Fatty liver disease in children: eat now pay later

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Abstract

Introduction With the recent epidemic in childhood obesity, nonalcoholic fatty liver disease (NAFLD) has become an emerging problem and a common cause of chronic liver disease in children.

Methods In this review, the most recent insights on the pathogenesis, diagnosis, natural history, and treatment of NAFLD in children are discussed.

Keywords Obesity ·
Nonalcoholic fatty liver disease (NAFLD) ·
Nonalcoholic steatohepatitis (NASH) · Children

Introduction

In children, liver steatosis is a typical finding in a variety of inherited metabolic disorders affecting the liver. Other causes of steatosis are malnutrition, infections (hepatitis C), and drug toxicity (Table 1). Currently, fatty liver disease is often seen in children in the absence of an apparent inherited metabolic defect or a specific cause. The vast majority of

these children are found to be obese and insulin resistant. In 1980, Ludwig [1] first described a pattern of liver injury consistent with alcoholic hepatitis in adults but in whom alcohol use was denied: nonalcoholic steatohepatitis (NASH). These patients were found to be largely obese, and they often had hyperlipidemia. Three years later, NASH was first described in obese children [2]. Today, NASH is considered a part of a broader spectrum: nonalcoholic fatty liver disease (NAFLD). This all-embracing term ranges from simple steatosis to NASH and frank cirrhosis. With the recent epidemic in childhood obesity, NAFLD has become an emerging problem and a common cause of chronic liver disease in children. In this review, the most recent insights on the pathogenesis, diagnosis, and treatment of NAFLD in children are discussed.

Epidemiology and risk factors

The definition of childhood obesity most widely used is body mass index (BMI) of more than 95th percentile, with overweight being defined as BMI between 85th and 95th percentiles, the normal range of BMI varying with age and sex. Age- and sex-specific BMI centile charts are available for different populations. Although BMI does not predict body fat content accurately, these definitions can still be applied adequately for most purposes in clinical practice, public health, and research when the cutoffs used are based on age- and sex-specific national reference data [3].

The prevalence of childhood obesity in the UK is increasing rapidly in the 2000s. Data from the National Study of Health and Growth and the Health Survey for UK show an increase in prevalence of overweight/obesity from 11.3%/1.8% to 22.6%/6% in boys and 9.6%/1.3% to 23.7%/6.6% in girls aged 5–10 years between 1974 and

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Table 1 Differential diagnosis of steatosis

General/nutritional
Acute systemic disease
Acute starvation
Protein-energy malnutrition
Total parenteral nutrition
Inflammatory bowel disease
Celiac disease
Mauriac syndrome
Infections
Hepatitis C
Metabolic
Cystic fibrosis
Wilson disease
α_1 -Anti-trypsin deficiency
Galactosemia
Fructosemia
Cholesterol ester storage disease (Wolman disease)
Glycogen storage disease
Mitochondrial and peroxisomal defects of fatty acid oxidation
Lipodystrophies
Abetalipoproteinemia
Weber-Christian disease
Schwachman-Diamond syndrome
Drug toxicity
Amiodarone
Methotrexate
Prednisolone
L-Asparaginase
Vitamin A
Valproate
Tamoxifen
Zidovudine and antiretrovirals
Ethanol

2002–2003 [4]. Similar results are seen in the USA, where data from the National Health and Nutrition Examination Survey (NHANES) showed a tripling of the prevalence of obesity among adolescents from 5% in 1960 to 17.1% in 2003/2004 [5]. Determining the true prevalence of NAFLD in children is difficult. Liver biopsy is the gold standard for diagnosis, but this is not feasible in large epidemiological studies. Hence, epidemiological studies often use surrogate markers such as serum alanine/aspartate aminotransferases (ALT/AST) or ultrasonographic (US) findings to diagnose NAFLD. However, the sensitivity, specificity, and predictive value of these biochemical and radiological markers are uncertain. Reference ranges of serum transaminases are derived from population samples, including individuals with undiagnosed liver disease such as NAFLD [6, 7].

