Chapter 5

Animal Models of Obesity Characterized by Non-alcoholic Fatty Liver Disease (NAFLD)

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http://dx.doi.org/10.5772/intechopen.72707

Abstract

Obesity is one of the major risk factors for the Nonalcoholic Fatty Liver Disease (NAFLD) development, as the leading cause of chronic liver disease. NAFLD is intrinsically related to obesity disorders, especially insulin resistance and dyslipidemia. Interaction between NAFLD and obesity still needs further clarification, and it is necessary to dertemine the mechanisms of these disorders in animal models of disease. Such models are usually the result of genetic and/or nutritional modifications, considering metabolic and histological changes commonly seen in humans. Obesity induced in rodents occur mainly through HFD, HCD, FFD or genetic alterations like in Lep, Acox, KKy models. These models are analogous to NAFLD development, since the increasing visceral fat is highly associated with the accumulation of fat in the form of triglycerides in the liver. Inflammatory markers such as TNF-alpha and IR are active in the predisposition of lipolysis. Hepatic inflammation during NAFLD can also be unleashed by oxidative stress. However, the mechanisms involved in the progression from NAFLD to NASH are not yet elucidated, as some models have shown unexpected outcomes such as severe malnutrition or obesity markers absence and IR after the use of Minimal-change disease (MCD) therapies and drugs, respectively. Thus, it is important to evaluate different animal models of obesity able to induce the profile of NAFLD and NASH disease in humans, assessing their mechanisms of action. The aim of this chapter is to have a comparative analysis of animal models commonly used in the pathophysiology of obesity that present NAFLD/NASH.

Keywords: animal models, obesity, NAFLD, NASH, fatty liver

1. Introduction

The rapid increase in the rate of obesity is a health problem critical in developed countries. Obesity is associated with a number of health problems that are often summarized together as metabolic syndrome and involve the development of insulin resistance, type 2 diabetes, cardiovascular disease and fatty liver disease [1].

Nonalcoholic Fatty Liver Disease (NAFLD) is the leading cause of chronic liver disease affecting 20–30% of the world's adult population, and is characterized by a buildup of fat, mainly in the form of triglycerides, in the hepatocyte cytoplasm, exceeding 5–10% of the cell weight, verified histologically or by imaging techniques. It requires exclusion of other causes of steatosis, such as excessive alcohol consumption, drugs or genetic diseases [2, 3]. About 20–30% of individuals with NAFLD can develop Non-alcoholic steatohepatitis (NASH) [4], a more severe disease condition related to metabolic abnormalities associated with obesity, namely hyperinsulinemia, dyslipidemia, and ectopic lipid accumulation [5]. More specifically, NASH is associated with lobular inflammation, hepatocellular damage and/or hepatic fibrosis [4].

NAFLD is common in Western countries, usually associated with the main characteristics of the metabolic syndrome, such as obesity, insulin resistance and hyperlipidemia [6]. Data from the U.S. National Health and Nutrition Examination Surveys, collected from 1988 to 2008, show a 2-fold higher prevalence of NAFLD, concomitant with the increased or increasing prevalence of metabolic conditions such as obesity and insulin resistance [7].

In this context, obesity induction with the development of hepatic steatosis in animal models is discussed. Animal models of obesity focused on NAFLD and NASH, as well as pathophysiological aspects related to obesity and liver diseases were systematically addressed in this chapter.

2. NAFLD pathophysiology aspects

The main mechanism related to the development of hepatic steatosis are: increased supply of free fatty acids due to increased lipolysis of visceral/subcutaneous adipose tissue and/or increased lipid intake; reduction of free fatty acid oxidation; increased hepatic de novo lipogenesis; and decreased hepatic secretion of very low density lipoprotein (VLDL). In addition, during NAFLD there is an imbalance between the intrahepatic production of triglycerides (TG) (derived mainly from plasma fatty acids delivered to the liver and not oxidized as a fuel) and the removal of intrahepatic TG (mainly exported from the liver to very low density lipoproteins, VLDL-TG) [8, 9], as shown in **Figure 1**.

