Nonalcoholic Fatty Liver Disease in children

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List of Abbreviations: NAFLD= Non-alcoholic fatty liver disease; NAFL=Non alcoholic fatty liver; NASH= Non-alcoholic steatohepatitis;

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Abstract

Non-alcoholic steatohepatitis (NASH), a progressive form of non-alcoholic fatty liver disease (NAFLD), is one of the most common hepatic diseases in children who present with particular risk factors, including obesity, sedentary lifestyle and/or a predisposing genetic background. The prevalence of NAFLD in children worldwide is a worrying phenomenon because this disease is closely associated with the development of both cirrhosis and cardiometabolic syndrome in adulthood. To date the etiopathogenesis of primary NAFLD in children is still unknown. Understanding the pathogenetic mechanisms provide the basis to characterize early predictors of the disease, noninvasive diagnostic tools and design novel specific treatments and possible management strategies. Despite a few clinical trials on the use of antioxidants combined with lifestyle intervention

for NAFLD, no treatment exist for children with NAFLD. In this Review, we provide an overview of current concepts in epidemiology, histological features, etiopathogenesis, diagnosis and treatment of NAFLD in pediatric population.

Keyword: NAFLD, obesity, children, NASH

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disorder closely associated with the metabolic syndrome and is the most common cause of abnormal liver function tests (LFT) in children. NAFLD is set to become the top cause for liver transplantation in adults and, whilst it is rare for children to develop end-stage liver disease from NAFLD, they may become cirrhotic as adults. Understanding and managing NAFLD in children may represent a method to intervene early and alter the disease process. Furthermore, children with steatosis require careful assessment as it may be secondary to other conditions (e.g. Wilson disease). For these reasons, paediatric NAFLD is of high importance to the all gastroenterologists, hepatologists, and paediatricians.

NAFLD refers to a spectrum of disease, ranging from hepatic steatosis ('simple steatosis', or non-alcoholic fatty liver (NAFL)), non-alcoholic steatohepatitis

(NASH) with or without fibrosis, and end-stage liver disease. Diagnosis requires radiological (or histological) demonstration of steatosis, exclusion of secondary causes, and no significant alcohol intake. Children present in three main ways: incidental abnormal LFT/imaging, screening in obesity, and during investigation of abdominal pain, the cause of which is not clear.

In this review we aim to provide an overview of paediatric NAFLD and discuss management in the context of recent guidance.

Epidemiology

Paralleling the dramatic rise in pediatric obesity worldwide, nonalcoholic fatty liver disease (NAFLD) has become a leading cause of chronic liver disease during the developmental age, and the main determinant on pediatric liver disease in Western countries[1]. Several studies have demonstrated a prevalence of 3–10% in general pediatric populations, which increases up to 60–70% in individuals with metabolic comorbidities[2]. However, NAFLD prevalence varies widely depending on geographical area and diagnostic methods used.

Initial population based studies, which estimated the prevalence of paediatric NAFLD by determining aminotransferases or by ultrasonography in several countries, have indicated a prevalence range of 3–7%[1]. In an autoptic study, conducted in unselected children deceased for accidents in California, the prevalence of histological NAFLD ranged from 0.7% in 2–4 years old, to 17.3% in 15–19 years old subjects, but increased to 38% in obese children[3]. In cohorts of children of various nationalities selected for overweight or obesity, the prevalence of elevated ALT was higher and ranging from 8 to 42%, whereas the prevalence of bright liver ranged from 1.7 to 77%[4]. A recent attempt to examine NAFLD prevalence based on a meta-analysis of studies conducted in 76 different populations led to an estimate of 7.6% (95% CI 5.5-10.3%) in the general population, and 34.2% (27.8-41.2%) in obesity clinics[5]. However, there was a huge heterogeneity (I^2=98%), which was partly accounted for by the sex distribution, difference in BMI and ethnicity, with NAFLD prevalence being higher in males, individuals with more severe adiposity, and in Asians.

Importantly, use of liver enzymes instead of imaging to diagnose NAFLD led a significant underestimation of disease prevalence[5].

Indeed, obesity and metabolic syndrome features are the major risk factors for paediatric NAFLD. The prevalence of this condition is higher in overweight (gender and age specific body mass index BMI >85th percentile) or obese (>95th) children as compared with normal weight pairs. However, due to the closest link to insulin resistance, central adiposity, that is accumulation of fat in visceral organs, plays a specific contribution in determining disease risk[6,7]. Indeed, waist circumference, an easily available index of visceral fat, correlates with NAFLD independently of BMI[1,8].

