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A narrative review of factors associated with the development and progression of non-alcoholic fatty liver disease

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Summary

Background: With the obesity pandemic, non-alcoholic fatty liver disease (NAFLD) has become the most prevalent liver disease. NAFLD can progress to non-alcoholic steatohepatitis (NASH), a potential cause of liver failure. It remains difficult to identify patients at risk for NASH, despite evolving insights in contributing factors, including genetic variance, hormones, adipokines, diet and body-fat distribution. We aimed to present a broad perspective on these risk factors associated with NAFLD development and progression with a focus on their contribution in different age groups and susceptible high-risk populations, hereby giving insight in the pathophysiology of NAFLD.

Methods: Literature was searched for relevant articles on the pathophysiology of NAFLD in different age groups.

Results: Our review underscores large contributions of diet, with particularly fructose promoting NASH development, and sex hormones, with oestrogens exerting protective effects and androgens negatively influencing NAFLD development. Genetic variation in corresponding pathways might further determine NAFLD progression.

Conclusions: Changes throughout the transition from childhood to adulthood show that variations in diet, hormone levels and metabolism are related to NAFLD progression. The human body uses different strategies to handle excessive nutrients, but each comes at a price. When corresponding pathways are strained by hormonal or genetic factors, NASH or other symptoms of the metabolic syndrome ensue. Potentially, stratification based on sex, body-fat distribution, diet, lifestyle, microbiome, adipokines, sex hormones, blood concentrations of liver enzymes, liver histology and genetic pre-disposition might help to identify patients at increased risk of NASH.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in the developed world.[1] The disease has a varying severity, ranging from reversible hepatic steatosis, characterised by excessive accumulation of triglycerides (TG) in the liver,[2,3] to non-alcoholic steatohepatitis (NASH), consisting of macrovesicular steatosis, lobular inflammation and hepatocyte injury.[3,4] NASH can progress to fibrosis, cirrhosis, liver failure and hepatocellular carcinoma. Globally, 25% of adults have hepatic steatosis. Obesity and type 2 diabetes mellitus significantly raise this risk; NAFLD rates of 70% and higher have been reported in these populations. NASH occurs in 1.5 to 6.45% of adults.[5] Furthermore, 8% of children in the developed world and 34% of obese children suffer from hepatic steatosis, which has become the most common liver abnormality in children.[6] Approximately 23% of children with steatosis have NASH.[7]

The heritability of NAFLD approaches 40%[3,8] and mutations in certain genes associated with lipid metabolism are known to cause NAFLD in patients with and without obesity.[3] However, the determinants and mechanisms driving progression of simple steatosis to more severe forms of NASH, sometimes resulting in liver fibrosis already in childhood, are incompletely understood.

To make the next step in clinical care for patients with NAFLD, a deep understanding of the pathogenesis of NAFLD and modifiers determining progression of liver disease is crucial. We set out to deepen this understanding by studying patients with severe forms of NAFLD and search for specific disease modifying factors. We considered children with NASH of particular interest since they have an early disease onset and are subject to large biological fluctuations in hormones, metabolism, insulin resistance (IR) and lifestyle. Likewise, severe phenotypes of NAFLD are seen with specific genetic variants providing insight in the contribution of affected pathways to NASH development.[9] Understanding the factors that pre-dispose to rapid disease progression can help to identify patients at increased risk for developing severe liver pathology and develop novel prevention and treatment strategies. In this narrative review, we will discuss the pathogenesis of NAFLD, elaborating on the relationship with IR and lipid metabolism. Subsequently, we will analyse the important factors in progression to NASH such as dysbiosis and TLR4 activation, the role of adipokines and how lipotoxicity is translated in oxidative and endoplasmic reticulum stress, leading to apoptosis. We will elaborate on risk factors that are subject to change during the transition from childhood to adulthood and on genetic variants that pre-dispose to NAFLD and NASH.

Highlights

- Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease, but factors contributing to progression to severe non-alcoholic steatohepatitis (NASH) are insufficiently known for targeted prevention or intervention strategies.
- Exploration of NAFLD development in various age groups, from childhood to late adulthood gives insight in specific disease contributing factors.
- Hormonal factors (androgens opposed to oestrogens) and dietary fructose appear most harmful in promoting NAFLD progression and the latter represents an easy target for intervention.
- The human body has different ways to handle excessive nutrients, but eventually each way leads to a burden such as NAFLD or cardiovascular disease.
- Stratification based on sex, body-fat distribution, diet, lifestyle, microbiome, adipokines, sex hormones, blood concentrations of liver enzymes, liver histology and genetic pre-disposition might help to identify patients at increased risk to develop NASH, and current genetic screening possibilities might reveal novel or more specific targets for prevention or intervention strategies.

2 | METHODS

PubMed electronic database from 2003 to 2018 was searched for relevant publications using the following terms: "NAFLD" OR "Non-alcoholic fatty liver disease" OR "NASH" OR "Non-alcoholic steatohepatitis" AND "pathogenesis" OR "mechanism" OR "pathology." This review focuses on risk factors in the transition from childhood to adulthood, for which we repeated the previous search combined with the following terms: AND "Child" OR "Childhood" OR "Children" Or "Adolescent" OR "Puberty" OR "Pubertal" OR "Paediatric" OR "Pediatric." The inclusion criteria were (a) Peer-reviewed academic journals published in English (b) research that focused on NAFLD development and factors associated with NAFLD development (c) articles with accessible abstracts and full text. Articles were read and assessed for relevance. Hand searching of the references of retrieved literature was done. Backward snowballing was used to complement the database search.

3 | PATHOGENESIS OF STEATOSIS AND INSULIN RESISTANCE

Central to hepatic steatosis are TG accumulation and IR. Hepatocytes accumulate TGs through fatty acid (FA) acquisition from dietary fat, adipose tissue lipolysis and hepatic lipogenesis. They clear TGs through FA β-oxidation and TG export as very low-density lipoprotein (VLDL) via the endoplasmic reticulum (ER) (Figure 1A).[3,10,11] Insulin influences these processes by inducing lipogenesis and antagonising tissue lipolysis.