TGs are the lipids that accumulate most in hepatocytes in NAFLD. TGs are synthesized through various enzymatic steps of glycerol and fatty acids condensation after activation thereof to their acyl-CoA esters. The liver obtains fatty acids from the circulation from the hydrolysis (lipolysis) of triglycerides in adipocytes in the post-absorptive state and, to a lesser degree, the postprandial lipolysis of triglyceride-rich particles (chylomicrons and VLDL). Fatty acids are released from triglyceride stores in adipose tissue through the action of the sensitive hormone lipase (located in adipocytes). These fatty acids upon their release into circulation are bound to

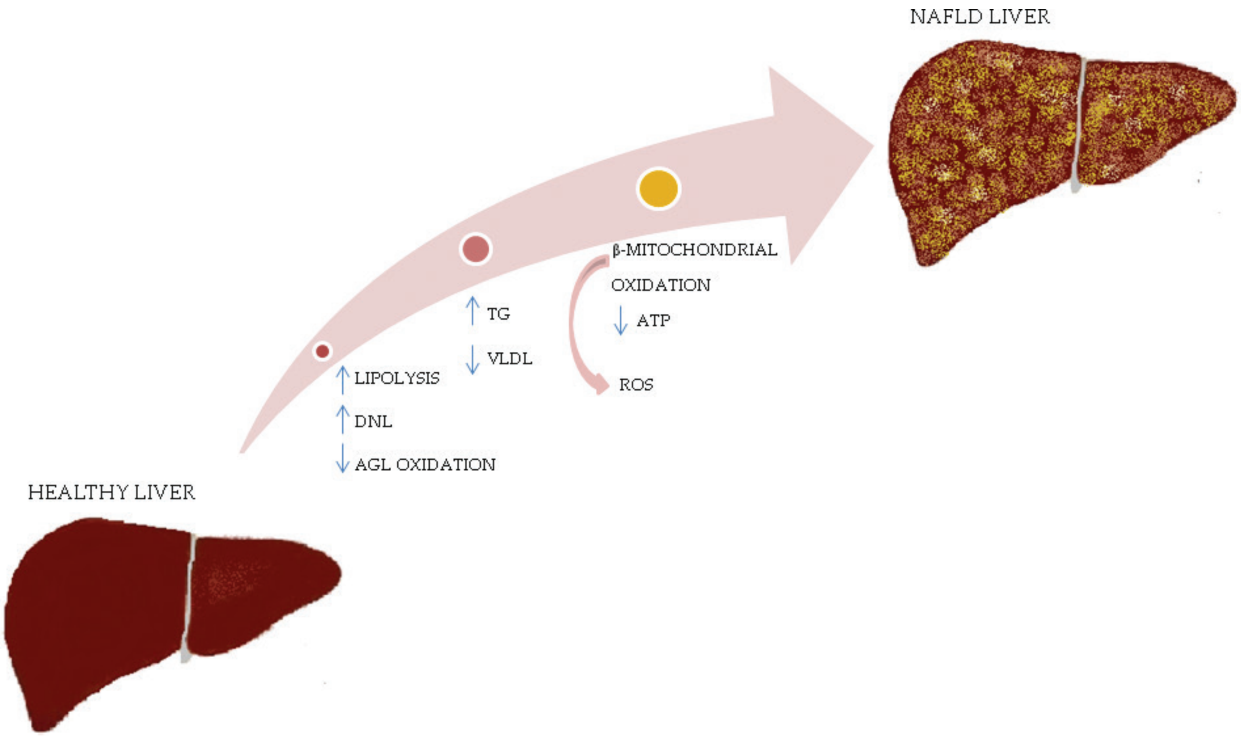

Figure 1. NAFLD mechanisms. Associated factors such as increased lipolysis and de novo hepatic lipogenesis (DNL), decreased free fatty acid oxidation and hepatic secretion of low density lipoprotein (VLDL) aggravate the onset of the disease. The action of Mitochondrial β-oxidation contributes to the synthesis of adenosine triphosphate (ATP), however results in the increase of reactive oxygen species.

albumin. The peripheral tissues, in turn, receive fatty acids as substrates for oxidation (mainly muscle) and storage (adipose tissue), through the action of endothelial lipoprotein lipase on particles rich in circulating triglycerides, either VLDL secreted by the liver, or chylomicrons delivered to lymphatic circulation after intestinal absorption of lipids [10].

During fat digestion, medium and short chain fatty acids are absorbed directly into the portal circulation. Whereas long chain fatty acids ($C > 14$) are mainly reesterified in chylomicrons, but a proportion of long chain unsaturated free fatty acids (FFA) enters the portal circulation [10].