There is significant variation in the normal ranges of transaminases between different laboratories and studies. Biopsy-proven NAFLD has been found in children with normal transaminases [8, 9]. Data based on these biochemical or radiological findings can therefore only underestimate the real epidemiological burden of pediatric NAFLD. Furthermore, most prevalence studies carry a high selection and referral bias because they are conducted in overweight or obese populations and children/adolescents who come to medical attention. A recent population-based prevalence study in the USA analyzed data of 5,586 adolescents (aged 12–19 years) in the 1999–2004 NHANES. The prevalence of elevated ALT levels (>30 U/L) in the absence of other causes of liver disease was 8% [10]. This result is remarkably higher than the estimated prevalence in the few other large-scale, population-based studies available. Tominaga et al. [11] reported a 2.6% prevalence of NAFLD among 810 Japanese children 4–12-year-old (based on US). Park et al. [12] found a 3.2% prevalence of NAFLD in 1,543 Korean adolescents 10–19-year-old (using ALT >40 U/L). An autopsy study in San Diego County studied liver histology in 742 children (aged 2–19 years) who died of unnatural death. The estimated population prevalence of NAFLD based on these findings was estimated to be 9.6%, proving that NAFLD is the most common liver abnormality in children aged 2–19 years [13]. The prevalence of NAFLD increases with age and the most important rise coincides with early puberty [13–15]. Multiple studies show a significant male predominance, suggesting that boys are more at risk to develop NAFLD than girls [13, 15, 16].

Major ethnic variations in the prevalence of NAFLD exist. Hispanic children and adolescents have a greater incidence than the white population, whereas black non-Hispanics are significantly less susceptible to NAFLD despite a susceptibility to insulin resistance [13, 15, 17]. Both genetic and environmental factors are likely to be involved in this ethnic distribution. Indeed, familial clustering of the condition, in association with insulin resistance, has been frequently reported [18, 19]. There is evidence that the occurrence of single-nucleotide polymorphisms may be associated with the distribution of NASH. *PNPLA3* (the gene for adiponutrin, an insulin-regulated phospholipase) is associated with liver fat content but not with insulin sensitivity or inflammatory change in the liver biopsy [20]. Other polymorphisms described are associated with the inflammatory component of NASH rather than with steatosis per se, for example, polymorphisms in interleukin (IL) 6 (IL-6) (174G/C) [21] and tumor necrosis factor alpha (TNF- α) [22]. Liver fibrosis in NAFLD patients was found to be associated with a splice mutation in the tumor suppressor gene Kruppel-like factor 6 (KLF6) in a genome-wide study [23].

Dietary chemical composition of fatty acids (FA) may be critical factors in lipotoxicity observed in the setting of insulin resistance. Palmitic acid rather than oleic acid results in lower steatosis but higher cell death and impaired insulin signaling [24]. Fructose is also implicated in pathogenesis [25]. Musso et al. [26] reported a study of 25 adult patients with NASH compared with controls as having higher intake of saturated fat and cholesterol and poorer intake of polyunsaturated fat, fiber, and antioxidant vitamins.