Nutritional factors such as excessive intake of calories, processed food and a sedentary lifestyle play a key role in the predisposition to liver fat accumulation, NAFLD and progressive liver disease[9,10]. Fructose intake has emerged as an important determinant of NAFLD risk, independently of total caloric intake, probably due to the ability to stimulate *de novo* lipogenesis[11,12]. This has recently been demonstrated to translate into increased risk of NASH[13]. The quality of fat, and specifically a reduced omega-3/omega-6 ratio, has also been reported to predispose to NAFLD in children at higher risk[14]. On the other hand, high levels of physical activity are associated with protection from NAFLD[8].

Inherited factors account for a large proportion of the inter-ethnic and inter-individual variability in the predisposition to NAFLD. Genetic studies have now identified the specific common variants that influence hepatic fat metabolism as important determinants of NAFLD in children and adults[15–17]. Also in the developmental age, NAFLD is more prevalent in individuals of Hispanic and Asian ethnicity as compared to Europeans, whereas those of African ancestry are relatively protected[5,18]. The most validated genetic risk factors are the PNPLA3 I148M and TM6SF2 E167K mutations, which influence lipid droplet remodeling and the secretion of lipids from hepatocytes[19–21], GCKR P446L regulating lipogenesis[22], and genetic variation in the *MBOAT7* that affects acyl chain remodeling of phosphatidyl inositol[17]. Evaluation of these genetic risk variants

increases the ability to stratify the risk of NAFLD[23]. Recent studies also suggest a link between NAFLD and epigenetic modifications, stable changes in expression of DNA related to chemical modifications of DNA and chromatin structure, caused by exposure to environmental factors[24]. In fact, accumulating evidence suggests that an adverse intrauterine environment as detected by low birth weight is associated with increased risk of pediatric and adult NAFLD[23,25]

Histology

From a histopathological point of view, NAFLD encompasses a disease spectrum ranging from "simple steatosis" (nonalcoholic fatty liver), to nonalcoholic steatohepatitis (NASH), which is characterized by the presence of hepatocellular damage under the form of ballooning and mixed lobular inflammation, is associated with activation of pericellular-perisinusoidal fibrogenesis and evolve in a variable proportion of cases to fibrosis, cirrhosis, and hepatocellular carcinoma[26]. Other common features include Mallory-Denk's bodies, megamitochondria, acidophil bodies, and iron accumulation[27].
NASH is associated with faster progression of liver fibrosis as compared to simple steatosis[28]. Importantly, fibrosis stage is the main determinant of the prognosis of patients with NAFLD, both concerning liver-related and overall mortality[29–32].

Pediatric NAFLD displays some peculiar histological features compared to the adult form, and, unlike the adult disease, are rarely influenced by some secondary lifestyle factors (e.g., alcohol, drugs), whereas other may have a more prominent role (e.g. fructose intake). Indeed, two different types of histological damage have been described in children with NAFLD[33]. These have been called "Type 1" and "Type 2" NAFLD. Type 1 NAFLD refers to the histological features classically associated with NASH in obese adults. These are represented by steatosis, that is accumulation of neutral lipids within intracellular lipid droplets, which is generally more severe in the centrilobular area. This is accompanied by hepatocellular damage under the form of ballooning, lobular inflammation

and/or perisinusoidal fibrosis. In contrast, Type 2 NAFLD has been described more frequently during the developmental age, and is characterized by steatosis with portal inflammation and/or periportal fibrosis. This form is more commonly observed in males and in children of Hispanic or Asian ethnicity as compared to Europeans[33]. However, in the majority of series it was found that, although Type 2 features are more frequent, most pediatric patients display overlapping features of Type 1 and Type 2 NAFLD, which can be considered the two extremes of a pathological spectrum[34–36]. Importantly, portal inflammation, which is typical of Type 2 NAFLD, has been associated with more severe fibrosis stage[37].

In a recent series, of 440 Caucasian children with histological NAFLD, 12% had Type 1, 22% type 2 Type 2, and 66% overlapping features[38]. Remarkably, those with type 2 had a more severe metabolic phenotype, with higher central adiposity and dyslipidemia. Furthermore, the presence of portal inflammation, a feature of Type 2 NAFLD, was independently associated with waist circumference and clinically significant fibrosis, suggesting that this histological feature is involved in mediating faster progression of the disease.

Pathogenesis of NAFLD in children

The pathogenesis of non-alcoholic fatty liver disease (NAFLD) in adults has been well-defined[39–42]. In contrast, the pathogenesis of NAFLD in children has not been the object of comparable attention and, thus, its contributing factors have yet to be mapped comprehensively and fully understood. Below, we describe the key pre-natal and post-natal factors that have been shown to affect the pathogenesis of NAFLD in children.

