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Pathophysiology of NASH in endocrine diseases

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the industrialized world. NAFLD encompasses a whole spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. The latter can lead to hepatocellular carcinoma. Furthermore, NASH is the most rapidly increasing indication for liver transplantation in western countries and therefore represents a global health issue. The pathophysiology of NASH is complex and includes multiple parallel hits. NASH is notably characterized by steatosis as well as evidence of hepatocyte injury and inflammation, with or without fibrosis. NASH is frequently associated with type 2 diabetes and conditions associated with insulin resistance. Moreover, NASH may also be found in many other endocrine diseases such as polycystic ovary syndrome, hypothyroidism, male hypogonadism, growth hormone deficiency or glucocorticoid excess, for example. In this review, we will discuss the pathophysiology of NASH associated with different endocrinopathies.

Keywords: NAFLD; NASH; endocrine diseases; insulin resistance.

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Introduction

Nonalcoholic steatohepatitis (NASH) is part of a disease spectrum, nonalcoholic fatty liver disease (NAFLD), which ranges from simple steatosis to fibrosis and ultimately cirrhosis [1-4]. NASH is therefore a progressive subtype of NAFLD that can result in cirrhosis, hepatocellular carcinoma and liver-related mortality. Importantly, hepatic fibrosis is the only histologic feature of NASH independently associated with long-term overall mortality, liver transplantation and liver-related mortality [5]. Validated drugs to treat NASH are still lacking, although numerous studies are underway [6]. Interestingly, numerous endocrine diseases other than type 2 diabetes are also associated with NAFLD and NASH [7-9]. The aim of this review is to present different endocrine diseases that may result in the development of NASH and discuss their underlying pathophysiology.

Epidemiology

NAFLD is now the most common cause of chronic liver disease in western countries, affecting approximately 30% of the general population, and its worldwide prevalence continues to increase concurrent due to the growing obesity epidemic [10]. As such, NAFLD is projected to become the most common indication leading to liver transplantation in the United States soon [11]. The prevalence of NAFLD can reach 90-95% in obese individuals and affects up to 70% of patients with type 2 [12-14].

The estimation of NASH prevalence at the population level is difficult because diagnosis requires a liver biopsy, which is infrequently performed. Indirect estimates suggest that NASH affects 3% to 6% of the US population, with an increased prevalence in patients with metabolic diseases and obesity. Although often clinically silent, NASH progresses to cirrhosis in approximately 20% of cases and is associated with increased rates of liver-specific and overall mortality [15].

Diagnosis

Liver biopsy is the gold standard for the diagnosis of NAFLD [10]. NAFLD is defined by the accumulation of fat in the liver with ≥ 5% of hepatocytes containing visible intracellular triglycerides or steatosis, affecting at least 5% of the liver volume or weight. NAFLD is a diagnosis of exclusion. Indeed, alcohol consumption should be below 30 g (= 3 units) per day for men and 20 g (= 2 units) per day for women. As a remainder, one unit of alcohol (= 10 g) is defined as 1 glass of beer (25 cl), 1 glass of wine (20 cl) or 1 glass of spirit (3 cl). Other diseases have to be considered before the diagnosis of NAFLD/NASH can be made, such as autoimmune liver disease, viral hepatitis infection, hemochromatosis, Wilson's disease, or drug consumption [16]. Simple hepatic steatosis can progress to NASH if the causative factors persist. NASH is characterized morphologically by steatosis, ballooning hepatocytes, inflammation, with or without fibrosis. NASH itself can continue to progress to cirrhosis and hepatocellular carcinoma [17, 18].

NAFLD can be considered as the hepatic manifestation of the metabolic syndrome [3]. The Metabolic Syndrome is defined by the presence of any 3 of the 5 following risk factors: elevated waist circumference, elevated triglycerides (≥ 1.7 mmol/l), reduced HDL-cholesterol (<1.0 mmol/l in males and < 1.3 mmol/l in females), elevated blood pressure (systolic ≥ 130 and/or diastolic ≥ 85 mmHg) and elevated fasting glucose (≥ 100 mg/dl) [3, 19, 20].

