation and is regulated by PPARγ agonists, insulin, and FAs. The functions of cytoplasmic FABPs include enhancement of FFA solubility and transport to specific enzymes and cellular compartments (to the mitochondria and peroxisomes for oxidation; to the endoplasmic reticulum [ER] for reesterification; into lipid droplets for storage; or to the nucleus for gene expression regulation).[71,72] Disruption or pharmacological blockade of FABP4 protects mice from dyslipidemia, atherosclerosis, IR, and fatty liver in the context of either a high-fat diet or genetically induced obesity.[73] The definitive biology and function of FABPs in human physiology and disease are still not fully clarified.[69,73] Few studies have assessed the involvement of hepatic FABP4 expression in NAFLD. Greco et al and Taskinen et al have described FABP4 as being upregulated in subjects with high liver fat content.[67,74] The expression of FABP4 and FABP5 in the liver was correlated with hepatic fatty infiltration in NAFLD patients.[75]

Recent studies have also suggested that hepatic FA uptake via FATPs can be a novel therapeutic strategy for NAFLD. Adenovirus-mediated knockdown of FATP2 or FATP5 reduced hepatic TG accumulation in high-fat fed mice.[76,77] Moreover, both deoxycholic and ursodeoxycholic acid have shown promise as inhibitors of FATP5-mediated FA uptake, suggesting that they may improve hepatic steatosis in high-fat fed mice.[78]

PPARγ

PPARγ is a master transcriptional regulator of adipogenesis and plays an important role in the process of lipid storage.[79] Thus, PPARα and PPARγ have opposing functions in the regulation of fat metabolism; PPARα promotes utilization, while activation of PPARγ promotes storage. Indeed, as increased PPARγ expression has been found in steatotic livers, it has been suggested that the role of PPARγ in the activation of lipogenic genes may contribute to the development of steatosis. Nevertheless, several studies have shown that PPARγ overexpression can prevent the progression of hepatic steatosis in murine models, and treatment with the PPARγ agonist rosiglitazone has been shown to have similar effects. The protective effects of PPARγ could be due to higher insulin sensitivity in adipose tissue and skeletal muscle leading to a reduction in FFA deposition in the liver. Adiponectin has also been shown to be increased by PPARγ, which also contributes to insulin sensitivity as well as upregulating PPARα expression, which leads to further hepatic FA oxidation. Furthermore, PPARγ expression has been shown to have anti-inflammatory and anti-fibrotic effects in stellate cells, macrophages, and epithelial cells. Westerbacka et al have described that PPARγ was overexpressed in the fatty liver of obese human subjects.[75]

Activation of PPARγ in adipose tissue has been proposed to promote the relocalization and storage of fat in adipose tissue, protecting peripheral tissues from lipotoxicity.

Regarding this, the TZDs have proven to be effective drugs for improving insulin sensitivity and treating type 2 diabetes. Moreover, pioglitazone and rosiglitazone are highly effective in improving NAFLD outcomes in patients.[80] Unfortunately, the clinical use of TZDs against NAFLD has been hampered by side effects.

De novo lipogenesis

The process in which the liver synthesizes endogenous FAs is called de novo lipogenesis. This includes de novo synthesis of FAs through a complex cytosolic polymerization in which glucose is converted to acetyl-CoA by glycolysis and the oxidation of pyruvate. Acetyl-CoA carboxylase (ACC1) then converts acetyl-CoA into malonyl-CoA. Finally, FA synthase (FAS) catalyzes the formation of palmitic acid from malonyl-CoA and acetyl-CoA.[81–83] Depending on the metabolic state, FAs are then processed to TGs and stored or rapidly metabolized.

Dietary fats are packed in chylomicrons and hydrolyzed, releasing FAs of which approximately 20% are delivered to the liver.[7] In the fasting state, a decline of insulin levels stimulates adipocyte TG hydrolase, thereby releasing FFAs that are transported to the liver. In the liver, FFAs derived from peripheral tissue, endogenous synthesis, or diet, can be used for: 1) energy and ketone body production via mitochondrial β-oxidation; 2) sterified and stored as TGs in lipid droplets; or 3) packaged with apolipoprotein B into VLDL that is secreted into the circulation.[83,84] In NAFLD patients, enhanced acquisition of FAs through uptake and de novo lipogenesis are not compensated by FA oxidation or production of VLDL particles (Figure 1).

The rate of de novo lipogenesis is regulated primarily at the transcriptional level. Several nuclear transcription factors are involved such as LxRα, sterol regulatory element-binding protein 1c (SREBP1c), carbohydrate-responsive element-binding protein (ChREBP), and farnesoid X receptor (FxR); and enzymes (ACC1, FAS, and steroyl CoA desaturase 1 [SCD1]). Postprandially, plasma glucose and insulin levels rise, which promote activation of lipogenesis through the activation of ChREBP and SREBP1c, respectively.[34,85] In humans, NAFLD has been associated with increased hepatic expression of several genes involved in de novo lipogenesis.[86,87]

LXRs

LXRs are ligand-activated transcription factors that belong to the nuclear receptor (NR) superfamily.[88] There are two LXR isoforms termed α and β. LxRα is mainly expressed in the liver, adipose tissue, and intestine, whereas LxRβ is ubiquitously expressed.[89] In addition to modulating cholesterol metabolism, LXRs have been characterized as major regulators of hepatic FA biosynthesis.[90] A major function of LxRα in the liver is the stimulation of de novo lipogenesis, through the induction of SREBP1c, ACC1, FAS, and SCD1 (Figure 2).[91–93]

Several authors have described an enhanced expression of LxRα and SREBP1c in NAFLD.[87,94,95]

SREBP1c

SREBPs are a family of membrane-bound transcription factors. SREBPs are synthesized as 125 kD precursors embedded in the ER. Proteolytic cleavage then allows the accumulation of active SREBP in the nucleus.

There are different SREBP isoforms: SREBP1c and SREBP2 are expressed in the liver, while SREBP1a is expressed only at very low levels in the liver of adult mice, rats, and humans.[96] SREBP1c, the predominant isoform in the liver, preferentially affects the transcription of genes that regulate de novo lipid synthesis, although SREBP2 regulates

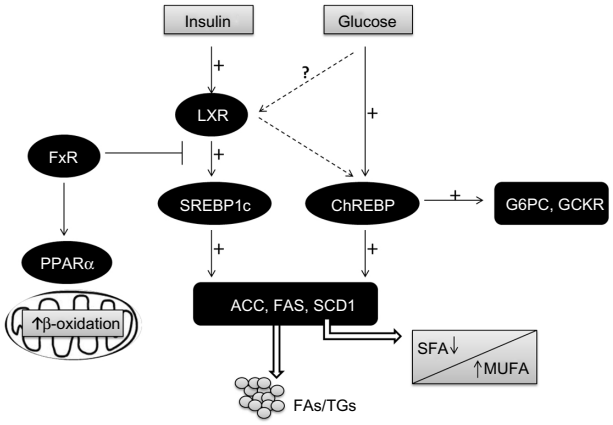

Figure 2 Transcriptional control of lipogenesis and glycolysis.
Notes: The conversion of glucose into FAs through de novo lipogenesis is nutritionally regulated by glucose and insulin signaling pathways, which induce the expression of glycolytic and lipogenic genes synergistically in response to dietary carbohydrates. Insulin activates the transcription factor SREBP1c, which induces lipogenic enzymes (ACC1, FAS, SCD1), while glucose activates the transcription factor ChREBP, which induces both lipogenic (ACCl, FAS, SCD1) and glycolytic (G6PC, GCKR) enzymes. ChREBP is also a direct target of LXRs, and modifies the ratio of MUFA/SFA in favor of MUFA by stimulating SCD1 activity. Recently, glucose was also identified as activating LXR's genes. Hepatic FxR activation inhibits FA/TG synthesis by suppressing SREBP1c and LXRα activation, and inducing the expression of PPARα, which promotes mitochondrial oxidation of FAs.
Abbreviations: ACC, acetyl-CoA carboxylase; ChREBP, carbohydrate-responsive element-binding protein; FA, fatty acid; FAS, fatty acid synthase; FFAs, free fatty acids; FxR, farnesoid X receptor; G6PC, glucose 6-phosphatase; GCKR, glucokinase regulatory protein; LXR, liver X receptor; MUFA, monosaturated fatty acids; PPARα, peroxisomal proliferator-activated receptor alpha; SCD1, steroyl CoA desaturase 1; SFA, saturated fatty acids; SREBP1c, sterol regulatory element-binding protein 1c; TG, triglyceride.

genes involved in cholesterol biosynthesis and metabolism. The SREBP1a isoform, despite its very low levels in the liver, transactivates both lipogenic and cholesterol genes.[97]

To date, the main regulation demonstrated for SREBP1c is at the transcriptional level. SREBP1c transcription is induced by two quite disparate stimuli: insulin, a hormone released in response to carbohydrate intake and leading to a parallel increase in both the membrane-bound precursor and the mature nuclear form, and LxRα, a transcription factor that acts as a cholesterol sensor.[92,93,98–100] In response to feeding, SREBP1c binds to its lipogenic genes, such as ACC1, FAS, and SCD1, and to its own gene, thereby stimulating hepatic lipogenesis (Figure 2).[96,101–105]

Different authors have described an enhanced expression of SREBP1c and LxRα in NAFLD.[87,94,95] However, Nagaya et al demonstrated the downregulation of the hepatic SREBP1c-mediated lipogenic pathway in advanced NASH patients; SREBP1c mRNA levels were inversely correlated with the fibrosis stage.[106] These discrepancies might be explained by differences in the cohort of studied patients. For example, Higuchi et al[94] included normal weight patients with NAFLD and Lima-Cabello et al[95] included patients with NAFLD and with steatosis related to chronic hepatitis C virus infection in mildly overweight men and women. Moreover, Higuchi et al did not evaluate either histological findings nor protein levels or intracellular localization of SREBP1c.[94]

ChREBP

De novo lipogenesis is regulated by glucose and insulin signaling pathways in response to dietary carbohydrate intake to induce glycolytic and lipogenic gene expression. SREBP1c has emerged as a major mediator of insulin action on lipogenic genes. However, SREBP1c activity alone is not sufficient for the stimulation of glycolytic and lipogenic gene expression.[107,108] Over recent years, it has been reported that the liver transcription factor ChREBP is required for the induction of glycolytic gene expression by glucose and that it acts together with SREBP1c to stimulate lipogenic genes.[109–111] Interestingly, ChREBP was also identified as a direct target of LXRs, which are an important regulator of the lipogenic pathway through the transcriptional control of SREBP1c, ACC1, FAS, and SCD1.[112–117] Oxysterols are known as ligands of LXRs, but glucose was also shown to activate LXRs and to induce their target genes, including ChREBP (Figure 2).[107,118]

Postprandial hyperglycemia raises the hepatic concentrations of phosphorylated intermediates, causing activation of ChREBP, which binds to the promoter of its target genes as a heterotetramer with its ubiquitously expressed partner Max-like protein X (Mlx). ChREBP target genes include not only enzymes of glycolysis and lipogenesis that predispose to hepatic steatosis, but also glucose 6-phosphatase (G6PC), which catalyzes the final reaction in glucose production, and glucokinase regulatory protein (GCKR), which inhibits hepatic glucose uptake.[119,120] Transcriptional induction of G6PC and GCKR manifests as hepatic glucose intolerance or IR.[121] Studies using a dominant negative variant of Mlx identified target genes of ChREBP–Mlx that promote hepatic glucose intolerance when overexpressed.[120]

Study results of the role and impact of ChREBP in glucose and lipid metabolism have been confusing. Global ChREBP deficiency in C57BL/6J mice results in IR.[122] On the other hand, ChREBP deficiency[123,124] or expression of a dominant negative Mlx isoform[125] in an obese *ob*/*ob* background decreases hepatic steatosis and other related metabolic alterations, including IR. Benhamed et al[126] hypothesized that these opposite phenotypes in these two murine models may reside in the fact that ChREBP controls both glycolysis and lipogenesis, and that the beneficial effect of ChREBP deficiency may only be apparent in the context of lipid overload. The authors showed that mice overexpressing ChREBP, on a standard diet, remained insulin sensitive, despite increased lipogenesis resulting in hepatic steatosis. However, mice that overexpress ChREBP, on a high-fat diet, showed normal insulin levels and improved insulin signaling and glucose tolerance compared with controls, despite having greater hepatic steatosis. This effect seems to be mediated by the fact that ChREBP modifies the monounsaturated FAs/saturated FAs (MUFA/SFA) balance in favor of MUFA, by stimulating SCD1 activity. Taken together, these results demonstrated that increasing ChREBP, by buffering detrimental FAs and favoring lipid partitioning, can dissociate hepatic steatosis from IR, with beneficial effects on both glucose and lipid metabolism. Interestingly, ChREBP expression in liver biopsies from patients with NASH was higher when steatosis was greater than 50% and lower in the presence of severe IR,[126] supporting this conclusion.

Furthermore, because insulin induces enzymes of lipogenesis by activation of SREBP1c and represses G6PC through other transcriptional regulators, a mechanism of "selective IR" has been proposed to explain the simultaneous elevation of hepatic glucose production (or G6PC expression) and lipogenesis in human type 2 diabetes or models of IR.[127]

FxR

The FxR is a member of the NR superfamily and a receptor for bile acids. FxR activation leads to alterations in pathways involved in energy metabolism. It is mainly expressed in the liver, intestine, kidneys, and the adrenal glands, with less expression in adipose tissue and heart.[128–130] FxR has emerged as a master regulator of lipid and glucose homeostasis in the liver and of inflammatory processes at hepatic and extrahepatic sites. Also, a number of synthetic FxR agonists are being used for the treatment of different hepatic and metabolic disorders, resulting in a lower inflammatory and fibrogenic process.[131,132]

The generation of FxR knockout mice showed a clear role for FxR as the master regulation of bile acid homeostasis.[133,134] However, FxR knockout mice also revealed elevated levels of cholesterol and TGs in both the plasma and liver, suggesting a key role for FxR lipid metabolism as well. In fact, it was demonstrated that FxR needs to be activated in order to reduce the expression of SREBP1c.[135] More recently, in addition to bile acid and lipid metabolism, it has been shown that FxR also plays an important role in glucose metabolism, improving insulin sensitivity and glucose tolerance in a diabetic mice model.[136,137]

Regarding its role in lipid metabolism, the majority of literature seems to point to the fact that FxR activation is beneficial in situations of excess, such as obesity and diabetes. FxR activation seems to reduce TGs levels by: 1) reducing FA synthesis in the liver, through the reduction of SREBP1c and LxR expression;[138] 2) inducing the expression of PPARα, which promotes FFA catabolism via β-oxidation; 3) increasing TG clearance; and 4) increasing adipose tissue storage and altering adipokine patterns (Figure 2).[139,140]

Another hepatic protective mechanism of FxR activation has been shown to be maintenance of gut integrity against gut-derived endotoxins through the induction of antibacterial factors such as angiogenin, inducible NO synthase, and interleukin-18 (IL18).[131,132]

Patients with NAFLD have lower protein and mRNA FxR levels, which has been attributed to higher TG synthesis and induced expression of SREBP1c and LxRα.[138]

ACC1 and FAS

In the process of FA synthesis, ACC1 converts acetyl-CoA, an essential substrate of FAs, to malonyl-CoA. FAS then utilizes both acetyl-CoA and malonyl-CoA to form palmitic acid (C16:0). Both are highly regulated by a transcriptional factor, SREBP1c, and play important roles in the energy metabolism of FAs. They are currently considered an attractive target for regulating the human diseases of obesity, diabetes, cancer, and cardiovascular complications. Dorn et al found that FAS expression was impaired in SS, while the absence of SS in hepatic inflammation did not affect FAS expression.[141] In agreement with Dorn et al, several authors have described an enhanced expression of FAS in NAFLD.[142] These authors have also described that ACC1 mRNA expression was higher in NAFLD. In support of increased FA synthesis in NAFLD, Morgan et al found that ACC1 and FAS mRNA expression were significantly higher in high-fat mice.[143] All these findings suggest that ACC1 and FAS might be a new diagnostic marker or therapeutic target for NAFLD.

FoxO1

FoxO1 is a transcription factor with an important role not only in glycogenolysis and gluconeogenesis, but also in lipid metabolism.