Pre-natal factors

Recent evidence has shown that there are some pre-natal factors that are responsible for the pathogenesis of paediatric NAFLD such as maternal obesity, metabolic syndrome during pregnancy, gestational diabetes and low birth weight[43–46]. This is supported by a series of well-conducted *in vitro* and *in vivo*

mechanistic studies. It is a multifactorial process that results from a combination of biochemical factors (foetal insulin, lipid profile) and epigenetic modification, which influences hepatic *de novo* lipogenesis, mitochondrial function, and oxidative stress in hepatocytes, macrophages, and adipocytes[47–53]. It is generally agreed that a 'second hit' is required to initiate development of NAFLD as an adolescent or adult, for which most is a positive energy balance in the form of a high-fat/-carbohydrate ('Western') diet[46,51,52,54]. Recent human studies showed that there is an association between maternal pre-pregnancy body mass index (BMI) and infant ectopic fat deposition in the liver and in the intra-abdominal cavity[45] (Table 1). Magnetic resonance imaging has been used to measure adiposity and hepatic liver fat in the new-born infant of obese mothers and women with gestational diabetes[44,45] and in one of these studies a correlation was shown between gestational BMI and offspring hepatic lipid accumulation[45]. Though it is unclear whether these same infants go on to develop progressive NAFLD. A possible explanation for the ectopic hepatic fat deposition in these newborn infants may be that immature foetal adipocytes are not sufficiently developed to accommodate and store lipids crossing the placenta in excess, throughout pregnancy. Consequently, in the presence of maternal obesity or gestational diabetes mellitus, two states where non-esterified fatty acid concentrations might be expected to be increased, excess transference of maternal lipid will result in accumulation of fetal ectopic fat as the foetus is not able to expand adipose depots to buffer the increased transplacental lipid delivery[55]. The presence and persistence of liver fat after birth has also be shown by us in a mouse model developed to investigate developmental programming of offspring NAFLD[56]. In this study, we showed that increased dietary maternal fat intake in the mother from before conception, primed development of increased liver fat in the offspring mice in adulthood, even if the offspring mouse had only ever eaten a normal chow (carbohydrate-rich, low fat) diet from weaning until adulthood. Furthermore, in adult mice that had been exposed in utero to increased dietary maternal fat intake in the mother from before conception, coupled with only consuming the same high fat diet from weaning until adulthood, these mice developed a florid form of NASH, that was a considerably more severe form of NAFLD than comparator mice who had only been exposed

to the high fat diet from weaning until adulthood. Additionally, mice exposed to gestational high fat diet had decreased mitochondrial electron transport chain function, suggesting that a stressor in early live has caused dysfunctional mitochondria that are less efficient at preforming mitochondrial β-oxidation, predisposing the mouse to hepatic fat accumulation and programming the development of NAFLD in adulthood. Another interesting pre-natal factor associated with the pathogenesis of paediatric NAFLD is the perturbation of intrauterine environment during pregnancy. According to the "thrifty phenotype" hypothesis, intrauterine growth retardation can lead to several chronic conditions and metabolic disorders (such as NAFLD) later in life[57]. We have shown that the "small for gestational age" state was significantly associated with severe liver steatosis (NAFLD Activity Score >5) in childhood[58]. Moreover, in a large epidemiological study, Sandboge et al. has also shown that body weight at 2 years was negatively associated with NAFLD, after adjusting for age, sex and gestational age. Thus the current evidence to date suggests that the intrauterine environment has the potential to have a powerful effect on the offspring's future risk developing NAFLD later in life.

Study	**Methodology**	**Findings**
Ayonrinde et al., 2017[59]	N=1,170 Mean age 17 years Prospective. USS-diagnosed NAFLD	NAFLD independently associated with: – Maternal pre-pregnancy obesity (OR 2.3) – Breast feeding 64 months (OR 0.6) – Adolescent obesity (OR 9.1)
Bugianesi et al., 2017[58]; Nobili et al., 2009[60]; and Nobili et al., 2007[61]	N=288 Mean age 13 years Retrospective. Biopsy-diagnosed NAFLD	– Low-birth weight associated with increased severe steatosis and portal inflammation, independent of insulin resistance – Each month of breastfeeding reduced NASH (OR 0.7) and fibrosis (OR 0.9) – SGA associated with insulin

		resistance
Suomela et al., 2016[23]	N=2,042 Mean age 42 years. Prospective. USS-diagnosed NAFLD	NAFLD independently associated with: – Preterm birth (OR 2.4) – Small for gestation age (OR 1.8) – Birth weight (OR 0.8) – Insulin at 11 years (OR 1.3)
Breij et al., 2014[62]	N=268 Mean age 21 years Retrospective. FLI-diagnosed NAFLD	– Rapid catch-up growth in the first 3 months associate with high FLI score – No association of FLI with SGA
Sandboge et al., 2013[63]	N=1,587 Mean age 62 years. Retrospective. NAFLD liver fat score/equation-diagnosed NAFLD	– Weight at 2 years negatively correlated with NAFLD score – Low weight at 2 years with later adult obesity had high risk of NAFLD (OR 19.5)
Fraser et al., 2007[64]	N=2,101 Mean age 68 years Retrospective. Abnormal LFT.	– Birth weight negatively correlated with ALT, GGT, and ALP

Table 1. Human evidence for pre-natal and infant risk factors associated with NAFLD.