3.1 | Obesity-induced systemic IR and FA release

Obese patients become less responsive to insulin, which is often attributed to chronic inflammation of adipose tissue. In obesity,

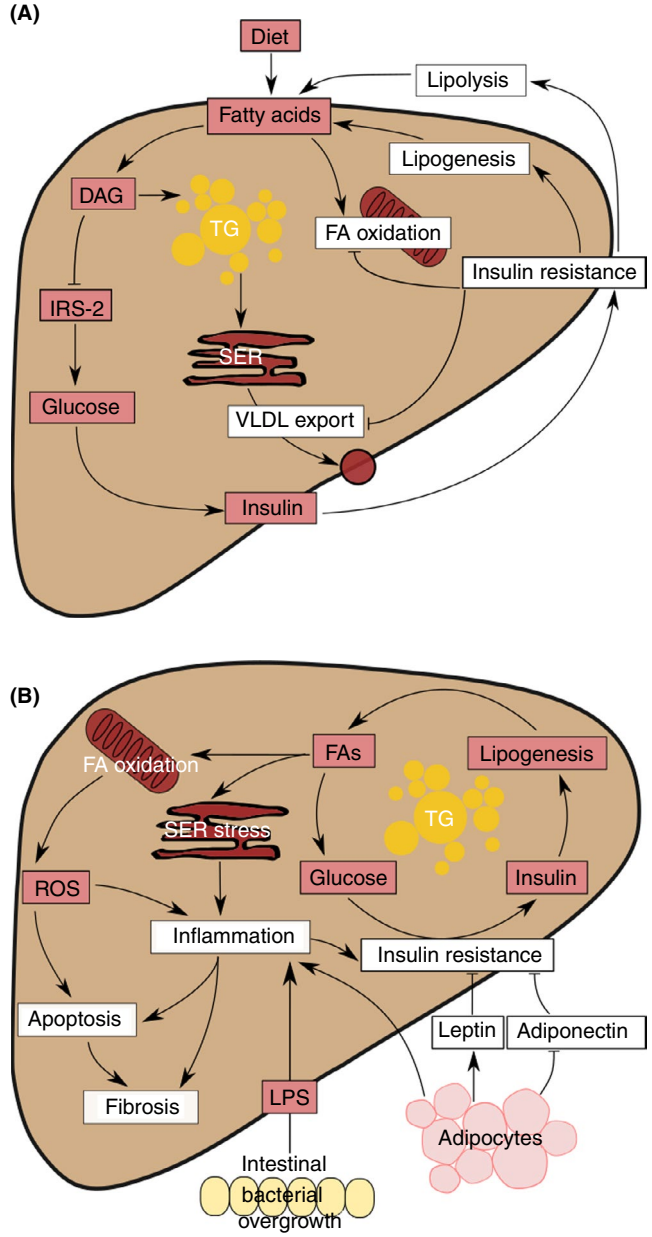

FIGURE 1 Interpretation of the current literature on the pathogenesis of steatosis (A) and factors contributing to the progression to NASH (B). FA = fatty acid; SER = smooth endoplasmic reticulum; VLDL = very low-density lipoprotein; DAG = diacylglycerol; TG = triglyceride; IRS-2 = insulin receptor substrate 2, ROS = Reactive oxygen species, LPS = lipopolysaccharides. A. Increased FA influx from lipolysis in adipose tissue, diet and lipogenesis causes increased IR via DAG and decreased phosphorylation of IRS-2, which leads to elevated glucose via enhanced gluconeogenesis and reduced glycogen synthesis. Elevated glucose levels trigger more insulin release. Insulin resistance stimulates lipolysis of adipose tissue and de novo lipogenesis, while it decreases VLDL export, further contributing to increased FA concentrations and steatosis. B. Elevated β-oxidation of FAs generates ROS. FAs are incorporated in the ER membrane, leading to the unfolded protein response. Both pathways cause hepatocyte apoptosis and contribute to inflammation. Inflammation is further triggered by intestinal bacterial overgrowth and adipose tissue inflammation. Inflammatory cytokines increase IR. Adipose tissue ameliorates insulin sensitivity via increased production of leptin and adiponectin. Adiponectin production is decreased in NASH. Although leptin production is increased in NAFLD, this does not lead to lower insulin concentrations, suggesting leptin resistance. With this resistance, the protective effect of leptin against hepatic steatosis is lost

storage of excessive TGs leads to hypertrophic adipose tissue, which enhances the production of pro-inflammatory cytokines TNF-α, IL-1, IL-6 and monocyte chemoattractant (MCP-1).[11] These pro-inflammatory factors antagonise the lipogenic effects of insulin, thereby contributing to systemic IR, which increases adipose tissue lipolysis. Together, this leads to increased hepatic FA concentrations.[11] Furthermore, MCP-1 attracts macrophages, creating low-grade inflammation. These macrophages and other resident cells subsequently produce even higher levels of inflammatory cytokines, which promote leptin production, and reduce adiponectin production[10,11] and affect IR.[12]

Adipose tissue inflammation, IR and increased FA influx in the liver stimulate each other, creating a positive feedback loop. More than half of hepatic fat is derived from lipolysis from adipose tissue[3] and this correlates with total fat mass in humans, resulting in increased FA release in obese patients. Normally, insulin inhibits adipose tissue lipolysis, but this is impaired in patients with systemic IR, resulting in elevated efflux of FAs from adipose tissue.[11,13] In the liver, the hyperinsulinaemia associated with IR inhibits β-oxidation and causes an upregulation of transcription factor SREBP-1c leading to enhanced lipogenesis.[3,11,13] These combined effects lead to hepatic steatosis.[11,13] Within this positive feedback loop, it is unclear whether IR precedes or follows steatosis.[3]

Body fat distribution is important, as visceral adipose tissue produces more pro-inflammatory cytokines, pro-hyperglycaemic factors and less adiponectin than subcutaneous adipose tissue.[14] Hence, IR and NAFLD have a stronger correlation with visceral adiposity than with BMI BMI.[15] Also, IR seems to decrease with higher skeletal muscle mass, which can be attributed to insulin-mediated glucose utilisation. A 7-year follow-up of patients with NAFLD showed a correlation between increased skeletal muscle mass and improvement or prevention of NAFLD, which might contribute to a stratification for at-risk disease-susceptible individuals.[14]