Diagnosis

Children with NAFLD are often asymptomatic but may present with vague nonspecific symptoms such as abdominal pain and/or fatigue. Most children are overweight (gender- and age-specific BMI >85th percentile) or obese (>95th centile) [27]. Hepatomegaly is often present but can be missed at clinical examination. Acanthosis nigricans, a black pigmentation of the skin folds, axillae, and neck, which is often seen in children with insulin resistance, is found in 30–50% of children with NAFLD [8, 17]. Often, these children have a positive family history of NAFLD, insulin resistance, or type 2 diabetes mellitus [16]. Various screening tools, such as serum transaminases and imaging techniques [US, computed tomography (CT), and magnetic resonance imaging (MRI)], are used for the detection of NAFLD. None of these has proven to be reliable and the sensitivity, specificity, and predictive values remain undetermined [6]. A mild-to-moderate elevation in the level of serum transaminases is often seen in NAFLD, but the sensitivity remains poor. Franzese et al. [28] studied the incidence of liver involvement in 72 obese children, using both US and serum transaminases [28]. Fifty-three percent of these children had a US image of bright liver consistent with liver steatosis, whereas only 25% had elevated levels of transaminases. Normal transaminases do not exclude NAFLD or even NASH and abnormal levels of transaminases in overweight or obese children are not necessarily caused by NAFLD. Serum transaminases are not good discriminators of histological severity [9]. Additional biochemical findings in childhood NAFLD are hypertriglyceridemia and low titers of autoantibodies (mainly anti-smooth muscle antibodies). Most children with NAFLD have elevated fasting insulin levels, with normal fasting glucose and homeostatic insulin resistance (HOMA-IR) and QUICKI indices consistent with insulin resistance [17]. Because of the low cost, the absence of radiation exposure, and the wide availability, US is often used in the screening for NAFLD. The accumulation of fat in the liver causes the liver to appear hyperechoic (“bright”) compared with the kidney. This finding, however, is nonspecific and does not

differentiate from other chronic liver diseases. When compared with histological findings, the sensitivity of US to detect fat infiltration below 30% of the liver is low [29].

Computed tomography is rarely used for the assessment of NAFLD in children because of its ionizing radiation exposure. Magnetic resonance imaging and spectroscopy are the imaging techniques with the greatest accuracy to determine hepatic fat content [30, 31]. However, aside from liver fat, other features of NASH cannot be assessed. No imaging technique reliably discriminates between simple steatosis and NASH. In the diagnostic workup of NAFLD, alternative causes of chronic liver disease, including chronic hepatitis B and C infection, Wilson disease, α_1 -antitrypsin deficiency, autoimmune hepatitis, cystic fibrosis, and drug toxicity, should be excluded. Table 1 gives the differential diagnosis of steatosis. In contrast to adults, alcoholic hepatitis is almost nonexistent in children. However, alcohol abuse is rising in the adolescent population, and this should always be questioned. Figure 1 is a flow chart of suggested investigations for suspected NAFLD/NASH. The definite diagnosis of NAFLD requires liver biopsy. This is the only way to assess the histological severity of the disease (degree of steatosis, inflammation, and fibrosis or cirrhosis) and to differentiate between simple steatosis and NASH. Furthermore, it is necessary to exclude these alternative causes of liver disease. However, liver biopsy has important limitations and is not suitable as a screening test. Therefore, simple, reproducible, noninvasive tests are urgently needed that distinguish NAFLD from NASH and determine histological severity. These tests would be useful in diagnosing NAFLD/NASH as well as monitoring disease progression and treatment response [32].

Fig. 1 Diagnostic flow chart for children with suspected NAFLD/NASH

Histology

NAFLD is defined as macrovesicular steatosis in more than 5% of hepatocytes. NAFLD comprises a histological spectrum ranging from simple steatosis to NASH and frank cirrhosis. Several studies have shown that children with NASH often present different histopathological features compared with adults [8, 9, 33]. In adults, the inflammatory changes typically consist of ballooning degeneration of hepatocytes or focal hepatocyte dropout with a polymorphonuclear infiltrate. Inflammation and fibrosis are most severe in the perivenular zone. Fibrosis is often pericellular, and Mallory's bodies can be present. This is known as type 1 NASH. In children, the inflammatory infiltrate is often mononuclear and inflammation is periportal. Fibrosis is also periportal and rarely pericellular, whereas Mallory bodies are rare [34]. This is type 2 NASH. Although type 1 NASH is most prevalent in adults and type 2 NASH in children, there is some crossover. A summary of the histopathological features of both subtypes is given in Table 2. Schwimmer et al. [9] reviewed the histologic findings in a cohort of 100 children (aged 2–18 years) with biopsy-proven NAFLD. Type 1 NASH was present in 17%, and type 2 NASH in 51% of the children. Sixteen percent of biopsies had overlapping features of both type 1 and 2 diseases, and the remaining 16% showed simple steatosis. Children with type 2 NASH were younger and had greater severity of obesity than those with type 1 NASH. Boys were more likely to have type 2 NASH than girls. Type 2 NASH was predominant among children of Asian or Native American race and Hispanic ethnicity, whereas type 1 NASH was more common in White children. The mechanism leading to these different phenotypes of NAFLD is not yet understood, but this finding might underline the need to address adult and pediatric NAFLD as two different entities. Care should be taken with extrapolation of data regarding pathogenesis, natural history, and treatment of NAFLD from adults to children.

