REVIEW

Pediatric non-alcoholic fatty liver disease: Recent solutions, unresolved issues, and future research directions

Maria Grazia Clemente, Claudia Mandato, Marco Poeta, Pietro Vajro

Maria Grazia Clemente, Pediatric Clinic, Department of Surgical, Microsurgical and Medical Sciences, University of Sassari, 07100 Sassari, Italy

Claudia Mandato, Pediatrics of AORN Santobono-Pausilipon, 80123 Naples, Italy

Marco Poeta, Pietro Vajro, Pediatrics, Department of Medicine, Surgery and Dentistry - "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Pietro Vajro, ELFID European Laboratory for the Investigation of Food Induced Disease, 80138 Naples, Italy

Abstract

Non-alcoholic fatty liver disease (NAFLD) in children is becoming a major health concern. A "multiple-hit" pathogenetic model has been suggested to explain the progressive liver damage that occurs among children with NAFLD. In addition to the accumulation of fat in the liver, insulin resistance (IR) and oxidative stress due to genetic/epigenetic background, unfavorable lifestyles, gut microbiota and gut-liver axis dysfunction, and perturbations of trace element homeostasis have been shown to be critical for disease progression and the development of more severe inflammatory and fibrotic stages [non-alcoholic steatohepatitis (NASH)]. Simple clinical and laboratory parameters, such as age, history, anthropometrical data (BMI and waist circumference percentiles), blood pressure, surrogate clinical markers of IR (acanthosis nigricans), abdominal ultrasounds, and serum transaminases, lipids and glucose/insulin profiles, allow a clinician to identify children with obesity and obesity-related conditions, including NAFLD and cardiovascular and metabolic risks. A liver biopsy (the "imperfect" gold standard) is required for a definitive NAFLD/NASH diagnosis, particularly to exclude other treatable conditions or when advanced liver disease is expected on clinical and laboratory grounds and preferably prior to any controlled trial of pharmacological/surgical treatments. However, a biopsy clearly cannot represent a screening procedure. Advancements in diagnostic serum and imaging tools, especially for the non-invasive differentiation between NAFLD and NASH, have shown promising results, *e.g.*, magnetic resonance elastography. Weight loss and physical activity should be the first option of intervention.

Effective pharmacological treatments are still under development; however, drugs targeting IR, oxidative stress, proinflammatory pathways, dyslipidemia, gut microbiota and gut liver axis dysfunction are an option for patients who are unable to comply with the recommended lifestyle changes. When morbid obesity prevails, bariatric surgery should be considered.

Key words: Non-alcoholic fatty liver disease; Childhood obesity; Non-alcoholic steatohepatitis; Hepatic metabolic syndrome; Non-alcoholic fatty liver disease diagnosis

Core tip: Due to the high prevalence of obesity worldwide, non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome, represents the most common chronic liver disease in children living in industrialized countries. The present review summarizes the currently available knowledge on NAFLD pathogenesis, diagnosis and treatment, highlights new research achievements that will likely influence therapeutic strategies and discusses possible future directions in pediatric research.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and liver-related morbidity and mortality worldwide. NAFLD has an estimated prevalence of 20%-30% in Western Countries and 5%-18% in Asia and is associated with the pandemics of obesity and excessive fructose consumption. Remarkably, the overall prevalence of NAFLD in children has reached approximately 10%, including up to 17% in teenagers and 40%-70% among obese children. While often benign and self-limiting, steatosis can progress with hepatocyte injury into non-alcoholic steatohepatitis (NASH) in 3%-5% of patients. NASH is characterized by lobular and/or portal inflammation, varying degrees of fibrosis, hepatocyte death and pathological angiogenesis[1]. Although NAFLD progression to advanced fibrosis and cirrhosis is mainly a chronic phenomenon, liver transplantation has been described in youths[2].

NAFLD PATHOGENESIS: OLD AND NEW FACTS

The "multiple-hit" hypothesis largely explains NAFLD pathogenesis and progression[3]. At disease onset, NAFLD is characterized by fat accumulation in the liver (steatosis) and insulin resistance (IR), which is heavily influenced by a sedentary lifestyle, hypercaloric diets, genetic susceptibility, and epigenetics; however, the interactions between these pathomechanisms remains poorly understood[4,5]. Other associated comorbidities can develop in addition to liver damage. NAFLD is the hepatic manifestation of metabolic syndrome (MetS), which has been linked with obesity, IR, hypertension and hyperlipidemia.

Our review presents recent insights into NAFLD pathogenesis, diagnosis and treatment, underlines a number of new research achievements that are likely to impact therapeutic strategies and discusses possible future perspectives in pediatric research.

FAT ACCUMULATION IN THE LIVER AND ADIPOSE TISSUE

The liver does not normally store triglycerides (TG), but TG accumulation in the cytoplasm of liver cells is not necessarily toxic *per se*. Overeating causes a breakdown of physiological mechanisms that regulate energy harvesting in liver and adipose tissues (AT). In conditions of excessive feeding, an overload in hepatic fat accumulation exacerbates IR by interfering with the phosphorylation of insulin receptor substrates. Specifically, a key aspect of fat metabolism imbalance is the dysregulation of insulin signaling pathways, *e.g.,* sterol regulatory element binding protein 1; fatty acid translocase cluster differentiation protein 36 (FAT/CD36); and hormone-sensitive lipase, which leads to TG imbalance, fatty acid mitochondrial oxidation, and lipoprotein excretion and transport[6].

Recent insights into lipotoxicity showed that fatty hepatocytes release large quantities of extracellular vesicles that transport bioactive molecules, including mRNA, non-coding RNAs, proteins, DNA and lipids, to target cells. The signals shuttled by extracellular vesicles control inflammation and fibrogenesis by recruiting or activating macrophages and quiescent hepatic stellate cells (HSCs), respectively[1].

AT has emerged as an active and crucial player in the development of hepatic steatosis and chronic low-grade inflammation, and it constitutes a key link between obesity and metabolic dysregulation. TG-rich chylomicrons are mainly transported to peripheral tissues (80%), where free fatty acids (FFAs) are released and available for uptake *via* lipoprotein lipase (LPL). One of the strongest inhibitors of LPL is apolipoprotein C-III (ApoC-III)[6]. In individuals with IR, insulin fails to efficiently suppress ApoC-III in the liver, which inhibits LPL action in peripheral tissues and favors the hepatic uptake of TGs-rich chylomicrons remnants.

In normal conditions, AT protects the body from excessive exposure to fatty acids. However, exhausting adipocyte expandability can produce lipotoxicity,

oxidative stress, and peripheral IR. Consequently, AT acquires a proinflammatory profile characterized by adiponectin suppression and the promotion of other adipocytokines, *e.g.,* adiponectin, leptin, resistin, and tumor necrosis factor-alpha (TNF-α), which leads to progressive liver damage. In particular, leptin can activate HSCs and suppress their apoptosis. Furthermore, peripheral IR independently predicts hepatic histological characteristics and alters the cardio metabolic risks in non-diabetic biopsy-proven NAFLD patients[7].

OXIDATIVE STRESS

Excessive FFA influx to the liver, a known cause of hepatic IR, overwhelms the mitochondria and causes the accumulation of incompletely oxidized substrates, such as fatty acids, ceramide and diacylglycerides. Increased beta-oxidation reduces the availability of oxidized cofactors (NAD and FAD) and decreases the outflow from the respiratory chain, which leads to the accumulation of electrons, ROS production, and cellular damage. Mitochondria become the targets of ROS-induced damage. Oxidative changes in respiratory complexes impair their catalytic functions and cause mutagenesis of the mitochondrial DNA. This further worsens oxidative damage, leading to hepatocellular death and NASH progression[8]. In addition, mito-chondrial proliferation and differentiation, mostly regulated by the transcription coactivator peroxisome proliferator-activated receptor (PPAR)-γ-coactivator-1α, can be impaired in NASH.

The oxidative stress induced by fatty acid over-load in hepatocytes is derived from mitochondria, peroxisomes and microsomes. IR significantly in-creases peroxisomal oxidation because insulin is the principal inhibitor of cytochrome P450 4A (CYP4A), a key enzyme in this pathway. This inhibition amplifies cytotoxic ROS and lipid peroxidation. These products can diffuse into the extracellular space, influence Kupffer cells and HSCs and induce the nuclear-factor κB (NFκB) pathway, which causes the subsequent synthesis of TNF-α and several other proinflammatory and fibrogenic cytokines[6].

SUGARS AND FAT ACCUMULATION

Over the last few decades, the progressively increased intake of added sweeteners has been associated with obesity, hypertension, and other components of MetS, including steatosis and severe liver damage. Obese children with NAFLD consume more carbohydrates when compared with their obese counterparts without NAFLD. High blood glucose levels play a major role in liver fat accumulation by activating carbohydrate response element-binding protein (ChREBP), which physiologically regulates glycolysis and *de novo* lipogenesis independently from insulin. Saturated fat stimulates fatty acid oxidation *via* a PPARα-dependent mechanism and *de novo* lipogenesis in the liver; however, the prominence of these effects depends on the sucrose content in the diet[6]. Hypercaloric diets enriched in fat and fructose/sucrose may act either by favoring the occurrence of systemic IR and, in turn, a dangerous hepatic FA accumulation or by causing visceral fat deposition and abdominal obesity, which are independent risk factors for MetS[9].

Fructose, a highly lipogenic sugar, is found in fruits and vegetables with a high fructan content (*e.g.,* artichokes, wheat, leeks, garlic) and honey. Fructose, sucrose and high-fructose corn syrup are used as added sweeteners, and the high consumption of these compounds has sparked increasing concern. Fructose metabolism differs from that of glucose because it is metabolized almost completely in the liver *via* GLUT5 and not by the insulin-dependent transporters GLUT1 and GLUT4; thus, fructose metabolism is relatively unregulated by insulin. Once in hepatocytes, fructose is mainly converted by fructokinase to fructose 6-phosphate, which is then hydrolyzed into fructose 1-6 bisphosphate by fructose aldolase in order to enter glycolytic/gluconeogenesis pathways. Fructose metabolism induces a hepatic depletion of ATP with a consequent increase in AMP and uric acid. Although fructose does not acutely increase insulin, fructose can ultimately increase IR and fasting glucose and insulin levels. However, the mechanism of action remains to be determined[10].

The metabolic destiny of ingested fructose is prevalently towards oxidation and conversion to glucose rather than to TG. Nonetheless, postprandial lipedema is higher after fructose consumption. Fructose determines the insulin-independent induction of several hepatic lipogenic enzymes (*e.g.,* pyruvate kinase, NADP+-de-pendent malate dehydrogenase, citrate lyase, acetyl CoA carboxylase, fatty acid synthase, and pyruvate dehydrogenase) and an increase in VLDL production and hepatic fat storage[11]. High fructose intake is linked with hepatocyte apoptosis, hepatic fibrosis and dyslipidemia[10].