3.2 | FA influx in the liver and hepatic IR

In the liver, FA are metabolised through β-oxidation, exported as VLDL or stored as TGs (Figure 1A). Elevated FA concentrations lead to accumulation of intermediate products such as diacylglycerol (DAG). Cytosolic DAG enhances the translocation of protein kinase Cε (PKCε) to the cell membrane, where it inhibits insulin signalling via reduced phosphorylation of the insulin receptor substrate 2 (IRS-2).[16] This in turn suppresses glycogen synthase activity, leading to decreased glycogen synthesis. In parallel, reduced IRS-2 activity causes translocation of Forkhead box protein O1 to the nucleus, where it enhances the expression of various gluconeogenic enzymes.[16]

Cytosolic DAG thus inhibits IRS-2, which leads to hepatic IR and enhanced gluconeogenesis and inhibited glycogen synthesis.[17]

4 | PATHOGENESIS OF NASH

Elevated hepatic FA concentrations increase the susceptibility of the liver to injury and are seen as the first hit. Multiple hits including IR, nutritional factors, adipokines, gut microbiota and genetic factors are now thought to cause the liver to progress from steatosis to NASH (Figure 1B).[10,13,18]

4.1 | Inflammation

Pro-inflammatory cytokines play a crucial role in the progression from steatosis to NASH.[3] First, it has been demonstrated in rodents that specific cytokines can elicit the same response in liver tissue as seen in NASH, including neutrophil chemotaxis, hepatocyte apoptosis/necrosis, Mallory body formation and stellate cell activation.[11] Second, the enhanced expression of TNF-α and IL-6 in adipose tissue was detected in obese subjects before liver inflammation was present,[19] suggesting that adipose tissue mediated cytokine release precedes, and might lead to liver inflammation. Third, inhibition of TNF-α led to amelioration of IR and histological parameters of NASH in an obese mouse model on a high-fat diet.[20]

Cytokines lead to NASH development through the activation of the nuclear factor κB (NF-κB) and C-Jun NH_2-terminal kinase (JNK) pathway. FAs, particularly saturated FA, were found to activate the toll-like receptor 4 (TLR4), activating both the NF-κB and JNK pathway.[21,22] In the liver, TLR4 is expressed by hepatocytes, Kupffer cells and stellate cells. Upon phagocytosis of cholesterol, Kupffer cells are activated and TLR4 expression is upregulated.[23] Activated Kupffer cells produce transforming growth factor β (TGF-β), which triggers the fibrogenic state of stellate cells.[24]

The importance of TLR4 signalling in the pathogenesis of NASH is illustrated by TLR4 mutant mice, that neither develops extreme adiposity and IR in response to a high saturated fat diet nor NASH in response to a methionine/choline-deficient diet.[25,26] Thus, inflammation through TLR4 receptor activation appears to be a major factor in murine models in the progression from steatosis to NASH. In humans, these mechanisms still require confirmation.

4.2 | Adipokines

In addition to being a major source of FAs and cytokines (IL-6 and TNF-α), adipose tissue releases adipokines, such as leptin and adiponectin.[10] Leptin and adiponectin enhance hepatic insulin sensitivity[12] and appear to be the important factors in the pathogenesis of NASH.

Under non-obese conditions, leptin protects against NAFLD. Leptin acts centrally to decrease food intake and has anti-hyperglycaemic effects.[27] In response to lower glucose concentrations, insulin concentrations decrease, reducing hepatic lipogenesis and increasing lipolysis. Similarly, in the liver, leptin stimulates FA β-oxidation and suppresses lipogenesis in vitro, decreasing the fat accumulation and lipoapoptosis.[10,27] Leptin is markedly increased in obesity and hepatic steatosis, and even more in NASH.[27] High leptin levels do not decrease insulin concentrations or IR in obese humans. Potentially, the protective effects of leptin are lost in obesity due to excessive leptin exposure and leptin resistance. This might be caused by the suppressor of cytokine 3 (SOCS-3) which is activated by leptin and inhibits leptin signalling when overexpressed. SOCS-3 expression is also stimulated by insulin and SOCS-3 overexpression leads to IR, whereas the inhibition of SOCS-3 ameliorates insulin sensitivity and hepatic steatosis.[27] In obesity, leptin enhances the pro-inflammatory signalling cascades via activation of NF-κB.[28] Moreover, prolonged hyperleptinaemia may lead to fibrosis via activated stellate cells.[27] Activated stellate cells, Kupffer cells and sinusoidal cells all express the leptin receptor during fibrosis development.[27,28] Leptin facilitates stellate cell proliferation. Once activated, stellate cells contribute to leptin expression, creating a positive feedback loop (Figure 2). Hence, in normal non-obese conditions, leptin protects against hepatic steatosis by decreasing lipid intake, carbohydrate intake and synthesis, and increasing lipid metabolism. When these signals are ignored, leptin is excessively secreted, putatively leading to leptin resistance and hepatic inflammation and fibrosis (Figure 2).

Adiponectin promotes insulin sensitivity and reduces inflammation, thereby protecting against NAFLD progression. Total body fat is inversely correlated with adiponectin, and adiponectin blood concentrations are lower in patients with NASH than with steatosis.[19,28] Adiponectin increases FA β-oxidation and decreases lipogenesis, thereby reducing FA concentrations.[18,28] Adiponectin upregulates IRS-2 in an obese mouse model, thereby promoting insulin sensitivity.[29] Insulin sensitivity is further increased by adiponectin via antagonising TNF-α and IL-6 effects, and enhancing the secretion of anti-inflammatory cytokines IL-10 and IL-1 receptor antagonists in human leucocytes in vitro.[30] Adiponectin downregulates TLR4-induced NF-κB activation and leads to reduced TNF-α production in rat Kupffer cells in vitro and in mice upon exposure to lipopolysaccharides (LPS).[31] Finally, adiponectin has been shown to inhibit stellate cell proliferation in vitro, possibly via inhibition of ROS production[30] (Figure 2). Summarising, adiponectin promotes insulin sensitivity, protects from liver inflammation and limits fibrosis (Figure 2). Decreased adiponectin levels might pre-dispose NAFLD patients to progress to NASH.