According to the hypothesis on hepatic lipotoxic lesions, certain FFAs and their metabolites flow through the liver and cause NAFLD/NASH. Dietary intake plays an important role in the generation of FFA. Excess carbohydrate consumption, particularly fructose, leads to *de novo* lipogenesis. Excess calories and fats can result in accumulation of lipids in adipose tissue, and finally, the stored fatty acids are released through lipolysis. Free fatty acids generated by lipogenesis, lipolysis or other mechanisms have three potential destinations: triglycerides formation, oxidation and elimination, or intermediate lipid formation [11].

Hepatic mitochondria play an important role in the oxidation of fatty acids and in the synthesis of ATP. Mitochondrial β-oxidation is a pathway for the elimination of fatty acids, but results in the generation of reactive oxygen species (ROS). In most circumstances, the endogenous antioxidant mechanisms are able to protect against cellular damage caused by ROS. However, in the configuration of impaired mitochondrial function related to obesity and chronic lipid overload, ROS lead to peroxidation of fatty acids, further interfering in mitochondrial function through oxidative damage to mitochondrial DNA and proteins [12].

The reason some obese individuals are able to regulate mitochondrial function and compensate lipid overload, while others are not, are still unclear. Multiple pathways involving a complex interaction between excess lipids, systemic inflammation and cellular stress probably contribute to the development and progression of NAFLD [13].

2.1. Crosstalk between obesity and NAFLD

Obesity is an important risk factor for non-alcoholic fatty liver disease (NAFLD). Although it is not a risk factor present in all obese individuals, a minority of patients with NAFLD are lean. In the survey conducted by the National Health and Nutrition Examination Survey III, 7.4% of lean adults and 27.8% of overweight/obese adults had hepatic steatosis that could be detected by ultrasound, which highlights the higher prevalence in overweight or obessed individuals. [14].

The pathophysiology of NAFLD is complex, since it is a multifactorial disease, whose disorders contribute to the metabolic syndrome, involving obesity, diabetes mellitus, hypertension and dyslipidemia [15]. Patients with NAFLD and metabolic syndrome (MS) have a higher prevalence and severity of fibrosis and necroinflammatory activity, compared to individuals of NAFLD without MS. In addition, the presence of MS is associated with a high risk of NASH among NAFLD individuals, after correction for gender, age and body mass [15].

Insulin resistance, an important feature of MS and type 2 diabetes mellitus (DM 2), is classified as peripheral insulin resistance and/or hepatic insulin resistance [16, 17]. Peripheral insulin resistance refers primarily to decreased insulin-mediated glucose uptake in skeletal muscle and adipocytes, whereas hepatic insulin resistance relates to the inability of insulin to decrease hepatic glucose production. The accumulation of fat in skeletal muscle has been considered the main pathogenic event leading to resistance to peripheral insulin. Briefly, the accumulation of arachidonic acid fatty acid metabolites in the muscle initiates a series of signaling reactions that increase the phosphorylation of specific serine residues (e.g, S307) on the insulin-1 receptor substrate (IRS-1) in the muscle. However, several researchers have begun to focus on the mechanism of hepatic insulin resistance [16, 17].

The IRS-1 is critical in its signaling, since it is an important target in the inflammatory process and, following serine phosphorylation of IRS-1, there is a reduction in the activation of phosphotidylinositol-3-kinase (PI3k), as well as other proteins involved in the normal insulin signaling process, such as protein kinase B (Akt). These deleterious events are shown to be mediated by proteins that activate inflammatory pathways, such as the c-Jun NH2-terminal kinase (JNK), the kinase IκB (Iκκβ) and PKCθ [18–21]. NFκB is a gene transcription factor that alters insulin signaling and after stimulation, Iκκβ is phosphorylated, leading to the translocation of NFκB to the cell nucleus and subsequent activation of proinflammatory cytokine genes, such as TNF-α, IL- 6 and IL-1β [22–24].

The mechanism of resistance to peripheral insulin point to the fat-induced induction of several inflammatory signaling kinases (PKCθ, IKK-β and JNK1), which may in turn increase serine phosphorylation in IRS-1, thus preventing their participation in the insulin signaling cascade. As a result, the insulin capacity to increase GLUT4 translocation is impaired. The mechanisms of fat-induced hepatic insulin resistance are similar to peripheral resistance.

Thus, accumulation of fat within the liver results in a blockage in insulin signaling in IRS-2. This proximal blockade in the insulin signaling cascade may limit the ability of insulin to activate hepatic glycogen synthesis and suppress hepatic glucose production, involving the role of PKC, IKK-β and/or JNK1 in the pathogenesis of hepatic insulin resistance induced by fat [17]. Based on a study of patients with diabetes, accumulation of fat in the liver may also be associated with increased hepatic gluconeogenesis [25].