With regard to lipid metabolism, liver-specific transgenic expression of active FoxO1 induces the expression of genes involved in lipid transport and decreases the expression of important genes for glycolysis and lipid/sterol synthesis, resulting in lower postprandial TG concentrations compared to in wild-type mice.[144] However, using a similar murine model, another group observed enhanced lipogenesis and liver steatosis.[145] Similarly, adenoviral delivery of an active FoxO1 variant to the liver results in lipogenesis, hepatic steatosis, and reduced FA oxidation. These increases in lipogenesis result from a feedback loop that enhances insulin signaling, thereby modulating lipid metabolism through SREBP1c in a FoxO1-independent manner.[146]

FoxO1 not only inhibits SREBP1c expression but also suppresses the expression of genes directly involved in FA synthesis, including FAS and adenosine triphosphate (ATP) citrate lyase.[144]

With regard to glucose metabolism, under fasting conditions, the liver provides energy by releasing glucose into the bloodstream. Initially, this results from the breakdown of liver glycogen stores (glycogenolysis), whereas with prolonged fasting, the primary source of glucose is gluconeogenesis. Studies with adenoviral vectors in isolated hepatocytes confirm that FoxO1 stimulates the expression of gluconeogenic genes and suppresses the expression of genes involved in glycolysis, the shunt pathway, and lipogenesis, including glucokinase and SREBP1c. Taken together, these results indicate that FoxO1 proteins promote hepatic glucose production through multiple mechanisms and contribute to the regulation of other important metabolic pathways in adapting to fasting and feeding in the liver, including

glycolysis, the pentose phosphate shunt, and lipogenic and sterol synthetic pathways.[144]

Chronic expression of an active FoxO1 mutant in the liver leads to increased expression of genes involved in gluconeogenesis, resulting in elevated plasma glucose and insulin levels, which are not able to maintain normal glycemia.[144] Valenti et al suggest that increased FoxO1 activity may play a role in the pathogenesis of hepatic IR associated with NAFLD.[147]

Reduction of FoxO1 in both liver and white adipose tissue using an antisense oligonucleotide-mediated approach improves glucose tolerance and both hepatic and peripheral insulin action in mice with diet-induced obesity.[148] Consistent with these studies, FoxO1 haploinsufficiency is able to rescue the loss of insulin sensitivity in insulin receptor–haploinsufficient mice partly by reducing the hepatic expression of gluconeogenic genes.[149]

FA oxidation

Oxidation of FAs occurs within the mitochondria, peroxisomes, and the ER. It facilitates the degradation of activated FAs to acetyl-CoA. FAs are activated by acyl-CoA-synthetase to acyl-CoA in the cytosol, which is indispensable for enabling FAs to cross membranes and enter organelles. Short- and medium-chain FAs pass the mitochondrial membrane without activation. However, activated LCFAs are shuttled across the membrane via carnitine palmitoyltransferase-1 (CPT1). Malonyl-CoA, an early intermediate of de novo lipogenesis, is an inhibitor of CPT1. In the fed state, FA oxidation is inhibited and de novo lipogenesis promoted, allowing for storage and distribution of lipids. In general, short-, medium-, and long-chain FAs are oxidized within mitochondria (β-oxidation), while toxic, very-long-chain FAs are oxidized within peroxisomes. In diabetes or FA overload, cytochrome P450 (CYP4A)-dependent ω-oxidation of LCFAs occurs in the ER and induces ROS and lipid peroxidation. During the process of β-oxidation, electrons are indirectly donated to the electron transport chain to drive ATP synthesis. Acetyl-CoA can be further processed via the tricarboxylic acid cycle, or in the case of FA abundance, be converted into ketone bodies. PPARα and insulin signaling are again involved in the regulation of FA oxidation and the formation of ketone bodies via transcriptional regulation of mitochondrial 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase.[82]

PPARα

In the liver, PPARα plays a pivotal role in FA metabolism by upregulating the expression of numerous genes involved in mitochondrial FA and peroxisome FA oxidation, as well as numerous other aspects of FA metabolism in the cell.[150] As a consequence, activation of PPARα can prevent and decrease hepatic fat storage.[151–154] When PPARα sensing is inefficient, overnight or prolonged fasting leads to severe hepatic steatosis, as seen in PPAR-$\alpha^{-/-}$ mice.[155,156] PPAR$\alpha^{-/-}$ mice fail to upregulate FA oxidation systems in the liver and cannot oxidize the influxed FAs, and thus develop severe hepatic steatosis. PPAR$\alpha^{-/-}$ mice also develop severe steatohepatitis when maintained on a diet deficient in methionine and choline.[153,157,158] Also of importance is that administering PPARα agonists to rats not only prevents the development of methionine- and choline-deficient diet-induced steatohepatitis by preventing intrahepatic lipid and lipoperoxide accumulation, but also reverses hepatic fibrosis by decreasing the expression of fibrotic markers and reducing the number of stellate cells.[153,157–159] The efficacy of these agonists in the treatment of NAFLD in human subjects has not yet been studied in depth. From the available data, no definitive conclusion can be made on the efficacy of PPARα agonists on NAFLD due to study limitations, such as small sample size, incomplete data, and the use of agonists in combination with other strategies.[160]

Besides governing metabolic processes, PPARα also regulates inflammatory processes, mainly by inhibiting inflammatory gene expression. Hepatic PPARα activation has been repeatedly shown to reduce hepatic inflammation elicited by acute exposure to cytokines and other compounds.[161–165]

In conclusion, PPARα activation plays a role in the modulation of hepatic steatosis due to its effects: upregulation of FA oxidation systems and the ensuing burning of energy, reduction in the toxicity of FAs, and its anti-inflammatory effect (Figure 3).[153,155,156,158]

CPT1

CPT1 is a regulatory enzyme in the mitochondria that transfers FAs from the cytosol to the mitochondria prior to β-oxidation (Figure 3). Inhibition of CPT1 has been shown to prevent IR induced by a high-fat diet, partly due to a reduction in some of the deleterious intermediates generated by incomplete FA oxidation and partly to a shift toward increased glucose oxidation for energy production.[166] Kohjima et al showed that CPT1 expression in humans is reduced by 50% in NAFLD compared with that in the normal liver.[142]

Inflammation and NAFLD

It is well-known that the balance between pro- and anti-inflammatory acting cytokines is fundamental in the control of systemic and hepatic insulin action, and as a consequence, in the development of NAFLD. IR is an important feature

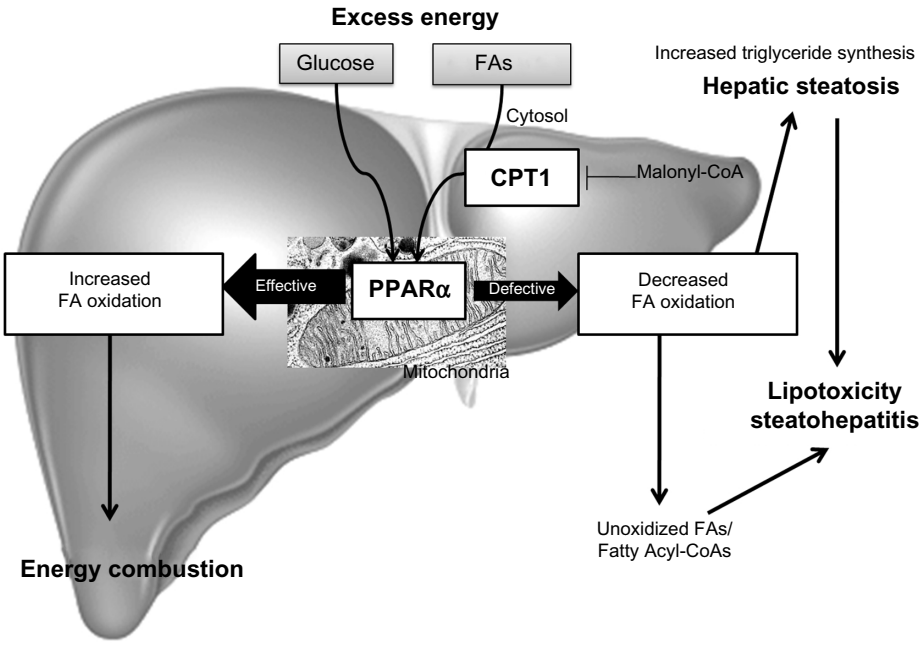

Figure 3 Fatty acid oxidation.
Notes: In the liver, mitochondrial, peroxisomal, and microsomal FA oxidation are regulated by PPARα and metabolize energy. Increased sensing of PPARα results in energy burning and reduced fat storage. Decreased sensing of PPARα leads to a reduction in energy utilization and increased lipogenesis, resulting in steatosis and steatohepatitis.
Abbreviations: CPT1, carnitine palmitoytransferase-1; FA, fatty acid; PPARα, peroxisomal proliferator-activated receptor alpha.

of NAFLD and is caused by a variety of factors, including soluble mediators derived from adipose tissue and/or immune cells: the adipocytokines (Figure 4).[167]

Adiponectin

Adiponectin, one of the major products of adipocytes, is a prototypic anti-inflammatory and anti-diabetic adipocytokine,

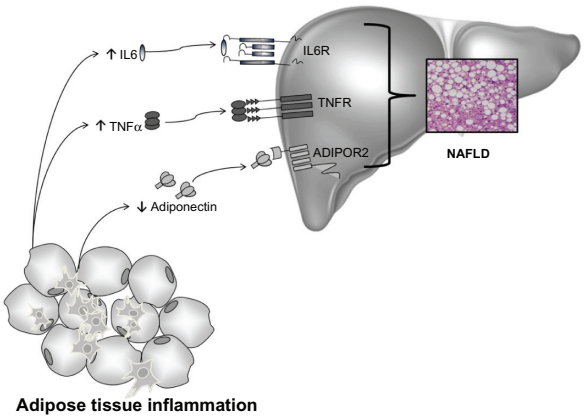

Figure 4 Cytokines and NAFLD.
Notes: The balance/imbalance of pro- and anti-inflammatory cytokines secreted by adipose may profoundly affect the liver. Hepatic adiponectin mRNA expression was lower in individuals with NAFLD. However, TNFα and IL6 mRNA expression were higher in these patients. NALFD is associated with more proinflammatory cytokines and with fewer anti-inflammatory cytokines.
Abbreviations: ADIPOR2, adiponectin receptor type 2; IL6, interleukin-6; NAFLD, non-alcoholic fatty liver disease; TNFα, tumor necrosis factor alpha; TNFR, tumor necrosis factor receptor; IL6R, interleukin-6 receptor.

the actions of which are mainly exerted by the activation of adenosine monophosphate (AMP)-activated kinase and PPARα. Adiponectin has two specific receptors: adiponectin receptor type 1 and 2 (ADIPOR1 and 2). ADIPOR1 is widely expressed, whereas ADIPOR2 can be mainly observed in the liver.[168] Serum levels of adiponectin are lower in individuals with obesity, type 2 diabetes, and in conditions of IR,[169] whereas adiponectin synthesis is induced by weight loss and PPARγ activation by its ligands, TZDs.[28] In general, studies have suggested that adiponectin exerts anti-inflammatory effects, stimulates secretion of anti-inflammatory cytokines such as IL10 or IL1 receptor antagonist (IL1Ra), blocks nuclear factor κB (NF-κB) activation, and inhibits the release of TNFα, IL6, and chemokines.[170,171]

The liver is not a relevant source of circulating adiponectin, but it is a major target organ for many of its effects. In mice with alcoholic and non-alcoholic fatty liver disease, administering recombinant adiponectin ameliorated necroinflammation and steatosis, partly via inhibition of the hepatic production of TNFα and the decrease in plasma concentration of this proinflammatory cytokine.[172]

In humans, adiponectin serum levels were lower in patients with NASH in comparison to matched controls and to patients with SS, independently of IR or the waist–hip ratio. IR and low adiponectin serum levels were associated with increased steatosis and necroinflammation, but not

with severe fibrosis, which was predicted only by IR.[173] Another study has shown that morbidly obese patients with IR undergoing bariatric surgery have lower mRNA adiponectin expression in adipose tissue and lower serum levels of adiponectin than those without IR. This low expression of adiponectin may predispose patients to the progressive form of NAFLD or to NASH.[174]

Low mRNA expression of adiponectin and ADIPOR2 was found in the liver of patients with NASH compared with those with SS. Moreover, ADIPOR2 expression was inversely related to alanine aminotransferase and the fibrosis stage.[175] More recently, Moschen et al demonstrated in a prospective study that rapid weight loss after bariatric surgery results in a significant improvement of both histological and biochemical liver parameters, which is accompanied by an increase of adiponectin serum levels, as well as hepatic mRNA adiponectin expression.[176]

Altogether, there is now strong evidence that circulating adiponectin levels are lower in obesity and related human disorders, including NAFLD.[176,177]

TNFα and IL6

TNFα and IL6 are two important proinflammatory adipocytokines and the expression of both is hugely increased in the fat cells of obese human subjects and patients with IR.[178,179] In patients with severe obesity, the mRNA expression of IL6 and TNFα is clearer in adipose compared to liver tissue.[180]

TNFα was identified more than two decades ago as the first inflammatory molecule linked with IR.[181] Higher serum levels of TNFα and soluble TNFα receptor 2 (TNFR2) have been found in patients with NASH compared with healthy subjects and these differences were independent of higher IR. However, no significant differences in TNFα and TNFR2 were found between SS and NASH patients.[173]

Enhanced TNFα hepatic expression was recently demonstrated in a group of obese patients with NAFLD. Crespo et al reported increased hepatic expression of TNFα and TNFR2 in patients with NASH compared to patients with SS. In these patients, more advanced fibrosis was also accompanied by the increased hepatic expression of TNFα.[182] In line with these results, TNFα plasma levels have been shown to correlate positively with the grade of liver fibrosis assessed by ultrasound-guided liver biopsy in patients with advanced stages of NAFLD.[183]

Furthermore, certain TNFα polygenetic polymorphisms have been found to have higher IR indices, a higher prevalence of impaired glucose tolerance, and higher susceptibility to the development of NAFLD and NASH.[184,185]

IL6 is a pleiotropic cytokine expressed in many inflammatory cells in response to different types of stimuli, regulating a number of biological processes including IR and the regulation of inflammation. It is known to be the main stimulating factor for hepatocyte synthesis and the secretion of C-reactive protein in humans,[186] and for this reason, it has been proposed as a potential mediator leading to NAFLD. However, the true mechanisms driving IL6 induced NAFLD remain unclear.

Preliminary studies have found that IL6 plays a protective role in liver fibrosis by promoting hepatocyte proliferation and by protecting against oxidative stress and mitochondrial dysfunction.[187] On the other hand, Wieckowska et al demonstrated markedly increased IL6 expression in the liver of patients with NASH compared to those with SS or normal liver. Hepatic IL6 expression also correlated positively with the severity of inflammation and fibrosis. IL6 plasma levels that were measured in parallel in this study correlated well with liver IL6 expression.[6] In another study, IL6 was evaluated among several serum markers in NAFLD patients, and IL6 circulating levels were significantly increased in patients with NAFLD as compared to healthy controls, but not in NASH compared to SS.[188]

Weight loss resulted in a dramatic decrease of IL6 subcutaneous and hepatic expression with a subsequent reduction in expression of the hepatic suppressor of cytokine signaling 3 (SOCS3) and improved insulin sensitivity. On the other hand, TNFα expression after weight loss only decreased in adipose tissue, not in hepatic tissue.[180] This would suggest that the liver might be a key organ for adipose tissue-derived IL6 and TNFα because continuous TNFα/IL6 exposure affects hepatic IR.[189]

Visfatin

Visfatin, also termed pre-B cell colony enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMPT) was first identified in 1994 as a protein secreted by activated lymphocytes, synergizing with IL7 and stem cell factor in early B cell formation.[190] Although the first discovery of this molecule suggested primarily a cytokine function, its rediscovery as the key enzyme in generating nicotinamide adenine dinucleotide has considerably widened its biological perspective.[191] Its extracellular functions (cytokine-like) are mainly proinflammatory as it potently induces various other proinflammatory cytokines such TNFα and IL6. Its intracellular functions concentrate on regulating the activity of NAD-consuming enzymes such as various sirtuins, thereby also affecting TNFα biosynthesis, cell lifespan, and longevity.

Only a few reports have so far addressed the role of this adipocytokine in human NAFLD. In patients with NAFLD, visfatin shows higher serum concentrations and weight loss is associated with both a decrease in serum levels and a reduction in liver mRNA expression, suggesting that the fatty liver might indeed contribute to an observable increase in serum visfatin levels.[176] In the same study, immunohistochemistry staining for visfatin was carried out in 18 paired liver biopsies. The staining showed that visfatin was abundantly expressed in hepatocytes; weight loss decreased this expression dramatically. Another report has demonstrated the correlation of visfatin serum levels with liver histology in NAFLD, and such high circulating levels could predict the presence of portal inflammation in NAFLD patients.[192]

A protective role of visfatin against hepatocyte inflammatory damage was suggested by Jarrar et al.[193] In that study, serum visfatin circulating levels in NAFLD patients were higher than in healthy control individuals, both lean and obese without NAFLD. Furthermore, when NASH occurred, visfatin concentration decreased significantly compared with SS, but was still significantly higher than in obese or lean healthy subjects without NAFLD.

Our findings are in line with data reporting that circulating levels and hepatic expression of visfatin are significantly higher in a group of morbidly obese women compared to lean controls and morbidly obese women with normal liver histology. Moreover, serum visfatin correlated well with IL6 and C-reactive protein.[194]

All these findings suggest that visfatin is a molecule with an important role in the pathophysiology of NAFLD and indicate that the liver could be a major source for this cytokine.

PPARδ

The PPARs family consists of three members: namely, PPARα, PPARβ/δ, and PPARγ. These receptors act as FA sensors that control many metabolic programs that are essential for systematic energy homeostasis. Today, due to its ubiquitous profile, much less is known about PPARδ than the other two in relation to human obesity and NAFLD.[195]

Oliver et al showed that IR obese rhesus monkeys normalized fasting glucose and insulin, increased high-density lipoprotein cholesterol and reduced low-density lipoprotein (LDL) cholesterol after treatment with a potent and specific PPARδ agonist, the GW501516.[196] Other studies in an animal model of adenovirus-mediated hepatic PPARδ overexpression showed that PPARδ regulates lipogenesis and glucose utilization for glycogen synthesis. These effects could result in hepatic protection from FFA-mediated damage, possibly due to the generation of protective MUFA and the lowering of lipotoxic SFA levels.[197]

Overweight and obese men subjected to the PPARδ agonists, GW501516 or MBX-8025, exhibited improved insulin sensitivity and decreased fasting plasma TGs, non-esterified FAs, apolipoprotein B-100, and LDL-cholesterol, with diminished liver fat content quantified by magnetic resonance imaging.[198,199]

However, the final mechanisms underlying PPARδ effects in the liver of NAFLD patients still need further study.

NAFLD and lipotoxicity

The pathogenesis of NAFLD is often interpreted by the "double-hit" hypothesis. The primary insult or the "first hit" is lipid accumulation in the liver,[8,200] followed by a "second hit" in which proinflammatory mediators induce inflammation, hepatocellular injury, and fibrosis.[201] This paradigm suggested TG accumulation to be the "first hit" that predisposes to further liver damage in the pathogenesis of NASH, but has recently been replaced by a more complex model as emerging evidence points to FAs and their metabolites as the true lipotoxic agents.[202] Interestingly, lipid accumulation and altered composition of phospholipids within ER membranes further promotes ER stress and IR in obese mice.[203] Cytosolic TGs are therefore now considered to be inert, and in fact, lipid droplet accumulation seems to be hepatoprotective.[204] However, TG accumulation and lipid droplet formation go hand in hand with pathophysiological mechanisms in NASH. FAs, as well as acyl-CoA and acetyl-CoA, have been identified as potential causes of lipotoxicity.[205] FAs have been found to initiate the extrinsic apoptosis cascade and also to interfere with NR signaling, which might influence the extent of hepatocyte damage and further promote IR and ER stress.[206] Accordingly, β-oxidation of LCFA within peroxisomes and ω-oxidation within the ER are upregulated in NASH and contribute to lipotoxicity and ROS formation.[142] This might be secondary to inhibition of mitochondrial β-oxidation due to an accumulation of malonyl-CoA and the inhibition of CPT1. In fact, recent studies indicate that activation of mitochondrial FA oxidation protects from steatosis and IR.[207]

It is known that FAs induce the production of TNFα. Hepatic TNF receptor expression correlates with the severity of NAFLD disease.[182] Also, TNF receptor activation increases expression of SREBP1c, which induces hepatic lipogenesis and lipid accumulation.[208] TNFα activation is further paralleled by death-receptor expression, which facilitates activation of the extrinsic apoptosis cascade. Apoptosis is indeed

the predominant form of hepatocellular injury in NASH. In fact, apoptotic activity within the unhealthy liver correlates with disease severity, and thus, cleaved cytokeratin-18 fragments in the serum of NAFLD could effectively be utilized as surrogate markers for the progression of NAFLD.[209] As previously mentioned, FA accumulation also leads to the induction of ER stress and ROS formation, which again promotes hepatic injury.[210]

On the other hand, other studies indicate that metabolic oxidative stress, autophagy, and inflammation are hallmarks of NASH progression. In this sense, CYP2E1, the principal isoform of the CYP450 enzyme, seems to be critically important in NASH development by promoting oxidative/nitrosative stress, protein modifications, inflammation, and IR.[211,212] Moreover, Das et al suggest that purinergic receptor X7 (P2X7), upregulated by CYP2E1, might have a key role in autophagy induced by metabolic oxidative stress in NASH.[213]