In NAFLD patients, ingested fructose may alter the microbiome, which increases the movement of endotoxins into the portal system because of increased tight junction permeability. These changes increase liver inflammation and IR *via* Toll-like receptor (TLR) 4 activation. Moreover, obese children with NAFLD showed an increased absorption and exaggerated metabolic response to fructose administration when compared with lean controls. This effect may be due to the altered intestinal bacterial flora fermentation of fructose into hydrogen or an up-regulation of fructose transporter GLUT5 in the intestinal epithelium[12].

GUT-LIVER AXIS AND GUT MICROBIOTA

The intestinal ecosystem has numerous physiological and pathological interactions with the host, including regulation of mucosal/systemic immunity and metabolic

and trophic functions. Intestinal microorganisms produce highly conserved molecules, such as pathogen associated molecular patterns (PAMPs) (*i.e.*, lipopolysaccharides/endotoxins), which are recognized by specific pattern recognition receptors (PRRs), including TLRs and NOD-like receptors. In NAFLD, an alteration in gut microbiota and enhanced gut permeability increase the exposure of the liver to PAMPs and/or other products of intestinal tissue injury, such as damage associated molecular patterns. Therefore, diet- and/or gut-microbiota-dependent increases in gut-derived products can cross the impaired gut permeability barrier, activate molecular mechanisms of innate immune response, and act as possible inductors of NAFLD progression[13,14].

Conflicting qualitative/quantitative differences in bacterial flora composition have been found in obese subjects, possibly due to differences in methodology and/or host related factors (*e.g.,* immune system response and diabetes)[15]. In obese adults, a functional study showed that the microbiota break down otherwise non-absorbable polysaccharides into monosaccharides and short chain fatty acids (SCFAs), which may provide extra calories. Monosaccharides activate the hepatic carbohydrate response element binding protein (ChREBP), which increases hepatic lipogenesis and fat accumulation; furthermore, SCFAs induce leptin production[13].

Recent research suggests a critical role of the Farnesoid X receptor (FXR) in carbohydrate and lipid metabolism, regulation of insulin sensitivity and NAFLD pathogenesis. FXR is a nuclear bile acid (BA) receptor highly expressed in tissues that participate in BA metabolism, such as the liver, intestine, and kidneys. Gut microbiota can change the BA composition of the host, particularly taurine-conjugated BA, which can antagonize intestinal FXR and lead to metabolic dysfunction, including obesity and IR. BA can also influence NAFLD *via* activation of hepatic FXR and G protein-coupled receptor TGR5[16,17].

ENDOGENOUS ALCOHOL PRODUCTION

The gut microbiota of healthy subjects produces trace amounts of endogenous ethanol (EE) from unabsorbed dietary sugars. In the liver, EE generates acetaldehyde, which in turn is oxidized to non-toxic acetate. In small intestinal bacteria overgrowth-related conditions, EE concentrations are significantly higher than in control subjects[18]. Furthermore, recent pediatric studies suggest that EE in NAFLD patients correlates with increased intestinal permeability[14]. Increased blood ethanol levels in patients with NAFLD might also result from an insulin-dependent impairment of alcohol dehydrogenase activity in liver tissue rather than an increase in EE synthesis[19].

NAFLD-RELATED GENES

Mutations in several genes involved in lipid and glucose metabolism, redox cellular state and inflammation can lead to hepatic steatosis. A single nucleotide polymorphism (SNP) of the patatin-like phospholipase 3 gene (*PNPLA3*), which encodes the insulin-regulated phospholipase adiponutrin, has been shown to be associated with both hepatic steatosis and inflammatory changes/fibrosis. Recent studies in pediatric cohorts showed that patients with the genetic variant *PNPLA3 I48M* (rs738409; C→G) are more likely to have NAFLD but not IR[20]. Furthermore, a high sugar diet strongly interacts with the *PNPLA3GG* homozygous variant and predicts increased hepatic fat[21].

A second gene particularly related to the progression of NAFLD to NASH is the G-protein-coupled-receptor 120 (*GPR120*), a receptor for polyunsaturated fatty acids (PUFAs) expressed by adipocytes, Kupffer cells and hepatocytes. The interaction between PUFAs and *GPR120* expression in macrophages reduces inflammation by inhibiting NF-κB activity. The *GPR120 270H* allele reduces the anti-inflammatory action of the GPR120 receptor. Therefore, *270H* carriers present pathological ALT levels due to liver injury caused by oxidative stress, mitochondrial dysfunction and overproduction of proinflammatory cytokines[22]. Alimentary supplementation of docosahexaenoic acid (DHA), an n-3 PUFA in fish oil, has been recently suggested as a potential therapeutic. DHA activates the GPR120 receptor, which exerts potent anti-inflammatory and insulin-sensitizing activities[23].

Under conditions of increased hepatic fatty acid influx or decreased efflux, PPARα activation prevents the accumulation of TGs by increasing the rate of fatty acid catabolism. The down regulation of the PPARα gene induced by the *Val227Ala* SNP was suggested to influence NAFLD pathogenesis; however, this effect was not histologically confirmed in an adult Italian population[24].

PPARγ, a molecular target of glitazones, is highly expressed in AT and regulates adipocyte differentiation and FA uptake and storage. Controversial evidence has suggested a role for the *Pro12Ala* loss-of-function SNP in PPARγ in IR and liver disease progression[24].

OTHER MECHANISMS INVOLVED IN NAFLD PATHOGENESIS

Iron and copper in pediatric obesity and NAFLD

Trace elements are critical in regulatory, immunologic, and antioxidant functions by protecting against inflammation and peroxidation. Disruption of the metal detoxification processes located in the liver are plausibly related to NAFLD development *via* oxidative stress. Perturbations of iron and copper (Cu) homeostasis have been shown to contribute to NAFLD pathogenesis[25].

Ghrelin-ghrelin O-acyltransferase system in the pathogenesis of NAFLD

The ghrelin-ghrelin *O*-acyltransferase (GOAT) system

has been recently reported to play a crucial role in both the development of steatosis and its progression to NASH. The ghrelin-GOAT system is involved in IR, lipid metabolism dysfunction, and inflammation, all of which play important roles in the pathogenesis of NAFLD. The ghrelin-GOAT system is linked to energy and lipid metabolism, IR, inflammation, and apoptotic cell death, which are common to both obesity and NAFLD. Therefore, the role of the ghrelin-GOAT system in NAFLD has recently become a subject of considerable interest. The role of ghrelin in appetite regulation (orexigenic function) and energy metabolism is well established and is now recognized as a promising target for the treatment of obesity and NAFLD[26].

Implication of vitamin D metabolism

Low levels of vitamin D are associated with obesity and NAFLD[27]. Vitamin D receptors are expressed in a wide range of tissues, including the liver, and the immune system (*e.g.*, T and B cells, macrophages, and monocytes). Vitamin D has a paracrine/autocrine role in the regulation of cell proliferation, differentiation, apoptosis and immunity. A growing body of evidence suggests that low levels of 25(OH)D are strongly associated with MetS. These data are summarized in a recently published meta-analysis. NAFLD subjects were 26% more likely to be vitamin D deficient when compared with controls[28]. It has been supposed that the progression of NAFLD caused by western diet is exacerbated by vitamin D deficiency. Specifically, activation of TLR2 and TLR4 *via* CD14/LBP and stimulation of downstream inflammatory signaling molecules leads to steatosis and inflammation. The metabolic, anti-inflammatory and antifibrotic properties of vitamin D provide plausible mechanisms by which vitamin D may influence disease progression and severity.

Obstructive sleep apnea syndrome

Obesity-related obstructive sleep apnea syndrome (OSAS) is considered to be a risk factor for more severe NAFLD. OSAS is a sleep disorder characterized by a complete or partial upper airway obstruction due to pharyngeal collapse during sleep, snoring, frequent nocturnal awakenings, sleep deprivation, and daytime sleepiness. Recently, several studies showed a correlation between OSAS and the progression of simple steatosis to steatohepatitis. This progression may be induced by a chronic intermittent hypoxia that promotes liver inflammation and fibrosis. Liver injury occurs through the enhancement of oxidative stress *via* the ischemia-reperfusion damage process and several molecular mechanisms, including the promotion of inflammatory cytokines (IL-1 and IL-6) in hepatocytes and macrophages by hypoxia-inducible factor and NFκB, which then modulates fibrogenesis and angiogenesis in Kupffer cells and HSCs[29,30].

NAFLD DIAGNOSIS

The initial step of identification requires that primary care pediatricians calculate and record the BMI at every visit for every child that is potentially "at risk" for obesity and NAFLD. NAFLD is generally "a silent liver disease" because it can present without any warning signs, and only those who develop NASH with more severe liver damage will have some symptoms of chronic liver disease. In clinical practice, NAFLD is therefore usually suspected based on the findings of hypertransaminasemia and/or an ultrasonographic bright liver in an otherwise healthy child who is overweight (BMI between 85th and 94th percentile) or obese (BMI ⩾ 95th percentile). However, the full spectrum of histologic NAFLD can also be present with normal liver tests. Data from mainly adult studies[31] show that NAFLD is frequently found in conjunction with other conditions, such as visceral obesity, hypertension, hyperinsulinemia, IR or diabetes, dyslipidemia with an atherogenic lipid profile, or fructose rich diet-related hyperuricemia. These conditions are also predictors of liver involvement and/or fibrotic progression. The available diagnostic procedures for NAFLD include a number of clinical signs, blood tests and imaging techniques[32,33].

CLINICAL SIGNS

At clinical examination, the presence of acanthosis nigricans, increased waist circumference (WC) and hepatomegaly should be recorded as they may represent surrogate markers of IR (one of the pathogenic hits), central/visceral obesity and liver involvement, respectively. Furthermore, the combination of these features can suggest NAFLD risk.

Regarding WC, Lin *et al*[34] found that for every 5 cm increase in waist circumference there was an odds ratio of 1.4 for predicting ultrasonographic liver steatosis. Increased WC is also associated with increased hepatic fibrosis[35,36].

Simple screening questionnaires or more specific polysomnography can be used to confirm/rule out OSAS, a novel pathogenetic factor of NAFLD in children[37].

In female adolescents with NAFLD, a screening for polycystic ovary syndrome (PCOS) is recommended, especially when history and clinical examination show signs of hyper-androgynism (*i.e.*, acne, hirsutism, and irregular menstrual cycles). Pre-menopausal women with NAFLD present a high prevalence of PCOS, which may be because both diseases share common pathogenic mechanisms linked to MetS and obesity[38].

Furthermore, the detection of NAFLD may be helpful for the early identification of individuals with an increased cardiovascular (CV) risk. In obese patients, liver involvement by itself may promote CV diseases independently of other MetS components.

Indeed, children with ultrasound-diagnosed NAFLD and hypertransaminasemia present functional and morphological vascular changes [*i.e.*, endothelial dysfunction, impaired flow-mediated dilation of the brachial artery, increased carotid intima-media thickness, and altered ventricular functions]. Patients with NASH are at higher risk for atherosclerosis due to peripheral IR, pro-atherogenic lipid profile, oxidative stress and systemic inflammation[39]. Thus, blood pressure evaluation, control, and monitoring should be an integral component of the clinical management of children with NAFLD[40].