In addition, the hormonal and biological activity of adipose tissue through the secretion of adipokines by white adipose tissue (WAT) contributes to the insulin and metabolic resistance of the disease, including NASH. This is due to the release of inflammatory cytokines by macrophages in the adipose tissue and the increased release of free fatty acids from adipocytes [12].

In the context of chronic overfeeding, white adipose tissue becomes expanded with lipids, leading to adipocyte hyperplasia or hypertrophy. The remodeling of white adipose tissue helps to accommodate adipocyte hypertrophy, but, eventually, impaired innervation and vascularization result in hypoxia and adipocyte dysfunction [26]. This dysfunction would increase adipocyte secretion mediated by JNK1 - inflammatory cytokines [27], resulting in increases and changes of immune cells in adipose tissue and eventual systemic metabolic stress with mitochondrial dysfunction, lipolysis, decreased lipid storage capacity and signaling rupture of insulin [28].

In addition, the Western dietary pattern with excessive intake of high-calorie, rich in fat, sugar and cholesterol and sedentary lifestyle are risk factors for hepatic steatosis. Such factors have an impact on lipid metabolism related to liver diseases, which include decreased conversion of cholesterol to bile acids; increased hydrolysis of cholesterol esters to free cholesterol [29]; increased endogenous cholesterol synthesis [30]; increased absorption of cholesterol-rich lipoproteins [29]; and decreased cholesterol excretion [31]. Excess cholesterol influences the fluidity of the membrane, affecting the function of its proteins [32]. It is also worth mentioning that this excessive consumption activates Kupffer cells and hepatic stellate cells, exacerbating liver inflammation, increasing extracellular matrix synthesis and eventually accelerating progress for NASH [33, 34].

3. Developing animal models of non-alcoholic hepatic steatosis

The progress of obesity with the presence of the NAFLD disorder is characterized as a very complex inflammatory process. Despite the search for new studies in the area, there are a number of challenges in conducting research with humans, especially to investigate genetic and dietary aspects. Controlling the diet and environment of humans becomes difficult for long periods of time, and ethical restrictions limit access to biological samples. Such problems can be circumvented with the use of animal models of disease [35].

Animal models are extremely important for elucidating the etiology of diseases in humans, besides having an integrated view of the pathogenic mechanisms, as well as monitoring the natural evolution of the disease under controlled genetic and environmental conditions. These models constitute important resources in the identification of targets and therapeutic agents that can prevent or revert diseases [36].

The choice of the animal model for studies of obesity is comprised mostly of small animals such as mice, rats, guinea pigs, hamsters, with the genetic, neuroendocrine and dietary alterations.

In these models, the disease can be induced through specific diets or with the use of chemical substances and may result in non-alcoholic hepatic steatosis [37–39].

Despite the diversity of existing models, it is of utmost importance that they can replicate the histological patterns and pathophysiological mechanisms characteristic of each stage of NAFLD in humans, develop comorbidities associated with this disease such as increase of body weight, peripheral resistance to insulin, dyslipidemia, release of adipokines by adipose tissue, among others [40].

Mice and rats are mammals belonging to the Muridae family and the Murinae subfamily, order Rodentia and genus of the mouse *Mus*, whose scientific name *Mus musculus/domesticus* and the rat of the genus *Rattus* and the species *Rattus Norvegicus/rattus*. Both are heavily used in research due to their practical handling and playback performance in short period of time. Its lineage classification may be as genetically variable (heterozygous) termed outbreed or genetically defined inbreed. Outbreds are non-consanguineous and heterogeneous, which brings them closer to representing natural populations, larger litters and lower mortality rate, for example the Swiss colony (NIH, Webster, ICR and CD-1) [41]. Conversely, inbreds are consanguineous and isogenic, from crossbreeding between siblings which determine them to be identical, such as C57BL/ J6, BALB/ C, C3H, FVB, 129, DBA and CBA [42, 43].

Controlled expression of some genes results in the animal model called transgenic, they are susceptible to genome changes with specific DNA fragments, may have a mutated or increased gene, and result in a genetic modification that is transmissible to their offspring. “Knockout” animals, i.e. animals having a knockout gene, have a total or partial sequence of a withdrawn gene, contributing to determine a pathology. And “knock-in” animals acquire a total or partial sequence of a gene introduced in their genome predisposing it to a certain pathology by excess of the gene product [44, 45].