In summary, while hepatic TG accumulation seems to be a benign symptom of hepatic steatosis, FA metabolites contribute to the progression of NAFLD to NASH. IR promotes the recruitment of FFAs from the serum pool as well as intrahepatic FA accumulation, which induces apoptosis and ROS formation. FAs themselves also promote hepatic IR via TNF receptor activation, indicating a vicious cycle of lipid accumulation (Figure 1D). Other mechanisms could also contribute to liver damage. Regarding that, some authors have even suggested a "multiple parallel hits hypothesis" to explain the pathophysiology of NAFLD.[41]

Cannabinoid receptors (CB1, CB2) in NAFLD

The endocannabinoid (EC) system consists of cannabinoid receptors, endogenous cannabinoid ligands, and their biosynthetic and degradative enzymes, and has recently emerged as a ubiquitous system with key functions in a variety of physiological settings. Over the last decade, the EC system has emerged as a pivotal mediator of acute and chronic liver injury. ECs regulate appetite behavior and are lipid mediators that produce similar effects to those of marijuana by acting on membrane-bound receptors.[214] Cannabinoid receptors are localized mainly in the brain, but are also present in minor amounts in the liver and some other peripheral tissues (CB1) and in immune and hematopoietic cells (CB2).[215,216]

ECs may also regulate peripheral energy metabolism, as demonstrated by their CB1-mediated effect on lipoprotein lipase activity in adipocytes[217] and their ability to stimulate lipogenesis in hepatocytes.[218,219] Cannabinoids exert their effects through two different cannabinoid receptors: CB1 and CB2. Under physiological conditions, the EC system is silent, since CB1 and CB2 receptors are faintly expressed. In contrast, induction of CB receptors and/or increased levels of ECs are common features of liver injuries of diverse origins.[220] Both receptors have been implicated in the development of liver fibrosis secondary to various etiologies. CB1-mediated EC tone is enhanced in experimental diet-induced or genetic models of NAFLD, and is characterized by upregulation of adipose tissue and hepatocyte CB1 receptors, and by increased liver synthesis of anandamide. The pathogenic role of CB1 receptors in NAFLD is supported by the resistance to steatosis of obese mice bearing a global or hepatocyte-specific CB1 deletion, or of rodents administered rimonabant or AM6545, a CB1 antagonist.[221–223] Studies with cultured hepatocytes and liver slices further indicate that the steatogenic properties of CB1 arise from altered hepatic lipid metabolism, consisting of a combination of hepatocyte activation of SREBP1c-mediated lipogenesis, reduction of FA oxidation via inhibition of AMP kinase, and decreased release of TG-rich VLDL.[221,222,224] In addition, the adipose tissue may largely contribute to the steatogenic process via CB1-induced release of FFAs by adipocytes (Figure 5).[225]

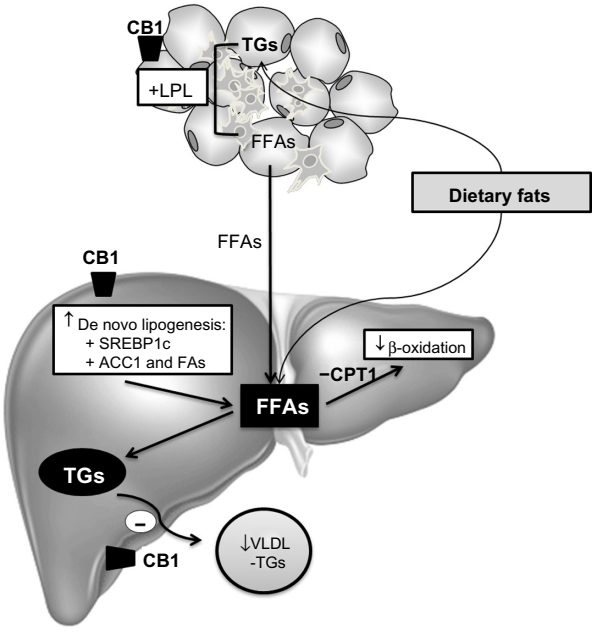

Figure 5 Mechanisms of CB1 involved in hepatic lipid accumulation.
Notes: The activation of CB1 receptors in adipose tissue promotes LPL activity, which results in increased FFA release into the liver. The activation of hepatic CB1 receptors contributes to liver fat accumulation by increased de novo hepatic lipogenesis, decreased FA oxidation, and decreased secretion of TG-rich VLDL.
Abbreviations: ACC1, acetyl-CoA carboxylase; CB, cannabinoid; CPT1, carnitine palmitoyltransferase-1; FA, fatty acid; FAS, fatty acid synthase; FFA, free fatty acid; LPL, lipoprotein lipase; SREBP1c, sterol regulatory element-binding protein 1c; TG, triglyceride; VLDL, very-low-density lipoprotein.

Also, a potential impact of CB1 receptors on the inflammatory response associated with NASH has been suggested by experiments in obese rats showing that rimonabant reduces liver inflammation.[222,223] Although the underlying mechanism remains to be delineated, in hepatocytes, CB1 receptors could contribute to the acute phase response, via activation of cAMP responsive element-binding protein (CREBH), a liver-specific transcription factor that upregulates acute phase response genes.[226] In addition, fat CB1 receptors reduce the production of adiponectin, an adipokine which reduces hepatic inflammation.[222,223]

With regard to liver fibrosis, it has been shown that marijuana use may correlate with the progression of fibrosis in patients with hepatitis C.[227] However, each receptor seems to have opposing roles in the liver. The CB2 receptor has been shown to be upregulated in the livers of cirrhosis patients and to ameliorate the progression of fibrosis.[228] In contrast, CB1 receptor activation has been linked to the progression of fibrosis, and CB1 antagonists have been shown to inhibit the progression of fibrosis.[229] Indeed, clinical trials with a CB1 receptor antagonist have shown that antagonism of CB1 can result in weight loss and improved metabolic and cardiac parameters in overweight and obese populations.[230] In summary, enhanced CB1 tone promotes liver fibrogenesis and cardiovascular alterations associated with cirrhosis, and contributes to the pathogenesis of NAFLD. On the other hand, upregulated CB2 signaling displays hepatoprotective effects, reducing liver inflammation, and improving liver fibrogenesis. Antagonism of CB1 and agonism of CB2 receptors have been identified as promising therapeutic strategies for the management of liver diseases.

Conclusion

NAFLD is characterized by IR, which leads to the deposition of fat, predominantly TGs, in the liver. The steatotic liver exhibits low-grade liver injury. However, a number of patients develop progressive liver injury with hepatocyte apoptosis, greater oxidative stress, and liver inflammation. The factors that lead to the progression of steatosis to steatohepatitis, are likely to be multiple and complex. We proposed a model of hepatocyte injury in fatty liver: in the susceptible steatotic hepatocyte, circulating FFAs can activate ER stress and apoptosis. While hepatic TG accumulation seems to be a benign symptom of hepatic steatosis, FA metabolites might contribute to the progression of NAFLD to NASH. IR promotes the recruitment of FFAs from the serum pool as well as intrahepatic FA accumulation through altered hepatic lipid metabolism, which finally induces apoptosis and ROS formation. Better understanding of the molecular pathways of liver injury should promote the development of diagnostic and therapeutic interventions aimed at reducing the morbidity and mortality associated with NAFLD.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Angulo P. Obesity and nonalcoholic fatty liver disease. *Nutr Rev*. 2007;65(6 Pt 2):57–63.
2. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis*. 2008;28(4):339–350.
3. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology*. 2011;140(1):124–131.
4. Nakamuta M, Kohjima M, Morizono S, et al. Evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. 2005;16(4):631–635.
5. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. *J Hepatol*. 2006;45(4):600–606.
6. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2008;103(6):1372–1379.
7. Ekstedt M, Franzén LE, Mathiesen UL, et al. Long-Term Follow-up of Patients with NAFLD and Elevated Liver Enzymes. *Hepatology*. 2006;44(4):865–873.
8. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology*. 2006;43(2 Suppl 1):S99–S112.
9. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2011;33(5):525–540.
10. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37(4):917–923.
11. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol*. 2009;104(4):861–867.
12. Adams LA, Feldstein AE. Non-invasive diagnosis of nonalcoholic fatty liver and nonalcoholic steatohepatitis. *J Dig Dis*. 2011;12(1):10–16.
13. Yan E, Durazo F, Tong M, Hong K. Nonalcoholic fatty liver disease: pathogenesis, identification, progression, and management. *Nutr Rev*. 2007;65(8 Pt 1):376–384.
14. Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol*. 1999;94(4):1018–1022.
15. Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ*. 2005;172(7):899–905.
16. Festi D, Schiumerini R, Marzi L, et al. Review article: the diagnosis of non-alcoholic fatty liver disease – availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther*. 2013;37(4):392–400.
17. Federico A, Trappoliere M, Loguercio C. Treatment of patients with non-alcoholic fatty liver disease: current views and perspectives. *Dig Liver Dis*. 2006;38(11):789–801.
18. Pacifico L, Celestre M, Anania C, Paolantonio P, Chiesa C, Laghi A. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. *Acta Paediatr*. 2007;96(4):542–547.

19. Lewis JR, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. *Dig Dis Sci*. 2010;55(3):560–578.
20. Yilmaz Y, Ulukaya E, Dolar E. A "biomarker biopsy" for the diagnosis of NASH: promises from CK-18 fragments. *Obes Surg*. 2008;18(11):1507–1508; author reply 1509–1510.
21. Schwenger KJ, Allard JP. Clinical approaches to non-alcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(7):1712–1723.
22. McCarthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic fatty liver disease. *J Acad Nutr Diet*. 2012;112(3):401–409.
23. Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology*. 2010;51(1):121–129.
24. St George A, Bauman A, Johnston A, Farrell G, Chey T, George J. Effect of a lifestyle intervention in patients with abnormal liver enzymes and metabolic risk factors. *J Gastroenterol Hepatol*. 2009;24(3):399–407.
25. Haukeland JW, Konopski Z, Eggesbø HB, et al. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol*. 2009;44(7):853–860.
26. Oh MK, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2008;28(5):503–522.
27. Van Wagner LB, Rinella ME. The role of insulin-sensitizing agents in the treatment of nonalcoholic steatohepatitis. *Therap Adv Gastroenterol*. 2011;4(4):249–263.
28. Lutchman G, Modi A, Kleiner DE, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. *Hepatology*. 2007;46(2):424–429.
29. Aithal GP, Thomas JA, Kaye PV, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology*. 2008;135(4):1176–1184.
30. Maroni L, Guasti L, Castiglioni L, et al. Lipid targets during statin treatment in dyslipidemic patients affected by nonalcoholic fatty liver disease. *Am J Med Sci*. 2011;342(5):383–387.
31. Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol*. 2007;47(1):135–141.
32. Tandra S, Vuppalanchi R. Use of statins in patients with liver disease. *Curr Treat Options Cardiovasc Med*. 2009;11(4):272–278.
33. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology*. 2012;55(6):2005–2023.
34. 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):1–26.
35. Petta S, Muratore C, Craxì A. Non-alcoholic fatty liver disease pathogenesis: the present and the future. *Dig Liver Dis*. 2009;41(9):615–625.
36. Utzschneider KM, Kahn SE. Review: the role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*. 2006;91(12):4753–4761.
37. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860–867.
38. Savage DB, Semple RK. Recent insights into fatty liver, metabolic dyslipidaemia and their links to insulin resistance. *Curr Opin Lipidol*. 2010;21(4):329–336.
39. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest*. 2008;118(3):829–838.
40. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev*. 2002;23(2):201–229.
41. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;52(5):1836–1846.
42. Koliwad SK, Streeper RS, Monetti M, et al. DGAT1-dependent triacylglycerol storage by macrophages protects mice from diet-induced insulin resistance and inflammation. *J Clin Invest*. 2010;120(3):756–767.
43. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology*. 2007;45(6):1366–1374.
44. Amaro A, Fabbrini E, Kars M, et al. Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. *Gastroenterology*. 2010;139(1):149–153.
45. Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology*. 2004;40(1):185–194.
46. Marí M, Caballero F, Colell A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab*. 2006;4(3):185–198.
47. Cheung O, Sanyal AJ. Abnormalities of lipid metabolism in nonalcoholic fatty liver disease. *Semin Liver Dis*. 2008;28(4):351–359.
48. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis*. 2008;28(4):360–369.
49. Arner P. Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab*. 2005;19(4):471–482.
50. Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care*. 2007;10(2):142–148.
51. Berk PD. Regulatable fatty acid transport mechanisms are central to the pathophysiology of obesity, fatty liver, and metabolic syndrome. *Hepatology*. 2008;48(5):1362–1376.
52. Martin G, Nemoto M, Gelman L, et al. The human fatty acid transport protein-1 (SLC27A1; FATP-1) cDNA and gene: organization, chromosomal localization, and expression. *Genomics*. 2000;66(3):296–304.
53. Ge F, Zhou S, Hu C, Lobdell H, Berk PD. Insulin- and leptin-regulated fatty acid uptake plays a key causal role in hepatic steatosis in mice with intact leptin signaling but not in ob/ob or db/db mice. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(4):G855–G866.
54. Zhou SL, Stump D, Sorrentino D, Potter BJ, Berk PD. Adipocyte differentiation of 3T3-L1 cells involves augmented expression of a 43-kDa plasma membrane fatty acid-binding protein. *J Biol Chem*. 1992;267(20):14456–14461.
55. Zhou SL, Stump D, Kiang CL, Isola LM, Berk PD. Mitochondrial aspartate aminotransferase expressed on the surface of 3T3-L1 adipocytes mediates saturable fatty acid uptake. *Proc Soc Exp Biol Med*. 1995;208(3):263–270.
56. Trigatti BL, Anderson RG, Gerber GE. Identification of caveolin-1 as a fatty acid binding protein. *Biochem Biophys Res Commun*. 1999;255(1):34–39.
57. Doege H, Stahl A. Protein-mediated fatty acid uptake: novel insights from in vivo models. *Physiology (Bethesda)*. 2006;21:259–268.
58. Doege H, Baillie RA, Ortegon AM, et al. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. *Gastroenterology*. 2006;130(4):1245–1258.
59. Auinger A, Valenti L, Pfeuffer M, et al. A promoter polymorphism in the liver-specific fatty acid transport protein 5 is associated with features of the metabolic syndrome and steatosis. *Horm Metab Res*. 2010;42(12):854–849.
60. Fernández MA, Albor C, Ingelmo-Torres M, et al. Caveolin-1 is essential for liver regeneration. *Science*. 2006;313(5793):1628–1632.
61. Mastrodonato M, Calamita G, Rossi R, et al. Altered distribution of caveolin-1 in early liver steatosis. *Eur J Clin Invest*. 2011;41(6):642–651.
62. Miquilena-Colina ME, Lima-Cabello E, Sánchez-Campos S, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut*. 2011;60(10):1394–1402.
63. Inoue M, Ohtake T, Motomura W, et al. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem Biophys Res Commun*. 2005;336(1):215–222.

64. Buqué X, Martínez MJ, Cano A, et al. A subset of dysregulated metabolic and survival genes is associated with severity of hepatic steatosis in obese Zucker rats. *J Lipid Res*. 2010;51(3):500–513.
65. Degrace P, Moindrot B, Mohamed I, et al. Upregulation of liver VLDL receptor and FAT/CD36 expression in LDLR−/− apoB100/100 mice fed trans-10, cis-12 conjugated linoleic acid. *J Lipid Res*. 2006;47(12):2647–2655.
66. Zhou J, Febbraio M, Wada T, et al. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology*. 2008;134(2):556–567.
67. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(5): G1281–G1287.
68. Bechmann LP, Gieseler RK, Sowa J-P, et al. Apoptosis is associated with CD36/fatty acid translocase upregulation in non-alcoholic steatohepatitis. *Liver Int*. 2010;30(6):850–859.
69. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov*. 2008;7(6):489–503.
70. Karakas SE, Almario RU, Kim K. Serum fatty acid binding protein 4, free fatty acids, and metabolic risk markers. *Metabolism*. 2009;58(7): 1002–1007.
71. Zimmerman AW, Veerkamp JH. New insights into the structure and function of fatty acid-binding proteins. *Cell Mol Life Sci*. 2002;59(7): 1096–1116.
72. Chmurzyńska A. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *J Appl Genet*. 2006;47(1):39–48.
73. Krusinová E, Pelikánová T. Fatty acid binding proteins in adipose tissue: a promising link between metabolic syndrome and atherosclerosis? *Diabetes Res Clin Pract*. 2008;82 Suppl 2:S127–S134.
74. Taskinen MR, Adiels M, Westerbacka J, et al. Dual metabolic defects are required to produce hypertriglyceridemia in obese subjects. *Arterioscler Thromb Vasc Biol*. 2011;31(9):2144–2150.
75. Westerbacka J, Kolak M, Kiviluoto T, et al. 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(11):2759–2765.
76. Falcon A, Doege H, Fluitt A, et al. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *Am J Physiol Endocrinol Metab*. 2010;299(3):E384–E393.
77. Doege H, Grimm D, Falcon A, et al. Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J Biol Chem*. 2008;283(32): 22186–22192.
78. Nie B, Park HM, Kazantzis M, et al. Specific bile acids inhibit hepatic fatty acid uptake in mice. *Hepatology*. 2012;56(4):1300–1310.
79. Okamura M, Inagaki T, Tanaka T, Sakai J. Role of histone methylation and demethylation in adipogenesis and obesity. *Organogenesis*. 2010;6(1):24–32.
80. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. 2010;362(18): 1675–1685.
81. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology*. 2010;51(2):679–689.
82. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J Hepatol*. 2012;56(4):952–964.
83. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol*. 2013;48(4): 434–441.
84. Fuchs M. Non-alcoholic fatty liver disease: the bile acid-activated farnesoid x receptor as an emerging treatment target. *J Lipids*. 2012;2012:934396.
85. Ferré P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab*. 2010; 12 Suppl 2:83–92.
86. Mitsuyoshi H, Yasui K, Harano Y, et al. Analysis of hepatic genes involved in the metabolism of fatty acids and iron in nonalcoholic fatty liver disease. *Hepatol Res*. 2009;39(4):366–373.
87. Kohjima M, Higuchi N, Kato M, et al. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int J Mol Med*. 2008;21(4):507–511.
88. Baranowski M. Biological role of liver X receptors. *J Physiol Pharmacol*. 2008;59 Suppl 7:31–55.
89. Faulds MH, Zhao C, Dahlman-Wright K. Molecular biology and functional genomics of liver X receptors (LXR) in relationship to metabolic diseases. *Curr Opin Pharmacol*. 2010;10(6):692–697.
90. Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem*. 2002;277(11):9520–9528.
91. Peet DJ, Turley SD, Ma W, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell*. 1998;93(5):693–704.
92. Repa JJ, Liang G, Ou J, et al. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev*. 2000;14(22):2819–2830.
93. Schultz JR, Tu H, Luk A, et al. Role of LXRs in control of lipogenesis. *Genes Dev*. 2000;14(22):2831–2838.
94. Higuchi N, Kato M, Shundo Y, et al. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol Res*. 2008;38(11):1122–1129.
95. Lima-Cabello E, Garcia-Mediavilla MV, Miquilena-Colina ME, et al. Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. *Clin Sci (Lond)*. 2011;120(6):239–250.
96. Horton JD, Goldstein JL, Brown MS. Critical review SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest*. 2002;109(9):1125–1131.
97. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest*. 1997;99(5):838–845.
98. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc Natl Acad Sci U S A*. 1999;96(24):13656–13661.
99. Foretz M, Pacot C, Dugail I, et al. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol*. 1999;19(5):3760–3768.
100. Azzout-Marniche D, Bécard D, Guichard C, et al. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. *Biochem J*. 2000;350 Pt 2: 389–393.
101. Osborne TF. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem*. 2000;275(42):32379–32382.
102. Chakravarty K, Leahy P, Becard D, et al. Sterol regulatory element-binding protein-1c mimics the negative effect of insulin on phosphoenolpyruvate carboxykinase (GTP) gene transcription. *J Biol Chem*. 2001;276(37):34816–34823.
103. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res*. 2001;40(6):439–452.
104. Horton JD, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci U S A*. 1998;95(11):5987–5992.
105. Kim JB, Sarraf P, Wright M, et al. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest*. 1998;101(1):1–9.
106. Nagaya T, Tanaka N, Suzuki T, et al. Down-regulation of SREBP-1c is associated with the development of burned-out NASH. *J Hepatol*. 2010;53(4):724–731.