SERUM LEVELS OF HEPATOBILIARY ENZYMES AND MOLECULES

Alanine aminotransferase: Although NAFLD is the most common cause of hypertransaminasemia in children and adolescents[41], elevated Alanine aminotransferase (ALT) is not a sensitive marker of disease existence and/or severity at ordinarily used thresholds[42]. According to the Screening ALT for Elevation in Today's Youth study, normal values of transaminases for teenagers and children are presently set too high to detect liver steatosis. The 95th percentile levels for ALT in healthy weight, metabolically normal, liver disease-free children should be 25.8 U/L for boys and 22.1 U/L for girls. With this cut-off, the diagnostic sensitivity raised from 32% to 80 % in boys and from 36% to 92% in girls[43].

Aspartate aminotransferase: The evaluation of both Aspartate aminotransferase (AST) and ALT values is essential because an increased AST/ALT ratio can reflect a progressive and more severe condition, such as fibrotic NASH.

γ-glutamyl transpeptidase: High serum levels of γ-glutamyl transpeptidase (GGT) represent a risk factor for advanced fibrosis in NAFLD[44].

Bile acids: Serum bile acids (BA) levels decrease in early NAFLD and increase during its progression to fibrosis. Given that BA levels are increased in cirrhotic adults, it has been postulated that the continuous rise in BA as NAFLD advances may have a value as a noninvasive biomarker for pediatric NAFLD progression[45].

Glucose, insulin, the homeostatic model assessment-IR and lipid profiles: These should be evaluated in all children with suspected or diagnosed NAFLD.

IMAGING TECHNIQUES

Imaging methods, such as ultrasounds (US) and magnetic resonance imaging (MRI) ± chemical shift imaging or spectroscopy, have been increasingly approved as noninvasive alternative methods to diagnose and monitor NAFLD/NASH[36,46]. (1) US is safe, but it is limited by the inability to detect fatty liver (liver brightness *vs* kidney parenchymal echogenicity) when steatosis involves < 30% of hepatocytes[47]. US has several advantages for use as a screening tool: relatively low cost, large diffusion in medical community and feasibility. US has been used to assess the outcome efficacy in pediatric trials with good compliance in children and parents[36,48]; (2) generally, MRIs are not cost-effective, even with certain modifications that could enable rapid and reproducible measurements of steatosis and fibrosis. With modern MR spectroscopy software, the measure of intracellular water and lipid content can be used to define hepatic steatosis if the hepatic TG/water ratio is > 0.5. Recently, advanced MRI for the quantitative assessment of hepatic steatosis was validated in a pediatric study that confirmed the correlation and diagnostic accuracy of MRI-estimated liver proton density fat fraction as a biomarker for hepatic steatosis when compared with histologic steatosis grade. Although magnitude-based MRI has the potential for clinical utility in the evaluation of NAFLD, there is no current single threshold value with sufficient accuracy for diagnosis of children[49]; and (3) other radiologic imaging methods can estimate liver stiffness as a surrogate for liver fibrosis, including transient elastography (TE), magnetic resonance elastography (MRE), and acoustic radiation force impulse imaging (ARFI)[50]. Perhaps the most promising imaging tool for differentiation between hepatic steatosis and NASH is MRE. A recent study underlined the utility of MRE for discriminating advanced fibrosis (stage 3-4) from mild fibrosis (stage 0-2) with excellent sensitivity (0.86) and specificity (0.91). In addition, magnetic resonance scanning allows an estimate of total fat tissue[51].

LIVER BIOPSY HISTOLOGY

The histological spectrum of NAFLD ranges from simple steatosis to NASH and cirrhosis. Pediatric fatty liver disease often displays a pattern that is distinct from adults. Schwimmer *et al*[52] categorized NASH into three types according to the histological characteristics: adult type, pediatric type, and overlap type. The first was characterized by steatosis, ballooning degeneration, and perisinusoidal fibrosis. The second and third types (mainly found in children) were characterized by steatosis, portal inflammation, and portal fibrosis.

Liver histology remains the mainstay measure for clinical trials. Biopsies are used both for enrollment and determining the outcome of clinical trials. End points, such as reversion of NASH or regression of fibrosis, may require a clear histological definition. However, liver biopsies are not exempt from possible sampling errors, which can result in substantial misdiagnosis and staging inaccuracies because histological lesions of NASH may be unevenly distributed throughout the liver parenchyma. In 2012 the ESPGHAN panel[36] indicated

Table 1 Non-invasive diagnostic tests of non-alcoholic fatty liver disease		
Hepatic fibrosis scores	**Advanced biochemical markers**	**Newly proposed markers**
AST/ALT ratio	Cytokeratin 18 fragment levels (CK-18)	Serum potassium
Platelet ratio index (APRI)	Extracellular matrix turnover biomarkers:	Soluble Fas and Fas Ligand (sFasL)
Fibrosis (FIB)-4 index	Enhanced liver fibrosis (ELF) test	Plasma catepsin D (CatD)
NAFLD Fibrosis score (NFS)	Amino-terminal propeptide III procollagen (PIIINP)	Circulating zonulin
Pediatric NAFLD fibrosis score	Hyaluronic acid (HA)	Adipokines (*e.g.,* chemerin)
		Serum Uric Acid (UA)
		Vitamin D
		Proteomics signature
		Metabolomic signature

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Non-alcoholic fatty liver disease.

that although a liver biopsy is required for a definitive NAFLD diagnosis, its invasive nature and high cost prohibit the use of biopsies as a screening procedure. The same panel recently[53] highlighted that indications for liver biopsy in NAFLD are still controversial and there is limited available evidence for the formulation of guidelines. Indications for liver biopsy are only based on expert opinions that must take into consideration a differential diagnosis and the risk of progression of liver disease to cirrhosis. For a differential diagnosis, a liver biopsy should be considered after noninvasive biochemical and metabolic tests have been completed.

The ESPGHAN panel generally accepted the criteria of Roberts *et al*[54] and summarized the indications for liver biopsy: to exclude other treatable disease, in cases of clinically suspected advanced liver disease, before pharmacological/surgical treatment, and as part of a structured intervention protocol or clinical research trial. In the most frequent age range of pediatric presentation (children older than 10 years), a liver biopsy should be considered if hypertransaminasemia or US hyperechogenicity persists after attempts at weight reduction and lifestyle changes for 3-6 mo and if the laboratory workup is still inconclusive. A liver biopsy should be completed even earlier in patients with a family history of NASH, hepatosplenomegaly, comorbidities, hypothalamic expansive processes, marked hypertransaminasemia, or elevated serum fibrosis markers.

NON-INVASIVE INDIRECT DIAGNOSIS OF NAFLD

A number of non-invasive diagnostic tests for NAFLD have been proposed and are summarized in Table 1.

Hepatic fibrosis scores: Noninvasive methods for diagnosing liver fibrosis in patients with NAFLD include simple fibrosis scores that use readily available laboratory tests (AST/ALT ratio, AST to platelet ratio index, fibrosis-4 index, and NAFLD fibrosis score), These scores were developed in adults and have poor performance in diagnosing advanced fibrosis in children with NAFLD. Recently, Alkhouri *et al*[55] developed a

new "Pediatric NAFLD fibrosis score" (based on ALT, alkaline phosphatase, platelet counts and GGT) that appears more capable of predicting advanced fibrosis.

Advanced biochemical markers: hepatocyte cell death and extracellular matrix turnover: Biomarkers of hepatocyte cell death, such as cytokeratin 18 (CK-18) fragment levels, can be used. Markers of extracellular matrix turnover, such as the enhanced liver fibrosis test that consists of tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type III procollagen and hyaluronic acid (HA), show good correlation with fibrosis stage[56].

Newly proposed markers: Serum potassium levels show an inverse relationship with the presence of aggressive disease (NASH and fibrosis) in children with NAFLD[57]. Markers of the extrinsic pathway of hepatocyte apoptosis [*e.g.,* circulating soluble Fas and Fas ligand (sFasL) levels] that are elevated in children with NASH may be potential novel biomarkers[58]. Plasma cathepsin D (CatD) holds a high diagnostic value for distinguishing pediatric patients with hepatic inflammation from children with simple steatosis. A reduction in CatD correlates better than ALT and CK-18 with pediatric disease progression, in terms of liver inflammation severity, steatosis grade, hepatocellular ballooning, NAFLD activity score, and this correlation reaches almost maximum accuracy after factoring in the CK-18 levels[59]. Circulating zonulin is associated with the stage of liver disease in obese children with biopsy-confirmed NAFLD. Increased zonulin values correlated with steatosis severity[60]. Serum levels of classic adipokines (*e.g.,* leptin, adiponectin, and resistin) are altered in patients with NAFLD. Recently, the role of novel adipokines (*e.g.,* chemerin, omentin, and vaspin) has emerged in NAFLD pathogenesis; however, their serum concentrations in obese children with NAFLD have been poorly studied. Chemerin appears to be the most suitable non-invasive biomarker for predicting intrahepatic lipid content[61]. Serum uric acid (UA), as a surrogate marker for fructose intake, is significantly increased in children with NASH[62]. Furthermore, elevated UA levels positively correlate with IR and the number of

Table 2 Fatty liver disease: selection of possible causes in children and adolescents[41]

General or systemic	Genetic-metabolic causes	Drugs/chemicals
Anorexia nervosa (± refeeding)	α- and β-oxidation defects	Corticosteroids
Celiac disease	Abeta or hypobetalipoproteinemia	Diltiazem
Diabetes mellitus type 1	Alpha 1 -antitrypsin deficiency	Ecstasy, Cocaine, Solvents
Hepatitis C	Cholesterol ester storage disease/LAL	Estrogens
Hypothalamic–pituitary disorders	Citrin deficiency	Ethanol
Inflammatory bowel disease	Congenital disorders of glycosylation	Methotrexate
Obesity/Metabolic syndrome	Cystic fibrosis/Shwachman syndrome	Nifedipine
Obstructive sleep apnea	Familial hyperlipoproteinemias	Pesticides
Polycystic ovary syndrome	Glycogen storage disease (I , VI and IX)	Prednisolone
Protein calorie malnutrition	Hereditary Fructose Intolerance	Solvents
Rapid weight loss	Lipodystrophy	Valproate
Small intestine bacterial overgrowth	Mitochondrial and peroxisomal defects	Vitamin A
Thyroid disorders	Organic acidosis	Zidovudine and HIV treatments
	Porphyria cutanea tarda	
	Turner syndrome	
	Urea cycle disorders	
	Wilson's disease	

LAL: Lysosomal acid lypase.