Post-natal factors

The post-natal factors predisposing to paediatric NAFLD are similar to those for adult NAFLD and include common factors such as obesity, hyperinsulinaemia and insulin resistance. In the presence of obesity, the potential for good adipose tissue expansion is fundamental for maintaining lipid homeostasis and insulin sensitivity and for protecting the lipid from increased lipid and inflammatory fluxes that have the potential for promoting development and subsequent progression of NAFLD. When the process of the adipose tissue expansion fails, there is an associated low-grade inflammation associated with adipocyte autophagy and adipokine production. In this low grade inflammatory state, there

is increased flux of free fatty acids from the adipose tissue to the liver together with increased pro-inflammatory cytokines causing the recruitment of macrophages in adipose tissue, stimulating the production of TNF-α, IL-6, and reactive oxygen species and increasing adipocyte lipolysis[65]. In the liver, the combined effect of free fatty acids and adipokines coming from the adipose tissue, increases endoplasmic reticulum stress with consequent activation of Kupffer cells; this triggers liver inflammation, potentially promoting development of NASH[66]. This inflammatory state, triggers a cascade of hepatocyte injury and increases oxidative stress and mitochondrial dysfunction with consequent impairment of liver metabolic capacity. Hyperinsulinemia and insulin resistance also contribute to the development of NAFLD in adolescence as during puberty, children experience a physiological state of insulin resistance. In a large cross-sectional study, Moran et al. studied 357 healthy children and adolescent who underwent hyperinsulinaemic-euglycemic clamps. These authors showed that there are significant differences in insulin resistance between boys and girls; and that insulin resistance increases significantly at Tanner stages 2, 3, 4, but decreased to near prepubertal levels at Tanner stage 5. Furthermore, whilst insulin resistance was related to BMI and anthropometric measures of fatness, these factors did not completely explain the insulin resistance that occurs during the Tanner stages of puberty[67]. Moreover, in further work, these authors showed there are sex-related developmental changes in insulin resistance, which are independent of changes in adiposity[68]. During the transition from late childhood through adolescence, insulin resistance in males increased in association with increased triglyceride concentrations and decreased high-density lipoprotein cholesterol levels. This phenomenon was noted despite a concurrent reduction in body fatness in male children, whereas the opposite effect was observed occurred in female children. It is interesting to speculate that these sex-related developmental changes in insulin resistance may underpin, not only differences in NAFLD prevalence between males and females in adulthood, but also differences in cardiovascular risk between males and females in adult life.

In childhood a physiological state of insulin resistance, plus a sedentary lifestyle and the consumption of unhealthy food[69] may increase obesity, decrease skeletal muscle oxidation of lipids, and promote hepatic *de novo* lipogenesis (DNL). In the presence of hyperinsulinaemia that is associated with insulin resistance, acetyl-CoA carboxylase is activated by insulin and acetyl-CoA is converted into malonyl-CoA, which is the committed step in hepatic fatty acid synthesis. There are two important transcription factors that regulate DNL: sterol regulatory element-binding protein-1 (SREBP-1c) and carbohydrate response element-binding protein (ChREBP). These transcription factors regulate enzymes involved in fatty acid and tri-acyl glycerol synthesis. In addition, a high-carbohydrate diet and particularly one that contains high levels of dietary fructose can increase hepatic *de novo* lipogenesis by increasing substrate supply, and promoting the expression of SREBP-1c and ChREBP[70,71]. DNL is an energy-expensive process, consuming 7 ATP and 14 NADPH to generate each palmitate from acetyl-CoA[72]. Consistent with this, fructose causes hepatic ATP depletion with resultant oxidative stress and potential for mitochondrial dysfunction[73,74].