The Pathology Committee of the NASH Clinical Research Network recently proposed a histological scoring system that could be useful in studies of both pediatric and adult NAFLD. The scoring system comprises the

evaluation of steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–4). The NAFLD activity score (NAS) is the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores. A NAS of 5 or more correlates with the diagnosis of NASH, whereas a NAS less than 3 is defined as “not NASH”. Because this system is typically developed for adult type 1 NASH, the interobserver agreement for pediatric NASH is not as strong as adult type 1 NASH [35]. The issue “if or when to perform a liver biopsy” in children with suspected NAFLD remains controversial. In case of suspected NAFLD, we certainly recommend a biopsy in obese children presenting with raised levels of transaminases when despite attempts at gradual weight loss, enzyme abnormalities persist after 3–6 months. A biopsy should be performed earlier in nonobese children, children with persistently elevated levels of serum transaminases for more than 1–3 months in the absence of another etiology, such as viral hepatitis, in the case of splenomegaly, or when alternative liver disease is suspected.

Natural history

The natural history of NAFLD varies according to the histological pattern of the disease. Simple fatty liver without inflammation or fibrosis seems to have a remarkably benign course, whereas NASH is a potentially serious condition that can progress to cirrhosis. Cirrhosis secondary to NASH has been reported in children as young as 10 years [8]. A recent study by Feldstein et al. [36] describes the long-term outcomes and survival of 66 children with NAFLD followed for up to 20 years. During this period, two children underwent transplantation for decompensated cirrhosis. Of 13 children who underwent follow-up biopsy, four showed progression of fibrosis [36]. In adult studies, the variables most commonly associated with fibrosis are as follows: presence of diabetes, increasing age, increased HOMA-IR, increased AST/ALT ratio, hyaluronic acid, and BMI, and decreased platelet count [37]. Similarly, in children, severity of obesity and insulin resistance seem to be predictors of advanced fibrosis [17].

Pathogenesis

NAFLD is considered as the hepatic manifestation of the metabolic syndrome. This syndrome, also called “syndrome X” or the “insulin resistance syndrome,” links obesity, type 2 diabetes mellitus, hypertension, hyperlipidemia, and NAFLD. Insulin resistance is known to be an almost universal finding in adults with NAFLD. Several

Table 2 Histological features of types 1 and 2 NASH

	Type 1	Type 2
Ballooning degradation	+	–
Perisinusoidal fibrosis	+	–
Steatosis	+	+
Portal inflammation	–	+
Portal fibrosis	–	+

Adapted from Schwimmer et al. [9]

studies also show a high prevalence of insulin resistance in obese children and adolescents with NAFLD [17, 38, 39]. The pathogenesis of NAFLD is still incompletely understood. The “two-hit hypothesis” proposed in 1998 [40] consists of a first hit of liver fat accumulation, which is caused by an imbalance in uptake and synthesis of hepatic lipids on one side and export and oxidation on the other side. The steatotic liver becomes then more vulnerable to “second hits” leading to hepatocyte injury, inflammation, and fibrosis. It is widely accepted that insulin resistance and the resulting hyperinsulinemia seem to play a major role in the development of hepatic steatosis and perhaps steatohepatitis. The molecular mechanism leading to insulin resistance is complex and has not yet been fully elucidated. Several molecules appear to interfere with the insulin signaling pathway (TNF- α , PC-1 membrane glycoprotein, leptin, and FA) [41].