MetS features[63]. Recent data suggested the possible protective role of vitamin D against NAFLD and/or its progression in children. These findings underline the need to test for NASH as an early, obesity-related complication when serum vitamin D levels are persistently low in obese children. The serum level of vitamin D, even when within the normal range, has been found to inversely correlate with NAFLD severity, independently of known metabolic risk factors[64]. Candidate proteomic biomarkers were identified in recent studies by mass spectrometry techniques. These studies, recently reviewed by Lădaru *et al*[65], have identified 251 candidate proteomic biomarkers: thirty-three biomarkers were confirmed, 14 were found in liver samples, 21 in serum samples, and two from both serum and liver samples[65]. A urinary metabolomic signature of pediatric obesity related liver disease, obtained by gas chromatography-mass spectrometry, showed distinct patterns that especially differed in the levels of metabolites involved in energy, peptide and organic acid metabolism, and intestinal bacteria metabolism[66].

DIFFERENTIAL DIAGNOSIS

With the rising prevalence of childhood obesity, the proportion of children with both an underlying primary liver disease and NAFLD has also increased. Therefore, it is essential to identify conditions that are treatable with specific therapies, such as Wilson's disease[67], autoimmune hepatitis[68] or celiac disease[69]. When pediatric NAFLD is suspected, other liver diseases should be excluded based on an age-driven algorithm[36].

Abnormal serum aminotransferases in overweight or obese children are not always diagnostic of NAFLD/NASH, and other causes should be ruled out, including muscle diseases[70] and treatable liver diseases[67-69].

The ESPGHAN panel highlighted that hepatosple-

nomegaly is suggestive of an unusually advanced liver disease in pediatric NAFLD, and this condition requires a rapid and complete assessment, including an early liver biopsy, to exclude other etiologies[36,54].

In general, obesity related-NAFLD does not occur in extremely young children (younger than 3 years of age) and is rare in children younger than 10 years of age. A differential diagnosis should be based first on clinical features and then on blood tests. As a final step, a liver biopsy must be considered. The remaining NASH-related conditions should also be carefully considered, especially in early-onset NAFLD among young children[36,41]. Table 2 shows the main differential diagnoses in children and adolescents[41].

TREATMENT

Lifestyle interventions (*i.e.*, diet and exercise) represent the mainstay treatment; however, compliance in both adults and children is poor. Research on the pathogenesis, genetic markers, and the role of gut microbiome in NAFLD has led to development of several medical and surgical therapeutic approaches. Figure 1 summarizes the currently available information.

Lifestyle changes

Lifestyle changes are the first line of intervention[71,72]. This goal is not easy for most patients; thus, the recommended behavior is commonly unsuccessful. However, when successful, it represents the most safe and efficacious cure for overweight/obese children with NAFLD.

Diet and exercise act synergistically by improving both hepatic and extra-hepatic insulin sensitivity and restoring insulin metabolism pathways, especially those related to glucose and lipid homeostasis. Moreover, long-term lifestyle changes (24 mo) improve liver histology in terms of the grade of steatosis,
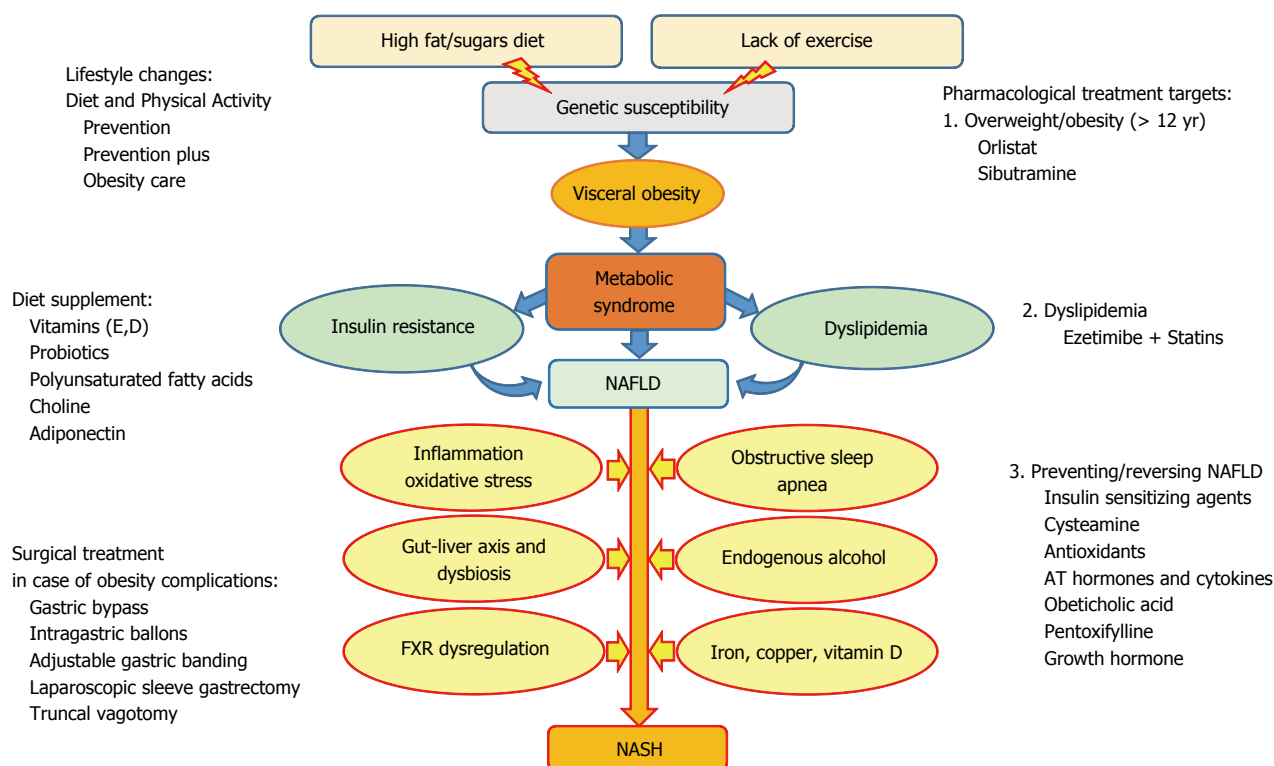
Figure 1 Simplified cartoon of the multiple pathomechanisms involved in development and progression of non-alcoholic fatty liver disease, and proposed stepwise treatment options. NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steato-hepatitis; FXR: Farnesoid X receptor; AT: Adipose tissue.

hepatic lobular inflammation, hepatocyte ballooning, and NAFLD activity score[72].

Family involvement and effective interaction with the primary care pediatrician are critical for the success of lifestyle changes. In 2007, an expert committee nominated by the American Academy of Pediatrics (AAP) developed new recommendations for pediatric obesity prevention and treatment[73]. The committee pointed out that awareness of the problem among primary care pediatricians who work with patients and parents is essential for the early identification of at-risk patients and for taking adequate preventive or corrective actions.

The expert committee highlighted the need for a radical change to the treatment of pediatric obesity, suggesting a "stepwise approach" that includes BMI assessments and the early identification of the risk factors for all children.

After identification and assessment, primary care pediatricians should proceed with the recommended measures of prevention or treatment, depending on the patient's age and BMI. The AAP expert committee identified three different plans of action: "prevention", "prevention plus" and "obesity care". These stages increase in intensity; thus, patients can begin with the less intensive plan and gradually move to the most intensive plan, depending on the response over an established period. "Prevention" applies to normal-weight children. It requires the yearly evaluation of high risk nutritional/activity behaviors and the

recommendations for both diet (*e.g.,* 5 or more servings of fruits and vegetables per day and avoiding sugar-sweetened beverages) and physical activity (*e.g.,* 2 or fewer hours of screen time per day, no television in the room where the child sleeps, and 1 h or more of daily physical activity).

"Prevention plus" applies to overweight children. The goal should be weight maintenance, which will reduce their BMI as the child's age increases. Recommendations for eating behavior include at least 5 to 6 family meals per week, allowing the child to self-regulate his or her meals and avoiding restrictive behaviors. The general rule to follow is "parents provide, child decides". Physical activity needs to be structured: 60 min of at least moderate physical activity per day and 20 min of vigorous activity 3 times a week. Referring families to community activity programs can be helpful and may encourage the development of family activities. Pedometer use can be recommended.

"Obesity care" (if BMI ≥ 95th percentile) is a multidisciplinary team task involving several professional figures, such as a physician, nurse, dietician, exercise trainer, social worker and psychologist. In these cases, obese children must be referred to a pediatric tertiary weight management center with expertise in childhood obesity.

The goal is weight maintenance or gradual weight loss until their BMI is < 85th percentile and should not exceed 1 pound (= 453.6 g) per month. In cases

of severe obesity in older children (> 5 years) and adolescents (BMI $\geqslant$ 99th percentile), weight loss may be more rapid with a maximum rate of 2 pounds (= 907.2 g) per week.

The expert committee identified in the following seven the behaviors that most successfully create energy balance: limit consumption of sugar sweetened beverages, limit TV (0 h for children < 2 years of age and < 2 h for children > 2 years-old), remove TV from primary sleeping area, eat breakfast daily, limit eating out, encourage family meals, and limit portion size[73].

Micronutrient diet supplementation

The benefits from adding otherwise insufficient micronutrients to the diet, including vitamin E and D, probiotics, polyunsaturated fatty acids, choline, and adiponectin, appear to be short-term. The long-term effects of micronutrient supplementation are controversial and further studies are required.

Vitamin E was the first diet supplement used in pediatric NAFLD and NASH because of its anti-oxidant properties[74,75]. Recent results from the "Treatment of NAFLD in Children" (TONIC) multicenter randomized placebo-controlled trial showed that vitamin E was not superior to the placebo at attaining the primary outcome of sustained reduction in ALT levels in patients with pediatric NAFLD[76]. However, children treated with vitamin E demonstrated significant improvements in the resolution of NASH in patients with NASH or borderline NASH at baseline when compared with placebo. Therefore, vitamin E treatment in children who failed to change their lifestyle and have biopsy-proven NASH can be recommended[76].

Diet supplementation with PUFAs, such as the omega-3 fatty acids DHA and eicosapentaenoic acid (EPA), restore insulin sensitivity and have anti-inflammatory actions[77,78]. A six-month diet with long chain omega-3 fatty acids induced a significant decrease in AST and GGT, but not ALT[79]. TG and ALT levels respond to DHA. The benefit of diet supplementation with PUFAs was confirmed in adolescents with NAFLD and genetic LPL deficiency, which has a high prevalence among the French-Canadian population in Quebec and is associated with a high risk of atherosclerosis early in life[80].

Vitamin D deficiency has been suggested to promote NASH by accelerating hepatic fibrogenesis[28]; thus, supplementation with vitamin D has been recommended[81].

Healthy humans are unable to synthesize enough choline *de novo* to prevent deficiency. Decreased choline intake is associated with worse fibrosis in a subset of patients with NASH. Studies have shown a reversal of NASH after choline supplementation in choline-deficient patients on long-term parenteral nutrition. Additional studies are needed to evaluate if low choline concentrations are associated with the initiation or progression of NAFLD or NASH[82].