Animals with genetic obesity or induced obesity (**Table 1**) present non-alcoholic hepatic steatosis, either by genetic alteration (**Table 2**) and increased lipid synthesis as in *ob/ob* mice, the *db/db* rat, agouti (obese yellow); and environmental factors such as diets deficient in methionine or choline, rich in carbohydrates or lipids and can also be combined with genetic alterations [46].

3.1. Genetic models

3.1.1. ob/ob mice

The *ob/ob* mice are spontaneous, obese mutants that do not have the Leptin gene (ob) which is autosomal recessive located on chromosome 6. From 4 weeks of age they are able to triple the normal weight when they are exposed to open offer of food due to lack of satiety caused by the absence of the hormone leptin. They are hypothermic and unable to stay warm. Its characteristics go beyond obesity, including hyperphagia, diabetes and non-alcoholic hepatic steatosis that presents with 10–12 weeks of life. The *ob/ob* is considered a good model of obesity linked hepatic steatosis, since metabolism of lipids and carbohydrates is related to the amount of white adipose tissue (WAT), which is increased in obesity. WAT in abundance reflects on increased expression of TNF-alpha and lipolysis, whose fatty acids are released to the liver for deposition [47, 48]. In this model, for hepatic steatoectomy, regarded as a second stimulus or agent (“second hit” such as ischemia induction) specific drugs or methionine and choline-deficient diets, is needed [46, 49, 50].

Diet	Concentration/nutrient	Animal model	Follow-up	Obesity	NAFLD	IR	NASH
HFD SFAs +Cholesterol	15% +1%	C57BL/J6	30 weeks	Yes	Yes	No	Yes
	30% +2%	SD	48 weeks	Yes	Yes	Yes	Yes
	49%	C57BL/J6	3 months	Yes	Yes	No	No
HCD	30% fructose	C57BL/J6	4 months	Yes	Yes	Yes	No
	70% fructose	Wistar	5 weeks	Yes	Yes	No	Yes
	65% sacarose	C57BL/J6	8 weeks	Yes	Yes	Yes	No
	70% sacarose	Wistar	4 weeks	Yes	Yes	No	No
	30% fructose/glicose/sacarose	Sprague Dawley	4 months	Yes	Yes	Yes	No
FF	40% HCD + 2% cholesterol e fructose drink	C57BL/J6	25 weeks	Yes	Yes	Yes	Yes

Table 1. Characteristics of obesity/NAFLD diet composition in various animal models.

The ob/ob model behaves similarly to the methionine and choline deficiency models, since they affect oxidative stress, lipid peroxidation, and cell death. They are characterized by a chronic subclinical inflammatory condition with constant release of proinflammatory cytokines, monocyte infiltration of reactive oxygen species (ROS), oxidation of lipid molecules, cholesterol and proteins present in low density lipoprotein (LDL). This is in addition to mitochondrial DNA damage (measured by the mitochondrial levels of 8-hydroxy-2′-deoxyguanosine) and reduced expression of the DNA mismatch repair enzyme [51, 52]. Lipid peroxidation of polyunsaturated fatty acids generates by-products of toxic aldehydes, including malondaldehyde and hydroxynonenal, which are more persistent than ROS and damage more distant intracellular organelles which can cause cell death. These products activate fibrous hepatic stellate cells and are chemotactic for neutrophils [53, 54].

Genetic models	Genetic alteration	Characteristics	Considerations	"Second hit": NASH
ob ob	Absence of the leptin gene	Hyperphagia, Obesity, diabetes, IR and NAFLD	10–12 weeks is confirmed NAFLD.	Hepatotoxic stimuli, specifics diets
db db	Deficient in leptin receptor	Obesity, IR, and NAFLD	3 months is confirmed NAFLD. Features similar to Nafld in humans.	Diet HFD or MCD
KK-AY	knockout for melanocortin receptor 4	Obesity, hyperphagia, hyperinsulinemia, hyperglycaemia and NAFLD	Altered gene expression of lipid metabolism.	Diet MCD, a HFD or use of endotoxin
ACOX	knockout mice for the enzyme acyl-coenzyme A oxidase	Accumulation of fat at liver	1 week presented microvesicular steatosis. At 2 months, livers show extensive steatosis.	Not necessary

Table 2. Characteristics of mouse genetic models for the development of NAFLD.