107. Denechaud PD, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. *FEBS Lett*. 2008;582(1):68–73.
108. Foufelle F, Ferré P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem J*. 2002;366(Pt 2):377–391.
109. Dentin R, Pégorier JP, Benhamed F, et al. Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. *J Biol Chem*. 2004;279(19):20314–20326.
110. Ishii S, Iizuka K, Miller BC, Uyeda K. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc Natl Acad Sci U S A*. 2004;101(44):15597–15602.
111. Ma L, Tsatsos NG, Towle HC. Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. *J Biol Chem*. 2005;280(12):12019–12027.
112. Cha JY, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem*. 2007;282(1):743–751.
113. Ulven SM, Dalen KT, Gustafsson JA, Nebb HI. LXR is crucial in lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2005;73(1):59–63.
114. Chen G, Liang G, Ou J, Goldstein JL, Brown MS. Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. *Proc Natl Acad Sci U S A*. 2004;101(31):11245–11250.
115. Joseph SB, Laffitte B, Patel PH, et al. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biol Chem*. 2002;277(13):11019–11025.
116. Zhang Y, Yin L, Hillgartner FB. SREBP-1 integrates the actions of thyroid hormone, insulin, cAMP, and medium-chain fatty acids on ACCalpha transcription in hepatocytes. *J Lipid Res*. 2003;44(2):356–368.
117. Chu K, Miyazaki M, Man WC, Ntambi JM. Stearoyl-coenzyme A desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. *Mol Cell Biol*. 2006;26(18):6786–6798.
118. Mitro N, Mak PA, Vargas L, et al. The nuclear receptor LXR is a glucose sensor. *Nature*. 2007;445(7124):219–223.
119. Arden C, Petrie JL, Tudhope SJ, et al. Elevated glucose represses liver glucokinase and induces its regulatory protein to safeguard hepatic phosphate homeostasis. *Diabetes*. 2011;60(12):3110–3120.
120. Ma L, Robinson LN, Towle HC. ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J Biol Chem*. 2006;281(39):28721–28730.
121. de la Iglesia N, Mukhtar M, Seoane J, Guinovart JJ, Agius L. The role of the regulatory protein of glucokinase in the glucose sensory mechanism of the hepatocyte. *J Biol Chem*. 2000;275(14):10597–10603.
122. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A*. 2004;101(19):7281–7286.
123. Iizuka K, Miller B, Uyeda K. Deficiency of carbohydrate-activated transcription factor ChREBP prevents obesity and improves plasma glucose control in leptin-deficient (ob/ob) mice. *Am J Physiol Endocrinol Metab*. 2006;291(2):E358–E364.
124. Dentin R, Benhamed F, Hainault I, et al. Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes*. 2006;55(8):2159–2170.
125. Iizuka K, Takeda J, Horikawa Y. Hepatic overexpression of dominant negative Mlx improves metabolic profile in diabetes-prone C57BL/6J mice. *Biochem Biophys Res Commun*. 2009;379(2):499–504.
126. Benhamed F, Denechaud P, Lemoine M, et al. The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. *J Clin Invest*. 2012;122(6):2176–2194.
127. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*. 2008;7(2):95–96.
128. Forman BM, Goode E, Chen J, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell*. 1995;81(5):687–693.
129. Lu TT, Makishima M, Repa JJ, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell*. 2000;6(3):507–515.
130. Zhang Y, Kast-Woelbern HR, Edwards PA. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. *J Biol Chem*. 2003;278(1):104–110.
131. Downes M, Verdecia MA, Roecker AJ, et al. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol Cell*. 2003;11(4):1079–1092.
132. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov*. 2008;7(8):678–693.
133. Kok T, Hulzebos CV, Wolters H, et al. Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *J Biol Chem*. 2003;278(43):41930–41937.
134. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell*. 2000;102(6):731–744.
135. Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest*. 2004;113(10):1408–1418.
136. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. *J Clin Invest*. 2006;116(4):1102–1109.
137. Zhang Y, Lee FY, Barrera G, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A*. 2006;103(4):1006–1011.
138. Yang ZX, Shen W, Sun H. Effects of nuclear receptor FXR on the regulation of liver lipid metabolism in patients with non-alcoholic fatty liver disease. *Hepatol Int*. 2010;4(4):741–748.
139. Teodoro JS, Rolo AP, Palmeira CM. Hepatic FXR: key regulator of whole-body energy metabolism. *Trends Endocrinol Metab*. 2011;22(11):458–466.
140. Modica S, Gadaleta RM, Moschetta A. Deciphering the nuclear bile acid receptor FXR paradigm. *Nucl Recept Signal*. 2010;8:e005.
141. Dorn C, Riener MO, Kirovski G, et al. Expression of fatty acid synthase in nonalcoholic fatty liver disease. *Int J Clin Exp Pathol*. 2010;3(5):505–514.
142. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. 2007;20(3):351–358.
143. Morgan K, Uyuni A, Nandgiri G, et al. Altered expression of transcription factors and genes regulating lipogenesis in liver and adipose tissue of mice with high fat diet-induced obesity and nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2008;20(9):843–854.
144. Zhang W, Patil S, Chauhan B, et al. FoxO1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. *J Biol Chem*. 2006;281(15):10105–10117.
145. Qu S, Altomonte J, Perdomo G, et al. Aberrant Forkhead box O1 function is associated with impaired hepatic metabolism. *Endocrinology*. 2006;147(12):5641–5652.
146. Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest*. 2006;116(9):2464–2472.
147. Valenti L, Rametta R, Dongiovanni P, et al. Increased expression and activity of the transcription factor FOXO1 in nonalcoholic steatohepatitis. *Diabetes*. 2008;57(5):1355–1362.
148. Samuel VT, Choi CS, Phillips TG, et al. Targeting foxo1 in mice using antisense oligonucleotide improves hepatic and peripheral insulin action. *Diabetes*. 2006;55(7):2042–2050.

149. Nakae J, Biggs WH, Kitamura T, et al. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet*. 2002;32(2):245–253.
150. Mandard S, Müller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci*. 2004;61(4):393–416.
151. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest*. 1999;103(11):1489–1498.
152. Harano Y, Yasui K, Toyama T, et al. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. *Liver Int*. 2006;26(5):613–620.
153. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology*. 2003;38(1):123–132.
154. Chou CJ, Haluzik M, Gregory C, et al. WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *J Biol Chem*. 2002;277(27):24484–24489.
155. 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(37):28918–28928.
156. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr*. 2001;21:193–230.
157. Kashireddy PV, Rao MS. Lack of peroxisome proliferator-activated receptor alpha in mice enhances methionine and choline deficient diet-induced steatohepatitis. *Hepatol Res*. 2004;30(2):104–110.
158. Rao MS, Papreddy K, Musunuri S, Okonkwo A. Prevention/reversal of choline deficiency-induced steatohepatitis by a peroxisome proliferator-activated receptor alpha ligand in rats. *In Vivo*. 2002;16(2):145–152.
159. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology*. 2004;39(5):1286–1296.
160. Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. *Triglyceride Metab Dis*. 2012;1821(5):809–818.
161. Vanden Berghe W, Vermeulen L, Delerive P, De Bosscher K, Staels B, Haegeman G. A paradigm for gene regulation: inflammation, NF-kappaB and PPAR. *Adv Exp Med Biol*. 2003;544:181–196.
162. Delerive P, De Bosscher K, Besnard S, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappa B and AP-1. *J Biol Chem*. 1999;274(45):32048–32054.
163. Delerive P, Gervois P, Fruchart JC, Staels B. Induction of IkappaBalpha expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators. *J Biol Chem*. 2000;275(47):36703–36707.
164. Gervois P, Vu-Dac N, Kleemann R, et al. Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor alpha agonists via inhibition of CCAAT box/enhancer-binding protein beta. *J Biol Chem*. 2001;276(36):33471–33477.
165. Gervois P, Kleemann R, Pilon A, et al. Global suppression of IL-6-induced acute phase response gene expression after chronic in vivo treatment with the peroxisome proliferator-activated receptor-alpha activator fenofibrate. *J Biol Chem*. 2004;279(16):16154–16160.
166. Huang YY, Gusdon AM, Qu S. Nonalcoholic fatty liver disease: molecular pathways and therapeutic strategies. *Lipids Health Dis*. 2013;12:171.
167. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med*. 2008;14(3–4):222–231.
168. Tilg H. Adipocytokines in nonalcoholic fatty liver disease: key players regulating steatosis, inflammation and fibrosis. *Curr Pharm Des*. 2010;16(17):1893–1895.
169. Maeda N, Takahashi M, Funahashi T, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*. 2001;50(9):2094–2099.
170. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun*. 2004;323(2):630–635.
171. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol*. 2006;6(10):772–783.
172. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest*. 2003;112(1):91–100.
173. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology*. 2004;40(1):46–54.
174. Baranova A, Gowder SJ, Schlauch K, et al. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. *Obes Surg*. 2006;16(9):1118–1125.
175. Kaser S, Moschen A, Cayon A, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut*. 2005;54(1):117–121.
176. Moschen AR, Molnar C, Wolf AM, et al. Effects of weight loss induced by bariatric surgery on hepatic adipocytokine expression. *J Hepatol*. 2009;51(4):765–777.
177. Marra F, Bertolani C. Adipokines in liver diseases. *Hepatology*. 2009;50(3):957–969.
178. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259(5091):87–91.
179. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest*. 1995;95(5):2111–2119.
180. Moschen AR, Molnar C, Geiger S, et al. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor alpha expression. *Gut*. 2010;59(9):1259–1264.
181. Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology*. 1992;130(1):43–52.
182. Crespo J, Cayón A, Fernández-Gil P, et al. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology*. 2001;34(6):1158–1163.
183. Lesmana CRA, Hasan I, Budihusodo U, et al. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. *J Dig Dis*. 2009;10(3):201–206.
184. Valenti L, Fracanzani AL, Dongiovanni P, et al. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. *Gastroenterology*. 2002;122(2):274–280.
185. Zhou YJ, Li YY, Nie YQ, et al. Influence of polygenetic polymorphisms on the susceptibility to non-alcoholic fatty liver disease of Chinese people. *J Gastroenterol Hepatol*. 2010;25(4):772–777.
186. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340(6):448–454.
187. Cressman DE, Greenbaum LE, DeAngelis RA, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science*. 1996;274(5291):1379–1383.
188. Haukeland JW, Damås JK, Konopski Z, et al. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J Hepatol*. 2006;44(6):1167–1174.
189. Senn JJ, Klover PJ, Nowak IA, et al. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem*. 2003;278(16):13740–13746.
190. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol*. 1994;14(2):1431–1437.

191. Moschen AR, Gerner RR, Tilg H. Pre-B Cell colony enhancing factor/NAMPT/visfatin in inflammation and obesity-related disorders. *Curr Pharm Des*. 2010;16(17):1913–1920.
192. Aller R, de Luis DA, Izaola O, et al. Influence of visfatin on histopathological changes of non-alcoholic fatty liver disease. *Dig Dis Sci*. 2009;54(8):1772–1777.
193. Jarrar MH, Baranova A, Collantes R, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2008;27(5):412–421.
194. Auguet T, Terra X, Porras JA, et al. Plasma visfatin levels and gene expression in morbidly obese women with associated fatty liver disease. *Clin Biochem*. 2013;46(3):202–208.
195. Videla LA, Pettinelli P. Misregulation of PPAR functioning and its pathogenic consequences associated with nonalcoholic fatty liver disease in human obesity. *PPAR Res*. 2012;2012:107434.
196. Oliver WR Jr, Shenk JL, Snaith MR, et al. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci U S A*. 2001;98(9):5306–5311.
197. Liu S, Hatano B, Zhao M, et al. Role of peroxisome proliferator-activated receptor {delta}/{beta} in hepatic metabolic regulation. *J Biol Chem*. 2011;286(2):1237–1247.
198. Risérus U, Sprecher D, Johnson T, et al. Activation of peroxisome proliferator-activated receptor (PPAR)delta Promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes*. 2008;57(2):332–339.
199. Ooi EMM, Watts GF, Sprecher DL, Chan DC, Barrett PHR. Mechanism of action of a peroxisome proliferator-activated receptor (PPAR)-delta agonist on lipoprotein metabolism in dyslipidemic subjects with central obesity. *J Clin Endocrinol Metab*. 2011;96(10):E1568–E1576.
200. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J Clin Endocrinol Metab*. 2006;91(4):1578–1581.
201. Tacke F, Luedde T, Trautwein C. Inflammatory pathways in liver homeostasis and liver injury. *Clin Rev Allergy Immunol*. 2009;36(1):4–12.
202. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology*. 2010;52(2):774–788.
203. Fu S, Yang L, Li P, et al. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature*. 2011;473(7348):528–531.
204. Ricchi M, Odoardi MR, Carulli L, et al. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. *J Gastroenterol Hepatol*. 2009;24(5):830–840.
205. Han MS, Park SY, Shinzawa K, et al. Lysophosphatidylcholine as a death effector in the lipoapoptosis of hepatocytes. *J Lipid Res*. 2008;49(1):84–97.
206. Nolan CJ, Larter CZ. Lipotoxicity: why do saturated fatty acids cause and monounsaturates protect against it? *J Gastroenterol Hepatol*. 2009;24(5):703–706.
207. Orellana-Gavalda JM, Herrero L, Malandrino MI, et al. Molecular therapy for obesity and diabetes based on a long-term increase in hepatic fatty-acid oxidation. *Hepatology*. 2011;53(3):821–832.
208. Endo M, Masaki T, Seike M, Yoshimatsu H. TNF-alpha induces hepatic steatosis in mice by enhancing gene expression of sterol regulatory element binding protein-1c (SREBP-1c). *Exp Biol Med (Maywood)*. 2007;232(5):614–621.
209. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology*. 2009;50(4):1072–1078.
210. Feldstein AE. Novel insights into the pathophysiology of nonalcoholic fatty liver disease. *Semin Liver Dis*. 2010;30(4):391–401.
211. Abdelmegeed MA, Banerjee A, Yoo SH, et al. Critical role of cytochrome P450 2E1 (CYP2E1) in the development of high fat-induced non-alcoholic steatohepatitis. *J Hepatol*. 2012;57(4):860–866.
212. Seth RK, Das S, Kumar A, et al. CYP2E1-dependent and leptin-mediated hepatic CD57 expression on CD8+ T cells aid progression of environment-linked nonalcoholic steatohepatitis. *Toxicol Appl Pharmacol*. 2014;274(1):42–54.
213. Das S, Seth RK, Kumar A, et al. Purinergic receptor X7 is a key modulator of metabolic oxidative stress-mediated autophagy and inflammation in experimental nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol*. 2013;305(12):G950–G963.
214. Di Marzo V, Piscitelli F, Mechoulam R. Cannabinoids and endocannabinoids in metabolic disorders with focus on diabetes. *Handb Exp Pharmacol*. 2011;(203):75–104.
215. Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res*. 2011;50(2):193–211.
216. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev*. 2006;58(3):389–462.
217. Cota D, Marsicano G, Tschap M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest*. 2003;112(3):423–431.
218. Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest*. 2005;115(5):1298–1305.
219. Jeong W, Osei-Hyiaman D, Park O, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metab*. 2008;7(3):227–235.
220. Mallat A, Teixeira-Clerc F, Deveaux V, Manin S, Lotersztajn S. The endocannabinoid system as a key mediator during liver diseases: new insights and therapeutic openings. *Br J Pharmacol*. 2011;163(7):1432–1440.
221. Osei-Hyiaman D, Liu J, Zhou L, et al. Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest*. 2008;118(9):3160–3169.
222. Tam J, Vemuri VK, Liu J, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest*. 2010;120(8):2953–2966.
223. Gary-Bobo M, Elachouri G, Gallas JF, et al. Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats. *Hepatology*. 2007;46(1):122–129.
224. Jourdan T, Demizieux L, Gresti J, et al. Antagonism of peripheral hepatic cannabinoid receptor-1 improves liver lipid metabolism in mice: evidence from cultured explants. *Hepatology*. 2012;55(3):790–799.
225. Jourdan T, Djaouti L, Demizieux L, Gresti J, Vergès B, Degrace P. CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes*. 2010;59(4):926–934.
226. Chanda D, Kim DK, Li T, et al. Cannabinoid receptor type 1 (CB1R) signaling regulates hepatic gluconeogenesis via induction of endoplasmic reticulum-bound transcription factor cAMP-responsive element-binding protein H (CREBH) in primary hepatocytes. *J Biol Chem*. 2011;286(32):27971–27979.
227. Hézode C, Roudot-Thoraval F, Nguyen S, et al. Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. *Hepatology*. 2005;42(1):63–71.
228. Muñoz-Luque J, Ros J, Fernández-Varo G, et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther*. 2008;324(2):475–483.
229. Teixeira-Clerc F, Julien B, Grenard P, et al. CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nat Med*. 2006;12(6):671–676.
230. Van Gaal L, Pi-Sunyer X, Després JP, McCarthy C, Scheen A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. *Diabetes Care*. 2008;31 Suppl 2:S229–S240.