Probiotics

The type of food ingested has an enormous influence on gut microbiota composition. Approximately 10 years ago, research studies began to demonstrate how gut microbiota are involved in significantly regulating energy balance, including reducing AT and cholesterol blood levels[83]. Compared to slim subjects, the obese have different gut microbiota, which also varies depending on the geographical area. Changes in the gut microbiota using prebiotics (substances useful to the growth of good gut microbiota) and probiotics (live microorganisms) are beneficial for weight reduction[83,84]. Extensive experiments conducted on animals showed strain-specific effects. In NAFLD, diet supplements studies included in particular the genera *Bifidobacteria* and *Lactobacillus*[85,86]. *Lactobacillus rhamnosus* has been extensively studied in NAFLD both *in vitro* and *in vivo*. This strain modulated gut microbiota, reversed small intestinal barrier impairments, reduced hepatic inflammation, improved lipid metabolism and, probably most importantly, increased the production of certain anorexigenic gut hormones[87,88]. The results of a recent double-blind clinical trial showed the benefits of the multi-strain probiotic *VSL#3* on weight reduction and liver fibrosis in obese children with NAFLD aged 6-12 years[89]. A double-blind clinical trial demonstrated that obese children with NAFLD treated with Lactobacillus GG showed a significant decrease (up to normalization in 80% of cases) in serum ALT values[90]. A recent meta-analysis confirmed that experimental treatment with probiotics is more effective than placebo at normalizing transaminase levels[91].

MEDICAL THERAPY

The pharmacological treatments currently available in pediatric NAFLD are mainly aimed at (1) reducing body weight; (2) preventing or reversing hepatic steatosis, inflammation and fibrosis; and (3) treating hypercholesterolemia.

Pharmacological treatment of the overweight/obese status

Candidates for pharmacotherapy are very high-risk children. These are children in the 99th percentile BMI at their first evaluation and children with a lower BMI who failed to change their lifestyle over a period of at least 6 mo. Tertiary care centers with specific expertise should take the care of children in these categories. The only two products currently approved by the Food and Drug Administration (FDA) for the treatment of pediatric obesity are orlistat and sibutramine[73].

Orlistat: The human intestine does not absorb orlistat. The mechanism of action for this drug is based on the inhibition of endoluminal lipase. Orlistat was approved by the FDA for children above 12 years of age[73]. Apart

from the common side effects of abdominal cramps and flatulence due to the amount of not absorbed fat in the fecal mass, orlistat can lead to chronic kidney disease due to secondary hyperoxaluria[92]. Therefore, the use of orlistat must be carefully monitored, and patients must follow a low oxalate and high calcium diet with abundant daily water intake up to 1.5 L/m^2 body surface area[92]. Probiotics able to eliminate oxalate and medications increasing the urinary solubility of crystals (*e.g.,* potassium citrate), should also be considered.

Sibutramine: A nonspecific reuptake inhibitor for serotonin, norepinephrine and dopamine reduces appetite. Its use is approved for adolescents over 16 years of age for a period of no more than 2 years[73]. The main side effect of vasoconstriction precludes its use in children with blood hypertension.

Medical therapy for preventing/reversing hepatic steatosis, inflammation and fibrosis
Treatments for NAFLD and NASH include insulin sensitizers, antioxidants and hepatoprotective agents. Their efficacy remains controversial in adults and in children as well[93-95].

Metformin: It is an insulin sensitizer that could also improve weight control. The results from the TONIC study, a recent large multicenter randomized double-blind trial, showed no significant improvement of ALT levels or liver histology in NAFLD children aged 8-17 years on metformin *vs* placebo[76].

Cysteamine: It's a coenzyme A catabolism product, has antioxidant properties and an insulin-sensitizing effect by up-regulating adiponectin levels. It reduced ALT and AST levels in children with NAFLD without reducing their body mass index. A randomized clinical trial is currently evaluating the potential effects of cysteamine bitartrate delayed-release capsules on the histologic severity of NAFLD in children aged 8-17 years; however, the results of this trial have not yet been published[96].

Antioxidants: Vitamin E studies were previously mentioned in the *"Micronutrient diet supplementation"* section of this review. Among the plant extract polyphenols, resveratrol reduces hepatic inflammation and ameliorates lipid metabolism in adults[97]. An ongoing clinical trial is evaluating the ability of resveratrol to reduce hepatic TG content in 13-18 year-old adolescents with NAFLD and metabolic syndrome[98].

Silibinin: It's a drug available on most of the markets, is of particular relevance since its clinical potential has been extensively demonstrated in various experimental models[99], and in a randomized clinical trial in adults with histologically documented NAFLD

where treatment was associated with improvement in liver enzymes, insulin resistance, and liver histology, without increases in body weight[100].

AT hormones and cytokines: Adipocytes are now recognized to play an active role in MetS *via* the production of hormones, such as adiponectin and resistin, and adipocytokines, including TNF-α. These molecules are under investigation as possible therapeutic targets[101].

Pentoxifylline: Pentoxifylline is a phosphodiesterase inhibitor that increases cyclic AMP and decreases TNF-α gene transcription[102]. The results of a clinical trial showed that pentoxifylline is well tolerated and improves hepatic histology in adults with NASH[103].

Obeticholic acid: The FLINT (FXR Ligand Obeticholic Acid in NASH Treatment) trial provided promising results on the efficacy of the obeticholic acid, an FXR agonist, at improving the histological features of hepatic inflammation and fibrosis in adults with NASH[104].

Growth hormone: There is evidence that growth hormone (GH) replacement therapy improves serum liver enzyme levels and hepatic histology in adults that suffer from acquired GH deficiency and NASH[105]. There is also a pediatric case report of an 11 year-old child suffering from acquired GH deficiency and NASH whose liver enzymes levels improved during GH treatment[106].

Medical therapy for the treatment of hypercholesterolemia
Ezetimibe: Selectively inhibits cholesterol absorption from small intestine by binding to the brush border. The FDA has recently approved its use for treatment of hypercholesterolemia[107]. Studies on *Ezetimibe* in NAFLD have shown improvement in hepatic histology but with worsening or no effects on insulin sensitivity and HbA1c levels[108,109]. Recent studies on its safety and effectiveness in adult patients reported promising results when used in combination with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (*statins*)[109,110].

SURGICAL THERAPIES FOR WEIGHT LOSS: BARIATRIC SURGERY

The number of surgical options has grown over the past decades, and these procedures are now used for weight loss in adults. Among the variety of techniques, there are strategies aimed to reduce gastric volume, food absorption and induce early satiety (gastric bypass, intragastric balloons, adjustable gastric banding, laparoscopic sleeve gastrectomy, *etc.*). Other strategies

create gastric stasis with an early sense of satiety, such as the truncal vagotomy[111-113]. Even if bariatric surgery is still considered investigational in adults, it has been suggested for adolescents with moderate/severe obesity when complicated by diabetes, OSAS, pseudotumor cerebri or NASH or when an impaired quality of life and daily activities exists[114,115].

FUTURE RESEARCH DIRECTIONS, PERSPECTIVESAND CONCLUSIONS

The epidemics of pediatric obesity and obesity-related liver disease (NAFLD and NASH) represents a serious problem. Increasing evidence indicates that affected children are at risk of significant progressive hepatopathy if inflammation and/or advanced fibrosis are already present.

The large diffusion of NAFLD does not consent a precise disease assessment of inflammation and fibrosis through liver biopsy in all individuals. Several non-invasive surrogate methods of diagnosis and surveillance are being developed with increasing sensitivity and specificity. Identification of NAFLD is also paramount for the early detection of other obesity related severe complications, *e.g.,* cardiovascular problems.

The existence of multiple pathogenic mechanisms may explain the resistance of NAFLD to standard treatments. A combination of several concomitant environmental and genetic factors, including the gut microbiota, leads to excessive accumulation of lipids in the liver (steatosis), which can often result in lipotoxicity, hepatocyte cell death, liver inflammation, fibrosis, and pathological angiogenesis.

Adult and pediatric NAFLD does not have a globally efficacious treatment. Lifestyle interventions (*i.e.,* dieting and exercise) represent the mainstay treatment, although effective interventions in adults and children are difficult due to a lack of compliance. Multiple medical treatments targeting IR, oxidative stress, gut liver axis and microbiota, proinflammatory pathways, and dyslipidemia can be proposed in patients that cannot adhere to the recommended lifestyle changes. Prevailing cases of morbid obesity may require bariatric surgery.

Knowledge on the pathogenesis, genetic markers, and the role of the gut microbiome in NAFLD continues to increase, which will likely lead to more effective treatment strategies. Obeticholic acid in adults appears to be a promising treatment; however, long term results are not yet available. In all cases, this type of therapy will require caution in adults and children due to the high expression of FXR in several tissues other than liver possibly showing beneficial and/or detrimental effects.

Last but not least, the prevention of obesity in both children and adults remains obviously imperative[73,116,117].

REFERENCES

1   **Povero D**, Feldstein AE. Novel Molecular Mechanisms in the Development of Non-Alcoholic Steatohepatitis. *Diabetes Metab J* 2016; **40**: 1-11 [PMID: 26912150 DOI: 10.4093/dmj.2016.40.1.1]

2   **Fitzpatrick E**, Hadzic N. Paediatric Non-Alcoholic Fatty Liver Disease: An Emerging Threat. *Paediatrics Today* 2015; **11**: 1-9 [DOI 10.5457/p2005-114.104]

3   **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; Epub ahead of print [DOI: 10.1016/j.metabol.2015.12.012]

4   **Berardis S**, Sokal E. Pediatric non-alcoholic fatty liver disease: an increasing public health issue. *Eur J Pediatr* 2014; **173**: 131-139 [PMID: 24068459 DOI: 10.1007/s00431-013-2157-6]

5   **Goyal NP**, Schwimmer JB. The Progression and Natural History of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2016; **20**: 325-338 [PMID: 27063272 DOI: 10.1016/j.cld.2015.10.003]

6   **Vacca M**, Allison M, Griffin JL, Vidal-Puig A. Fatty Acid and Glucose Sensors in Hepatic Lipid Metabolism: Implications in NAFLD. *Semin Liver Dis* 2015; **35**: 250-261 [PMID: 26378642 DOI: 10.1055/s-0035-1562945]

7   **Rosso C**, Mezzabotta L, Gaggini M, Salomone F, Gambino R, Marengo A, Saba F, Vanni E, Jouness RI, Saponaro C, Buzzigoli E, Caviglia GP, Abate ML, Smedile A, Rizzetto M, Cassader M, Gastaldelli A, Bugianesi E. Peripheral insulin resistance predicts liver damage in nondiabetic subjects with nonalcoholic fatty liver disease. *Hepatology* 2016; **63**: 107-116 [PMID: 26473614 DOI: 10.1002/hep.28287]

8   **Nassir F**, Rector RS, Hammoud GM, Ibdah JA. Pathogenesis and Prevention of Hepatic Steatosis. *Gastroenterol Hepatol* (N Y) 2015; **11**: 167-175 [PMID: 27099587]