Whereas liver biopsy is required for the diagnosis of NASH, NAFLD can also be evaluated non-invasively by imaging techniques such as ultrasound, computed tomography or magnetic resonance imaging. Transient elastography (eg. FibroScan) is being widely used combined with different scores, such as NAFLD fibrosis score (NFS) or Fibrosis-4 (FIB4) index, to better predict the severity of hepatic injury. FibroScan has a sensitivity of 85% for detecting advanced fibrosis and 92% for detecting cirrhosis [21]. Current standard of care regarding NAFLD/NASH diagnosis is reviewed elsewhere [4, 10, 15, 16, 22].

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Etiology

As mentioned above, NAFLD is a diagnosis of exclusion, so its workup must exclude other causes such as alcohol consumption, hepatitis B and C infection, drug abuse, autoimmune liver disease, hemochromatosis and Wilson's disease.

The principal risk factors to develop NAFLD and NASH are the presence of insulin resistance and obesity. However, NAFLD and NASH are associated with other extrahepatic manifestations, adding to the burden of disease. These manifestations notably include: obstructive sleep apnea, hypertension, dyslipidemia, gut microbiota alterations, genetic predisposition (notably polymorphisms in PNPLA3 and TM6SF2 genes), sedentary lifestyle, and consumption of certain foods (e.g., fructose, saturated fatty acids, overconsumption of carbohydrates leading to *de novo* lipogenesis) [2, 13, 23-26]. Nevertheless, some endocrine diseases are also associated with NAFLD and NASH (Table 1), and their pathophysiology will be discussed.

General pathophysiology of NASH

The pathophysiology of NAFLD is complex and multifactorial with multiple systemic alterations involved [27]. The traditional "two hits" theory consists of a first "hit" with intrahepatic accumulation of fatty acids, followed by a second "hit" including other factors such as oxidative stress or mitochondrial dysfunction. However, this theory has been considered too simplistic to adequately represent the pathogenesis of NAFLD. Therefore, it has been replaced by the "multiple-parallel hits" model that seems more accurate to represent the process of NAFLD development and progression, where various factors act in parallel and in a synergic manner in subjects with genetic predisposition [27-29]. This multiple hits hypothesis is based on the concept that genetic and environmental factors associated with dietary habits lead to obesity, insulin resistance development, and alteration of intestinal microbiome [29]. Insulin resistance promotes hepatic *de novo* lipogenesis and adipose tissue lipolysis, leading to an increased

flux of fatty acids to the liver [30]. Insulin resistance will also lead to adipose tissue dysfunction inducing secretion of inflammatory cytokines [31].

Intrahepatic accumulation of fatty acids will induce the development of several deleterious phenomena such as mitochondrial dysfunction, endoplasmic reticulum stress, oxidative stress and production of reactive oxygen species [32]. In addition, the alteration of intestinal microbiome induces an increased production of intestinal fatty acids and increased intestinal permeability, altogether leading to the activation of cytokines production such as TNF-α and IL-6 [33]. These elements will subsequently lead to a chronic hepatic inflammatory state promoting the development and progression of NAFLD and NASH, as summarized in Figure 1.

Endocrine causes of NASH

Type 2 diabetes/insulin resistance

NAFLD is a major risk factor for the development of type 2 diabetes, most likely because of its strong association with hepatic insulin resistance [34]. This is notably due to the fact that some lipid intermediates are more likely to cause hepatic insulin resistance. Indeed, while triglycerides are usually considered inert, other lipids such as diacylglycerols and ceramides have been clearly involved in the development of insulin resistance [1, 3, 20, 34]. Both diacyglycerols and ceramides interact with insulin signaling. Whereas ceramides inhibit Akt2 phosphorylation and downstream insulin signaling [2], diacylglycerols activate protein kinase Cε (PKCε) as key pathway responsible for causing NAFLD-associated hepatic insulin resistance [34]. Confirmation of this interaction between diacylglycerols, PKCε activation and hepatic insulin resistance has been demonstrated in numerous human and rodent models of NAFLD-associated hepatic insulin resistance [20, 35-49]. There are multiple causes for the accumulation of diacylglyerols in the liver and PKCε activation resulting in hepatic insulin resistance. Notably, diacylglycerols can accumulate following an increased delivery of chylomicrons remnants