3.1.2. *db/db* mice and Zucker fa/fa rats and Koletsky *f/f*

Obese Zucker fa/fa rats and the *db/db* mouse are deficient in leptin receptor function, resulting from mutations in the leptin gene which occur on chromosome 4. Quite close phenotypically to these models is the obese Koletsky *f/f* rat which has the leptin deficiency with reduced energy expenditure and neuropeptidergic alterations of the hypothalamus [55].

Obese fa/fa Zucker rats fed with high fat diet (HFD) made with 60% of saturated lipids for 8 weeks, confirmed the occurance of hyperglycemia and hepatic steatosis. NADPH oxidase activity increased 2.5-fold leading to hepatic liver injury of the animals thus contributing to the progression of NASH [56].

3.1.3. KK-ay mice and knockout for melanocortin receptor 4

The KK-Ay genotype results from the crossing of KK diabetic rats with yellow coat and agouti background (Ay) [57], they develop obesity due to the antagonistic action of the agouti protein in the central nervous system capable of promoting alimentary hyperphagia and consequent obesity [58]. Changes in the genes of the agouti rat and in the encoding gene of the melanocortin receptor 4 (MC4R) are related to the involvement of the melanocortins system in the pathogenesis of NAFLD [59]. They present indicators of resistance to insulin and leptin, conditions that favor the appearance of hepatic steatosis. Also the genetic depletion of MC4R is associated with a severe obesity phenotype such as hyperphagia, hyperinsulinemia, hyperglycemia and hyperleptinemia [60]. To progress to NASH, a “second hit” is needed again as an example: diet MCD, a HFD or use of endotoxin [61].

3.1.4. ACOX and knockout mice for the enzyme acyl-coenzyme A oxidase

The enzyme acyl-coenzyme A oxidase-1 (ACOX 1) is the first enzyme of peroxisomal β-oxidation of long chain fatty acids. Peroxisomal and mitochondrial fatty acid beta-oxidation occuring in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase (ACOX−/−) exhibits a wide range of microvesicular steatohepatitis. These animals develop NAFLD due to the accumulation of fat generated by impaired β-oxidation [62]. At 1 week of age, the liver of the AOX−/− mice already presented microvesicular steatosis, which intensifies at 2 months of age to inflammatory infiltration [63]. However, when they are 6 to 8 months old, the liver of these mice exhibits reversal of steatosis from hepatocyte regeneration and also exhibits growth retardation [64].

3.2. Animal models induced by diets

3.2.1. High fat diet (HFD)

High fat diet (HFD) treated animals acquire obesity and also increased epididymal fat, hyperglycemia and insulin resistance, because these associated comorbidities are also widely used as a method of inducing hepatic steatosis, as they cause liver changes similar to that of human disease. Usually fat sources such as lard and soybean oil are used, different concentrations of lipids are taken into account in the preparation, varying in proportion from 40 to 60%. The most commonly used rodents are C57BL/6, Swiss, Sprague Dawley, Wistar and SHR [62, 65].

A higher fat supply leads to increased adiposity in adipose tissue, frequent stimulation of pro-inflammatory cytokines secretion, increased free fatty acids, insulin resistance, and lipolysis, with consequent increase in the transport of free fatty acids to the liver via the portal vein and increased intake of fatty acids. In the liver, hyperinsulinemia inhibits beta-oxidation, reducing the output of fatty acids, leading to unanticipated the accumulation of triglycerides in the cytoplasm of hepatocytes which hallmarks NAFLD. Both actions favor the accumulation of fat in the hepatocytes, a condition that promotes hepatic resistance to the action of insulin. Loss of insulin's ability to suppress hepatic glucose production aggravates overall insulin resistance and exacerbates the manifestation of metabolic syndrome components [66–69].

The addition of cholesterol along with saturated fatty acids has shown to have a good disease predisposition, with progression of NAFLD to NASH. Savard et al. [70] tested the effect of this diet on the respective proportions of saturated fatty acids and cholesterol on the C57BL/J6 model for 30 weeks: control (4% fat and 0% cholesterol); high cholesterol[HC] (4% fat and 1% cholesterol); high fat[HF] (15% fat and 0% cholesterol); and high fat, high cholesterol[HFHC] (15% fat and 1% cholesterol). The animals treated with HCHC showed the highest weight gain, hepatic lipid content, inflammation of adipose tissue and reduction in adiponectin plasma levels, leading to NAFLD in a profound way, as they developed macrovesicular steatosis (grade 3) associated with inflammatory spots (grade 2) and peripheral fibrosis, with these effects being twice as large as in the HC and HF groups [70].