9   **Després JP**, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; **444**: 881-887 [PMID: 17167477]

10 **Vos MB**, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. *Hepatology* 2013; **57**: 2525-2531 [PMID: 23390127 DOI: 10.1002/hep.26299]

11 **Moore JB**, Gunn PJ, Fielding BA. The role of dietary sugars and de novo lipogenesis in non-alcoholic fatty liver disease. *Nutrients* 2014; **6**: 5679-5703 [PMID: 25514388 DOI: 10.3390/nu6125679]

12 **Sullivan JS**, Le MT, Pan Z, Rivard C, Love-Osborne K, Robbins K, Johnson RJ, Sokol RJ, Sundaram SS. Oral fructose absorption in obese children with non-alcoholic fatty liver disease. *Pediatr Obes* 2015; **10**: 188-195 [PMID: 24961681 DOI: 10.1111/ijpo.238]

13 **Vajro P**, Paolella G, Fasano A. Microbiota and gut-liver axis: their influences on obesity and obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2013; **56**: 461-468 [PMID: 23287807 DOI: 10.1097/MPG.0b013e318284abb5]

14 **Guercio Nuzio S**, Di Stasi M, Pierri L, Troisi J, Poeta M, Bisogno A, Belmonte F, Tripodi M, Di Salvio D, Massa G, Savastano R, Cavallo P, Boffardi M, Ziegenhardt D, Bergheim I, Mandato C, Vajro P. Multiple gut-liver axis abnormalities in children with obesity with and without hepatic involvement. *Pediatr Obes* 2016; In press

15 **Kirpich IA**, Marsano LS, McClain CJ. Gut-liver axis, nutrition, and non-alcoholic fatty liver disease. *Clin Biochem* 2015; **48**: 923-930 [PMID: 26151226 DOI: 10.1016/j.clinbiochem.2015.06.023]

16 **Fuchs M**. Non-alcoholic Fatty liver disease: the bile Acid-activated farnesoid x receptor as an emerging treatment target. *J Lipids* 2012; **2012**: 934396 [PMID: 22187656 DOI: 10.1155/2012/934396]

17 **Xu JY**, Li ZP, Zhang L, Ji G. Recent insights into farnesoid X receptor in non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 13493-13500 [PMID: 25309079 DOI: 10.3748/wjg.v20.i37.13493]

18 **de Medeiros IC**, de Lima JG. Is nonalcoholic fatty liver disease an endogenous alcoholic fatty liver disease? - A mechanistic hypothesis. *Med Hypotheses* 2015; **85**: 148-152 [PMID: 25956735 DOI: 10.1016/j.mehy.2015.04.021]

19 **Engstler AJ**, Aumiller T, Degen C, Dürr M, Weiss E, Maier

IB, Schattenberg JM, Jin CJ, Sellmann C, Bergheim I. Insulin resistance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. *Gut* 2016; **65**: 1564-1571 [PMID: 26006114 DOI: 10.1136/gutjnl-2014-308379]

20   **Santoro N**, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, Calí AM, Narayan D, Shaw MM, Pierpont B, Savoye M, Lartaud D, Eldrich S, Cushman SW, Zhao H, Shulman GI, Caprio S. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; **52**: 1281-1290 [PMID: 20803499 DOI: 10.1002/hep.23832]

21   **Davis JN**, Lê KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, Allayee H, Goran MI. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am J Clin Nutr* 2010; **92**: 1522-1527 [PMID: 20962157 DOI: 10.3945/ajcn.2010.30185]

22   **Marzuillo P**, Grandone A, Perrone L, Miraglia Del Giudice E. Understanding the pathophysiological mechanisms in the pediatric non-alcoholic fatty liver disease: The role of genetics. *World J Hepatol* 2015; **7**: 1439-1443 [PMID: 26085904 DOI: 10.4254/wjh.v7.i11.1439]

23   **Della Corte C**, Mosca A, Ionata A, Nobili V. Docosahexaenoic Acid and Its Role in G-Protein-Coupled Receptor 120 Activation in Children Affected by Nonalcoholic Fatty Liver Disease. *Endocr Dev* 2016; **30**: 29-36 [PMID: 26683215 DOI: 10.1159/000439324]

24   **Dongiovanni P**, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm Des* 2013; **19**: 5219-5238 [PMID: 23394097]

25   **Feldman A**, Aigner E, Weghuber D, Paulmichl K. The Potential Role of Iron and Copper in Pediatric Obesity and Nonalcoholic Fatty Liver Disease. *Biomed Res Int* 2015; **2015**: 287401 [PMID: 26273604 DOI: 10.1155/2015/287401]

26   **Zhang SR**, Fan XM. Ghrelin-ghrelin O-acyltransferase system in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 3214-3222 [PMID: 25805927 DOI: 10.3748/wjg.v21.i11.3214]

27   **Hourigan SK**, Abrams S, Yates K, Pfeifer K, Torbenson M, Murray K, Roth CL, Kowdley K, Scheimann AO; NASH CRN. Relation between vitamin D status and nonalcoholic fatty liver disease in children. *J Pediatr Gastroenterol Nutr* 2015; **60**: 396-404 [PMID: 25710716 DOI: 10.1097/MPG.0000000000000598]

28   **Eliades M**, Spyrou E, Agrawal N, Lazo M, Brancati FL, Potter JJ, Koteish AA, Clark JM, Guallar E, Hernaez R. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2013; **38**: 246-254 [PMID: 23786213 DOI: 10.1111/apt.12377]

29   **Savransky V**, Nanayakkara A, Vivero A, Li J, Bevans S, Smith PL, Torbenson MS, Polotsky VY. Chronic intermittent hypoxia predisposes to liver injury. *Hepatology* 2007; **45**: 1007-1013 [PMID: 17393512 DOI: 10.1002/hep.21593]

30   **Parola M**, Vajro P. Nocturnal hypoxia in obese-related obstructive sleep apnea as a putative trigger of oxidative stress in pediatric NAFLD progression. *J Hepat* 2016; In press [DOI 10.1016/j.jhep.2016.05.042]

31   **NICE guideline**. Non-alcoholic fatty liver disease: Assessment and management of non-alcoholic fatty liver disease (NAFLD) in adults, children and young people. 2015-12, cited 2016-06. Available from: URL: https://www.nice.org.uk/guidance/GID-CGWAVE0692/documents/full-guideline-updated

32   **Socha P**, Wierzbicka A, Dhawan A. Non alcoholic fatty liver disease in children. In: Jara P, editor. International Hepatology Updates: Liver Diseases in Children. Barcelona, Spain: Perlmayer, 2009: 147-161

33   **De Bruyne RM**, Fitzpatrick E, Dhawan A. Fatty liver disease in children: eat now pay later. *Hepatol Int* 2010; **4**: 375-385 [PMID: 20305757 DOI: 10.1007/s12072-009-9160-z]

34   **Lin YC**, Chang PF, Yeh SJ, Liu K, Chen HC. Risk factors for liver steatosis in obese children and adolescents. *Pediatr*

Neonatol 2010; **51**: 149-154 [PMID: 20675238 DOI: 10.1016/S1875-9572(10)60028-9]

35   **Manco M**, Bedogni G, Marcellini M, Devito R, Ciampalini P, Sartorelli MR, Comparcola D, Piemonte F, Nobili V. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. *Gut* 2008; **57**: 1283-1287 [PMID: 18218674 DOI: 10.1136/gut.2007]

36   **Vajro P**, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, Durmaz O, Lacaille F, McLin V, Nobili V. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr* 2012; **54**: 700-713 [PMID: 22395188 DOI: 10.1097/MPG.0b013e318252a13f]

37   **McKenzie SA**, Bhattacharya A, Sureshkumar R, Joshi B, Franklin A, Pickering R, Dundas I. Which obese children should have a sleep study? *Respir Med* 2008; **102**: 1581-1585 [PMID: 18640017 DOI: 10.1016/j.rmed.2008.06.004]

38   **Michaliszyn SF**, Lee S, Tfayli H, Arslanian S. Polycystic ovary syndrome and nonalcoholic fatty liver in obese adolescents: association with metabolic risk profile. *Fertil Steril* 2013; **100**: 1745-1751 [PMID: 24034940 DOI: 10.1016/j.fertnstert.2013.08.015]

39   **Bonci E**, Chiesa C, Versacci P, Anania C, Silvestri L, Pacifico L. Association of Nonalcoholic Fatty Liver Disease with Subclinical Cardiovascular Changes: A Systematic Review and Meta-Analysis. *Biomed Res Int* 2015; **2015**: 213737 [PMID: 26273598 DOI: 10.1155/2015/213737]

40   **Schwimmer JB**, Zepeda A, Newton KP, Xanthakos SA, Behling C, Hallinan EK, Donithan M, Tonascia J; Nonalcoholic Steatohepatitis Clinical Research Network. Longitudinal assessment of high blood pressure in children with nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e112569 [PMID: 25419656 DOI: 10.1371/journal.pone.0112569]

41   **Vajro P**, Maddaluno S, Veropalumbo C. Persistent hypertrans-aminasemia in asymptomatic children: a stepwise approach. *World J Gastroenterol* 2013; **19**: 2740-2751 [PMID: 23687411 DOI: 10.3748/wjg.v19.i18.2740]

42   **Mofrad P**, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292 [PMID: 12774006]

43   **Schwimmer JB**, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, Sirlin CB. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010; **138**: 1357-1364, 1364.e1-2 [PMID: 20064512 DOI: 10.1053/j.gastro.2009.12.052]

44   **Tahan V**, Canbakan B, Balci H, Dane F, Akin H, Can G, Hatemi I, Olgac V, Sonsuz A, Ozbay G, Yurdakul I, Senturk H. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. *Hepatogastroenterology* 2008; **55**: 1433-1438 [PMID: 18795706]

45   **Jahnel J**, Zöhrer E, Alisi A, Ferrari F, Ceccarelli S, De Vito R, Scharnagl H, Stojakovic T, Fauler G, Trauner M, Nobili V. Serum Bile Acid Levels in Children With Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr* 2015; **61**: 85-90 [PMID: 25729888 DOI: 10.1097/MPG.0000000000000774]

46   **Koplay M**, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. *World J Hepatol* 2015; **7**: 769-776 [PMID: 25914777 DOI: 10.4254/wjh.v7.i5.769]

47   **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750 [PMID: 12198701]

48   **Pacifico L**, Celestre M, Anania C, Paolantonio P, Chiesa C, Laghi A. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. *Acta Paediatr* 2007; **96**: 542-547 [PMID: 17306008]

49   **Schwimmer JB**, Middleton MS, Behling C, Newton KP, Awai HI,

Paiz MN, Lam J, Hooker JC, Hamilton G, Fontanesi J, Sirlin CB. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *Hepatology* 2015; **61**: 1887-1895 [PMID: 25529941 DOI: 10.1002/hep.27666]