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observed secondary to increased dietary intake, or depending on diet composition, like a high-fat or high-fructose diet, or in the case of lipodystrophy or genetic predisposition. Also, increased fatty acids release from adipocytes can result in hepatic accumulation of diacylglycerols, for example in the case of certain gene variants or in cases of insulin resistance. Moreover, skeletal muscle insulin resistance, which can be seen in predisposed or sedentary individuals, can result in *de novo* lipogenesis, leading to increased hepatic diacylglycerol content. Finally, mitochondrial dysfunction, as discussed above, can also result in hepatic accumulation of diacylglycerols. Interestingly, studies in both humans [48] and rodents [38, 50-53] have clearly demonstrated that compartmentation of diacylglycerols within the hepatocyte is a major factor in determining PKCε activation and hepatic insulin resistance.

The liver is essentially an exocrine gland, secreting bile into the intestine, but can also be considered as an endocrine gland. Indeed, the liver produces some important hormones or hormone precursors, such as insulin-like growth factor 1 (IGF-1), angiotensinogen, thrombopoetin and hepcidin. More recently, numerous hepatokines have been described [54]. Among them, the liver produces Fibroblast Growth Factor 21 (FGF21), a hormone also produced by the white adipose tissue. FGF21 has recently emerged as a key regulator in the metabolism of glucose and lipids [55-57]. FGF21 levels are increased in NAFLD and correlate with hepatic triglyceride content [58], thus FGF21 is considered an emergent biomarker of NAFLD [59, 60]. In diet-induced obese mice, which already display increased levels of FGF21, suggesting a state of FGF21 resistance, chronic administration of FGF21 not only reverses hepatic steatosis, but also improves insulin sensitivity by notably decreasing hepatic diacylglycerol hepatic content and subsequent PKCε activation [61]. Mice lacking *Fgf21* (FGF21 KO) have hepatic insulin resistance and increased hepatic glucose production associated with an increase in plasma glucagon levels. FGF21 KO mice are also hypometabolic and display increased fat mass [47]. FGF21 may have a potential role as a therapeutic agent for conditions associated with insulin resistance as it has been shown that administration of a recombinant form of this hormone in obese mice and diabetic monkeys improves insulin sensitivity, body

weight, lipid profile and even hepatic insulin resistance [61-66]. Moreover, FGF21 administration could have the potential to modulate inflammation [67]. As NASH is associated with inflammation, FGF21 administration, for example by using FGF21 analogs, could be of interest in this context [68, 69].

Altogether, these data suggest that the accumulation of ectopic fat in the liver, leading to NAFLD, plays an important pathophysiological role in the development of insulin resistance and type 2 diabetes. Modulation of hepatokines released by the liver, such as FGF21, could represent a therapeutic role in the treatment of NAFLD and NASH.

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is an endocrine syndrome frequently encountered in young women of childbearing age, with a prevalence of 8% to 15% [70]. PCOS is best defined by the Rotterdam criteria, i.e. oligo/anovulation, clinical and/or biological signs of hyperandrogenism, and polycystic ovaries (by ultrasound) [70]. Genome-wide association studies have shown a relationship between PCOS and several genes involved in type 2 diabetes, such as THADA, INSR and HMGA2 [71], and insulin resistance occurs in about half of women with PCOS [72]. A meta-analysis of 17 studies revealed that there is a significantly higher risk of NAFLD in women with PCOS than in a control group (OR = 2.25, 95% CI = 1.95-2.60). Moreover, this association was independent of obesity and geographic region, but might be correlated with hyperandrogenism [73]. A retrospective longitudinal cohort study using a large primary care database in the United Kingdom, evaluating NAFLD rates in 63'120 women with PCOS and 121'064 control women, found that women with PCOS had an increased rate of NAFLD (HR = 2.23, 95% CI 1.86-2.66, $p < 0.001$), also after adjusting for body mass index or dysglycemia [74]. The prevalence of NAFLD in women with PCOS varies between 35% and 70%, depending on the diagnostic method used [75]. Regarding the association between PCOS and the histological severity of NAFLD, a study reported that among 200 women with PCOS, 6 of

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them had biopsy-proven fibrosing NASH. Compared with the 194 of 200 PCOS women who did not undergo biopsy, women with biopsy-documented NASH had lower HDL-cholesterol, higher triglycerides, higher fasting insulin, higher aspartate aminotransferase, and higher alanine aminotransferase [76]. Conversely, the prevalence of PCOS in women with NAFLD has been shown to reach 71% in one cohort [77]. However, it is not clear whether PCOS is an independent risk factor for NAFLD.