4.3 | Lipotoxicity

Previously, it was thought that accumulation of TGs in hepatocytes caused hepatic inflammation and IR. However, storage of FAs as inert lipids is not toxic[10,13] and the conversion of FAs to TGs functions as a protective mechanism.[16] This is supported by mouse models with deficient TG synthesis, resulting in decreased hepatic steatosis but increased liver injury and fibrosis.[32] Similarly, genetic defects that prevent the removal of TG from the liver cause steatosis, but not IR.[3,16] Thus, intrahepatic TG content is a marker for increased FA exposure—rather than a cause of hepatic IR.

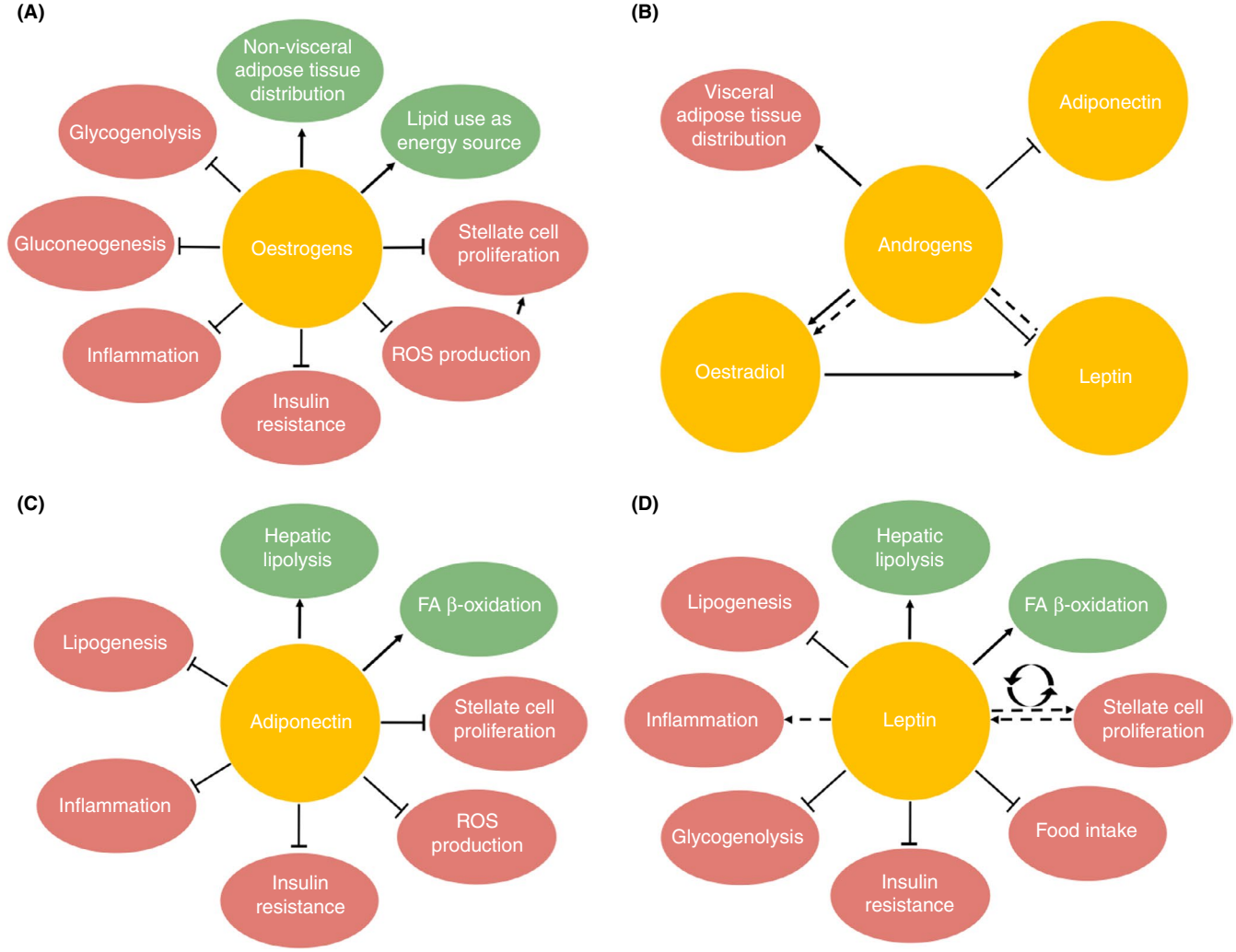

FIGURE 2 Schematic representation of the effects related to NAFLD/NASH of oestrogens, androgens, adiponectin and leptin. A. Combined effects of oestrogens reduce the chance of progression to NASH. B. Androgens promote visceral adipose tissue distribution and inhibit production of adipokines, thereby increasing the risk of progression to NASH. In obesity, androgen conversion to oestrogen is increased, but inhibition of leptin production by androgens is lost. C. Adiponectin inhibits several risk factors for progression to NASH and promotes hepatic lipolysis and FA β-oxidation. D. Leptin has similar effects to adiponectin, but these are partly lost in obesity, putatively related to leptin resistance. Moreover, high leptin levels trigger stellate cell activation and stimulate stellate cell proliferation, instigating a positive feedback loop with activated stellate cells producing leptin, thereby contributing to progression to NASH. —: effects under normal conditions; ---: pronounced or changed effects in obesity; ↑: stimulation; ⊥: inhibition

FA excess leads to hepatocyte dysfunction and ultimately apoptosis. Specifically, saturated FAs cause toxicity.[33] Mono-unsaturated FAs cause TG accumulation without leading to cell damage. Potentially, the ratio between mono-unsaturated and saturated FAs determines whether hepatocytes are damaged by high FA concentrations.[18] This ratio is determined by stearoyl-CoA desaturase-1 (SCD1), which converts saturated FA to mono-unsaturated FA.[34] Genetic inhibition of *SCD1* in mice leads to less steatosis, but more apoptosis, supporting the theory that saturated FAS are lipotoxic.[33]