Clin Invest Arterioscl. 2015;xxx(xx):xxx-xxx

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REVISIÓN

Papel de las lipasas metabólicas y la lipotoxicidad en el desarrollo de esteatosis hepática y esteatohepatitis no alcohólica

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Recibido el 4 de febrero de 2015; aceptado el 4 de marzo de 2015

PALABRAS CLAVE
Lipasas metabólicas;
Lipotoxicidad;
Enfermedad del hígado graso no alcohólica

Resumen La enfermedad del hígado graso no alcohólico (EHGNA) se ha convertido en el trastorno hepático más común en los países desarrollados, que abarca condiciones patológicas que van desde la esteatosis simple a la esteatohepatitis no alcohólica, cirrosis y hepatocarcinoma. A menudo la patogenia de la EHGNA ha sido interpretada por la hipótesis del «doble impacto», donde tras la acumulación de lípidos hepáticos tendría lugar la aparición de mediadores proinflamatorios que inducirían inflamación, lesión hepatocelular y fibrosis. Actualmente, el modelo propuesto sugiere que la constante exposición de los hepatocitos a ácidos grasos libres

Abreviaturas: ATP: adenosín trifosfato; AG: ácidos grasos; AGL: ácidos grasos libres; AGPAT: acil-glicerol-fosfato-acil transferasa; APOC3: apolipoproteína C3; ATGL: lipasa de triglicéridos del tejido adiposo; ChREBP: proteína de unión al elemento de respuesta a carbohidratos; CB1/2: receptor de canabinoides 1/2; CD36: clúster de diferenciación 36; DAG: diacilgliceroles; DGAT: diacilglicerol aciltransferasa; DGAT2: diacilglicerol aciltransferasa 2; DM2: diabetes mellitus tipo 2; EHGNA: enfermedad del hígado graso no alcohólica; EHNA: esteatohepatitis no alcohólica; FAS: sintasa de ácidos grasos; FABP: proteína de unión de ácidos grasos; FADH2: flavín adenín dinucleótido; FATP: proteína transportadora de ácidos grasos; G6Pasa: glucosa-6 fosfatasa; GPAT: glicerol-fosfato-acil-transferasa; GS: glucógeno sintasa; GSK3: quinasa glucógeno sintasa 3; GLUT: transportador de glucosa; IL-6: interleucina 6; NADH: nicotinamida adenina dinucleótido; OAS: oligonucleótidos antisentido; LpL: lipoproteína lipasa; LxRα, receptor X hepático; PAP: fosfatasa del ácido fosfatídico; PDK1: proteína quinasa D; PEPCK: fosfoenolpiruvato carboxiquinasa; PIP2: fosfatidilinositol 4,5-bifosfato; PIP3: fosfatidilinositol 3,4,5-trifosfato; PK: proteín cinasa; PKCε: proteína cinasa Cε; PI(3)K: fosfatidilinositol 3; PNPLA3: Patatin-like phospholipase domain-containing protein 3; PPARγ: receptor gamma activado por el factor proliferador de perixosomas; RI: resistencia a la insulina; ROS: especies reactivas de oxígeno; RTKI: receptor tirosina cinasa de la insulina; SREBP1c: proteína de unión al elemento regulador de esterol; SM: síndrome metabólico; SRI/II: sustrato del receptor de la insulina I/II; TAB: tejido adiposo blanco; TG: triglicéridos; TNF-α: factor de necrosis tumoral alfa.

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http://dx.doi.org/10.1016/j.arteri.2015.03.003

Cómo citar este artículo: Berlanga A, et al. Papel de las lipasas metabólicas y la lipotoxicidad en el desarrollo de esteatosis hepática y esteatohepatitis no alcohólica. Clin Invest Arterioscl. 2015. http://dx.doi.org/10.1016/j.arteri.2015.03.003

y sus metabolitos, agentes potencialmente lipotóxicos, estarían contribuyendo al desarrollo de EHGNA y resistencia hepática a la insulina; sugiriendo así un papel primordial para las lipasas metabólicas intracelulares en este proceso.

KEYWORDS
Metabolic lipases;
Lipotoxicity,
Non-alcoholic fatty liver disease

Role of metabolic lipases and lipotoxicity in the development of non-alcoholic steatosis and non-alcoholic steatohepatitis

Abstract Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in developed countries, covering a spectrum of pathological conditions ranging from single steatosis to non-alcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma. Its pathogenesis has been often interpreted by the ''double-hit'' hypothesis, where the lipid accumulation in the liver is followed by proinflammatory mediators inducing inflammation, hepatocellular injury and fibrosis. Nowadays, a more complex model suggests that free fatty acids and their metabolites could be the true lipotoxic agents that contribute to the development of NAFLD and hepatic insulin resistance, suggesting a central role for metabolic lipases in that process.

Introducción

La enfermedad del hígado graso no alcohólica (EHGNA) se ha convertido en el trastorno hepático más común en los países desarrollados, afectando aproximadamente a un 30% de adultos y a un 10% de niños[1,2]. Esta enfermedad abarca un espectro anatomopatológico de condiciones que van desde la simple acumulación de triglicéridos (TG) hepáticos (esteatosis simple) a la esteatosis con inflamación (esteatohepatitis), cirrosis e incluso hepatocarcinoma[2,3]. Aproximadamente el 20% de los pacientes con esteatohepatitis progresan hacia cirrosis e insuficiencia hepática[4,5]. De hecho, la esteatohepatitis asociada a cirrosis es actualmente la tercera causa más frecuente de trasplante hepático en los Estados Unidos[6].

La EGHNA es considerada como la manifestación hepática del síndrome metabólico (SM) y se encuentra fuertemente asociada a la diabetes mellitus tipo 2 (DM2) y la obesidad. Tanto la obesidad como la DM2 son consecuencias del estilo de vida moderno, que se caracteriza por el aumento de la ingesta de ácidos grasos (AG) saturados, trans-insaturados y fructosa en la dieta, así como el sedentarismo[7].

Aunque los mecanismos implicados en la patogénesis y progresión de la EHGNA no son del todo conocidos[3], la resistencia a la insulina (RI) en el músculo, tejido adiposo y en el hígado parece desempeñar un papel central[8,9]. El deterioro en la señalización de la insulina en el tejido adiposo provoca un aumento de la lipólisis, generando así un flujo de AG hacia el hígado que promueven la RI hepática, con el consiguiente aumento de la lipogénesis *de novo* y acumulación de TG en este órgano[10,11]. Aproximadamente el 60% de los AG que participan en el cúmulo de TG hepáticos en la EHGNA proceden de la lipólisis del tejido adiposo, el 15% directamente de la dieta (ingesta excesiva de grasa e hidratos de carbono) y el 25% restante proceden del incremento de la ratio de la lipogénesis *de novo* controlada por factores de transcripción como SREBP1c (proteína de unión al elemento regulador de esterol), ChREBP (proteína de unión al elemento de respuesta a hidratos de carbono), LxRα (receptor X hepático) y PPARγ (receptor gamma activado por el factor proliferador de perixosomas). Muchos son los estudios que han demostrado el incremento de la expresión hepática de genes involucrados en la lipogénesis *de novo* en pacientes con EHGNA[12–16]. En este sentido, nuestro grupo, al estudiar la expresión de genes involucrados en el metabolismo lípidico hepático en mujeres obesas mórbidas con EGHNA, observó que la expresión hepática de FAS (sintasa de ácidos grasos), importante enzima lipogénica bajo el control de LxRα[17], estaba significativamente aumentada en mujeres con esteatosis/esteatohepatitis no alcohólica, respecto a aquellas con histología hepática normal[18].

Recientemente se ha observado que la constante exposición de los hepatocitos a metabolitos lipídicos potencialmente tóxicos, tales como AG, ácido fosfatídico, ácido lisofosfatídico, ceramidas y diacilgliceroles (DAG), pueden dar lugar a efectos «lipotóxicos»[3], caracterizados por estrés en el retículo endoplásmico, inflamación, apoptosis, necrosis, *ballooning* y formación de cuerpos de Mallory-Denk, características histopatológicas propias de la esteatohepatitis[19]. Estas observaciones refuerzan la hipótesis de que los metabolitos derivados de los TG serían los verdaderos agentes tóxicos[20,21], y que la hidrólisis de los TG hepáticos a través de lipasas metabólicas estarían contribuyendo al desarrollo de la EHGNA[19].

Dado que actualmente no existen terapias efectivas para la EHGNA, a excepción de la pérdida de peso, los esfuerzos en la investigación actual se centran en la comprensión de la patogenia de esta enfermedad, con la finalidad de identificar nuevas dianas terapéuticas. En este sentido, esta revisión pretende actualizar los conocimientos sobre

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la contribución de las lipasas metabólicas hepáticas y mediadores lipotóxicos en el desarrollo de la esteatosis y esteatohepatitis no alcohólica.

Rol de las lipasas metabólicas en la patogénesis y progresión de la enfermedad del hígado graso no alcohólico

Debido a que la EHGNA se caracteriza principalmente por la acumulación hepática de lípidos[22], las lipasas metabólicas se han involucrado en la patogénesis y progresión de la enfermedad y se encuentran actualmente en el centro de interés.

Los AG liberados del tejido adiposo son transportados hacia el hígado y captados mediante transportadores celulares específicos como clúster de diferenciación 36 (CD36) y proteína transportadora de ácidos grasos (FATP) o por difusión pasiva[23]. Una vez dentro de los hepatocitos son esterificados y almacenados en forma de TG. Estudios en ratones modificados genéticamente que sobreexpresan diacilglicerol aciltransferasa 2 en el hígado, enzima que cataliza el último paso en la formación de TG, han objetivado un aumento de la acumulación hepática de TG sin inflamación o RI hepática. Sin embargo, la silenciación de la expresión de diacilglicerol aciltransferasa 2 previene el cúmulo hepático de TG, pero causa daño lipotóxico debido al exceso de AG libres[19]. Estos hallazgos sugieren que los TG podrían estar protegiendo contra la lesión hepática mediada por lípidos. Sin embargo, las propiedades antilipotóxicas de los TG se ven limitadas debido a que las lipasas metabólicas pueden actuar sobre ellos liberando AG libres, generando así una nueva fuente de intermediarios lipotóxicos[19].

Diacilgliceroles, resistencia a la insulina hepática y enfermedad del hígado graso no alcohólico

La acción de la insulina en las células hepáticas requiere un conjunto de señales intracelulares coordinadas, que se basan sobre todo en procesos de fosforilación y desfosforilación. Durante este proceso, la insulina se une a su receptor y activa la vía de la PI(3)K y Akt2, suprimiendo la producción hepática de la glucosa mediante 2 mecanismos principales: primero, la disminución de la expresión de las enzimas gluconeogénicas mediante la fosforilación y la exclusión nuclear del factor de transcripción FOXO1, y en segundo lugar, el aumento de la actividad glucógeno sintasa (GS), mediante la fosforilación e inactivación de la cinasa glucógeno sintasa 3 (GSK3)[24–26] (fig. 1 A).

El desarrollo de la EHGNA se encuentra fuertemente asociado a la resistencia a la insulina hepática. Esta relación ha sido demostrada en ratas sometidas a una dieta rica en grasa durante 3 días, las cuales desarrollaron esteatosis y RI hepática, sin cambios en el peso corporal, adiposidad o RI en el músculo esquelético[27]. Curiosamente, en el hígado de estos animales se observó un aumento de DAG. La conexión entre la acumulación de DAG y RI hepática podría atribuirse a la activación de la proteína quinasa Cε (PKCε), altamente expresada en el hígado. Estos cambios se han asociado con reducciones en la fosforilación del receptor de la insulina y en la actividad Akt2. Por lo tanto, en este modelo, la actividad de la insulina para inducir la síntesis de glucógeno e inhibir la gluconeogénesis se ve disminuida debido a la activación de PKCε mediada por DAG, promoviendo la unión de PKCε en el dominio intracelular del receptor de la insulina (fig. 1 B).

PKCε es un miembro de la familia PKC, compuesta por 3 grupos diferentes: convencional (α, βI, βII, y γ), novel (δ, ε, η, y θ) y atípico (ζ y λ)[28]. PKCε es una isoforma novel con una afinidad mucho mayor por el DAG que las isoformas PKC convencionales[29]. El papel específico de PKCε en la RI hepática fue examinado en un estudio en el que se utilizaron oligonucleótidos antisentido (OAS), que actúan preferentemente en el hígado y el tejido adiposo[30]. Samuel et al. demostraron en ratas que la disminución de la expresión hepática de PKCε mediante OAS específicos las protegía del desarrollo de RI hepática inducida por lípidos, a pesar del aumento en el contenido de lípidos hepáticos[31]. Estos resultados fueron replicados en ratones *knockout* para el gen PKCε, donde también se observó una protección frente al desarrollo de RI hepática inducida por una dieta rica en grasa[32]. Posteriormente, la interacción entre DAG, la activación de PKCε y la RI hepática ha sido demostrada en numerosos modelos de EHGNA asociada a resistencia hepática a la insulina[33–44].

Estos modelos para la resistencia hepática a la insulina inducida por lípidos han sido translacionados a humanos. Kumashiro et al. evaluaron los posibles mecanismos implicados en la RI hepática en un grupo de pacientes con obesidad mórbida y EHGNA. En este caso, el contenido de DAG hepático y la activación de PKCε fueron los predictores más fuertes de RI hepática[45]. En cambio, no encontraron asociación entre la sensibilidad a la insulina y otros factores implicados en el desarrollo de RI hepática, tales como ceramidas, marcadores de estrés del retículo endoplásmico o concentraciones de citoquinas inflamatorias. Estos resultados fueron replicados en otro estudio en el que se demostró también el contenido de DAG hepático como el mejor predictor de la RI hepática en humanos obesos, mientras que no hubo asociación con el contenido de ceramidas o marcadores de inflamación hepáticos[46]. De hecho, se ha sugerido que la inflamación hepática es una consecuencia, y no una causa, de la resistencia a la insulina. Por lo tanto, aunque el exceso de ingesta de calorías conduce a la obesidad, solo aquellos que desarrollan esteatosis hepática desarrollarán RI. Estos resultados sostienen la hipótesis de que el paso clave en la patogénesis de la resistencia a la insulina hepática consiste en la acumulación de DAG, dando lugar a la activación de PKCε.

Mecanismos implicados en la acumulación hepática de diacilgliceroles

Los DAG pueden acumularse en el hígado por diferentes motivos. En primer lugar, por un incremento del transporte de quilomicrones remanentes hacia el hígado. En segundo lugar, por un incremento de la liberación de AG por parte de los adipocitos. En tercer lugar, la hiperinsulinemia posprandial debido a la RI en el músculo esquelético puede derivar en un aumento de la lipogénesis de *novo* hepática, causando un aumento del contenido hepático de DAG. Finalmente, la disminución de la función mitocondrial puede también estar participando en la acumulación del contenido hepático de

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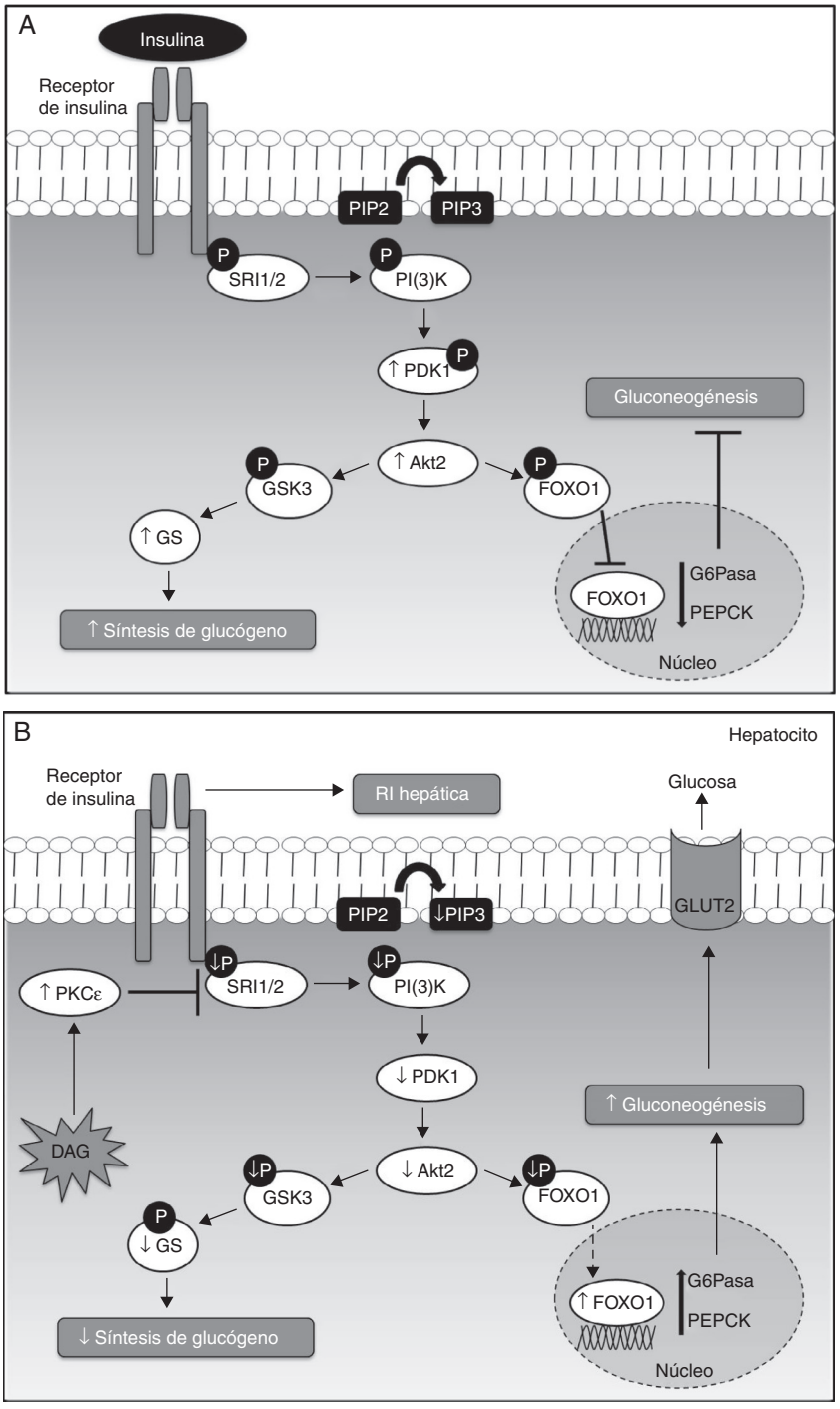

Figura 1 A. Señalización hepática de la insulina. La insulina, a su llegada al hepatocito, se une y activa el receptor tirosina cinasa de la insulina (RTKI), el cual promueve la fosforilación del sustrato del receptor de la insulina (SRI), siendo el SRI2 el más importante a nivel hepático. La fosforilación de SRI2 genera sitios de unión para la quinasa fosfatidilinositol 3 —PI(3)K—. La unión de PI(3)K a SRI2 convierte el lípido de membrana fosfatidilinositol 4,5-bifosfato (PIP2) en fosfatidilinositol 3,4,5-trifosfato (PIP3) que, a su vez, recluta Akt2. Bajo condiciones de estímulo de insulina, la proteína cinasa D (PDK1) es fosforilada y activa Akt2, que parece suprimir la producción hepática de glucosa mediante 2 mecanismos principales: primero, la disminución de la expresión de las enzimas gluconeogénicas mediante la fosforilación y la exclusión nuclear de la proteína FOXO1, que inhibe la activación de la expresión de proteínas gluconeogénicas como la glucosa-6 fosfatasa (G6Pasa) y la fosfoenolpiruvato carboxiquinasa (PEPCK), dando lugar a la supresión de la gluconeogénesis hepática; y en segundo lugar, el aumento de la actividad de la glucógeno sintasa (GS) mediante la fosforilación e inactivación de la quinasa glucógeno sintasa 3 (GSK3). En su forma inactiva (fosforilada), GSK3 no cataliza la fosforilación e inactivación de la GS, permitiendo así la síntesis de glucógeno hepática.B. Mecanismos moleculares de DAG-PKCε que median la resistencia a la insulina hepática. La acumulación hepática de DAG permite la activación y translocación de PKCε hacia la membrana plasmática, lo que provoca la inhibición del receptor quinasa de la insulina y su señalización intracelular.