Softic *et al.* investigated the differential effects of glucose and fructose in a mouse model and showed that the addition of fructose to a high fat diet was associated with: increased expression of SREBP-1c and ChREBP, increased fatty acid synthesis and also hepatic insulin resistance. In contrast, when glucose was added to the high fat diet these authors found an increase in total ChREBP and liver triglyceride accumulation, but not insulin resistance[70,75] (**see Figure 1**). In the same study, Softic et al. studied hepatic expression of ketohexokinase (KHK), an enzyme that catalyses the first step of intracellular fructose metabolism[76], in both mice and obese adolescents with NAFLD who were undergoing bariatric surgery. These authors found that KHK expression was increased 2-fold in the mice whose high fat diet was supplemented with fructose. This finding contrasted with their observations in mice whose high fat diet was supplemented with glucose, where KHK expression did not increase significantly. In the adolescents, KHK expression was 2-fold higher in obese patients with NASH, compared with obese patients without fatty liver. Thus, these data suggest that dietary fructose is associated with an increase expression

of KHK favouring the production of acyl-CoA that contributes to the development of NAFLD through increased DNL[75]. The high activity of KHK contributes to reduced cellular ATP by rapid phosphorylation of fructose and, via re-generation of ATP in oxidative phosphorylation, hepatocytes become depleted in inorganic phosphate. The net effect is elevated uric acid[77] production, which has been independently associated with advanced NASH histology in children[13].

Hepatic outcomes

The long-term hepatic outcomes of paediatric NAFLD are not clear due to a lack of prospective natural history data and few children undergo paired biopsied. The outcomes of NAFLD in adults are more defined, where data demonstrates that after over 30 years of follow-up NAFLD is associated with increased all-cause mortality (hazard ratio (HR) 1.3) and hepatocellular carcinoma in (OR 6.6)[30]. Overall, liver-related events occur in <10% of patients with NAFLD but there is a strong correlation with fibrosis stage and outcome[29]. In addition, it has been suggested that there are a group of 'rapid progressors' who may develop severe fibrosis within 5 years[78], though it remains to be established whether this is due to sampling variability on repeat biopsy. Liver-related causes of death are third behind cardiovascular disease and non-hepatic malignancy.

There are no robust data to determine whether this natural history holds true for paediatric NAFLD. It is also not well established what proportion of children with NAFLD continue to have NAFLD as adults. It is known that around a third of obese adolescents will become obese adults[79] but a follow-up study from paediatric to adult transition in NAFLD has not yet been reported.

Feldstein *et al.* reported a retrospective cohort of 66 children with NAFLD where two children underwent transplant for decompensated cirrhosis[80]. This study has the longest follow-up but its retrospective design and lack of data about method of diagnosis as children limit its conclusions. There have been other case

series to suggest that severe fibrosis is possible whilst still a child[81] however it is generally regarded that end-stage liver disease whilst under 16 years is unlikely to be secondary to NAFLD alone and should prompt a search for alternative diagnoses[82]. It is worthy of mention that there are some specific circumstances, particularly acquired hypothalamic-pituitary insufficiency, that may be associated with rapid progression of fibrosis[83].

In adults, fibrosis is the sole predictor of long-term liver-related events[31]. This finding has been reproduced in multiple cohorts and non-invasive fibrosis scores (NAFLD Fibrosis Score, Fib-4, BARD) correlate with mortality[84]. Whilst similar scores exist in paediatric NAFLD (for example, the Paediatric NAFLD Fibrosis Index[85]) it is not known whether they correlate with long-term outcome.

Data from randomised controlled trials demonstrates that stage 1 and 2 fibrosis is readily reversible within two years[86,87]. It is not known to what extent advanced fibrosis can regress in paediatric NAFLD.

Therefore, to accurate determine the long-term hepatic outcomes in paediatric NAFLD natural history studies are required. These have recently been established both sides of the Atlantic[88,89] and are likely to require more than 20 years follow-up to quantify rates of hepatocellular carcinoma development and liver failure.

Dangerous liaisons

There are several potential pit-falls in the diagnosis, monitoring, and management of paediatric NAFLD, which will be discussed in this section.

The most important point about diagnosing NAFLD in children is to exclude conditions masquerading as fatty liver. It is recognised that a variety of liver and

systemic disorders may cause secondary steatosis including those listed in table 2.

Secondary causes of NAFLD	**Test**	**Abnormality**
Hepatitis B & C, EBV, CMV	Viral hepatitis serology	IgG or IgM serology positive
Wilson disease	Serum caeruloplasmin	Low levels
	Liver biopsy	Raised dry weight copper
Autoimmune hepatitis	Autoantibodies	Positive ANA / ASMA
	Immunoglobulins	Increased IgG
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin levels and Pi type	PiZZ
Hereditary haemochromatosis	Serum iron studies	Raised ferritin and transferrin saturation
Lipodystrophy, and other insulin-resistance syndromes	Clinical examination	Reduced subcutaneous adipose
	Serum fasting insulin	Greatly elevated
Lysosomal acid lipase deficiency	Dry blood spot assay	Reduced LAL activity

Table 2. Some secondary causes of hepatic steatosis that should be excluded before making a diagnosis of paediatric NAFLD.