Similarly, adult Sprague Dawley rats fed HFD made with 30% lipids (lard and 2% cholesterol) were evaluated at 4, 8, 12, 16, 24, 36, 48 weeks intervals. At week 8 body weight and epididymal fat weight began to increase, which was associated with increased serum levels of free fatty acids, cholesterol and TNF-α, as well as the development of NAFLD fatty liver. Steatohepatitis occurred between weeks 12 and 48. Apparent hepatic fibrosis did not occur until week 24, and went from week 36 to 48 with insulin resistance reproducing the pathological sequence of events typical of human NASH [71].

Obesity models induced by HFD consumption have also been characterized by inflammation in peripheral tissues as well as in hypothalamic areas critical for energy homeostasis, in an attempt to interrupt body weight control and glucose homeostasis [72]. According to Thaler et al. [73] unlike inflammation in peripheral tissues, which develops as a consequence of obesity, inflammatory signaling of the hypothalamus is confirmed in rats and mice within 1 to 3 days after initiation of HFD treatment, ie before substantial weight gain. In addition, both reactive glucose and markers suggestive of neuronal injury were evident in the arched hypothalamic nuclei of rodents in the first week of dietary feeding, leading to the knowledge that obesity is associated with neuronal injury in an area of the brain, suggesting a crucial aspect for the control of body weight [73]. Induction of FHD obesity in AFasKO mice with death receptor Fas deficiency (also known as CD95), specifically in adipocyte cells, were protected from adipose tissue inflammation and also from hepatic steatosis (more sensitive to insulin, both at the level of the body and in the liver) and hepatic insulin resistance [74].

In C57BL/6 mouse fed with HF diet (49% of lipids) during gestation and/or lactation, or both, the presence of non-alcoholic fatty liver steatosis was verified by expression of protein-1c binding to the sterol regulatory element. There was an exacerbation of NAFLD phenotype in utero and during lactation, demonstrating the development of hepatic steatosis already in fetal life [75].

3.2.2. High carbohydrate diet (HCD)

Prolonged consumption of HCD causes obesity and non-alcoholic fatty liver disease (NAFLD), in addition to oxidative stress in the liver and insulin resistance. The most common sources of simple carbohydrates in the diet are fructose, glucose and sucrose, for example the addition of corn syrup (50–90% fructose) or refined sugar (50% fructose). Fructose is highly lipogenic and has been more widely used than sucrose and glucose, however, induction of chronic models in NAFLD requires longer treatment periods [76–78]. Fructose is primarily metabolized in the liver without the need for insulin, its phosphorylation consumes ATP and accumulates ADP thus stimulating the formation of uric acid and reactive oxygen species (ROS) which rapidly increases the synthesis and hepatic deposition of triglycerides leading to a fatty liver in rodents [79–81].

Treatment with a 30% fructose, glucose and sucrose diet for 4 months in Sprague Dawley rats demonstrated that such treatment induced metabolic syndrome, intrahepatic accumulation of uric acid and triglycerides, increased MCP-1 and TNF-alpha, as well as hepatic steatosis [82]. This simple carbohydrate intake is associated with a greater translocation of the endotoxin from the intestine to the portal vein, ROS formation in the liver (due to the greater oxidation of fatty acids in the hepatocytes) and induction of the TNF-alpha factor. TNF-alpha has been associated with the development of NAFLD because it is involved in the dysregulation of hepatic lipid metabolism and in insulin signaling [83, 84].

The C57BL/6 mouse model fed a high calorie and sucrose diet (65%) for 8 weeks showed obesity, insulin resistance and macrovesicular steatosis [85]. In another study with C57BL/J6 mice with TNF-alpha receptor 1/− TNFR1/− (sterol regulatory element-binding protein 1) were protected against the onset of hepatic steatosis and also the insulin resistance induced by HCD with fructose (30%), this result was associated with increased phosphate levels of adenosine monophosphate-activated protein kinase (AMPK) and protein kinase (AKT), decreased expression of SREBP-1, fatty acid synthesis in the liver and decreased levels of retinol binding protein (RBP4) that behaved differently from the control group [86].