Insulin resistance is a major player in PCOS, promoting hyperandrogenism through an increased release of androstenedione and testosterone [78]. Indeed, insulin acts as a co-gonadotropin to increase luteinizing hormone (LH), therefore stimulating androgens production. A concomitant decrease in sex hormone binding globulin (SHBG) mediated both by insulin resistance and hyperandrogenism further increases the levels of androgens, which leads to a vicious circle increasing insulin resistance. The potential mechanisms leading to insulin resistance in PCOS need to be further defined, but a post receptor defect in the insulin receptor signal transduction has been suggested as there is no structural abnormality in the insulin receptor [79]. Hyperandrogenism in PCOS is therefore a potential culprit in the development of NAFLD. Indeed, a recent meta-analysis showed that among women with PCOS, those with NAFLD had higher serum total testosterone as well as free androgen index [80]. The mechanism behind the association of androgen excess and NAFLD in PCOS has been shown to come from intra-adipose tissue androgen generation, which drives lipotoxicity, notably by increasing adipocyte hypertrophy and fatty acid overspill [81]. Among women with PCOS and androgen excess, circulating glycerophospholipids and lysoglycerophospholipids have been identified, and are known as potential biomarkers of NASH [26, 82]. Interestingly, systemic lipotoxicity is increased after an acute androgen challenge in women with PCOS, but not in body mass index-matched controls [81]. Finally, women with PCOS, obesity and NAFLD display an increased excretion of 5α-reduced steroids [83]. The role of 5α-reductase is further discussed in the glucocorticoid excess section.

Overall, women with PCOS show a high prevalence of NAFLD, even independently of obesity and dysglycemia. Hyperandrogenism and insulin resistance play a key role in the pathophysiology of PCOS-associated NAFLD, although the exact mechanisms remain elusive. Further studies are needed to better understand the complex endocrine regulations in the interconnections linking PCOS with NAFLD, in order to notably establish whether treatment with anti-androgenic drugs may reduce the risk of NAFLD in women with PCOS.

Hypothyroidism

Hypothyroidism is a frequent endocrine disorder defined by thyroid hormone insufficiency [84]. Primary overt hypothyroidism is defined by an elevated level of thyroid-stimulating hormone (TSH) in association with low serum free thyroxine (T4) levels, while subclinical hypothyroidism is characterized by elevated TSH levels in association with normal levels of T4. Thyroid hormones are involved in various metabolic processes, including body fat distribution, lipid utilization, energy expenditure, and glucose homeostasis. Altered thyroid hormone levels may, therefore, participate in the development of NAFLD [85]. Indeed, individuals with hypothyroidism are more at risk of developing components of the metabolic syndrome such as impaired fasting glucose levels, obesity, and hyperlipidemia that are clearly associated with the occurrence of NAFLD, thus suggesting a close link between hypothyroidism and NAFLD [86]. A meta-analysis including nearly 13'000 individuals revealed a prevalence of 15 to 36% of hypothyroidism among NAFLD patients [87]. This association has been confirmed in a single large study including more than 2'000 subjects with subclinical or overt hypothyroidism [88].