Saturated FA influx in the hepatocyte leads to concentration-dependent β-oxidation of FAs in the mitochondria.[35] Mitochondrial capacity to control the oxidative balance collapses under the continuous pressure of excessive influx of FAs, leading to generation of ROS.[34] ROS are also generated via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by various NAFLD associated stimuli, such as pro-inflammatory cytokines and leptin.[36] Concurrently, NADPH oxidase is elevated in patients with cirrhosis and in paediatric patients with NASH.[37] Excessive oxidative stress causes impaired mitochondrial respiration and hepatocellular apoptosis.[34] Not only hepatocytes are sensitive to oxidative stress: in Kupfer cells, enhanced uptake of FAs combined with high ROS concentrations leads to NF-κB activation. Similarly, oxidative stress in stellate cells results in the activation of fibrogenic status and autophagy.[24]

Saturated FAs exert further lipotoxic effects by changing the critical free cholesterol-to-phospholipid ratio of the ER membrane, affecting ER membrane fluidity and calcium homeostasis.[18,24] Disruption of this homeostasis leads to accumulation of misfolded

and unfolded proteins and ER stress. Consequentially, the unfolded protein response (UPR) is initiated, which triggers JNK activation, contributing to IR. Ongoing ER stress eventually exceeds ER capacity and apoptosis ensues.[34]

4.4 | Dysbiosis

The microbiome composition of patients with obesity or NAFLD is different from controls[23,38] and small intestinal overgrowth is more common in NAFLD.[39] A different microbiome composition may elevate absorption of calories and increased FA uptake in adipocytes and hepatocytes, contributing to NAFLD.[38,40,41] Small intestinal bacterial overgrowth correlates with severity of steatosis.[39] Bacterial overgrowth and the NAFLD microbiome composition are associated with increased gut permeability, caused by disrupted intercellular tight junctions.[23] This leads to the leakage of LPS, derived from Gram-negative bacteria in the gut into the circulation. LPS have pro-inflammatory effects on hepatocytes, Kupffer cells and stellate cells. Activation of TLR4 by LPS triggers pro-inflammatory gene expression, formation of inflammasomes and generation of ROS via NADPH oxidase 2, which lead to hepatic injury.[22,23,37] Adults with a high-fat intake[42] and children with NAFLD[37] both showed elevated levels of circulating LPS. High-fructose intake has been linked to increased gut permeability in mice via translocation of LPS and activation of TLR4.[43] Additionally, NAFLD patients have higher serum ethanol levels, which is attributed to more fermenting bacteria in their microbiome.[44] This increases the translocation of endotoxins, TLR4 activation, generation of ROS and TNF-α.[44] Concurrently, probiotic treatment of paediatric NAFLD leads to an improved lipid profile and decreased hepatic steatosis.[45-47] These data suggest that high-fat and high-fructose diets contribute to altered gut microbiome and translocation of LPS into the circulation, which stimulates hepatic inflammation via TLR4.

5 | PAEDIATRIC NAFLD

NAFLD already occurs in early childhood,[15] but is more common in adolescents.[8] Insight in paediatric NASH can be considered of specific relevance to identify factors affecting fast progression of NAFLD. In addition, paediatric patients offer the opportunity to evaluate the effects of lifestyle, growth, anthropometry and hormonal factors on the pathogenesis of NAFLD, since these factors all highly fluctuate during the transition from childhood to adulthood.

5.1 | Histology

Paediatric NASH differs in histological characteristics from adult NASH. Whereas adult NASH is characterised by perisinusoidal changes including steatosis, lobular inflammation, hepatocellular ballooning and fibrosis in the absence of portal changes (defined as type 1),[4] paediatric NASH predominantly includes portal steatosis, inflammation and fibrosis (defined as type 2),[48,49] or an overlap between both types. The portal pathology is most striking in boys, severe metabolic syndrome and severe obesity.[1,7,50,51] The portal area is exposed to blood first, receiving the highest concentrations of nutrients, oxygen, hormones and inflammatory or toxic substances, and harbours the highest concentration of Kupffer cells. Portal hepatocytes are specialised in oxidative liver functions such as gluconeogenesis, FA β-oxidation and cholesterol synthesis.[52] One might thus hypothesise that increased oxidative stress or high concentrations of harmful nutrients such as fructose result in portal injury and might accelerate progression from steatosis to NASH in children. This is supported by the finding of increased steatosis in the portal area in rats fed with both sucrose and fructose-enriched diets.[50] Similarly, Nobili *et al* recently showed that high-fructose intake in children correlated with increased portal steatosis, inflammation and hypothesis.[53]

5.2 | Lifestyle and diet

During childhood, children change their lifestyle habits, which influence NAFLD progression in a gender dependent manner. During adolescence, physical activity decreases, while sugar and fat intake increase.[54] Girls are less active than boys and already have reduced levels of physical activity before the onset of puberty,[55,56] which does not correspond with the observation of lower NASH prevalence in girls. The high NASH prevalence in boys correlates with the high-carbohydrate intake in boys.[56] High-carbohydrate intake in humans has been linked to increased IR and portal FA concentrations, upregulation of lipogenesis and inhibition of β-oxidation, all contributing to increased FA load and steatosis.[57] More specifically, fructose intake has emerged as a determinant in NAFLD.[58] The average yearly consumption of fructose has significantly increased over the years.[59] Fructose consumption is higher in children from 9 to 18 years compared to adults[60] and two to threefold higher in NAFLD patients compared to controls.[58,61,62] While glucose mainly serves as a substrate for glycogenesis, fructose mainly serves as a substrate for lipogenesis.[63] High fructose instead of glucose intake was found to increase dyslipidaemia, intrahepatic TG accumulation, IR, hepatic inflammation and fibrosis in humans.[10,64,65] High-fructose diet elicited inflammatory changes in the JNK pathway[66] and enhanced the SOCS-3 expression inducing leptin resistance in rats.[59] High-fructose diet was further found to induce low-grade endotoxaemia via enhanced-intestinal permeability, thereby pre-disposing to NASH.[43,61] Overall, the effects of fructose on NAFLD are clearly illustrated by the decrease in liver transaminases induced by a low fructose and low-glycaemic index diet without caloric restriction in children with NAFLD.[67] Similarly, obese children showed reduced hepatic fat, visceral fat, lipogenesis and IR already after 9 days of fructose restriction.[63]