As with Wistar rats, the effect of the sucrose diet (70%) when compared to starch (70%) for 28 days resulted in a significant difference in the group that received sucrose, thus the amount of hepatic fat and serum fructosamine concentration was increased in sucrose diet group and in both hepatic steatosis confirmed in the two groups [87]. However, when using HCD with 70% fructose for 5 weeks, obesity, elevated levels of hepatic triglycerides, macrovesicular steatosis, lobular inflammation (as were observed [88]. Another report with the same animal model in (HCD) added 10% of fructose (corn syrup) in drinking water and evaluated at 7, 14 and 21 days. At the start of treatment, an increase in triglycerides,oxidative stress and hepatic sensitivity to hyperinsulinemia (β-cell reaction) were was observed in serum and liver, suggesting that this increase is related to metabolism that occurred in the liver and probably in the adipose tissue as well [89].

Epidemiological studies have shown that increasing the intake of fructose mono- or disaccharides by humans is a considerable risk factor for NALFD [90, 91], and it is estimated that patients with fructosemia present a fructose consumption about 2–3 times higher than healthy individuals or with other liver diseases [92]. These data stimulated the study of experimental models of NAFLD induced by fructose.

3.2.3. *Fast food diet (FF)*

Charlton et al. [93] proposed the mouse fast food diet model. C57BL/6 mice were fed for 25 weeks on a diet composed of 40% fat (12% saturated fatty acids, 2% cholesterol) and the water offered contained corn syrup, it was found that the animals developed obesity and resistance to insulin. I Other studies used the same model and also stated that the intake of FF diet in the form of emulsion for 6 weeks led to the onset of hepatic steatosis and inflammation, and elevated levels of endotoxemia and glycemia [86].

This diet model was very similar to the Western diet, since it presents high concentrations of fat, fructose and cholesterol, appearing as a good model related to human diets and with the capacity to induce obesity, NAFLD among other comorbidities in rodents.

3.3. Combined models (genetic and environmental)

The use of the C57BL LDLr male mouse (low density lipoprotein receptor deficient) treated for 21 weeks with HFD, was associated with weight gain, macrovesicular steatosis and lobular inflammation. Inflammation in adipose tissue and liver provides a positive attenuation for studies of obesity and associated cardiometabolic diseases such as NAFLD and atherosclerosis [94].

db/db mice subjected to methionine and choline deficient diet (MCD) showed macrovesicular steatosis, and increased hepatic collagen type 1 mRNA levels in comparison to the control group. This outcome suggests an important model for the study of NASH, i.e. establishing obesity, diabetes, insulin resistance and dietary MCD results in steatohepatitis indicating leptin activity in liver fibrosis. In this way, an interesting comparison is made with the *ob/ob* model that is deficient in leptin which also develops steatohepatitis but not hepatic fibrosis when fed with the MCD diet [95].

The verification in the obese and diabetic C57BI/6-A and KK-Ay models of diet treatment (MCD) for 8 weeks demonstrated that KK-Ay rats exhibited increased susceptibility to steatohepatitis and inflammatory infiltration as well as increased levels of TNFα mRNA and lipid peroxidation in the liver where hypoadiponectinemia probably played a key role in the exacerbation of inflammatory and profibrogenic responses [96].

The treatment of the KK-Ay (MC4R) model with hyperlipidic diets at concentrations of 35 and 60% of lipids derived from soybean oil and lard, develop severe hepatic steatosis and show liver changes in the lipogenic gene profile [97]. Another study has shown that the exposure of these animals to 60% HFD for 1 year leads to the appearance of more severe forms of NAFLD such as NASH, fibrosis and hepatocellular cancer, as well as leading to the development of systemic metabolic alterations very similar to those observed in humans [98]. This same model was used for a study of hepatic lesions induced by D-galactosamine/lipopolysaccharide LPS (GalN/LPS) endotoxin, there were significant increases in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood, apoptotic and necrotic changes in hepatocytes and/or showed a high degree of lethality. GalN/LPS-induced liver injury was more pronounced in KK-Ay obese than in the control group [61]. LPS is a key component of many bacteria present in the microbiota, plays a central role in innate immune responses and is considered the second hit in NASH models [99].

4. Conclusion

It is very clear that mechanisms responsible for the development of NAFLD, which can occur through a diet, typically western diet, rich in saturated fat and fructose, in addition to other simple sugars, promotes metabolic disorders. This initially affects processes such as liver lipogenesis, resistance to insulin, and even as it is now known, negative consequences due to an unbalanced microbiota, with greater release of LPS. Studies on the mechanisms involved in the development of NAFLD and progression to NASH has steadily increased, due to the increasing number of obesity and liver disorders worldwide. The combination of factors that interfere with etiology such as weight gain, dietary ingredients and genetics are factors that further instigate the urgency to elucidate its effects on the liver using animal models of human diseases.

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