The potential pathophysiological mechanisms supporting this epidemiological relationship comprise the frequent occurrence of insulin resistance in hypothyroidism, possibly mediated by an increase in the levels of several adipocytokines such as TNF-α, IL-1, or leptin;

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elevated oxidative stress; and increased lipid peroxidation that are often encountered in hypothyroidism and that may lead to the development of insulin resistance [89, 90]. Interestingly, TSH *per se* may promote liver *de novo* lipogenesis. Indeed, the receptor for thyrotropin is expressed on the surface of hepatocytes, where it can be activated by TSH, thereby resulting in stimulation of the peroxisome proliferator-activated receptor-α (PPARα) pathway that leads to the activation of sterol regulatory element-binding transcription factor 1 (SREBP-1c), which promotes hepatic lipogenesis [90, 91]. This mechanism may, in part, explain the observed association of NAFLD and subclinical hypothyroidism [92]. Due to shared features between hypothyroidism and the metabolic syndrome, and the tight relationship between NAFLD and the metabolic syndrome, screening for the presence of NAFLD should be considered for individuals with hypothyroidism, in particular when they are also overweight or obese.

Levothyroxine administration has been shown to be associated with reduction of the body mass index and the level of serum lipids, thus suggesting a potential beneficial impact on NAFLD [84]. In addition, several randomized controlled trials have shown a reduction in liver enzyme levels and hepatic fat content after levothyroxine administration in NAFLD patients who had euthyroidism or subclinical hypothyroidism [93-95]. Notably, thyroid hormones increase β-oxidation of fatty acids, which leads to a decrease in hepatic lipid content, at least in rodents [96]. Moreover, thyroid hormones analogs decrease hepatic steatosis in different rodent models of NAFLD [93, 97]. Also, TSH has been shown to increase hepatic triglyceride content through upregulation of SREBP-1c activity [91]. Therefore treating hypothyroidism would in theory reduce TSH and help improving NAFLD. Further studies are required to assess whether levothyroxine replacement in patients with concomitant hypothyroidism and NAFLD can have a positive impact on liver disease progression and outcomes.

Male hypogonadism

Male hypogonadism is a clinical syndrome defined by reduced testosterone secretion and/or spermatogenesis. Male hypogonadism can be caused by diseases of the testes (primary hypogonadism) or dysfunction of the hypothalamic-pituitary axis (secondary hypogonadism) [98]. The clinical features of male hypogonadism depend on the time of onset and the severity of the androgen deficiency, as well as whether it involves an insufficiency of spermatogenesis and/or testosterone secretion. The diagnosis of hypogonadism is based on the presence of signs and symptoms compatible with testosterone deficiency and several reduced morning testosterone serum level measurements.

Studies in males have reported an association between low testosterone and increased visceral adipose tissue, insulin resistance, and dyslipidemia [99, 100]. Accordingly, higher levels of testosterone are associated with reduced visceral abdominal adipose tissue [101]. The association between low testosterone levels and NAFLD has been observed in several epidemiological studies, and a meta-analysis including 16 studies has confirmed this association between lower testosterone levels and NAFLD in men [102-104].

Several mechanisms may play a role in the association between reduced testosterone levels and NAFLD, but they remain poorly described. An increase in abdominal adipose tissue with low testosterone levels may lead to hepatic steatosis and insulin resistance. Furthermore, low testosterone levels are associated with low-grade inflammation [99]. Preclinical studies have shown that low levels or the absence of testosterone may cause hepatic steatosis through increased *de novo* lipogenesis via upregulation of hepatic SREBP-1. This upregulation of SREBP-2 and ACC-1 appears to be due to reduced AMPKα-1 activity [105]. Additionally, testosterone may activate SR-B1 scavenger receptor and stimulate hepatic lipase by hydrolysis of phospholipids and by hydrolysis of triglycerides, thereby leading to an enhancement of specific cholesterol uptake of HDL-C lipids by the liver as well as cholesterol efflux from peripheral cells [106]. Therefore, low testosterone may favor uncontrolled hepatic lipid accumulation, thereby leading to the development of NAFLD.

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In the preclinical setting, testosterone replacement has been associated with a beneficial impact on hepatic fat content in animal models such as castrated rats [107, 108]. In humans, the benefit of testosterone administration in NAFLD is still a matter of debate, with conflicting results between randomized controlled trials. For example, one study found a significant reduction in hepatic fat content, while another study reported the absence of a reduction in hepatic fat [109, 110]. In light of the limitations of the available studies, testosterone replacement cannot currently be considered as a treatment for NAFLD in men with low testosterone levels. Further studies are needed to obtain a better understanding of the impact of testosterone administration on the development and outcomes of NAFLD, and these studies should take into account several long-term safety aspects of testosterone replacement therapy, such as cardiovascular outcomes, as these remain a matter of debate [111].