Steatosis

Traditionally, steatosis thought to arise from increased hepatic supply of free fatty acids (FFA) as a result of obesity and associated extrahepatic insulin resistance. Normally, the adipocytes of lean, insulin-sensitive people store fat after meals and release fat during fasting. In contrast, fat-laden, insulin-resistant adipocytes of obese people continue to release large amounts of glycerol and FA in the circulation, which gives rise to increased delivery of FFA to the liver. These increased FFA may then induce hepatic insulin resistance [42]. The resulting hyperinsulinemia gives rise to increased hepatic lipogenesis because insulin stimulates lipogenic enzymes via the transcription factors sterol receptor binding protein 1-c (SREBP-1c) and peroxisome proliferator-activated receptor γ (PPAR γ) even in the insulin-resistant state [43]. Increased glucose levels also stimulate lipogenesis through the activation of carbohydrate response element binding protein (ChREBP), a transcription factor activating the expression of key enzymes of glycolysis and lipogenesis [44, 45]. Furthermore, the hyperinsulinemic condition results in decreased triglyceride secretion in the form of very low-density lipoprotein because insulin lowers apolipoprotein B synthesis and stability [46, 47]. Hence, hepatic FFA uptake and lipogenesis outweigh FA oxidation and triglyceride secretion leading to hepatic fat accumulation.

Oxidative stress

Because the liver cannot enlarge indefinitely and a new steady state has to be reached, as a result of this accumulation, mitochondrial FA oxidation and ketogenesis are increased. In addition, the augmented pool of FFA activates PPAR α , a transcription factor that regulates the

expression of different genes encoding enzymes involved in mitochondrial, peroxisomal, and microsomal FA oxidation. However, mitochondrial and peroxisomal oxidations are major sources of reactive oxygen species (ROS) giving rise to oxidative stress. This leads us to the hypothesized “second hit” in the development of NASH. High β -oxidation rates increase electron delivery to the mitochondrial respiratory chain (RC). This RC, however, is put under stress by the release of large amounts of TNF- α by the fat-engorged adipocytes of obese persons that permeabilize hepatic mitochondria and partially release cytochrome *c* from the mitochondrial intermembrane space. This results in an imbalance between a high input and a restricted flow of electrons over the RC, creating overreduction of RC complexes that can react with oxygen to form ROS [48].

Mitochondrial function is impaired in patients with severe steatosis [49], and NASH patients have ultrastructural abnormalities of mitochondria as well as severe mitochondrial DNA depletion [50, 51]. The overload of the mitochondrial RC, the resulting formation of ROS, and subsequent lipid peroxidation products certainly give rise to mitochondrial damage. On the other hand, mitochondrial abnormalities could also be a preexisting condition enabling the excessive production of ROS in the setting of enhanced FFA β -oxidation [50]. This could explain why for the same amount of obesity, or for the same degree of insulin resistance, certain patients just have steatosis, whereas others develop NASH and cirrhosis. Genetic polymorphisms could also at least partially explain this difference in susceptibility because some of these could favor mitochondrial dysfunction [52]. Enhanced ROS formation in the vulnerable steatotic liver subsequently triggers lipid peroxidation and the formation of reactive aldehydes such as 4-hydroxynonenal and malondialdehyde. These further give rise to mitochondrial damage and ROS formation, resulting in a vicious cycle [48].

Endoplasmic reticulum (ER) stress is also postulated as an important player in the process because this membranous network is the location of folding of proteins [53]. As the work of ER increases with biological stress, transcription factors are activated that coordinate the unfolded protein response, which slows down protein synthesis and promotes protein degradation. An inadequate response leads to protein activation, which gives rise to insulin resistance, apoptosis, inflammation, and mitochondrial dysfunction.