Schwimmer *et al.* presented strong evidence for liver biopsy in children with suspected NAFLD. From 374 children referred from primary care, 255 underwent biopsy and 61 had a diagnosis other than NAFLD, most frequently autoimmune hepatitis. Children and adults with 'lean' NAFLD, with BMI and waist circumference <95% centile, may be more likely to have secondary causes

for steatosis or NAFLD-associated polymorphisms[90]. In very lean children with severe insulin resistance lipodystrophy should be considered[91].

Differentiating Wilson disease (WD) and NAFLD may be very challenging and some paediatricians would argue that biopsy is the only method to truly exclude WD. Caeruloplasmin is a good screening test for WD and, depending on the threshold used, may have a negative predictive value of 99% (Table 3)[92]. However, a subset of patients may have normal caeruloplasmin and missing the diagnosis can result in permanent neurological damage. Therefore, a liver biopsy with dry weight copper improves the reliability of diagnosis, but can give a false positive (for WD) as cholestatic disorders can cause copper accumulation[82]. Molecular genetics can be used to make a diagnosis of WD but ATP7B can be affected by a variety of mutations, therefore genetics cannot exclude the diagnosis[93].

Caeruloplasmin concentration cut-off (g/L)	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%)**	**Negative predictive value (%)**
0.20	98	56	48	99
0.14	93	100	100	97
0.10	79	100	100	92

Table 3. Data on the validity of use of 3 different cut-off thresholds of caeruloplasmin for diagnosis of Wilson disease in patients clinically suspected of having the condition.

Lysosomal acid lipase deficiency (LAL-D), also known as cholesterol ester storage disease, is a potential differential diagnosis for NAFLD. This rare, autosomal recessive lysosomal disorder has a wide spectrum of clinical presentation[94]. Wolman disease is the most severe form with rapidly progressive liver failure in infants. A milder form presents in childhood or adults with

hepatic steatosis, raised aminotransferases, raised low-density lipoprotein and low high-density lipoprotein. Diagnosis is theoretically important because of the potential for treatment with sebelipase alfa[95]. However, despite the possibility for misdiagnosis of LAL-D as NAFLD, or a role of LAL in NAFLD[96], to date there are no reports of identification of LAL-D in cohorts of children with fatty liver, therefore its relevance to routine clinical practice remains to be established.

It is not clear how best to monitor paediatric NAFLD, but it is well established that aminotransferases are a poor marker of disease activity. ALT and AST fluctuate throughout the disease course and do not correlate with fibrosis[97], though may show some correlation with NAFLD Activity Score[38]. Bloods often become most abnormal during the relatively insulin resistant state of adolescence[98], therefore a reduction of ALT without evidence of weight loss, should not be relied upon as a reassuring finding.

Though the long-term cardiovascular and metabolic outcomes of paediatric NAFLD have yet to be formally quantified, these are likely to the main burden of disease for patients in adult life[29,32]. Therefore, hepatologists must remember that weight loss and improvement of insulin resistance is the most important management goal, beyond the therapies targets at improving liver disease activity discussed below.

Management programme

The treatment of paediatric NAFLD can be divided into conservative, medical, and surgical approaches. As alluded to above, the determinants of successful management are not clear as the natural history of the condition is uncertain. The goals are multi-dimensional: improve the metabolic health of children to reduce their long-term cardiovascular risk, and reduce liver-related clinical events, presumably by targeting fibrosis. Results from the major controlled trials in paediatric NAFLD are summarised in Table 4.

Weight loss is the core therapy for paediatric NAFLD and for all children with obesity[99]. This may be achieved by dietary modification and/or physical activity. A variety of diets have been used in randomised and non-randomised clinical trials. The most frequently used diet is a 'low fat' hypocaloric diet at with 25-30 kcal/kg, 50-60% carbohydrate and 23-30% fat (with 1/3 saturated fat)[87,100,101]. A recent systematic review of dietary and physical activity interventions found that there was insufficient evidence to suggest any single method of weight loss, but that greater weight loss gave a larger improvement in non-invasive markers of NAFLD[102]. In studies with biopsy end-points, a reduction in age-sex-corrected BMI is associated with an improvement in features of NAS and fibrosis[87].

There is recent physiological evidence to suggest that fructose restriction improves the metabolic profile of obese children and potential for treatment of fatty liver[12]. Low carbohydrate[103] or low fructose[104] diets have only been used in pilot studies on paediatric NAFLD however there are several larger studies planned[105].