Growth hormone dysregulation

Growth hormone deficiency

Liver is thought to be one of the key organ targets of growth hormone (GH). GH exerts various physiological actions on glucose and lipid metabolism. GH stimulates glycogenolysis and gluconeogenesis, thereby inducing insulin resistance and promoting preferential release of free fatty acids from visceral adipose tissue, which in turn induces competition between fatty acids and glucose as a substrate, thereby reducing glucose metabolism [112]. Due to its pronounced impact on lipid and glucose metabolism, the level of GH appears to be related to lipid accumulation in the liver. Deficiency or excess of GH appears to be associated with the risk of developing NAFLD.

Adult growth hormone deficiency (GHD) is clinically characterized by decreased muscle strength, increased visceral adipose tissue, dyslipidemia, and an increased risk of cardiovascular disease [113, 114]. GHD in adults is usually caused by a pituitary adenoma or its treatment such as surgery. An association between GHD in adults and the development and

severity of NAFLD has been reported in several observational studies. Thus, several studies have reported that IGF-1 and GH levels are reduced in patients with NAFLD [115-118]. There are several mechanisms that link GH deficiency and NAFLD, although the precise pathogenesis remains to be elucidated. Mice with liver-specific deletion of GH receptor exhibit severe hepatic steatosis, increased hepatic *de novo* lipogenesis, and insulin resistance [119, 120]. Furthermore, the same mouse model exhibits other features of the metabolic syndrome such as low-grade inflammation, increased reactive oxygen species production, and mitochondrial dysfunction.

Several clinical studies have reported potential beneficial effects of GH administration on liver enzyme levels and hepatic steatosis [121]. GH replacement treatment in adults should only be provided in case of symptomatic and severe growth deficiency in order to decrease visceral adipose tissue, to improve lipid profile, and to reduce cardiovascular morbidity and mortality [122]. Due to limited data to date in this regard, GH supplementation therapy should not be considered as a specific treatment for NAFLD patients with or without GH deficiency. [123]

Growth hormone excess

Acromegaly is caused by the overproduction of GH and, consequently, IGF-1, and it is most often the result of somatotroph adenomas. Excess GH secretion results in deleterious effects on glucose metabolism and insulin signaling at hepatic and extra-hepatic sites by stimulation of gluconeogenesis and glycogenolysis. Excess GH causes an increase in lipolysis that leads to a high levels of free fatty acids, thereby further promoting the development of insulin resistance [124, 125].

Few studies to date have shown an association between acromegaly and NAFLD prevalence, and in particular the hepatic steatosis index (HSI). Interestingly, a reduction of GH and IGF-1 levels after treatment induction in acromegaly has been associated with a reduction of the HSI [126, 127]. However, due to the relatively low prevalence of acromegaly, there is still limited data regarding its association with NAFLD and the impact of control of the disease with

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conventional treatments such as octreotide long-acting release on NAFLD progression or regression.

Glucocorticoid excess

Excessive levels of glucocorticoids (GCs) have been linked to the risk of developing NAFLD, as GCs may modulate several key pathways involved in lipid and carbohydrate metabolism [128]. Excessive levels of endogenous GCs, as occurs in Cushing's syndrome, have been associated with a prevalence of 20% of NAFLD in a study assessing hepatic fat content with computed tomography in individuals with active Cushing's syndrome [129]. In accordance with this, the exogenous GC hydrocortisone has been shown to be associated, in a dose-dependent manner, with an increased risk of NAFLD independently of the body mass index or waist circumference [130]. Currently available data are still not in agreement regarding the presence or not of hypercortisolism in patients with metabolic syndrome and NAFLD and the potential increased risk of the occurrence of a metabolic disturbance such as type 2 diabetes or insulin resistance in the presence of elevated levels of cortisol [131-133].