Cytokines and inflammation

Free fatty acids can directly activate the nuclear factor kappa B (NF κ B) pathway. Increased production of inflammatory cytokines by hepatocytes resulting from

NF κ B activation leads to Kupffer cell activation with subsequent inflammatory mediator release and hepatic and systemic insulin resistance [54]. ROS also increases the expression of several cytokines (TNF- α , Fas ligand, TGF- β , IL-8) which are involved in the different lesions of NASH such as cell death, inflammation, and fibrosis [48]. Visceral adipose tissue is also a source of many inflammatory mediators such as leptin, adiponectin, TNF- α , and IL-6. These adipokines directly target the liver through the portal vein [55] and are generally proinflammatory and profibrogenic. Adiponectin has an anti-inflammatory role and levels are decreased with increasing severity of disease. Serum levels of these factors correlate well with the severity of disease in NASH [56].

Fibrosis

Development and progression of fibrosis are associated with inflammation, oxidative stress, and hepatocellular damage. Inflammatory cytokines (TNF- α , IL-6, and TGF- β) and oxidative stress-related molecules induce hepatic stellate cell (HSC) activation to myofibroblast. The myofibroblast is contractile and proliferative and produces cytokines and matrix components. Apoptotic hepatocytes also have a direct effect on HSC [57]. HSC also express pattern recognition receptor (e.g., Toll-like receptor 4) activation (by lipopolysaccharide, for example), which leads to the expression of proinflammatory cytokines and amplification of profibrogenic stimulus [58].

The exact sequence, however, of development of obesity, fatty liver, and NAFLD remains unclear and whether insulin resistance causes hepatic steatosis or whether the accumulation of fat in the liver is the primary event leading to hepatic and peripheral insulin resistance is uncertain [59].

Noninvasive biomarkers

On the basis of the current understanding of the pathogenesis of NAFLD, investigators are trying to identify novel biomarkers that could be used as noninvasive screening tools with the aim of identifying those with advanced disease or risk of progression and those with simple steatosis not requiring follow-up. Many markers of inflammation, hepatocyte apoptosis, fibrosis, and oxidative stress are under investigation. Promising new approaches that use proteomics, metabolomics, and genomics may help in the identification of these new biomarkers [32].

Markers of inflammation, including adipocytokines and cytokines, correlate well with disease [56]. In particular, high serum levels of TNF- α and low levels of adiponectin are associated with greater degree of liver damage [60].

Markers of apoptosis/cell death are very useful in differentiating simple steatosis from NASH. Activation of caspase 3 results in cleavage of cytokeratin 18 (CK18), which is a major intermediary filament in hepatocytes. CK18 fragments have recently been shown by a number of studies to correlate well with severity of NASH [61, 62].

Noninvasive markers of fibrosis may consist of simple bedside tests or indices that have been studied in large cohorts of patients, for example, the AST to platelet ratio index [63] and the Forn index [64]. Others measure extracellular matrix turnover [65, 66]. Combinations of both include the Fibrotest [67, 68] and the Hepascore [69]. A promising combination of markers is the European Fibrosis Score, which combines hyaluronic acid, matrix metalloproteinase, and tissue inhibitory of metalloproteinase-1 (TIMP1). This demonstrated AUROC of 0.92, 0.98, and 0.99 in distinguishing any, significant, and advanced fibrosis in 112 children with NAFLD [70]. Other panels of markers specific for NAFLD include NAFLD fibrosis score (incorporating age, glucose, AST, ALT, BMI, platelets, and albumin), which gives an AUROC of 0.88 for advanced fibrosis [71], and the BARD score (BMI, AST/ALT ratio, and diabetes), which was found to be useful in excluding patients without advanced disease [72]. Other such tools include the HAIR score (hypertension, ALT, and insulin resistance) [73], the NASH test (weight, triglycerides, glucose, alpha 2 macroglobulin, and apolipoprotein A [74], and a tool proposed by Palekar et al. [75], which composites the score of age, sex, BMI, AST/ALT ratio, and hyaluronic acid. These panels of markers have not been evaluated in children, however, and are not likely to give the same predictive power in this population. These biomarkers perform best at extremes of the spectrum and are not useful in distinguishing those with intermediate stages of disease.