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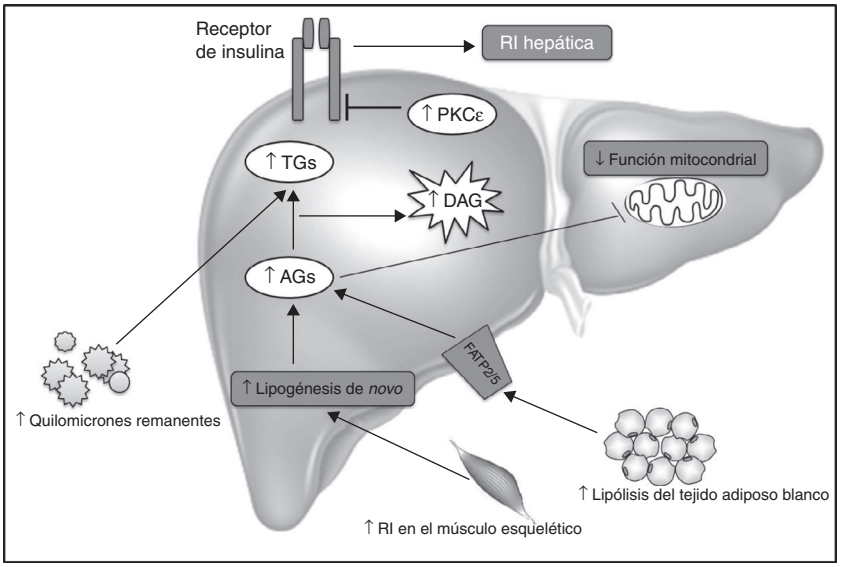

Figura 2 Mecanismos implicados en la acumulación hepática de DAG.
El aumento en el contenido hepático de DAG es el resultado de un desequilibrio entre la captación/liberación de ácidos grasos (AG), la tasa de oxidación mitocondrial de AG y la conversión de los DAG a triglicéridos (TG) durante la lipogénesis hepática. Que la ratio de la ingesta energética sea superior al gasto energético es una de las causas de la EHGNA y resistencia a la insulina (RI) hepática inducida por el mecanismo DAG-PKCε. La predisposición de factores genéticos, como las variantes genéticas de APOC3 que provocan un aumento de las concentraciones plasmáticas APOC3, dan lugar a la supresión de la actividad de la lipoproteína lipasa, el aumento de quilomicrones remanentes posprandiales, y el aumento de la captación hepática de AG, contribuyendo al incremento del contenido hepático de DAG. Defectos en el almacenamiento lipídico en los adipocitos, tales como las lipodistrofias, así como alteraciones genéticas o adquiridas en la oxidación mitocondrial de AG, pueden contribuir a la acumulación hepática de DAG y consecuente desarrollo de EHGNA y RI hepática. Finalmente, los ácidos grasos liberados durante la lipólisis en los adipocitos entrarían en el hígado mediante transportadores específicos de AG (FATP2/5), aumentando el contenido hepático de DAG.

DAG[47] (fig. 2). Cabe mencionar además que el sistema endocannabinoide parece tener un papel emergente en el desarrollo de RI hepática y acumulación de lípidos en el hígado.

Sistema endocannabinoide en el tejido hepático

Los endocannabinoides entran en las células hepáticas mediante transportadores específicos, tales como proteínas de unión de ácidos grasos (FABPs), FABP5 y FABP7, actuando[48,49] sobre los receptores de canabinoides 1 (CB1) y 2 (CB2). Tanto los receptores de endocanabinoides como endocanabinoides específicos, como el 2-aciletanolamida (2-AE), se encuentran incrementados en el hígado de modelos de ratón con obesidad inducida por la dieta[50,51]. Además, en un estudio propio realizado en mujeres con obesidad mórbida y EHGNA, observamos que la expresión hepática de CB1 se encuentra significativamente incrementada en mujeres con esteatohepatitis comparadas con las que presentan únicamente esteatosis hepática[52]. Parece que la activación de CB1 podría activar la lipogénesis hepática mediante la inducción de estrés en el retículo endoplasmático, así como la activación de factores de transcripción (por ejemplo SREBP1c)[53], proceso que estaría contribuyendo a la formación de DAG. Finalmente, el DAG acumulado podría ser transformado en 2-AE de nuevo, dando lugar a un bucle de retroalimentación positiva que estaría induciendo y agravando la esteatosis y la RI hepática[54].

Aumento de la ingesta calórica

La mayor causa de la HGNA en los países desarrollados se atribuye especialmente a un desequilibrio energético, donde la ingesta calórica excede al gasto calórico, conduciendo a un aumento de lípidos liberados hacia el hígado[55–57].

En este sentido, Jonayvaz et al. demostraron que los ratones que seguían una dieta cetogénica rica en grasa desarrollaban esteatosis hepática severa y RI a pesar de manifestar un incremento de gasto energético y pérdida de peso. En este caso, el contenido de DAG hepático fue incrementado en un 350% debido a la activación de PKCε, a la disminución de la fosfoliración de IRS2 inducida por insulina, y a la disminución de la supresión de la producción hepática de glucosa durante un clamp hiperinsulinémico-euglucémico.

En humanos diferentes estudios sugieren que la movilización regional de los TG circulantes y el transporte de ácidos grasos se encuentran alterados en los pacientes obesos que padecen EHGNA. La lipoproteína lipasa (LpL) hidroliza los TG circulantes, seguido por la absorción de estos en el tejido hepático a través de los transportadores celulares FATP y CD36[58]. La actividad LpL en el tejido adiposo en respuesta a la insulina parece estar disminuida en pacientes con obesidad[59], mientras que la EHGNA se ha asociado con el incremento de la expresión hepática de LpL, FATP y

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CD36[60–62]. En general, parece que la sobreexpresión hepática de LpL[63,64] o de CD36 provoca la acumulación hepática de lípidos, así como la resistencia a la insulina hepática[65], mientras que la supresión hepática de FATP protege contra el desarrollo de esteatosis hepática e IR[66].

En su conjunto, estos estudios sugieren que en la obesidad inducida por un aumento de la ingesta calórica los ácidos grasos son transportados desde el tejido adiposo hacia el hígado y el músculo esquelético, donde son reesterificados en DAG, induciendo así resistencia a la insulina en estos órganos.

Disminución de la función mitocondrial

En el hepatocito la β-oxidación mitocondrial es la principal ruta de oxidación de los ácidos grasos[67]. Las coenzimas reducidas flavín adenín dinucleótido y nicotinamida adenina dinucleótido, generadas en el propio proceso y en la oxidación del acetil-CoA mediante el ciclo de los ácidos tricarboxílicos, donan sus electrones a la cadena respiratoria, produciéndose adenosín trifosfato (ATP) por la fosforilación de la adenosín difosfato mediada por la ATP sintasa. Además, las mitocondrias son también la principal fuente de especies reactivas del oxígeno (ROS)[68]. Las ROS producidas en este y otros procesos son neutralizadas por sistemas enzimáticos, sobresaliendo la superóxido dismutasa, la catalasa y la glutatión peroxidasa, y por defensas celulares vitamínicas, principalmente la vitamina E y la C. En pacientes con EHGNA se ha descrito disfunción mitocondrial, anomalías ultraestructurales[69,70], actividad reducida de los complejos de la cadena respiratoria[68,71], fosforilación oxidativa deficiente, una menor capacidad para sintetizar ATP, un descenso en la concentración de ATP intracelular[72] y daño en el ADN mitocondrial[73].

Zhang et al.[44] demostraron que la disminución de la función mitocondrial hepática puede ser un factor predisponente de EHGNA y RI hepática. Este estudio observó que los ratones *Knockout* para acil-CoA deshidrogenesa de cadena larga, los cuales tienen reducida la función mitocondrial en el hígado, son propensos a padecer esteatosis hepática asociada a un incremento del contenido de DAG, a la activación de PKCε y al desarrollo de RI hepática cuando estos son alimentados con una dieta rica en grasa. En humanos la asociación de la disminución de la oxidación hepática con EHGNA es menos clara, ya que existen estudios discrepantes. Algunos han demostrado una disminución de la oxidación[74,75], mientras que otros han sugerido un incremento en el metabolismo mitocondrial hepático[76].

Defectos en el almacenamiento lipídico

Las lipodistrofias son un grupo de síndromes de carácter congénito u adquirido cuya característica clínica principal es la pérdida parcial o completa del tejido adiposo. A nivel metabólico se caracterizan por una severa resistencia a la insulina, hipertrigliceridemia grave, bajos niveles circulantes de leptina, adiponectina y colesterol HDL, causado por la acumulación ectópica de grasa, lo que incluye el desarrollo de la EHGNA[77]. El 80% de los pacientes con lipodistrofia cumplen los criterios definitorios de SM[78], sin embargo, presentan bajos niveles de hormonas derivadas de los adipocitos en comparación con pacientes con SM asociado a la obesidad[73]. Así, las lipodistrofias, situaciones en las que no existe expansión del tejido adiposo subcutáneo ni visceral, ofrecen una posibilidad única para evaluar de forma específica el papel del cúmulo hepático de grasa en el desarrollo de la EHGNA[47].

Los ratones que expresan *the dominant-negative protein A-ZIP/F1* carecen prácticamente de tejido adiposo blanco (ratones sin grasa) y desarrollan acumulación ectópica de grasa en el hígado y en el músculo esquelético, dando lugar a la aparición de una marcada RI periférica y hepática. Esta RI puede ser corregida mediante el trasplante de tejido adiposo blanco procedente de ratones sanos, lo que se traduce en una disminución de la cantidad grasa hepática acumulada y de la RI, tanto del tejido hepático como de tejidos periféricos[79]. Además, Shimomura et al., utilizando de nuevo un modelo murino de lipodistrofia, demostraron también su reversibilidad mediante la administración sistémica de leptina recombinante[80].

En humanos se han estudiado también pacientes con lipodistrofia relacionada con la mutación en la perilipina-1, responsable de la inhibición de la triglicérido-lipasa intracelular. En estos pacientes, debido al aumento de la lipólisis, se observa una reducción del tejido graso subcutáneo y el desarrollo de EHGNA[81]. En los pacientes con lipodistrofia también se ha podido demostrar beneficio de la administración exógena de leptina recombinante[82]. Petersen et al., observaron que la administración exógena de leptina en pacientes lipodistróficos reducía significativamente el contenido lipídico hepático y muscular, debido principalmente a la disminución de la ingesta calórica, con la consiguiente mejora en la sensibilidad a la insulina tanto hepática como de los tejidos periféricos[83]. Recientemente, se ha comprobado que, en pacientes con diferentes grados de lipodistrofia, la terapia sustitutiva con leptina mejoraba el daño histológico del tejido hepático[77].

En conjunto, los estudios realizados en modelos animales y en pacientes lipodistróficos demuestran una clara disociación entre la cantidad de tejido graso corporal global o la cuantía del tejido graso visceral, y el grado de RI hepática, sugiriendo que la distribución y acumulación específica del tejido graso al nivel del hígado y del músculo esquelético, no la cantidad grasa corporal total, es la que determina la RI hepática y muscular[84].

Este último concepto estaría apoyado por el papel que el receptor PPARγ estaría desempeñando en el desarrollo de la esteatosis hepática. PPARγ está altamente expresado en el tejido adiposo, ejerciendo un papel clave en la captación de los ácidos grasos por parte de los adipocitos, así como en su diferenciación celular. Existen pacientes con ciertas mutaciones que tienen disminuida la actividad de PPARγ. Estos desarrollan síndrome metabólico y EHGNA, causado por el aumento del flujo lipídico hacia el hígado[85]. A pesar de que PPARγ está altamente expresado en el tejido adiposo, se expresa también a nivel hepático pero en menor grado. Ratones deficientes en la actividad hepática de PPARγ están protegidos contra el desarrollo de esteatosis hepática, sugiriendo que PPARγ desempeña un papel importante en la regulación del cúmulo de lípidos hepáticos[86]. Pacientes en tratamiento con tiazolidindionas,

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fármacos agonistas de PPARγ, presentan una disminución de la acumulación de lípidos a nivel hepático y del músculo esquelético, favoreciéndose el depósito lipídico en el tejido graso subcutáneo y el aumento de la sensibilidad hepática a la insulina[87,88].

Así, los conocimientos aportados por los estudios de las lipodistrofias parecen apuntar a que la resistencia hepática a la insulina está determinada por el cúmulo específico de grasas a nivel hepático, y no por el grado de obesidad global, y en segundo lugar, resaltan la importancia del tejido adiposo y de su capacidad adaptativa para almacenar el exceso de grasas, hipertrofiándose, protegiendo al hígado de un cúmulo lipídico excesivo.

Resistencia a la insulina en el músculo esquelético

En este apartado se pretende dilucidar el papel de la RI muscular en la patogénesis de la EHGNA y la RI hepática.

Papel del exceso de ácidos grasos libres en la resistencia a la insulina muscular

El potencial de los ácidos grasos libres (AGL) para alterar el metabolismo glucídico muscular fue propuesto hace más de 50 años[89], y desde entonces ha sido ampliamente investigado. Diferentes autores[90–96] han confirmado que un incremento en la concentración plasmática de AGL altera la señalización de la insulina y causa insulinorresistencia muscular en individuos sanos. Además, se ha demostrado que una disminución de la concentración plasmática de AGL secundaria a acipimox, análogo del ácido nicotínico e inhibidor de la lipólisis del tejido adiposo, mejora rápidamente la sensibilidad a la insulina muscular[97].

Así mismo, numerosos estudios han demostrado una fuerte relación entre los lípidos intramiocelulares y la RI muscular[55,56,98]. En adultos no diabéticos con normopeso el contenido en TG intramiocelular es un predictor de RI muscular mucho más potente que los ácidos grasos circulantes[99], lo que sugiere que, en sujetos obesos, los lípidos intramiocelulares pueden estar desempeñando un papel causal en la RI muscular. De hecho, sujetos obesos insulinosensibles o insulinorresistentes se distinguen según el cúmulo lipídico muscular y hepático[100].

Por otro lado, es importante señalar que la RI muscular correlaciona con una variedad de metabolitos lipídicos tóxicos, consecuencia de una oxidación incompleta de ácidos grasos, como acilcarnitinas, ceramidas y DAG[96,101–104]. En modelos murinos, cuando se aumenta los AG en plasma mediante la infusión de Liposyn® + heparina para activar la LpL, la RI muscular aparece a las 3 h, a consecuencia del aumento de DAG y de la activación de PKCε[105]. Sin embargo, no se observan cambios en el contenido de TG o ceramida muscular en ese momento, por lo que es importante diferenciar entre los distintos lípidos que participan en la patogénesis de la RI muscular. Los hallazgos de RI muscular mediada por DAG han sido confirmados en humanos por Itani et al.[106]. Además, en la patogénesis de RI muscular, es importante tener en cuenta que el músculo esquelético también es el objetivo de citoquinas proinflamatorias circulantes como factor de necrosis tumoral alfa e interleucina 6. Finalmente, macrófagos inflamatorios M1 activados procedentes del tejido adiposo pueden infiltrar el músculo esquelético y causar RI de una manera similar a lo que ocurre en el tejido adiposo[107].

Insulinorresistencia en músculo esquelético en pacientes con esteatohepatitis no alcohólica

Según la literatura un aumento moderado en la adiposidad y en la concentración plasmática de AGL puede causar lipotoxicidad muscular. Por tanto, no es de extrañar que, en pacientes obesos con EHGNA, la RI muscular esté completamente establecida[108–110], e incluso en individuos no obesos con esteatohepatitis no alcohólica (EHNA) si presentan RI en el tejido adiposo[111–113]. En cambio, individuos obesos sin RI en el tejido adiposo se comportan desde el punto de vista metabólico como sujetos delgados, con casi normal insulinosensitividad muscular y habitualmente sin desarrollo de EHGNA[108,111,114].

La RI primaria en el músculo esquelético puede conducir a una redistribución de los sustratos hacia el hígado, dando lugar a la aparición de esteatosis hepática y, posteriormente, RI hepática a través del cúmulo hepático de DAG con la activación de PKCε. Esta hipótesis ha sido respaldada en estudios de modelos murinos que presentan una inactivación específica del gen del receptor de insulina (MIRKO)[115]. La RI muscular selectiva en estos ratones comporta una hiperinsulinemia compensatoria con redistribución de los sustratos hacia lugares ectópicos, tales como el hígado. Además, ratones que carecen del transportador de glucosa muscular (GLUT4) presentan una pérdida casi completa de la captación muscular de glucosa estimulada por la insulina, que se asocia a esteatosis hepática y RI[116]. Estos hallazgos se han corroborado también en humanos. Así, en individuos con RI muscular selectiva, observada en sujetos sanos, jóvenes, delgados o en individuos en el cuartil inferior de insulinosensibilidad global, los hidratos de carbono ingeridos se desvían de la síntesis de glucógeno muscular hacia la lipogénesis hepática, predisponiendo a estos individuos a RI hepática y aparición de EHGNA[117] (fig. 3). Por otra parte, un estudio reciente ha demostrado que una sola sesión de ejercicio revierte los defectos en el transporte de la glucosa muscular estimulada por insulina, así como los defectos en la síntesis de glucógeno[118]. Esta mejoría se evidencia por una disminución de la lipogénesis hepática *de novo* y por la reducción de la síntesis hepática de TG neta después de una dieta rica en hidratos de carbono. Por lo tanto, se demuestra que la RI muscular puede ser una diana terapéutica para la prevención y el tratamiento de la EHGNA[118]. En este sentido, Haufe et al. demostraron que el efecto de la mejora de la condición física sobre la insulinosensibilidad en pacientes con sobrepeso/obesidad está mediada a través de una reducción en el contenido de grasa hepática[119]. Además, se ha objetivado que el ejercicio crónico sin reducción en el peso corporal o en la grasa corporal total también conduce a una reducción en el contenido de grasa hepática[120].

En resumen, tanto estudios en ratones como en humanos apoyan el concepto de que la RI muscular selectiva, que es una de las alteraciones metabólicas más tempranas detectadas en jóvenes delgados descendientes de padres con diabetes tipo 2, puede ser un factor importante y temprano en la patogénesis de la EHGNA y la resistencia hepática a la insulina.