Trial	n	Intervention	End-points
Dietary intervention			
Vos et al., 2009[104]	10	Low-fructose vs low-fat diet	Liver enzymes: no effect
Ramon-Krauel et al., 2013[103]	16	Low-glycaemic load diet: vs low-fat diet	Liver enzymes and MRS: improved but no difference between groups
Jin et al., 2014[106]	21	Low-fructose vs standard diet	Liver enzymes and MRS: no effect
Antioxidants			
Vajro et al., 2004[100]	28	Lifestyle vs lifestyle + vitamin E	Liver enzymes: improved but no difference between groups Ultrasound: unchanged
Nobili et al., 2008[87]	53	Lifestyle vs lifestyle + vitamin E + vitamin C	Histological steatosis, lobular inflammation, ballooning, and NAS: improved but no difference between groups
Wang et al., 2008[107]	76	No intervention vs strict lifestyle vs unstructured lifestyle + vitamin E.	Liver enzymes: improved with strict lifestyle intervention Ultrasound: unchanged
Lavine et al., 2011[86]	173	Metformin vs vitamin E vs placebo	NAS and ballooning improved with vitamin E. Liver enzymes: improved but no difference between groups

Akcam et al., 2011[108]	67	Lifestyle vs lifestyle + metformin vs lifestyle + vitamin E	Liver enzymes: improved but no difference between groups
Shiasi Arani et al., 2014[109]	119	Metformin vs vitamin E vs placebo	Ultrasound: improved but no difference between drugs
Schwimmer et al., 2016[110]	169	CBDR vs placebo	Lobular inflammation: improved Liver enzymes: improved
Zohrer et al., 2017[111]	40	Lifestyle vs lifestyle + DHA-Cho-VE	Liver enzymes and ultrasound: improved with DHA-Cho-VE Histological improvement in DHA-Cho-VE, but no biopsy in placebo for comparison
Metformin			
Nadeau et al., 2009[112]	50	Lifestyle vs lifestyle + metformin	Liver enzymes: improved but no difference between groups Ultrasound: improved with metformin
Polyunsaturated fatty acids			
Nobili et al., 2013[113]	60	DHA vs placebo	Liver enzymes and ultrasound steatosis: improved
Boyraz et al., 2015[101]	108	Lifestyle vs lifestyle + PUFA	Liver enzymes and ultrasound: improved in lifestyle + PUFA group
Janczyk et al., 2015[114]	76	DHA/EPA vs placebo	Liver enzymes and ultrasound: improved but no difference between groups
Pacifico et al., 2015[115]	51	DHA vs placebo	Liver enzymes: improved but no difference between groups MRI hepatic fat: improved
Della Corte, et al., 2016[116]	41	DHA + vitamin D vs placebo	Liver enzymes: improved Histological improvement in DHA + Vit D, but no biopsy in placebo for comparison
Probiotics			
Vajro et al., 2011[117]	20	Lactobacillus vs placebo	Liver enzymes: improved Ultrasound: unchanged
Alisi et al., 2014[118]	44	Lifestyle vs lifestyle + VSL#3	Liver enzymes: unchanged Ultrasound: improved with VSL#3
Famouri et al., 2017[119]	64	*Prokid* probiotic vs placebo	Liver enzymes and ultrasound steatosis: improved
Bariatric surgery			
Manco et al., 2017[120]	93	Sleeve gastrectomy vs intragastric weight loss device (IGWLD) vs lifestyle (non-randomised)	Fibrosis and NASH: improved most in sleeve gastrectomy, and also in IGWLD

Table 4. Randomised controlled trials in paediatric NAFLD. CBDR, cysteamine bitartrate delayed release; DHA, docosahexaenoic acid; DHA-Cho-VE, docosahexaenoic acid with choline and vitamin E; EPA, eicosapentaenoic acid; IGWLD, intragastric weight loss device; PUFA, polyunsaturated fatty acids; US, ultrasound scan; and VSL#3, a probiotic mixture.

However, it must be remembered, that even with close follow-up in well-conducted clinical trials there is a relatively poor response to dietary and physical excise in childhood obesity[121]. Compliance is poor and participants with low-uptake of advice have greater rises in aminotransferases[100]. Therefore, NAFLD should ideally be managed in a multidisciplinary clinic that includes a dietician, clinical nurse specialist, and clinical psychologist to give the best chance of achieving weight loss.

There are no approved pharmacological therapies of paediatric NAFLD[122]. The main groups of agents that have been tested to date are: antioxidants, metformin, poly-unsaturated fatty acids (PUFA), probiotics, and vitamin D.