50   **Mansoor S**, Collyer E, Alkhouri N. A comprehensive review of noninvasive liver fibrosis tests in pediatric nonalcoholic Fatty liver disease. *Curr Gastroenterol Rep* 2015; **17**: 23 [PMID: 26031832 DOI: 10.1007/s11894-015-0447-z]

51   **Patel V**, Sanyal AJ, Sterling R. Clinical Presentation and Patient Evaluation in Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2016; **20**: 277-292 [PMID: 27063269 DOI: 10.1016/j.cld.2015.10.006]

52   **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649 [PMID: 16116629]

53   **Dezsőfi A**, Baumann U, Dhawan A, Durmaz O, Fischler B, Hadzic N, Hierro L, Lacaille F, McLin VA, Nobili V, Socha P, Vajro P, Knisely AS; ESPGHAN Hepatology Committee. Liver biopsy in children: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr* 2015; **60**: 408-420 [PMID: 25383787 DOI: 10.1097/MPG.0000000000000632]

54   **Roberts EA**. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? *J Hepatol* 2007; **46**: 1133-1142 [PMID: 17445934]

55   **Alkhouri N**, Mansoor S, Giammaria P, Liccardo D, Lopez R, Nobili V. The development of the pediatric NAFLD fibrosis score (PNFS) to predict the presence of advanced fibrosis in children with nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e104558 [PMID: 25121514 DOI: 10.1371/journal.pone.0104558]

56   **Fitzpatrick E**, Dhawan A. Noninvasive biomarkers in non-alcoholic fatty liver disease: current status and a glimpse of the future. *World J Gastroenterol* 2014; **20**: 10851-10863 [PMID: 25152587 DOI: 10.3748/wjg.v20.i31.10851]

57   **Tabbaa A**, Shaker M, Lopez R, Hoshemand K, Nobili V, Alkhouri N. Low Serum Potassium Levels Associated with Disease Severity in Children with Nonalcoholic Fatty Liver Disease. *Pediatr Gastroenterol Hepatol Nutr* 2015; **18**: 168-174 [PMID: 26473136 DOI: 10.5223/pghn.2015.18.3.168]

58   **Alkhouri N**, Alisi A, Okwu V, Matloob A, Ferrari F, Crudele A, De Vito R, Lopez R, Feldstein AE, Nobili V. Circulating Soluble Fas and Fas Ligand Levels Are Elevated in Children with Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2015; **60**: 2353-2359 [PMID: 25764498 DOI: 10.1007/s10620-015-3614-z]

59   **Walenbergh SM**, Houben T, Hendrikx T, Jeurissen ML, van Gorp PJ, Vreugdenhil AC, Adriaanse MP, Buurman WA, Hofker MH, Mosca A, Lindsey PJ, Alisi A, Liccardo D, Panera N, Koek GH, Nobili V, Shiri-Sverdlov R. Plasma cathepsin D levels: a novel tool to predict pediatric hepatic inflammation. *Am J Gastroenterol* 2015; **110**: 462-470 [PMID: 25732418 DOI: 10.1038/ajg.2015.29]

60   **Pacifico L**, Bonci E, Marandola L, Romaggioli S, Bascetta S, Chiesa C. Increased circulating zonulin in children with biopsy-proven nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 17107-17114 [PMID: 25493023 DOI: 10.3748/wjg.v20.i45.17107]

61   **Klusek-Oksiuta M**, Bialokoz-Kalinowska I, Tarasów E, Wojtkowska M, Werpachowska I, Lebensztejn DM. Chemerin as a novel non-invasive serum marker of intrahepatic lipid content in obese children. *Ital J Pediatr* 2014; **40**: 84 [PMID: 25399407 DOI: 10.1186/s13052-014-0084-4]

62   **Vos MB**, Colvin R, Belt P, Molleston JP, Murray KF, Rosenthal P, Schwimmer JB, Tonascia J, Unalp A, Lavine JE; NASH CRN Research Group. Correlation of vitamin E, uric acid, and diet composition with histologic features of pediatric NAFLD. *J Pediatr Gastroenterol Nutr* 2012; **54**: 90-96 [PMID: 22197855 DOI: 10.1097/MPG.0b013e318229da1a]

63   **Cardoso AS**, Gonzaga NC, Medeiros CC, Carvalho DF. Association of uric acid levels with components of metabolic syndrome and non-alcoholic fatty liver disease in overweight or obese children and adolescents. *J Pediatr* (Rio J) 2013; **89**: 412-418 [PMID: 23791233 DOI: 10.1016/j.jped.2012.12.008]

64   **Chung GE**, Kim D, Kwak MS, Yang JI, Yim JY, Lim SH, Itani M. The serum vitamin D level is inversely correlated with nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2016; **22**: 146-151 [PMID: 27044765 DOI: 10.3350/cmh.2016.22.1.146]

65   **Lădaru A**, Bălănescu P, Stan M, Codreanu I, Anca IA. Candidate proteomic biomarkers for non-alcoholic fatty liver disease (steatosis and non-alcoholic steatohepatitis) discovered with mass-spectrometry: a systematic review. *Biomarkers* 2016; **21**: 102-114 [PMID: 26632636 DOI: 10.3109/1354750X.2015.1118542]

66   **Troisi J**, Pierri L, Landolfi A, Marciano F, Bisogno A, Belmonte F, Palladino C, Guercio Nuzio S, Vajro P. A urinary metabolomic signature of pediatric obesity related liver disease. Proceedings of the 49th ESPGHAN Meeting; 2016 May 25-28; Athens, Greece. *J Pediatr Gastroenterol Nutr* 2016; **62** Suppl 1: S561

67   **Spindelboeck W**, Deutschmann A, Lackner K. Obesity and Wilson disease in childhood and adolescence. *J Pediatr Gastroenterol Nutr* 2009; **48** Suppl 3: E63

68   **Adams LA**, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2004; **99**: 1316-1320 [PMID: 15233671 DOI: 10.1111/j.1572-0241.2004.30444.x]

69   **Franzese A**, Iannucci MP, Valerio G, Ciccimarra E, Spaziano M, Mandato C, Vajro P. Atypical celiac disease presenting as obesity-related liver dysfunction. *J Pediatr Gastroenterol Nutr* 2001; **33**: 329-332 [PMID: 11593131 DOI: 10.1097/00005176-200109000-00019]

70   **Veropalumbo C**, Del Giudice E, Capuano G, Gentile C, Di Cosmo N, Vajro P. Duchenne and Becker muscular dystrophy presenting as nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 463-464 [PMID: 21970998 DOI: 10.1097/MPG.0b013e318217f5d9]

71   **Lassailly G**, Caiazzo R, Pattou F, Mathurin P. Perspectives on Treatment for Nonalcoholic Steatohepatitis. *Gastroenterology* 2016; **150**: 1835-1848 [PMID: 26971824 DOI: 10.1053/j.gastro.2016.03.004]

72   **Della Corte C**, Vajro P, Socha P, Nobili V. Pediatric non-alcoholic fatty liver disease: recent advances. *Clin Res Hepatol Gastroenterol* 2014; **38**: 419-422 [PMID: 24726273 DOI: 10.1016/j.clinre.2014.02.008]

73   **Barlow SE**, Expert Committee. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics* 2007; **120** Suppl 4: S164-S192 [PMID: 18055651]

74   **Rahimlou M**, Ahmadnia H, Hekmatdoost A. Dietary supplements and pediatric non-alcoholic fatty liver disease: Present and the future. *World J Hepatol* 2015; **7**: 2597-2602 [PMID: 26557952 DOI: 10.4254/wjh.v7.i25.2597]

75   **Vajro P**, Mandato C, Franzese A, Ciccimarra E, Lucariello S, Savoia M, Capuano G, Migliaro F. Vitamin E treatment in pediatric obesity-related liver disease: a randomized study. *J Pediatr Gastroenterol Nutr* 2004; **38**: 48-55 [PMID: 14676594]

76   **Lavine JE**, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, Scheimann AO, Sanyal AJ, Chalasani N, Tonascia J, Ünalp A, Clark JM, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NSCR Network. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* 2011; **305**: 1659-1668 [PMID: 21521847 DOI: 10.1001/jama.2011.520]

77   **Janczyk W**, Lebensztejn D, Wierzbicka-Rucińska A, Mazur A, Neuhoff-Murawska J, Matusik P, Socha P. Omega-3 Fatty acids therapy in children with nonalcoholic Fatty liver disease: a randomized controlled trial. *J Pediatr* 2015; **166**: 1358-1363.e1-3 [PMID: 25771388 DOI: 10.1016/j.jpeds.2015.01.056]

78   **Pacifico L**, Bonci E, Di Martino M, Versacci P, Andreoli G, Silvestri LM, Chiesa C. A double-blind, placebo-controlled randomized trial to evaluate the efficacy of docosahexaenoic acid supplementation on hepatic fat and associated cardiovascular risk

factors in overweight children with nonalcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2015; **25**: 734-741 [PMID: 26026214 DOI: 10.1016/j.numecd.2015.04.003]

79 **Boyraz M**, Pirgon Ö, Dündar B, Çekmez F, Hatipoğlu N. Long-Term Treatment with n-3 Polyunsaturated Fatty Acids as a Monotherapy in Children with Nonalcoholic Fatty Liver Disease. *J Clin Res Pediatr Endocrinol* 2015; **7**: 121-127 [PMID: 26316434 DOI: 10.4274/jcrpe.1749]

80 **Spahis S**, Alvarez F, Dubois J, Ahmed N, Peretti N, Levy E. Plasma fatty acid composition in French-Canadian children with non-alcoholic fatty liver disease: Effect of n-3 PUFA supplementation. *Prostaglandins Leukot Essent Fatty Acids* 2015; **99**: 25-34 [PMID: 26066299 DOI: 10.1016/j.plefa.2015.04.010]

81 **Luger M**, Kruschitz R, Kienbacher C, Traussnigg S, Langer FB, Schindler K, Würger T, Wrba F, Trauner M, Prager G, Ludvik B. Prevalence of Liver Fibrosis and its Association with Non-invasive Fibrosis and Metabolic Markers in Morbidly Obese Patients with Vitamin D Deficiency. *Obes Surg* 2016; Epub ahead of print [PMID: 26989059]

82 **Guerrerio AL**, Colvin RM, Schwartz AK, Molleston JP, Murray KF, Diehl A, Mohan P, Schwimmer JB, Lavine JE, Torbenson MS, Scheimann AO. Choline intake in a large cohort of patients with nonalcoholic fatty liver disease. *Am J Clin Nutr* 2012; **95**: 892-900 [PMID: 22338037 DOI: 10.3945/ajcn.111.020156]

83 **Bäckhed F**, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; **307**: 1915-1920 [PMID: 15790844]

84 **Paolella G**, Mandato C, Pierri L, Poeta M, Di Stasi M, Vajro P. Gut-liver axis and probiotics: their role in non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 15518-15531 [PMID: 25400436 DOI: 10.3748/wjg.v20.i42.15518]