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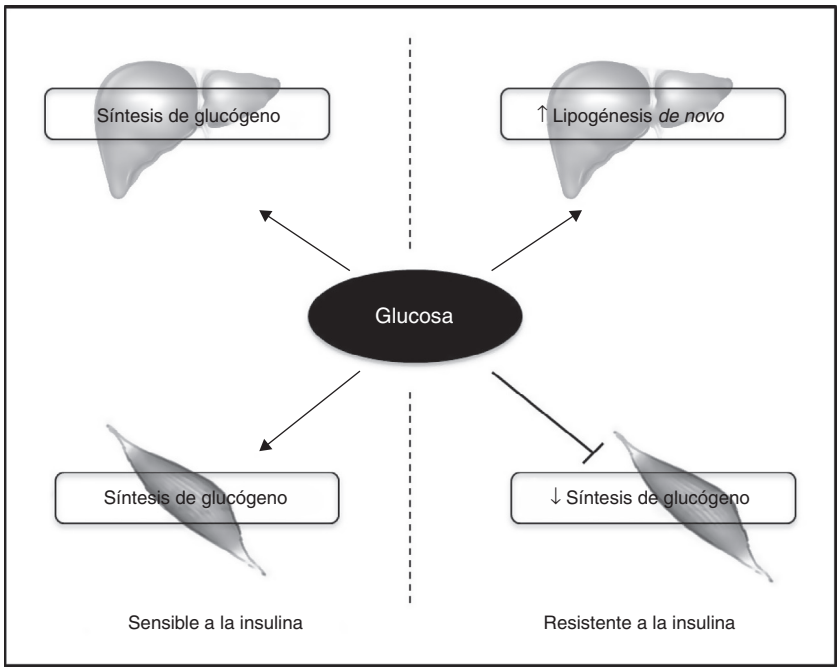

Figura 3 La resistencia a la insulina en el músculo esquelético contribuye a la lipogénesis hepática.
En los sujetos insulinosensibles la insulina estimula la síntesis de glucógeno tanto en tejido muscular como hepático. Sin embargo, en aquellos que presentan resistencia a la insulina en el músculo esquelético, esta hormona no es capaz de promover la síntesis de glucógeno, redistribuyendo el sustrato hacia el hígado, lo que contribuye a un incremento de la lipogénesis *de novo* hepática. El aumento de la síntesis de lípidos en el hígado de pacientes con resistencia a la insulina en el músculo esquelético promueve el desarrollo de EHGNA.

Factores genéticos

En los últimos años se han descrito variantes genéticas de distintos genes que hacen más suceptibles a los individuos portadores a desarrollar EHGNA y RI cuando se exponen a factores ambientales.

Apolipoproteína C3

La apolipoproteína C3 (APOC3) es una proteína que forma parte de las lipoproteínas de muy baja densidad, que se encarga de inhibir la actividad lipoproteín lipasa con el fin de regular la distribución de lípidos en los diferentes órganos y tejidos del cuerpo.

Recientemente, Petersen et al. han demostrado que un grupo de indios asiáticos, varones, sanos y delgados, que presentan una de las 2 variantes genéticas (C-482 T/T-455 C) en el elemento de respuesta a la insulina del gen APOC3 se encuentran en un riesgo más elevado de desarrollar EHGNA y RI. Las concentraciones plasmáticas de APOC3, así como la hipertrigliceridemia posprandial de los individuos que poseen este polimorfismo, son aproximadamente un 30% más elevadas comparadas con los sujetos homozigotos para APOC3 (C-482/T-455)[121]. Además, después de ser sometidos a una infusión intravenosa de lípidos, estos sujetos presentaron una disminución de la hidrólisis de triglicéridos y un aumento de la hipertrigliceridemia posprandial y quilomicrones remanentes, debido a la exagerada inhibición de la actividad LpL, permitiendo así el desarrollo de EHGNA y RI. También se pudo observar una reversión de la esteatosis hepática y RI en estos sujetos después de una modesta pérdida de peso[121]. Sin embargo, cabe destacar que las variantes genéticas de APOC3 no son la causa directa del desarrollo de esteatosis hepática, pero suponen una condición de predisposición en aquellos individuos que son portadores, siendo más susceptibles a desarrollar EHGNA y RI cuando se exponen a factores ambientales tóxicos, tales como una dieta hipercalórica y rica en grasas. De acuerdo a esta hipótesis, Lee et al. han demostrado recientemente que ratones que sobreexpresan APOC3 no presentan esteatosis hepática o RI cuando son alimentados con dieta control. Sin embargo, cuando esta es remplazada por una dieta rica en grasas desarrollan esteatosis hepática severa con un incremento del contenido hepático de DAG, de la activación de PKCε y RI[40].

Adiponutrin

Otro grupo en riesgo de desarrollo de EHGNA y RI hepática son adultos y niños hispanos[122]. En esta población se ha visto que la sustitución de isoleucina por metionina en la posición 148 (I148M) de la enzima PNPLA3, conocida también como adiponutrin, codificada por el gen PNPLA3, se encuentra fuertemente asociada al contenido hepático de TG y desarrollo de EHGNA, incluyendo esteatohepatitis, cirrosis y hepatocarcinoma[123–127]. Sin embargo, a diferencia de las variantes del gen APOC3 descritas en la sección anterior, las variantes genéticas de PNPLA3 no se encuentran asociadas a RI, sugiriendo que la enfermedad de EHGNA puede disociarse de la RI hepática[128–130].

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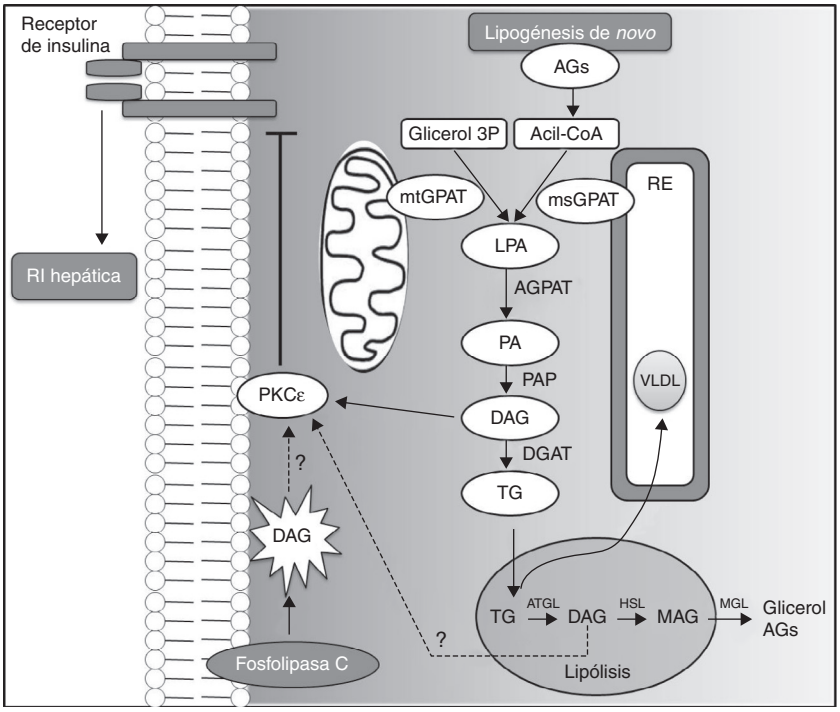

Figura 4 Vías metabólicas implicadas en la acumulación hepática de DAG.
La vía del glicerol-3-fosfato (glicerol 3P) representa la ruta de la lipogénesis *de novo* en la síntesis de triglicéridos y fosfolípidos. La glicerol-fosfato-acil-transferasa (GPAT) cataliza la acetilación de glicerol 3P con acil-CoA para generar ácido lisofosfatídico (LPA), lo que parece ser el paso limitante en la síntesis de triglicéridos (TG). Posteriormente, las enzimas acil-glicerol-fosfato-acil-transferasa (AGPAT), fosfatasa del ácido fosfatídico (PAP) y diacilglicerol aciltransferasa (DGAT) catalizan la formación de ácido fosfatídico (PA), diacilglicerol (DAG) y TG, respectivamente. Los DAG activan la translocación de PKCε hacia la membrana plasmática, inhibiendo la activación del receptor de la insulina. Los ácidos LPA y PA que no han sido sintetizados en el retículo endoplasmático (RE) requieren la translocación a través del citosol para la síntesis de TG en el RE. En el hígado los TG pueden depositarse en vacuolas intracelulares o ser exportados en lipoproteínas de muy baja densidad (VLDL). En las vacuolas, durante la lipólisis, la conversión de TG a DAG es mediada por la lipasa de triglicéridos del tejido adiposo (ATGL). Los DAG pueden ser hidrolizados a monoacilglicerol (MAG) por la hormona sensible a la lipasa (HSL) y, posteriormente, a glicerol por la lipasa de monoglicéridos (MGL), que puede ser utilizado como sustrato para la gluconeogénesis. Estas reacciones, al liberar ácidos grasos, contribuyen a su acumulación hepática. Los DAG provenientes de lipólisis intravacuolar y de la vía de la fosfolipasa C, que libera DAG a partir de lípidos de membrana, podrían activar la PKCε y la resistencia hepática a la insulina (RI). Sin embargo, estos 2 últimos mecanismos no están del todo dilucidados.

PNPLA3 pertenece a la familia de proteínas que comparten un dominio evolutivo identificado por primera vez en la patatina, la principal proteína de los tubérculos de patata[131]. El genoma humano codifica 9 proteínas que contienen el dominio de la patatina, de los cuales PNPLA3 está estrechamente relacionada con PNPLA2, la mayor hidrolasa de TG del tejido adiposo[132,133]. PNPLA3 se expresa predominantemente en el hígado y en el tejido adiposo humano[134], y se encuentra frecuentemente asociada a membranas y vacuolas lipídicas[135]. Esta proteína está altamente regulada en respuesta a estímulos nutricionales, tanto a nivel transcripcional como postranslacional. Su expresión se suprime en estado de ayunas, mientras que aumenta en respuesta a la presencia de glucosa e insulina, donde el factor de transcripción SREBP1c se encarga de estimular su transcripción[136].

Se sugiere que PNPLA3 presenta tanto función lipasa como de transacilación, y que la mutación I148M causa la pérdida de la función lipasa del gen[137], contribuyendo así al aumento del contenido de TG hepáticos. Yongcheng et al. demostraron que PNPLA3 cataliza la hidrólisis de los 3 mayores glicerolípidos (TG, DAG y MAG), con mayor preferencia por los ácidos grasos C18:1, y que la mutación I148M reduce la actividad hidrolasa contra los 3 glicerolípidos. Sin embargo, cabe destacar que, prácticamente, en la mayoría de estos estudios se han incluido individuos obesos como sujetos controles, los cuales presentan casi siempre algún grado de esteatosis hepática asociada a RI, haciendo difícil discernir si las variantes genéticas de PNPLA3 con EHGNA están participando o no en la RI hepática. Para identificar si la mutación confiere un riesgo independiente en el desarrollo de RI hepática, sería importante evaluar la sensibilidad a la insulina hepática en individuos delgados con EHGNA portadores de la mutación I148M[136].

En concordancia a los resultados obtenidos en humanos, la sobreexpresión del gen mutante I148 M de PNPLA3 en ratones se asoció a un aumento del tamaño de las vacuolas lipídicas y acumulación de TG hepáticos[135,136]. Sin embargo, la sobreexpresión de PNPLA3 no mutado no cambió el contenido de lípidos hepáticos en ratones[135,136,138]. Por otro

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lado, los ratones *Knockout* para PNPLA3 no desarrollaron esteatosis hepática ni trastornos en el metabolismo de la glucosa[139,140].

Rol de la compartimentación intracelular de diacilgliceroles: disociación de la enfermedad del hígado graso no alcohólico y la resistencia a la insulina hepática

El modelo descrito anteriormente, que explica la relación entre DAG, PKCε y RI hepática, se centra principalmente en la incapacidad de la insulina para alterar el metabolismo de la de glucosa hepática. Sin embargo, la capacidad de la insulina para activar la lipogénesis parece estar intacta en la mayoría de los modelos de EHGNA. Por ello, aunque la EHGNA se encuentra fuertemente asociada a RI hepática y DM2, recientemente se ha descrito la «paradoja de la resistencia hepática a la insulina selectiva», en la que se propone la existencia de una disociación entre la EHGNA y la RI hepática[141,142].

Experimentalmente es posible inducir resistencia a la insulina sin EHGNA, o inducir EHGNA sin resistencia a la insulina bajo ciertas condiciones. Por ejemplo, el bloqueo de la secreción hepática de lipoproteínas de muy baja densidad con una dieta deficiente en colina, o mediante la modificación genética de la maquinaria de exportación, aumenta las concentraciones de TG hepáticos pero no induce RI en ratones[143,144]. Por otro lado, los factores de transcripción ChREBP y SREBP1 han sido recientemente implicados, de forma independiente, en la disociación de EHGNA y RI hepática tanto en ratones como en humanos[145,146]. Para explicar esta paradoja y la RI hepática selectiva, Cantley et al. examinaron la localización subcelular de DAG en células hepáticas y observaron que el *knockdown* del gen CGI-58, activador de la lipasa de triglicéridos del tejido adiposo (ATGL) que participa en la hidrólisis de TG, promueve la acumulación de DAG en gotas lipídicas, evitando así la acumulación de DAG en la membrana celular y la translocación de PKCε hacia la membrana celular[147]. Estos resultados concuerdan con otros estudios realizados tanto en humanos[45] como en modelos animales[38,148,149], sugiriendo que la compartimentación de DAG en el hepatocito podría ser un factor importante en la patogénesis de la resistencia hepática a la insulina.

La acumulación hepática de DAG puede resultar de la vía glicerol 3-fosfato, que representa la ruta de la lipogénesis en la síntesis de TG y fosfolípidos. Por otro lado, los DAG intracelulares también pueden derivar de la hidrólisis de TG compartimentados en gotas lipídicas, mediada por la lipasa ATGL, así como de la activación de la fosfolipasa C, que libera DAG a partir de lípidos de membrana (fig. 4). De qué manera los DAG que derivan de estas 2 últimas vías pueden conducir a la activación PKCε y a la RI hepática no se ha determinado todavía. Sin embargo, los resultados obtenidos usando OAS específicos para CGI-58 indican claramente que los lípidos secuestrados en gotas lipídicas no promueven la activación PKCε y resistencia hepática a la insulina, y que la compartimentación de DAG y TG en compartimentos neutros, en los cuales se estaría evitando la activación de PKCε, podrían explicar por qué el contenido hepático de DAG no siempre se correlaciona con la RI hepática en algunos modelos animales[47].

Conclusiones

Las lipasas metabólicas son las enzimas responsables de la hidrólisis de TG tanto en tejido adiposo como hepático. La lipólisis en el tejido adiposo provoca un aumento del flujo de AG hacia el hígado, promoviendo así el desarrollo de esteatosis hepática[150]. Existe una creciente evidencia, no solo en modelos animales de EHGNA, sino también en humanos con EHGNA asociada a obesidad, a DM2 y a lipodistrofia, que la esteatosis hepática está fuertemente vinculada al desarrollo de RI hepática. Por lo tanto, si los lípidos hepáticos son mediadores importantes para la RI hepática, es de esperar que la reducción de la esteatosis hepática en pacientes con EHGNA y DM2 conlleve a la reducción de la RI hepática.

La EHGNA aparece cuando la ratio de lípidos suministrados hacia el hígado excede a la oxidación y a la exportación lipídica hepática. Por otro lado, un gran número de estudios, tanto en seres humanos como en modelos animales, han demostrado que la acumulación hepática de DAG permite la activación de PKCε, dando lugar a la aparición de resistencia hepática a la insulina. Además, la compartimentación del DAG acumulado parece ser un factor crucial para el desarrollo de RI hepática, pudiendo explicar por qué algunos pacientes y modelos murinos con EHGNA no desarrollan RI hepática. Así, el mecanismo DAG-PKCε parece desempeñar un papel primordial en el desarrollo de la EHGNA asociada a la RI hepática. Por todo ello, para el tratamiento de la EHGNA y la DM2 resulta de gran interés todas aquellas terapias que van dirigidas a reducir la liberación de ácidos grasos hacia el hígado, a suprimir la producción de DAG o a aumentar la oxidación mitocondrial de los AG[151].

Aparte de la pérdida de peso, no existen actualmente terapias efectivas para la EHGNA. Por dicho motivo, la investigación actual se centra en la comprensión de la enfermedad subyacente a la esteatosis hepática, con la finalidad de identificar nuevas dianas terapéuticas. En este sentido, se ha sugerido que nuevas terapias dirigidas a prevenir la acumulación hepática de DAG y la activación de PKCε podrían ser efectivas[84]. Por otro lado, pequeños péptidos dirigidos a revertir la función enzimática correcta de las variantes genéticas de PNPLA3 asociadas a la progresión de la EHGNA y la esteatohepatitis no alcohólica podrían proporcionar un enfoque innovador para el tratamiento de la EHGNA.

Financiación

Este trabajo ha recibido financiación del Fondo de Investigación Sanitaria (expediente n.° PI13/00468 de Teresa Auguet).

Autoría

Alba Berlanga y Esther Guiu-Jurado han contribuido por igual en este trabajo. Todos los autores han contribuido en la redacción del artículo. La versión final de este manuscrito está aprobada por todos los autores.