AASLD guidance recommends vitamin E as the only therapy that is potentially efficacious in paediatric NAFLD[99]. This is primarily based on data from the TONIC trial that demonstrated an improvement in ballooning and overall NAS with vitamin E use, without any major adverse events[123]. Other studies have used vitamin E in a variety of doses and durations but have been limited by not using protocolled paired biopsies[100,108], combination treatment[87,111], or differing weight loss between intervention arms[107]. Overall, vitamin E is probably safe and may improve NASH activity, though it remains to be established if this translates into improved liver-related outcomes.

Results from a randomised trial of cysteamine bitartrate delayed release (CBDR) have recently shown modest improvements in lobular inflammation and there was overall improvement in histology in participants weighing under 65kg[124]. It acts by increasing intracellular glutathione, which then scavenges oxygen free radicals. The evidence body for CBDR is smaller than that for vitamin E and it is likely that further studies will be required before it is incorporated into guidelines.

Metformin has had more modest results, with some reports of improved radiological evidence of steatosis[112,109], but randomised biopsy data is limited to

that from the TONIC trial[123]. However, aside from its effects on the liver, metformin may be used by paediatric endocrinologists in children with insulin resistance at risk of type 2 diabetes[121,125]. At this time, consensus guidelines do not recommend metformin as a primary treatment for NAFLD or NASH.

PUFA, mostly docosahexanoic acid (DHA) and eicosapentanoic acid, are given with the aim of alerting the composition of the hepatic lipidome and reducing lipotoxicity. Non-invasive data are encouraging, where use of at least 250 mg per day for 6 months may result in reduction of radiological steatosis but there is no biopsy data available to confirm these findings[101,115,113].

The evidence of use of probiotics is similar: a variety of regimens have been used in trials for paediatric NAFLD but there are no studies with biopsy end-points to date[119,118]. Although there is a large body of evidence that confirms the association of intestinal dysbiosis with obesity and NAFLD[126–128], there is little data to support modulation of the microbiome as a primary treatment strategy.

The association of vitamin D deficiency with paediatric obesity[129], NAFLD, and NASH[130] is relatively well established and the use of replacement therapy in deficient children is logical. Indeed, in children deficient in vitamin D, replacement in combination with DHA does improve histology[116]. However, it remains to be demonstrated whether children with acceptable 25-OH-D_3 (>20 ng/L) benefit from supplementary vitamin D. In addition, the risk of hypervitaminosis and resulting renal impairment must be considered.

Finally, bariatric surgery is a treatment option for severe paediatric obesity with co-morbidities. Expert consensus recommends that NAFLD should not be a primary indication for weight-loss surgery however evidence does suggest it to be of use, with potential for reversal of fibrosis[131,120]. These findings are consistent with data from adults, though there were insufficient un-biased studies for a Cochrane review to recommend it as a primary treatment[132]. However, the long-term data is clear that bariatric surgery improves the metabolic and cardiovascular outcomes for obese individuals[133] and therefore it

may be part of the care for patients with NAFLD. The psychological effects of bariatric surgery are not to be underestimated and there is emerging data on the impact that such operations have on patients later in adult life[134].

Therefore, weight loss is the primary treatment of paediatric NAFLD and it appears that the method by which this is achieved does not affect the outcome. Vitamin E is the only drug treatment that may be a direct hepatic benefit but pharmacological management of the liver is a small component of the overall care for children with the metabolic syndrome.

Implications & future directions

The implications of these data are that paediatric NAFLD is increasing and these patients will become adults with end-stage liver disease or HCC in 10-30 years time. Weight loss is the only intervention demonstrated to have significant efficacy. Further investigation into the pathogenesis of paediatric NAFLD is needed to complement translational studies with an aim to develop novel therapeutic strategies. There are only a few agents in trials in children and drug development is progressing at a much slower rate than in adults.

Future directions must focus on establishing on expanding our understanding NAFLD pathogenesis with a translational therapy in mind. For example, determining the microbiome phylogena associated with less severe fibrosis and trialling probiotic therapy. It may also involve exploring whether recent advances in adult hepatology are also applicable to paediatrics, such as the role of VAP-1 in gut-liver axis, which has not yet been investigated in children. There is much heterogeneity in investigations of NAFLD, particularly in regards to use of biopsy, establishing biomarkers that correlate with histology progression and clinical outcomes remains a major target. Finally, despite weight loss being the primary treatment for NAFLD, minimal progress has been made in guidance on determining the optimal regimen of lifestyle changes.

In conclusion, despite major advances in understanding, research in paediatric NAFLD has not yet translated into patient benefit. It is an important condition with unmet scientific and clinical needs that requires urgent attention to slow the future epidemic of end-stage liver disease in adults.

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