85 **Del Chierico F**, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric NAFLD and obese patients unveiled by an integrated meta-omics based approach. *Hepatology* 2016; Epub ahead of print [PMID: 27028797 DOI: 10.1002/hep.28572]

86 **Vajro P**, Mandato C, Veropalumbo C, De Micco I. Probiotics: a possible role in treatment of adult and pediatric non alcoholic fatty liver disease. *Ann Hepatol* 2013; **12**: 161-163 [PMID: 23293210]

87 **Vajro P**, Veropalumbo C, D'Aniello R, Mandato C. Probiotics in the treatment of non alcoholic fatty liver disease: further evidence in obese children. *Nutr Metab Cardiovasc Dis* 2013; **23**: e9-10 [PMID: 23177179 DOI: 10.1016/j.numecd.2012.10.006]

88 **Balasubramanian H**, Patole S. Early probiotics to prevent child-hood metabolic syndrome: A systematic review. *World J Methodol* 2015; **5**: 157-163 [PMID: 26413489 DOI: 10.5662/wjm.v5.i3.157]

89 **Alisi A**, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, Giammaria P, Reali L, Anania F, Nobili V. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2014; **39**: 1276-1285 [PMID: 24738701 DOI: 10.1111/apt.12758]

90 **Vajro P**, Mandato C, Licenziati MR, Franzese A, Vitale DF, Lenta S, Caropreso M, Vallone G, Meli R. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2011; **52**: 740-743 [PMID: 21505361 DOI: 10.1097/MPG.0b013e31821f9b85]

91 **Ma YY**, Li L, Yu CH, Shen Z, Chen LH, Li YM. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. *World J Gastroenterol* 2013; **19**: 6911-6918 [PMID: 24187469 DOI: 10.3748/wjg.v19.i40.6911]

92 **Kwan TK**, Chadban SJ, McKenzie PR, Saunders JR. Acute oxalate nephropathy secondary to orlistat-induced enteric hyperoxaluria. *Nephrology* (Carlton) 2013; **18**: 241-242 [PMID: 23432752 DOI: 10.1111/j.1440-1797.2012.01649.x]

93 **Vajro P**, Paolella G, Poeta M, Pizza C, Sangermano M, Massa G. Pediatric non alcoholic fatty liver disease: more on novel treatment targets. *BMC Pediatr* 2013; **13**: 109 [PMID: 23870449 DOI: 10.1186/1471-2431-13-109]

94 **Corey KE**, Vuppalanchi R, Vos M, Kohli R, Molleston JP, Wilson L, Unalp-Arida A, Cummings OW, Lavine JE, Chalasani N. Improvement in liver histology is associated with reduction in dyslipidemia in children with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2015; **60**: 360-367 [PMID: 25714579 DOI: 10.1097/MPG.0000000000000584]

95 **Hampp C**, Kang EM, Borders-Hemphill V. Use of prescription antiobesity drugs in the United States. *Pharmacotherapy* 2013; **33**: 1299-1307 [PMID: 24019195 DOI: 10.1002/phar.1342]

96 **Dohil R**, Schmeltzer S, Cabrera BL, Wang T, Durelle J, Duke KB, Schwimmer JB, Lavine JE. Enteric-coated cysteamine for the treatment of paediatric non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2011; **33**: 1036-1044 [PMID: 21395631 DOI: 10.1111/j.1365-2036.2011.04626.x]

97 **Chen S**, Zhao X, Ran L, Wan J, Wang X, Qin Y, Shu F, Gao Y, Yuan L, Zhang Q, Mi M. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: a randomized controlled trial. *Dig Liver Dis* 2015; **47**: 226-232 [PMID: 25577300 DOI: 10.1016/j.dld.2014.11.015]

98 **Faghihzadeh F**, Adibi P, Rafiei R, Hekmatdoost A. Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. *Nutr Res* 2014; **34**: 837-843 [PMID: 25311610 DOI: 10.1016/j.nutres.2014.09.005]

99 **Salamone F**, Galvano F, Marino Gammazza A, Paternostro C, Tibullo D, Bucchieri F, Mangiameli A, Parola M, Bugianesi E, Li Volti G. Silibinin improves hepatic and myocardial injury in mice with nonalcoholic steatohepatitis. *Dig Liver Dis* 2012; **44**: 334-342 [PMID: 22197629 DOI: 10.1016/j.dld.2011.11.010]

100 **Loguercio C**, Andreone P, Brisc C, Brisc MC, Bugianesi E, Chiaramonte M, Cursaro C, Danila M, de Sio I, Floreani A, Freni MA, Grieco A, Groppo M, Lazzari R, Lobello S, Lorefice E, Margotti M, Miele L, Milani S, Okolicsanyi L, Palasciano G, Portincasa P, Saltarelli P, Smedile A, Somalvico F, Spadaro A, Sporea I, Sorrentino P, Vecchione R, Tuccillo C, Del Vecchio Blanco C, Federico A. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: a randomized controlled trial. *Free Radic Biol Med* 2012; **52**: 1658-1665 [PMID: 22343419 DOI: 10.1016/j.freeradbiomed.2012.02.008]

101 **Vajro P**, Paolella G, Pierri L, D'Aniello R. Treatment of NASH with ursodeoxycholic acid: pros and cons. More information in children. *Clin Res Hepatol Gastroenterol* 2013; **37**: e93-e94 [PMID: 23562789 DOI: 10.1016/j.clinre.2013.02.012]

102 **Zein CO**, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, McCullough AJ. Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *Hepatology* 2011; **54**: 1610-1619 [PMID: 21748765 DOI: 10.1002/hep.24544]

103 **Sharma BC**, Kumar A, Garg V, Reddy RS, Sakhuja P, Sarin SK. A Randomized Controlled Trial Comparing Efficacy of Pentoxifylline and Pioglitazone on Metabolic Factors and Liver Histology in Patients with Non-alcoholic Steatohepatitis. *J Clin Exp Hepatol* 2012; **2**: 333-337 [PMID: 25755455 DOI: 10.1016/j.jceh.2012.10.010]

104 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]

105 **Matsumoto R**, Fukuoka H, Iguchi G, Nishizawa H, Bando H, Suda K, Takahashi M, Takahashi Y. Long-term effects of growth hormone replacement therapy on liver function in adult patients with growth hormone deficiency. *Growth Horm IGF Res* 2014; **24**: 174-179 [PMID: 25116471 DOI: 10.1016/j.ghir.2014.07.002]

106 **Fujio A**, Kawagishi N, Echizenya T, Tokodai K, Nakanishi C, Miyagi S, Sato K, Fujimori K, Ohuchi N. Long-term survival with growth hormone replacement after liver transplantation of pediatric nonalcoholic steatohepatitis complicating acquired hypopituitarism. *Tohoku J Exp Med* 2015; **235**: 61-67 [PMID: 25744617 DOI:

10.1620/tjem.235.61]

107 **Averna M**. The effect of ezetimibe on NAFLD. *Atheroscler Suppl* 2015; **17**: 27-34 [PMID: 25659874 DOI: 10.1016/S1567-5688(15)50007-X]

108 **Husain NE**, Hassan AT, Elmadhoun WM, Ahmed MH. Evaluating the safety of Liptruzet (ezetimibe and atorvastatin): what are the potential benefits beyond low-density lipoprotein cholesterol-lowering effect? *Expert Opin Drug Saf* 2015; **14**: 1445-1455 [PMID: 26134926 DOI: 10.1517/14740338.2015.1063613]

109 **Takeshita Y**, Takamura T, Honda M, Kita Y, Zen Y, Kato K, Misu H, Ota T, Nakamura M, Yamada K, Sunagozaka H, Arai K, Yamashita T, Mizukoshi E, Kaneko S. The effects of ezetimibe on non-alcoholic fatty liver disease and glucose metabolism: a randomised controlled trial. *Diabetologia* 2014; **57**: 878-890 [PMID: 24407920 DOI: 10.1007/s00125-013-3149-9]

110 **Musso G**. Ezetimibe in the balance: can cholesterol-lowering drugs alone be an effective therapy for NAFLD? *Diabetologia* 2014; **57**: 850-855 [PMID: 24554006 DOI: 10.1007/s00125-014-3192-1]

111 **Scheimann AO**, Nadler EE, Driscoll DJ, Butler MG, Miller JL, Markovic TP, Goldstone AP. Laparoscopic sleeve gastrectomy in 108 obese children and adolescents ages 5 to 21 years by Alqahtani AR, Antonisamy B, Alamri H, Elahmedi M, Zimmerman VA. *Ann Surg* 2015; **261**: e118 [PMID: 24045441 DOI: 10.1097/SLA.0b013e3182a7187c]

112 **van Mil SR**, Biter LU, Grotenhuis BA, Zengerink JF, Mannaerts GH. Laparoscopic Sleeve Gastrectomy versus Gastric Bypass in Late Adolescents: What Is the Optimal Surgical Strategy for Morbid Obesity? *Eur J Pediatr Surg* 2016; Epub ahead of print [PMID: 26745522 DOI: 10.1055/s-0035-1570104]

113 **Pedroso FE**, Gander J, Oh PS, Zitsman JL. Laparoscopic vertical sleeve gastrectomy significantly improves short term weight loss as compared to laparoscopic adjustable gastric band placement in morbidly obese adolescent patients. *J Pediatr Surg* 2015; **50**: 115-122 [PMID: 25598106 DOI: 10.1016/j.jpedsurg.2014.10.014]

114 **Fullmer MA**, Abrams SH, Hrovat K, Mooney L, Scheimann AO, Hillman JB, Suskind DL. Nutritional strategy for adolescents undergoing bariatric surgery: report of a working group of the Nutrition Committee of NASPGHAN/NACHRI. *J Pediatr Gastroenterol Nutr* 2012; **54**: 125-135 [PMID: 21857247 DOI: 10.1097/MPG.0b013e318231db79]

115 **Nobili V**, Vajro P, Dezsofi A, Fischler B, Hadzic N, Jahnel J, Lamireau T, McKiernan P, McLin V, Socha P, Tizzard S, Baumann U. Indications and limitations of bariatric intervention in severely obese children and adolescents with and without nonalcoholic steatohepatitis: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr* 2015; **60**: 550-561 [PMID: 25591123 DOI: 10.1097/MPG.0000000000000715]

116 **Poeta M**, Di Salvio D, Torsiello N, Massa G, Guercio Nuzio S, Savastano R, Alfano G, Pierri L, Tripodi M, Siano MA, Terminiello MR, Mottola P, Vajro P. Obesity prevention project in preschool-age: the 3P-Project effectiveness in short- and medium-term. Proceedings of the 49th ESPGHAN Meeting; 2016 May 25-28; Athens, Greece. *J Pediatr Gastroenterol Nutr* 2016; **62** Suppl 1: S809

117 **Paolella G**, Vajro P. Childhood Obesity, Breastfeeding, Intestinal Microbiota, and Early Exposure to Antibiotics: What Is the Link? *JAMA Pediatr* 2016; **170**: 735-737 [PMID: 27294594 DOI: 10.1001/jamapediatrics.2016.0964]