Fibroscan (transient elastography) is a useful method to detect fibrosis, using ultrasound and low-frequency (50 Hz) elastic waves with a propagation velocity directly related to the stiffness of the liver. A recent study has shown fibrosis in 52 children with NAFLD with an AUROC of 0.977, 0.992, and 1 for distinguishing any, significant, and severe fibrosis [76]. However, reproducibility of transient elastography may be affected in the setting of a BMI of more than 30 [77]. A combination both serum markers and transient elastography may give optimal predictive power for the detection or exclusion of fibrosis.

Treatment

Treatment of childhood NAFLD remains a largely unsolved question. Proposed strategies consist of lifestyle

modifications and pharmacological treatment. Weight reduction through diet and physical exercise is the only effective treatment of childhood NASH currently known. Weight loss should be gradual because rapid, extensive weight loss may exacerbate liver disease. Several case series and uncontrolled trials have demonstrated the effect of weight loss on improvement of transaminase levels or ultrasound abnormalities [28, 78, 79]. A recent prospective study carried out in 84 children (aged 3–18.8 years) who had elevated levels of transaminases and histologically proven NAFLD demonstrated a significant decrease in BMI, levels of fasting glucose, insulin, lipids, liver enzymes, and liver echogenicity on US after a 12-month program of lifestyle advice consisting of diet and physical exercise [80]. Up to now, there are no published data on the effect of diet and exercise on liver histology in children. Determination of the optimal dietary intervention and the optimal rate and degree of weight reduction should be subject for further research [6]. Only very few randomized, double-blinded, controlled trials are available for drugs used in the treatment of childhood NAFLD. On the basis of the current understanding of the pathogenesis of NAFLD, the main treatment options used in children are insulin-sensitizing agents and antioxidant therapy. A small, uncontrolled, open-label pilot study with vitamin E (400–1,200 IU/day) in 11 children with NASH showed improvement of serum transaminase levels despite no major changes in BMI or US appearance of the liver [81]. Other studies with vitamin E in children did not confirm these results [82, 83]. Ursodeoxycholic acid (UDCA) has been studied in both adult and pediatric NAFLD. A small study that evaluated the efficacy of UDCA in 31 obese children with abnormal transaminases [84] did not show any effect of UDCA treatment. Further studies, however, are needed to determine the efficacy of UDCA. On the basis of almost universal finding of insulin resistance in NAFLD patients, insulin-sensitizing agents have been used in the treatment of NAFLD. In children, an open-label pilot study of metformin (500 mg twice daily for 24 weeks) was conducted in ten nondiabetic children with biopsy-proven NASH and elevated ALT levels [85]. Significant improvement was observed in serum ALT levels and hepatic steatosis as assessed by MRI. A subsequent study conducted in children did not show any benefit of metformin compared with lifestyle advice [83]. A third study of metformin in insulin-resistant adolescents resulted in lower severity scores of fatty liver on US and a decrease in prevalence of fatty liver disease in the metformin group [86]. Treatment of adults with thiazolidinediones seems promising, but caution with the use of this drug in children is warranted in view of the lack of safety data in children with liver disease. Table 3 gives an overview of the currently available

clinical trials (both lifestyle changes and pharmacological interventions) in the field of pediatric NAFLD. Currently, a large, randomized, double-blind, placebo-controlled trial is under investigation by the Clinical Research Network in NASH, in which both vitamin E and metformin are used. The purpose of this study was to determine whether therapeutic modification of insulin resistance or oxidative stress leads to improvement in serum or histological indicators of liver injury and quality of life (TONIC trial) [6].

Prevention

Since treatment options are limited, prevention is the best strategy in the management of NAFLD. The environment (family, peers, neighborhood, and school) plays an extremely important role in the development of eating behavior and lifestyle. To change diet and lifestyle habits, intervention at different levels of society is indicated. Increasing public awareness, incorporating preventive measures in the school curriculum, and eradication of child poverty are important strategies to tackle this growing epidemiological problem.