5.3 | Growth (anabolism)

Insulin sensitivity fluctuates with sex and age[66,68] (Figure 3). At the beginning of puberty, IR is similar in both sexes and gradually rises

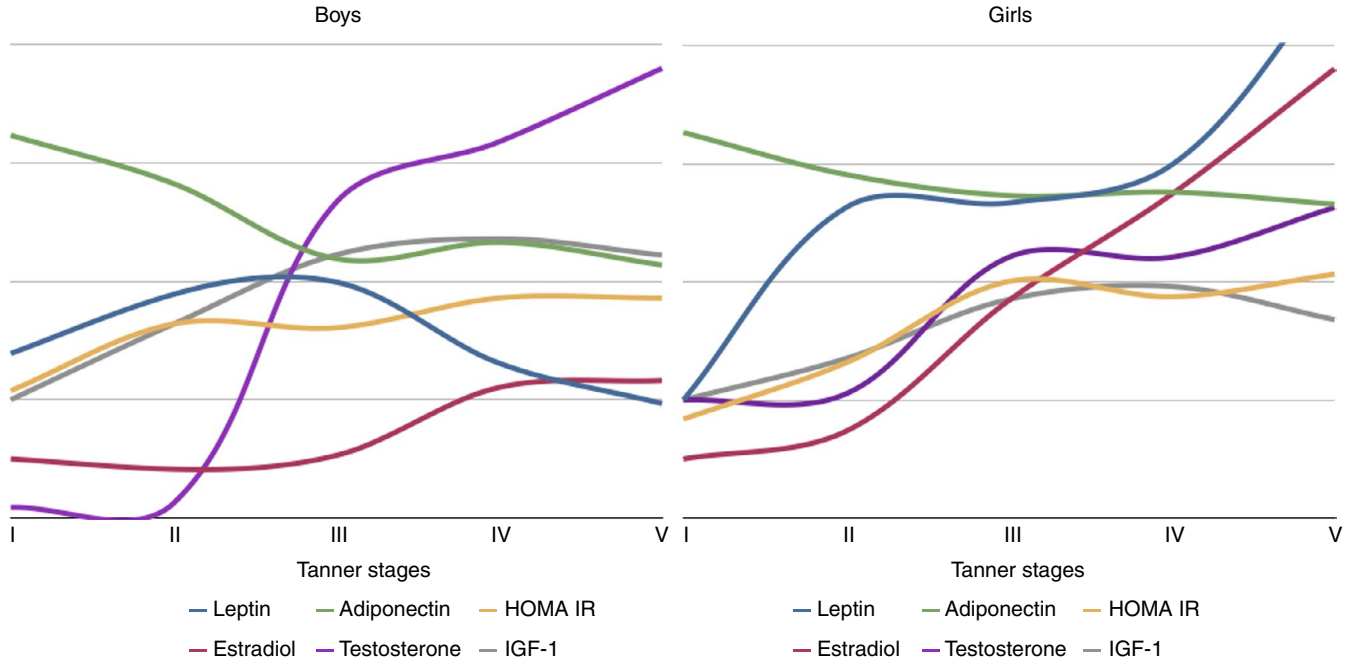

FIGURE 3 Fluctuations of potentially relevant factors in NAFLD pathogenesis in boys and girls during puberty. All trends are based on the general population. Leptin trends are derived from Xu *et al*, Horlick *et al*, and Ellis *et al*[68,99,100]; adiponectin from Xu *et al*, Martos-Moreno *et al*, Böttner *et al* and Andersen *et al*[68,80,101,102]; insulin resistance (HOMA IR) from Xu *et al*[68] and calculated by homeostasis model assessment of insulin resistance; oestradiol from Sehested *et al*,[103] Konforte *et al*[104] and LabCorp; testosterone from Konforte *et al*[104]; and IGF-1 from reference values from Mayo medical laboratories. Distributions were linearly transformed to display relative differences during pubertal stages for individual sexes

during puberty.[66,68] Growth hormone (GH) secretion at night leads to increased IR.[69] To compensate for IR, insulin secretion increases two to threefold during puberty.[69]

GH and its downstream primary mediator insulin-like growth factor 1 (IGF-1) play an important role in the regulation of lipid and glucose metabolism. GH stimulates adipose tissue lipolysis, increasing the hepatic influx of FAs. GH also enhances the hepatic lipogenesis through the activation of SREBP-1c.[70] GH signalling stimulates the hepatic TG secretion via increased VLDL export and enhances FA oxidation.[70,71] Overall, GH lowers fasting free FAs.[69] GH stimulates the hepatic glucose production, and triggers IR specifically in adipose tissue and muscle, leading to hyperinsulinaemia. Hyperinsulinaemia synergises with GH and IGF-1 to stimulate protein anabolism supporting growth.

The increase in GH, IGF-1 and insulin concentrations during adolescence ensure anabolism and thereby protect against steatosis. Circulating GH and IGF-1 levels are lower in NAFLD patients than in controls.[72,73] Patients with GH deficiency have an increased NAFLD prevalence.[72] Moreover, GH secretion is reduced in obesity,[74] without a proportional decrease in IGF-1 concentrations,[70] maintaining a negative feedback loop for GH. This consequently enhances the NAFLD progression. GH replacement therapy in a patient with GH deficiency reversed NASH.[70,74] Furthermore, treatment with GH reduced visceral adiposity and liver fat.[75] All these studies support the anabolic role for GH and IGF-1, protecting against IR and hepatic steatosis during puberty.[69,70]

5.4 | Hormonal sex differences during puberty

The role of hormonal influences in the development of NASH is implied by the studies showing that NAFLD is more common in boys than girls,[7] nearly reaching a ratio of 2:1 in obese children[6] and further diverges during adolescence, increasing in boys and decreasing in girls.[76] In obese children, liver steatosis increases from 40% to 51% during puberty in boys, while it decreases from 17% to 12% in girls (reaching 25% mid-puberty; Figure 4).[76] During puberty, oestrogen secretion increases steadily in girls and only slightly in boys (Figure 3). Oestrogens reduce gluconeogenesis and glycogenolysis.[77] Consequently, human subjects with deficient oestrogen synthesis exhibit increased IR, which can be resolved by oestrogen supplementation.[77] Oestrogens have anti-oxidant properties, as they inhibited ROS generation, lipid peroxide levels, hepatic inflammation, stellate cell proliferation and activation, apoptosis and fibrosis in rats[28] (Figure 2). Oestrogens stimulate the use of lipids as energy source and promote nonvisceral adipose tissue distribution[78] (Figure 2).