Excess GCs can mediate hepatic fat accumulation through several mechanisms, including the stimulation of *de novo* hepatic lipogenesis, increased gluconeogenesis, the stimulation of food intake, inhibition of hepatic β-oxidation, and enhancement of lipolysis of adipose tissue and free fatty acids uptake by the liver [128]. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive cortisone to active cortisol, appears to play a potentially important role in excess GC-mediated development of NAFLD and insulin resistance. This enzyme is upregulated in the visceral adipose tissue of obese individuals, thereby leading to increased hepatic exposure of cortisol by the splanchnic venous system [134]. In keeping with this, mice with adipose tissue-specific KO of 11β-HSD1 are protected against the development of the metabolic syndrome and, conversely, mice that overexpress 11β-HSD1 in adipose tissue are prone to developing NAFLD and insulin

resistance [135]. 11β-HSD1 inhibitors have been shown to have a beneficial effect on metabolic syndrome, and their use can, therefore, be considered as a potential future therapy for NAFLD [136, 137].

5α-reductase is another hormone involved in GC metabolism. Notably, it has been suggested that 5α-reductase activation might act as a protective mechanism preventing progression of metabolic disturbances in the liver through increased local GC clearance [138]. Also, mice knock-out for 5α-reductase are predisposed to insulin resistance and NAFLD [83]. Finally, 5α-reductase inhibition through the use of dutasteride has been shown to promote hepatic fat accumulation in humans [139].

Altogether, excess GCs levels appear to play an important role in the development of insulin resistance and NAFLD, although clinical applications to potentially counteract GCs action remain to be elucidated.

Miscellaneous

Vitamin D deficiency has been associated in various observational data with the development and severity of NAFLD in subjects with normal aminotransferases (AST/ALT) levels [140, 141]. In addition, NAFLD patients have an additional risk of low vitamin D of approximately 25% compared with subjects without NAFLD [142]. However, the association between the risk of future new-onset insulin resistance and low vitamin D remains unclear [143, 144].

Mechanistically, vitamin D acts through the vitamin D receptor (VDR) present in hepatocytes. Vitamin D has been shown to enhance insulin sensitivity *in vitro* through upregulation of GLUT4 and modification of free fatty acids metabolism [145]. Preclinical studies suggest potential anti-inflammatory, antiproliferative, and antifibrotic effects of vitamin D

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administration on the liver *in vivo* [146, 147]. Small-scale randomized controlled trials have shown that vitamin D supplementation improves the metabolic syndrome [148-150].

Several observational case-control or cross-sectional studies have assessed the association between NAFLD and bone mineral density (BMD). The findings to date of studies in this regard remain conflicting, with some studies showing a significant association between NAFLD and low BMD, while other studies have reported a significant association between NAFLD and increased BMD, and, lastly, some studies have observed no association [151-154]. Larger-scale prospective and mechanistic studies are warranted to better elucidate the potential link between bone demineralisation and NAFLD, with investigation of gender differences and any specific segment of the skeleton that can be affected.

Primary aldosteronism (PA) is the most frequent cause of secondary hypertension, accounting for approximately 10% of all cases [155]. PA can not only cause hypertension but also insulin resistance and dyslipidemia. [156] Activation of the mineralocorticoid receptor by aldosterone leads to impaired insulin sensitivity in skeletal muscle and adipocytes by stimulation of inflammatory and oxidative stress metabolic pathways [157, 158]. PA has been reported to increase the risk of metabolic syndrome and NAFLD [159-161]. Thus, there may be merit in screening patients with PA for NAFLD. The impact of therapeutic or surgical treatments of PA on the development and outcome of NAFLD remain to be determined.

Prolactin is a pituitary-derived hormone with potent enhancing effects on reproduction and lactation. Since the receptor of prolactin is also present in liver, it may play a role in hepatic metabolic regulation. A negative correlation between prolactin plasma levels and body weight, insulin resistance, and NAFLD development has been observed in observational studies [162, 163]. The prolactin receptor is down-regulated in obese subjects with NAFLD, and *in vitro* experiments indicate that prolactin, via its hepatic receptor, improves hepatic steatosis through a reduction of fatty acid translocase (FAT)/CD36, which is a major transporter for hepatic

uptake of free fatty acids [162]. Prolactin and its hepatic receptor may, therefore, represent an attractive approach to counteract the development of NAFLD.