Future research

The many unsolved questions regarding the diagnosis, pathogenesis, natural history, and management of pediatric NAFLD are topics for future research. There is a need to determine sensitive and specific surrogate noninvasive markers of NAFLD/NASH, allowing the screening of large, at-risk populations. Research should also focus on the further unraveling of the pathogenesis of NAFLD (role of environmental factors, identification of candidate genes, or polymorphisms involved in NAFLD). The differences in pathogenesis, natural history, and treatment of the two distinct subtypes of pediatric NASH (types 1 and 2) should be further investigated. Longitudinal follow-up studies are needed to better understand the natural history and outcome of NAFLD in children. Future clinical studies should help determine the optimal dietary intervention and pharmacological treatment of children with NAFLD.

Conclusion

With the recent epidemic of obesity, NAFLD is an emerging problem. The exact prevalence of NAFLD/NASH in the pediatric population is still unknown because current noninvasive screening methods lack sensitivity and

Table 3 Overview of treatment trials in pediatric NAFLD

Intervention	No. of patients	Design	Outcome
Diet and lifestyle			
Vajro et al. [79]	9	Open prospective single arm	Biochemical and US improvement
Franzese et al. [28]	75	Open prospective single arm	Biochemical and US improvement
Tock et al. [87]	73	Open prospective single arm	Reduction in visceral adiposity and US improvement
Nobili et al. [80]	84	Open prospective single arm	Decrease in BMI, fasting glucose, insulin, lipids, liver enzymes, liver echogenicity
Wang et al. [88]	38 no intervention 19 lifestyle 19 oral vitamin E	Open randomized	Both lifestyle and vitamin E decrease in BMI, lipids, liver enzymes, HOMA-IR, but lifestyle is more effective
Reinehr et al. [89]	43 no intervention 109 lifestyle	Open longitudinal study not randomized	Improvement in BMI and liver enzymes (persists 1 year after intervention)
Antioxidant therapy			
Lavine et al. [81]	11 oral vitamin E	Open prospective single arm	Biochemical improvement independent of weight loss
Vajro et al. [82]	14 diet + placebo 14 diet + vitamin E	Blinded randomized placebo controlled	Decrease in ALT with both vitamin E and diet only US improvement associated with weight loss only
Nobili et al. [83]	43 diet + placebo 45 diet + vitamin E + vitamin C	Blinded randomized placebo controlled	No beneficial effect from antioxidant therapy versus diet only on ALT and HOMA-IR
Metformin			
Schwimmer et al. [85]	10	Open prospective single arm	Improvement in ALT, liver fat, insulin sensitivity, and quality of life
Nobili et al. [90]	30 lifestyle + placebo 30 lifestyle + metformin	Open placebo controlled	No effect on transaminases, steatosis, histology
Nadeau et al. [86]	13 lifestyle + placebo 37 lifestyle + metformin	Double blind placebo controlled	Lower fasting insulin, fatty liver score on US and prevalence of steatosis
UDCA			
Vajro et al. [84]	13 diet only 7 UDCA only 7 UDCA + diet 6 control	Open randomized	No benefit compared with diet only

ALT alanine aminotransferase; BMI body mass index; HOMA-IR homeostatic insulin resistance; US ultrasonography

specificity and do not discriminate between steatosis and steatohepatitis. Liver biopsy remains the gold standard for diagnosis and staging of NAFLD. Because this is not feasible for screening large populations, there is a need for development of noninvasive surrogate markers. To date, the pathogenesis of NAFLD is incompletely understood, but insulin resistance seems to play a major role. Moreover, recent data suggest that pediatric NAFLD is a different entity from adult NAFLD, so vigilance is warranted with extrapolation of results. Weight reduction seems the only effective treatment. Large, multicenter, randomized, double-blind controlled trials are needed to evaluate the efficacy of pharmacological therapies (under way), but the most important intervention in childhood NAFLD should be the prevention of childhood obesity.

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