Leptin levels also show a sex-dependent pattern: in girls, leptin concentrations increase steadily during puberty, while decreasing from mid-puberty onwards in boys (Figure 3). Androgens inhibit leptin production, but this association is lost in obesity as leptin levels increase[79] (Figures 2 and 4). This might be explained by enhanced conversion of androgens to oestrogens in obesity.[77] Moreover, oestrogens are thought to increase the sensitivity to central leptin.[78] In obese boys and girls, leptin concentrations are high throughout puberty, with a

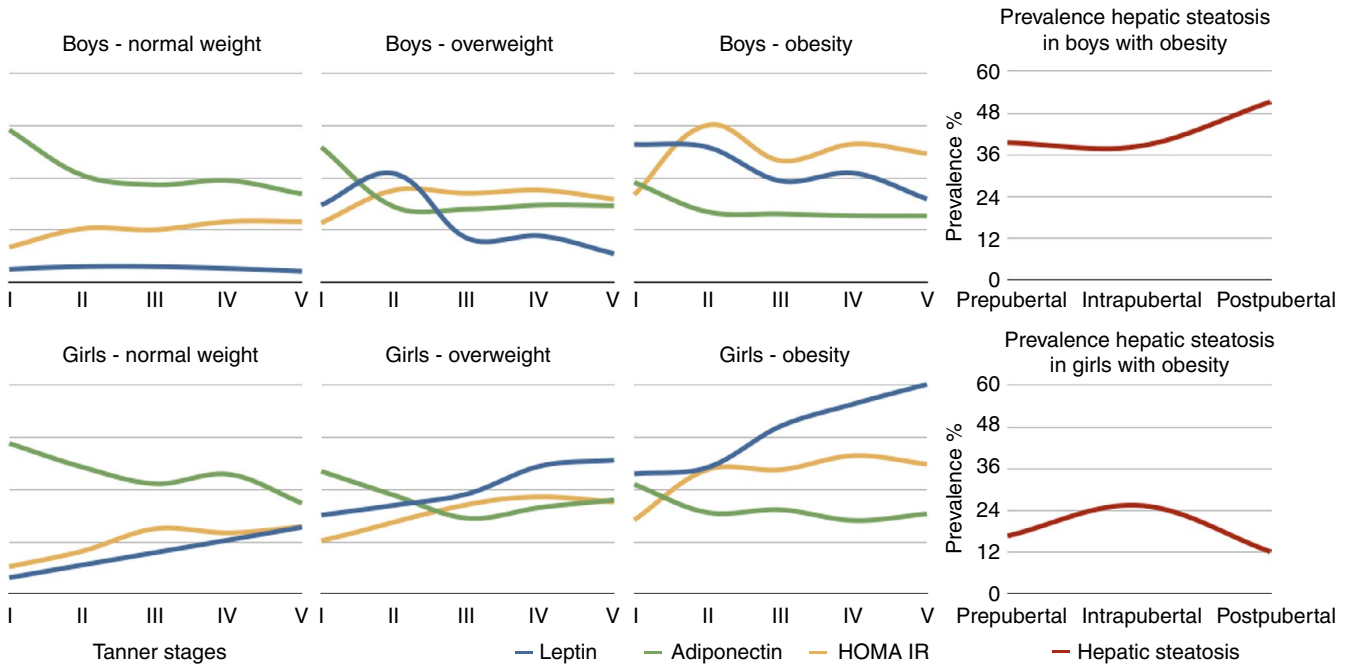

FIGURE 4 Leptin, adiponectin and insulin resistance trends in boys and girls according to weight status and prevalence of hepatic steatosis in boys and girls with obesity. Leptin, adiponectin and insulin resistance trends are derived from Xu *et al*,[68] with HOMA IR calculated by homeostasis model assessment of insulin resistance. Prevalence of hepatic steatosis, determined by ultrasound examination, is derived from Denzer *et al*.[76] Distributions were linearly transformed to display relative differences during pubertal stages for individual sexes

tendency to increase in puberty in girls and decrease in boys (Figure 4), potentially resulting in leptin resistance via increased SOCS-3 expression.[27] Leptin appears as an independent determinant of pubertal IR.[68]

At the beginning of puberty, adiponectin levels are similar in girls and boys and decrease significantly during puberty (Figure 3).[68] In girls, the decrease in adiponectin levels corresponds with the pubertal increase in adipose tissue. The more severe decrease in adiponectin levels in boys can be explained by increasing androgen levels since adiponectin concentrations are inversely correlated with androgen concentrations.[68,80] In overweight or obese children, adiponectin levels correlate inversely with IR[68] (Figure 4).

The prevalence of hepatic steatosis is twofold higher in obese boys than in obese girls. This cannot be explained by IR differences (Figure 4). The higher leptin concentrations and sensitivity, and slightly higher adiponectin concentrations[68] in girls from mid-puberty onwards might contribute to this difference. These differences can be enhanced by the sex hormones and exogenous factors such as diet. Female sex hormones contribute to a protective fat distribution for NAFLD progression, increased leptin and reduced inflammation and oxidative stress. In men, androgens pre-dispose to accumulation of visceral adipose tissue, low leptin and adiponectin concentrations and no direct NAFLD protective effects.