Summary and conclusion

NAFLD is the most common chronic liver disease in Western countries, with NASH being a more progressive subtype notably characterized by inflammation and hepatocyte injury, with or without fibrosis. The latter is the only histologic feature associated with long-term outcomes of patients with NAFLD. NAFLD is intimately entangled with various endocrine diseases, sharing the keystone pathophysiological mechanism of insulin resistance. However, our understanding of the pathophysiology of NAFLD in different endocrinopathies is far from being understood and therefore limits our capacity to more specifically treat NAFLD in this context. Moreover, the natural course of NAFLD secondary to endocrine disorders compared to the course of “primary” NAFLD is unknown. Therefore, in the coming years, it will be of importance to better understand the interrelationships between endocrine diseases and NAFLD in order to better target treatments.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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KG and FRJ wrote the paper and contributed equally.

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Figures Legends

Figure 1. Multiple parallel hits hypothesis for the progression of NAFLD

Genetic, dietary and environmental factors lead to the development of insulin resistance, adipocytes proliferation and dysfunction, and alteration of intestinal microbiota. Subsequently, insulin resistance leads to lipolysis, release of adipokines such as TNF-α or IL-6, and stimulates hepatic DNL.

As a consequence, the increased flux of hepatic FFAs induces the accumulation of TG, which cause mitochondrial dysfunction and ER stress. Intestinal permeability participates in the activation of hepatic inflammation and ER stress.

Altogether these multiple parallel hits lead to the development of NAFLD and its progression to fibrosis and cirrhosis.

Abbreviations: DNL, de novo lipogenesis ; ER , endoplasmic reticulum ; FFA, free fatty acids; IL-6, interleukin 6; NAFLD, non-alcoholic fatty liver disease ; TG, triglycerides ; TNF-α, tumor necrosis factor α.

Table 1: Endocrine causes of NAFLD

Hormone	Gland of origin	Example of disease
Cortisol	- Pituitary gland (ACTH) - Adrenal gland (cortisol)	- Cushing's disease - Cushing's syndrome - Exogenous corticoid administration
Thyroxine (T4)	- Pituitary gland (TSH) - Thyroid gland (free T4)	- Primary hypothyroidism (thyroid disease) - Secondary hypothyroidism (pituitary (TSH) or hypothalamic disease (TRH))
Growth hormone (GH)	- Pituitary gland (GH) - Ectopic secretion	- Acromegaly (pituitary adenoma (GH) or hypothalamic mass (GHRH)) - Ectopic secretion of GHRH or GH (Bronchial carcinoid, pancreatic islet-cell tumor, small cell lung cancer, adrenal adenoma, medullary thyroid carcinoma, pheochromocytoma) - Growth hormone deficiency
Testosterone	- Testicles	- Primary hypogonadism (congenital abnormalities, acquired diseases) - Secondary hypogonadism (pituitary disease (LH) or hypothalamic disease (GnRH))
Prolactin	- Pituitary gland	- Micro or macroprolactinoma - Stalk effect

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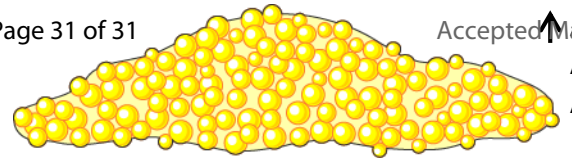


↑Adipose tissue
Adipose tissue dysfunction
Adipokines

↑Lipolysis

↑FFA

Genetic factors
Environmental factors
Dietary habits

De novo lipogenesis

↑TG

TNF-α
IL-6

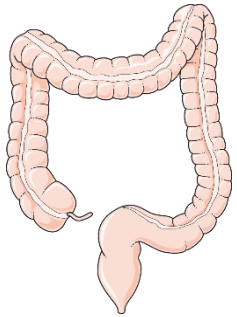
ER stress

Mitochondrial dysfunction

Skeletal muscle insulin resistance

INFLAMMATION
FIBROSIS

Gut Microbiota



Intestinal permeability

NAFLD