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Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

Bibliografía

1. WHO | Obesity and overweight [consultado 26 Ene 2015]. Disponible: http://www.who.int/mediacentre/factsheets/fs311/en/
2. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: Old questions and new insights. Science. 2011;332:1519–23.
3. Trauner M, Arrese M, Wagner M. Fatty liver and lipotoxicity. Biochim Biophys Acta. 2010;1801:299–310.
4. Hui JM, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K, et al. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. Hepatology. 2003;38:420–7.
5. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol. 2010;53:372–84.
6. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology. 2011;141:1249–53.
7. Stefan N, Staiger H, Häring H-U. Dissociation between fatty liver and insulin resistance: the role of adipose triacylglycerol lipase. Diabetologia. 2011;54:7–9.
8. Utzschneider KM, Review Kahn SE. The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2006;91:4753–61.
9. Bertolani C, Marra F. The role of adipokines in liver fibrosis. Pathophysiology. 2008;15:91–101.
10. Anderson N, Borlak J. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. Pharmacol Rev. 2008;60:311–57.
11. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest. 2008;118:829–38.
12. Higuchi N, Kato M, Shundo Y, Tajiri H, Tanaka M, Yamashita N, et al. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. Hepatol Res. 2008;38:1122–9.
13. Lima-Cabello E, García-Mediavilla MV, Miquilena-Colina ME, Vargas-Castrillón J, Lozano-Rodríguez T, Fernández-Bermejo M, et al. Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. Clin Sci (Lond). 2011;120:239–50.
14. Kohjima M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, et al. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. Int J Mol Med. 2008;21:507–11.
15. Kohjima M, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. Int J Mol Med. 2007;20:351–8.
16. Nakamuta M, Fujino T, Yada R, Yada M, Yasutake K, Yoshimoto T, et al. Impact of cholesterol metabolism and the LXRalpha-SREBP-1c pathway on nonalcoholic fatty liver disease. Int J Mol Med. 2009;23:603–8.
17. Baranowski M. Biological role of liver X receptors. 2008;59 Suppl 7:31–55.
18. Auguet T, Berlanga A, Guiu-Jurado E, Martinez S, Porras JA, Aragonès G, et al. Altered fatty acid metabolism-related gene expression in liver from morbidly obese women with non-alcoholic fatty liver disease. Int J Mol Sci. 2014;15:22173–87.
19. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology. 2010;52:774–88.
20. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology. 2010;52:1836–46.
21. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. Clin Exp Gastroenterol. 2014;7:221–39.
22. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest. 2004;114:147–52.
23. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, et al. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. Int J Endocrinol. 2012;2012:983814.
24. Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. Trends Endocrinol Metab. 2010;21:589–98.
25. Hanke S, Mann M. The phosphotyrosine interactome of the insulin receptor family and its substrates IRS-1 and IRS-2. Mol Cell Proteomics. 2009;8:519–34.
26. Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science. 1997;275:665–8.
27. Samuel VT, Liu Z-X, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem. 2004;279:32345–53.
28. Newton AC. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. Biochem J. 2003;370 Pt 2:361–71.
29. Dries DR, Gallegos LL, Newton AC. A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. J Biol Chem. 2007;282:826–30.
30. Crooke ST. Progress in antisense technology. Annu Rev Med. 2004;55:61–95.
31. Samuel VT, Liu Z-X, Wang A, Beddow SA, Geisler JG, Kahn M, et al. Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. J Clin Invest. 2007;117:739–45.
32. Frangioudakis G, Burchfield JG, Narasimhan S, Cooney GJ, Leitges M, Biden TJ, et al. Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: Regulation of lipogenesis by protein kinase C delta. Diabetologia. 2009;52:2616–20.
33. Birkenfeld AL, Lee H-Y, Guebre-Egziabher F, Alves TC, Jurczak MJ, Jornayvaz FR, et al. Deletion of the mammalian INDY homolog mimics aspects of dietary restriction and protects against adiposity and insulin resistance in mice. Cell Metab. 2011;14:184–95.
34. Choi CS, Savage DB, Abu-Elheiga L, Liu Z-X, Kim S, Kulkarni A, et al. Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. Proc Natl Acad Sci U S A. 2007;104:16480–5.
35. Erion DM, Ignatova ID, Yonemitsu S, Nagai Y, Chatterjee P, Weismann D, et al. Prevention of hepatic steatosis and hepatic insulin resistance by knockdown of cAMP response element-binding protein. Cell Metab. 2009;10:499–506.
36. Jornayvaz FR, Jurczak MJ, Lee H-Y, Birkenfeld AL, Frederick DW, Zhang D, et al. A high-fat, ketogenic diet causes hepatic insulin resistance in mice, despite increasing energy expenditure and preventing weight gain. Am J Physiol Endocrinol Metab. 2010;299:E808–15.
37. Jornayvaz FR, Samuel VT, Shulman GI. The role of muscle insulin resistance in the pathogenesis of atherogenic dyslipidemia and nonalcoholic fatty liver disease associated with the metabolic syndrome. Annu Rev Nutr. 2010;30:273–90.

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38. Jornayvaz FR, Birkenfeld AL, Jurczak MJ, Kanda S, Guigni BA, Jiang DC, et al. Hepatic insulin resistance in mice with hepatic overexpression of diacylglycerol acyltransferase 2. Proc Natl Acad Sci U S A. 2011;108:5748–52.
39. Jornayvaz FR, Lee H-Y, Jurczak MJ, Alves TC, Guebre-Egziabher F, Guigni BA, et al. Thyroid hormone receptor-α gene knockout mice are protected from diet-induced hepatic insulin resistance. Endocrinology. 2012;153:583–91.
40. Lee H-Y, Birkenfeld AL, Jornayvaz FR, Jurczak MJ, Kanda S, Popov V, et al. Apolipoprotein CIII overexpressing mice are predisposed to diet-induced hepatic steatosis and hepatic insulin resistance. Hepatology. 2011;54:1650–60.
41. Nagai Y, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, et al. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. Cell Metab. 2009;9:252–64.
42. Neschen S, Morino K, Hammond LE, Zhang D, Liu Z-X, Romanelli AJ, et al. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. Cell Metab. 2005;2:55–65.
43. Savage DB, Choi CS, Samuel VT, Liu Z-X, Zhang D, Wang A, et al. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. J Clin Invest. 2006;116:817–24.
44. Zhang D, Liu Z-X, Choi CS, Tian L, Kibbey R, Dong J, et al. Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance. Proc Natl Acad Sci U S A. 2007;104:17075–80.
45. Kumashiro N, Erion DM, Zhang D, Kahn M, Beddow SA, Chu X, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A. 2011;108:16381–5.
46. Magkos F, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. Gastroenterology. 2012;142:1444–6, e2.
47. Jornayvaz FR, Shulman GI. Diacylglycerol activation of protein kinase C and hepatic insulin resistance. Cell Metab. 2012;15:574–84.
48. Newberry EP, Kennedy SM, Xie Y, Luo J, Crooke RM, Graham MJ, et al. Decreased body weight and hepatic steatosis with altered fatty acid ethanolamide metabolism in aged L-Fabp -/- mice. J Lipid Res. 2012;53:744–54.
49. Kaczocha M, Vivieca S, Sun J, Glaser ST, Deutsch DG. Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. J Biol Chem. 2012;287:3415–24.
50. Liu J, Zhou L, Xiong K, Godlewski G, Mukhopadhyay B, Tam J, et al. Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. Gastroenterology. 2012;142, 1218-1228.e1.
51. Silvestri C, di Marzo V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab. 2013;17:475–90.
52. Auguet T, Berlanga A, Guiu-Jurado E, Terra X, Martinez S, Aguilar C, et al. Endocannabinoid receptors gene expression in morbidly obese women with nonalcoholic fatty liver disease. Biomed Res Int. 2014;2014:502542.
53. Chanda D, Kim Y-H, Kim D-K, Lee M-W, Lee S-Y, Park T-S, et al. Activation of cannabinoid receptor type 1 (Cb1r) disrupts hepatic insulin receptor signaling via cyclic AMP-response element-binding protein H (Crebh)-mediated induction of Lipin1 gene. J Biol Chem. 2012;287:38041–9.
54. Jeong W, Osei-Hyiaman D, Park O, Liu J, Bátkai S, Mukhopadhyay P, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. Cell Metab. 2008;7:227–35.
55. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. Hepatology. 2010;51:679–89.
56. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest. 2000;106:171–6.
57. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005–23.
58. Goldberg IJ, Eckel RH, Abumrad NA. Regulation of fatty acid uptake into tissues: Lipoprotein lipase- and CD36-mediated pathways. J Lipid Res. 2009;50 Suppl:S86–90.
59. Sadur CN, Yost TJ, Eckel RH. Insulin responsiveness of adipose tissue lipoprotein lipase is delayed but preserved in obesity. J Clin Endocrinol Metab. 1984;59:1176–82.
60. Westerbacka J, Kolak M, Kiviluoto T, Arkkila P, Sirén J, Hamsten A, et al. 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;56:2759–65.
61. Greco D, Kotronen A, Westerbacka J, Puig O, Arkkila P, Kiviluoto T, et al. Gene expression in human NAFLD. Am J Physiol Gastrointest Liver Physiol. 2008;294:G1281–7.
62. Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci U S A. 2009;106:15430–5.
63. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A. 2001;98:7522–7.
64. Merkel M, Weinstock PH, Chajek-Shaul T, Radner H, Yin B, Breslow JL, et al. Lipoprotein lipase expression exclusively in liver. A mouse model for metabolism in the neonatal period and during cachexia. J Clin Invest. 1998;102:893–901.
65. Koonen DPY, Jacobs RL, Febbraio M, Young ME, Soltys C-LM, Ong H, et al. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. Diabetes. 2007;56:2863–71.
66. Falcon A, Doege H, Fluitt A, Tsang B, Watson N, Kay MA, et al. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. Am J Physiol Endocrinol Metab. 2010;299:E384–93.
67. 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:G852–8.
68. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. Hepatology. 2004;40:1387–95.
69. 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.
70. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: Summary of an AASLD single topic conference. Hepatology. 2003;37:1202–19.
71. Solís Herruzo JA, García Ruiz I, Pérez Carreras M, Muñoz Yagüe MT. Non-alcoholic fatty liver disease. From insulin resistance to mitochondrial dysfunction. Rev Esp Enferm Dig. 2006;98:844–74.
72. Pessayre D, Mansouri A, Fromenty B. Nonalcoholic steatosis and steatohepatitis. V. Mitochondrial dysfunction in steatohepatitis. Am J Physiol Gastrointest Liver Physiol. 2002;282:G193–9.

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73. Haque WA, Shimomura I, Matsuzawa Y, Garg A. Serum adiponectin and leptin levels in patients with lipodystrophies. J Clin Endocrinol Metab. 2002;87:2395.
74. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: A pilot study. JAMA. 1999;282:1659–64.
75. Schmid AI, Szendroedi J, Chmelik M, Krssák M, Moser E, Roden M. Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. Diabetes Care. 2011;34:448–53.
76. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab. 2011;14:804–10.
77. Safar Zadeh E, Lungu AO, Cochran EK, Brown RJ, Ghany MG, Heller T, et al. The liver diseases of lipodystrophy: the long-term effect of leptin treatment. J Hepatol. 2013;59:131–7.
78. Gorden P, Lupsa BC, Chong AY, Lungu AO. Is there a human model for the metabolic syndrome with a defined aetiology? Diabetologia. 2010;53:1534–6.
79. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. J Biol Chem. 2000;275:8456–60.
80. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature. 1999;401:73–6.
81. Gandotra S, Le Dour C, Bottomley W, Cervera P, Giral P, Reznik Y, et al. Perilipin deficiency and autosomal dominant partial lipodystrophy. N Engl J Med. 2011;364:740–8.
82. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, et al. Leptin-replacement therapy for lipodystrophy. N Engl J Med. 2002;346:570–8.
83. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109:1345–50.
84. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014;59:713–23.
85. Savage DB, Tan GD, Acerini CL, Jebb SA, Agostini M, Gurnell M, et al. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. Diabetes. 2003;52:910–7.
86. Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, et al. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem. 2003;278:34268–76.
87. Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, et al. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. Diabetes. 2002;51:797–802.
88. Luedtke A, Boschmann M, Colpe C, Engeli S, Adams F, Birkenfeld AL, et al. Thiazolidinedione response in familial lipodystrophy patients with LMNA mutations: A case series. Horm Metab Res. 2012;44:306–11.
89. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;1:785–9.
90. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest. 2000;105:311–20.
91. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, et al. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes. 2003;52:2461–74.
92. Kashyap SR, Belfort R, Berria R, Suraamornkul S, Pratipranawatr T, Finlayson J, et al. Discordant effects of a chronic physiological increase in plasma FFA on insulin signaling in healthy subjects with or without a family history of type 2 diabetes. Am J Physiol Endocrinol Metab. 2004;287:E537–46.
93. Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, et al. Dose-response effect of elevated plasma free fatty acid on insulin signaling. Diabetes. 2005;54:1640–8.
94. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: A reexamination. Diabetes. 2000;49:677–83.
95. Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. Curr Diab Rep. 2006;6:177–81.
96. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: Unravelling the mechanism. Lancet. 2010;375:2267–77.
97. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E. Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. Am J Physiol Endocrinol Metab. 2007;292:E1775–81.
98. Cusi K. Nonalcoholic fatty liver disease in type 2 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes. 2009;16:141–9.
99. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: A 1 H NMR spectroscopy study. Diabetologia. 1999;42:113–6.
100. Stefan N, Kantartzis K, Häring H-U. Causes and metabolic consequences of fatty liver. Endocr Rev. 2008;29:939–60.
101. Muoio DM. Intramuscular triacylglycerol and insulin resistance: Guilty as charged or wrongly accused? Biochim Biophys Acta. 2010;1801:281–8.
102. Holland WL, Brozinick JT, Wang L-P, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab. 2007;5:167–79.
103. Watson ML, Coghlan M, Hundal HS. Modulating serine palmitoyl transferase (SPT) expression and activity unveils a crucial role in lipid-induced insulin resistance in rat skeletal muscle cells. Biochem J. 2009;417:791–801.
104. Amati F, Dubé JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, et al. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? Diabetes. 2011;60:2588–97.
105. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest. 1999;103:253–9.
106. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes. 2002;51:2005–11.
107. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010;72:219–46.
108. Lomonaco R, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, et al. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012;55:1389–97.
109. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355:2297–307.

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110. Lomonaco R, Ortiz-Lopez C, Orsak B, Finch J, Webb A, Bril F, et al. Role of ethnicity in overweight and obese patients with nonalcoholic steatohepatitis. Hepatology. 2011;54:837–45.
111. Kim HJ, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch Intern Med. 2004;164:2169–75.
112. 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.
113. Ortiz-Lopez C, Lomonaco R, Orsak B, Finch J, Chang Z, Kochunov VG, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes Care. 2012;35:873–8.
114. 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.
115. Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, et al. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. J Clin Invest. 2000;105:1791–7.
116. Kotani K, Peroni OD, Minokoshi Y, Boss O, Kahn BB. GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid utilization. J Clin Invest. 2004;114:1666–75.
117. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A. 2007;104:12587–94.
118. Rabøl R, Petersen KF, Dufour S, Flannery C, Shulman GI. Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. Proc Natl Acad Sci U S A. 2011;108:13705–9.
119. Haufe S, Engeli S, Budziarek P, Utz W, Schulz-Menger J, Hermsdorf M, et al. Cardiorespiratory fitness and insulin sensitivity in overweight or obese subjects may be linked through intrahepatic lipid content. Diabetes. 2010;59:1640–7.
120. Sullivan S, Kirk EP, Mittendorfer B, Patterson BW, Klein S. Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. Hepatology. 2012;55:1738–45.
121. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang X-M, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med. 2010;362:1082–9.
122. Liska D, Dufour S, Zern TL, Taksali S, Calí AMG, Dziura J, et al. Interethnic differences in muscle, liver and abdominal fat partitioning in obese adolescents. PLoS One. 2007;2:e569.
123. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40:1461–5.
124. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology. 2011;53:1883–94.
125. Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet. 2010;42:21–3.
126. Falleti E, Fabris C, Cmet S, Cussigh A, Bitetto D, Fontanini E, et al. PNPLA3 rs738409 C/G polymorphism in cirrhosis: Relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int. 2011;31:1137–43.
127. Krawczyk M, Bonfrate L, Portincasa P. Nonalcoholic fatty liver disease. Best Pract Res Clin Gastroenterol. 2010;24:695–708, http://dx.doi.org/10.1016/j.bpg.2010.08.005.
128. Kantartzis K, Peter A, Machicao F, Machann J, Wagner S, Königsrainer I, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes. 2009;58:2616–23.
129. Kotronen A, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia. 2009;52:1056–60.
130. Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology. 2010;52:904–12.
131. Park WD, Blackwood C, Mignery GA, Hermodson MA, Lister RM. Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH(2)-terminal sequence analysis. Plant Physiol. 1983;71:156–60.
132. Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. J Lipid Res. 2006;47:1940–9.
133. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science. 2004;306:1383–6.
134. Huang Y, He S, Li JZ, Seo Y-K, Osborne TF, Cohen JC, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. Proc Natl Acad Sci U S A. 2010;107:7892–7.
135. He S, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, et al. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. J Biol Chem. 2010;285:6706–15.
136. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148:852–71.
137. Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem. 2004;279:48968–75.
138. Qiao A, Liang J, Ke Y, Li C, Cui Y, Shen L, et al. Mouse patatin-like phospholipase domain-containing 3 influences systemic lipid and glucose homeostasis. Hepatology. 2011;54:509–21.
139. Basantani MK, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, et al. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. J Lipid Res. 2011;52:318–29.
140. Chen W, Chang B, Li L, Chan L. Patatin-like phospholipase domain-containing 3/adiponutrin deficiency in mice is not associated with fatty liver disease. Hepatology. 2010;52:1134–42.
141. Brown MS, Goldstein JL. Selective versus total insulin resistance: A pathogenic paradox. Cell Metab. 2008;7:95–6.
142. Chavez JA, Summers SA. Lipid oversupply, selective insulin resistance, and lipotoxicity: Molecular mechanisms. Biochim Biophys Acta. 2010;1801:252–65.
143. Niebergall LJ, Jacobs RL, Chaba T, Vance DE. Phosphatidylcholine protects against steatosis in mice but not non-alcoholic steatohepatitis. Biochim Biophys Acta. 2011;1811:1177–85.
144. Jacobs RL, Zhao Y, Koonen DPY, Sletten T, Su B, Lingrell S, et al. Impaired de novo choline synthesis explains why phosphatidylethanolamine N-methyltransferase-deficient mice are protected from diet-induced obesity. J Biol Chem. 2010;285:22403–13.
145. Ruiz R, Jideonwo V, Ahn M, Surendran S, Tagliabracci VS, Hou Y, et al. Sterol regulatory element-binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver. J Biol Chem. 2014;289:5510–7.
146. Benhamed F, Denechaud P, Lemoine M, Robichon C, Moldes M, Bertrand-michel J, et al. The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. J Clin Invest. 2012;122:2176–94.

Cómo citar este artículo: Berlanga A, et al. Papel de las lipasas metabólicas y la lipotoxicidad en el desarrollo de esteatosis hepática y esteatohepatitis no alcohólica. Clin Invest Arterioscl. 2015. http://dx.doi.org/10.1016/j.arteri.2015.03.003

147. Cantley JL, Yoshimura T, Camporez JPG, Zhang D, Jornayvaz FR, Kumashiro N, et al. CGI-58 knockdown sequesters diacylglycerols in lipid droplets/ER-preventing diacylglycerol-mediated hepatic insulin resistance. Proc Natl Acad Sci U S A. 2013;110:1869–74.
148. Birkenfeld AL, Lee H-Y, Majumdar S, Jurczak MJ, Camporez JP, Jornayvaz FR, et al. Influence of the hepatic eukaryotic initiation factor 2 alpha (eIF2alpha) endoplasmic reticulum (ER) stress response pathway on insulin-mediated ER stress and hepatic and peripheral glucose metabolism. J Biol Chem. 2011;286:36163–70.
149. Jurczak MJ, Lee A-H, Jornayvaz FR, Lee H-Y, Birkenfeld AL, Guigni BA, et al. Dissociation of inositol-requiring enzyme (IRE1α)-mediated c-Jun N-terminal kinase activation from hepatic insulin resistance in conditional X-box-binding protein-1 (XBP1) knock-out mice. J Biol Chem. 2012;287:2558–67.
150. Fuchs CD, Claudel T, Trauner M. Role of metabolic lipases and lipolytic metabolites in the pathogenesis of NAFLD. Trends Endocrinol Metab. 2014;25:576–85.
151. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. Nature. 2014;510:84–91.

Cómo citar este artículo: Berlanga A, et al. Papel de las lipasas metabólicas y la lipotoxicidad en el desarrollo de esteatosis hepática y esteatohepatitis no alcohólica. Clin Invest Arterioscl. 2015. http://dx.doi.org/10.1016/j.arteri.2015.03.003

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