6 | GENETIC VARIANTS CAUSING NAFLD/NASH

NAFLD development and progression is thought to result in a complex interplay between environmental, metabolic and genetic risk factors. Genome-wide association studies (GWAS) have revealed several overlapping genetic risk loci for NAFLD development in adults, children and animal models.[81] The I148M-variant (rs738409) in the *patatin-like phospholipase domain containing 3* (*PNPLA3*) gene is a common single nucleotide polymorphism (SNP) that is associated with an increased prevalence of hepatic steatosis, increased ALT concentrations, severe inflammation and fibrosis.[82] The PNPLA3 protein has lipase activity towards TGs and is highly expressed in hepatocytes and stellate cells.[83] Impaired TG hydrolysis in hepatocytes affects lipid droplet size[84] and leads to the accumulation of hepatic TGs[85] in people carrying the I148M variant without affecting IR.[86] Increased liver fat content was found in homozygote carriers of the *PNPLA3* variant already under the age of 10 years.[87] Rapid NAFLD progression in patients with *PNPLA3* SNPs underscores the importance of abnormal lipolysis in hepatocytes in NAFLD progression. In hepatic stellate cells, the I148M variant leads to the reduced hydrolysis of retinol esters, leading to lower free retinol levels and elevated intracellular retinol retention in stellate cells.[83] This activates hepatic stellate cells and eventually pre-disposes to fibrosis, but pathway connecting the I148M variant to inflammation and fibrogenesis remain unclear. Additionally, the *PNPLA3* I148M variant increases the susceptibility for hepatocellular carcinoma (HCC).[88]

Another strong genetic predictor for NAFLD in children and adults is the *glucokinase regulator* (*GCKR*) SNP (rs1260326) that inhibits *GCKR*'s response to fructose-6-phosphate and associates with liver fat content in NAFLD.[89,90]

Another common SNP that is associated with NAFLD is the *transmembrane 6 superfamily member 2* (*TM6SF2*) E167K variant (rs58542926). This variant decreases *TM6SF2* function, leading to

reduced VLDL secretion.[91] Consequently, hepatic TG content increases and circulating lipid concentrations decrease, leading to an improved overall cardiovascular risk profile, but increased risk of NASH.[92,93] The association between increased hepatic steatosis in patients with the TM6SF2 SNP and advanced liver disease has been found both in adults and children.[94] The association of this gene with NAFLD suggests a significant role for VLDL export in preventing NASH.

Other candidate genes associated with NAFLD have been proposed, including a variant (rs641738) in the *membrane bound O-acyltransferase domain containing 7-transmembrane channel-like 4* (*MBOAT7*) gene, which is associated with NAFLD in adults. Children carrying the minor allele of the *MBOAT7* gene have higher ALT plasma levels compared to non carriers.[95]

Genetic modifiers of oxidative stress, inflammatory response, IR, lipid metabolism, fibrogenesis and satiety all have been suggested to influence the pathology of NAFLD. The role of most of these genes is less established and requires further elucidation particularly in paediatric NAFLD. So far, potential NAFLD modifying genes were not reported to differ between children and adults. Moreover, it is likely that the risk of NAFLD progression is determined by the combined effect of risk genes.[96]

7 | CONCLUSION

We live in an era in which many persons have an unhealthy lifestyle including excess of food and insufficient physical activity. The harmful impact of this unhealthy lifestyle on disease development is becoming more and more apparent. Research on the contributing factors and mechanisms enabling identification of people at a particularly high risk for development of NAFLD is urgently needed. From the current literature, it can be concluded that diet, lifestyle, fat distribution and microbiome are partially influenceable factors that can pre-dispose to NASH, whereas gender, sex hormones and genetic pre-disposition are also risk factors, but cannot be influenced. All these factors, together with blood concentrations of liver enzymes and liver histology could potentially be used to stratify individuals with NAFLD into a low or high risk of progression to NASH.

Many of these factors that determine NAFLD progression, including lifestyle, body fat distribution, IR, adipokines and hormone concentrations are subject to enormous changes during the transition from childhood to adulthood. From studies on paediatric NAFLD, we deduce that oestrogens exert protective effects through a favourable fat composition. With rising oestrogen levels during puberty, leptin concentrations also increase, while androgens inhibit leptin secretion. The pre-pubertal gender differences in NAFLD underscore the involvement of other factors as well. Dietary intake seems an important contributing factor. The specific portal histological abnormalities in children suggest an important role for oxidative stress and toxic substances, which appear related to high-fructose intake. Awareness of the damaging effects of fructose is necessary to prevent further increase in NAFLD incidence and progression, especially because this might guide dietary advice in avoiding certain products that are generally considered healthy.

Moreover, genetic profiles will help to identify NAFLD patients prone to rapid NASH progression. Various genetic variants influencing the susceptibility to NAFLD and NASH development in both childhood and adulthood have now been identified. With the feasibility of large genetic screens, new insights can be expected from whole exome sequencing, for example in confirmed cases of NASH and their matched, healthy controls of same ethnicity, and also in the animal models of NAFLD.[97,98]

Taken together, factors associated with NAFLD development and progression become increasingly clear from the large number of studies focusing on NAFLD as an important health threat worldwide for now and future generations. Based on these studies, we conclude that the human body has different ways to handle excess nutrients in order to minimise damage. Whereas excessive glucose and sucrose lead to carbohydrate burden thereby leading to diabetes mellitus, carbohydrates like fructose result in lipid burden. FAs are stored in the liver and adipose tissue as TGs, lowering toxic FA intermediates. However, TG storage leads to adipose tissue inflammation, which increases the risk of developing the metabolic syndrome. Accordingly, elevated FA β-oxidation reduces FA concentrations, but increases oxidative damage, accelerating progression to NASH. Alternatively, FAs can be exported from the liver as VLDL, decreasing the risk of progression to NASH, but worsening the cardiovascular risk profile. Strain on specific pathways will determine the symptoms in individual patients. Because each strategy to handle excess nutrients comes at a price, optimising the balance between energy intake and expenditure still seems the most effective therapeutic option.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

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CONFLICT OF INTEREST

The authors have no further conflicts of interest to declare.

AUTHORSHIP

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