Non-alcoholic liver disease and childhood obesity

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) in children and adolescents has an estimated prevalence of 36.1% in the context of obesity. This figure is anticipated to increase in conjunction with the global obesity epidemic. Worryingly, NAFLD in childhood persisting into adulthood is likely to be harmful, contributing to significant hepatic and extra-hepatic morbidity. Early disease detection is required, although the optimum timing, frequency and mode of screening remains undetermined. Whilst the efficacy of several medications, antioxidants, fatty acid supplements and probiotics have been investigated in children, healthy eating and physical activity, remain the only prevention and treatment strategies for paediatric NAFLD. This short review discusses the epidemiology, diagnosis, pathogenesis and management of NAFLD in childhood obesity.

INTRODUCTION

The term “non-alcoholic fatty liver disease” can either be used to summarise a histopathological spectrum of liver disease, which progresses from steatosis to non-alcoholic steatohepatitis (NASH), or it can be used to describe early benign disease prior to the onset of NASH.

In adults, NAFLD not only increases the risk of cirrhosis and hepatocellular carcinoma, but also contributes to the development of type 2 diabetes mellitus, cardiovascular disease and chronic kidney disease and is therefore increasingly considered to be a multi-system disease[1]. In children and adolescents, NAFLD is similarly associated with significant extra-hepatic morbidity[2-4]. Although the long-term consequences of paediatric NAFLD remain largely unknown due to the lack of high-quality longitudinal data, it seems reasonable to assume that NAFLD in childhood persisting into adulthood is likely to be a harmful condition.

Adiposity, as defined by body mass index (BMI), is an important risk factor for NAFLD, with prospective studies in children demonstrating a clear association between adiposity gain in childhood and adverse liver outcomes in adolescence[5]. Adiposity-related risk is predominantly attributed to the development of insulin resistance and adipose tissue dysfunction in these individuals. The prevalence of NAFLD in children and adolescents is therefore anticipated to increase in conjunction with the global obesity epidemic that currently affects millions of young people worldwide.

This short review will discuss: a) the epidemiology of childhood NAFLD, including the effects of ethnicity; b) the diagnosis of childhood NAFLD including invasive and non-invasive testing; c) risk factors and pathogenesis of NAFLD in childhood; and d) the management and treatment of NAFLD in childhood.

a) The epidemiology of childhood NAFLD, including the effects of ethnicity

It has proved difficult to achieve a precise estimate of the prevalence of NAFLD in children and adolescents. Differences in study design and the methodology used to diagnose NAFLD, plus the changing prevalence and inconsistent definition of obesity in childhood, have all contributed to uncertainty regarding the exact prevalence of NAFLD in this age group. Many studies have defined NAFLD on the basis of screening biochemistry and/or imaging, rather than liver biopsy, which is the generally accepted "gold standard" diagnostic investigation[6,7].

Anderson et al (2015) conducted a systematic review and meta-analysis to estimate the prevalence of NAFLD in children and adolescents, aged ≥ 1 and ≤ 19 years[8]. Using data from nine general population studies, the authors reported NAFLD prevalence estimates of 2.3% (95% CI: 1.5% to 3.6%) in normal weight individuals, 12.5% (95% CI: 9.2% to 16.7%) in overweight individuals and 36.1% (95% CI: 24.6% to 49.4%) in obese individuals, with a higher prevalence reported in males compared to females[8].

The prevalence of NAFLD in obese populations shows geographical variation, partly reflecting the genetic contribution to NAFLD aetiology[8]. Nobili et al (2019) mapped the prevalence of paediatric NAFLD, reporting the highest prevalence in Central America and the Middle East, with figures of 42.5% in children aged 8 to 11 years determined by alanine aminotransferase (ALT) in Mexico and 16.9% in children aged 6 to 19 years determined by ultrasound in Iran[3].

b) The diagnosis of childhood NAFLD including invasive and non-invasive testing

<u>Screening</u>

The European and North American Societies for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN and NASPGHAN) have published clinical guidance for the diagnosis of paediatric NAFLD[6,7]. Some children and adolescents with NAFLD present with non-

specific symptoms, including generalised abdominal discomfort and fatigue, whilst others are asymptomatic. ESPGHAN recommends performing screening liver function tests (LFTs) and ultrasonography in all children and adolescents with obesity (BMI > 95th percentile), prior to stratifying further management according to age, history and physical examination[6]. By contrast, NASPGHAN recommends measuring ALT from the age of 9 years in all overweight (BMI $\geq$ 85th and < 94th percentile) and obese (BMI $\geq$ 95th percentile) children, provided the former have additional risk factors for NAFLD[7]. Both recommend re-screening should initial results return normal, although the optimum timing and frequency of re-screening remain undetermined[6,7].

Despite the pragmatic argument for using ALT as a screening investigation, clinicians should be aware that ALT has a low sensitivity for NAFLD and values do not correlate with ultrasound or biopsy findings – a 'normal' ALT value may therefore be falsely reassuring[9,10]. Age- and sex-related reference ranges of ALT have been developed in children. These should be considered when interpreting ALT results rather than necessarily using laboratory reported upper limits of normal[11,12]. Increasingly refined paediatric NAFLD plasma screening panels are in development, combining ALT with other metabolic data, to improve screening specificity[13].

It is also important to note that ultrasound has been shown to have a high sensitivity and specificity for moderate to severe steatosis in children and young people, however mild steatosis may not be detected[9]. By contrast, MRI estimated hepatic proton density fat fraction (PDFF) allows quantification of steatosis severity. MRI PDFF values show significant correlation with histological steatosis grade and may be more useful than ultrasound in defining early disease[14-16].

<u>Differential diagnoses</u>

NAFLD is a diagnosis of exclusion. Following the identification of an elevated ALT and/or hyper-echogenicity on ultrasound, clinicians should consider genetic, metabolic and systemic causes of fatty liver disease, particularly in young children and those with very high

or chronically elevated ALT values. Table 1 lists important secondary causes of hepatic steatosis in children and adolescents[6,7].

Table 1: Secondary causes of hepatic steatosis

Causes	
Endocrine	Growth hormone deficiency Hypothyroidism Hypogonadism Polycystic ovarian syndrome Type 1 diabetes mellitus
Hepatic	Wilson's disease Haemochromatosis Alpha 1 anti-trypsin deficiency
Gastrointestinal	Coeliac disease Inflammatory bowel disease
Metabolic	Inborn error of metabolism Disorders of lipid and lipoprotein metabolism Lipodystrophies
Renal	Nephrotic syndrome
Genetic	Alstrom syndrome Prader Willi syndrome Bardet-Biedl syndrome
Respiratory	Cystic fibrosis Obstructive sleep apnoea
Infection	Hepatitis C HIV Bacterial overgrowth
Medications	Corticosteroids Oestrogens Amiodarone Sodium valproate Nifedipine Diltiazem Methotrexate Antiretrovirals TPN
Other	Alcohol misuse Rapid weight loss Anorexia nervosa Protein energy malnutrition

<u>Invasive evaluation of hepatic fibrosis</u>

Liver biopsy remains the gold standard diagnostic investigation, as it is currently the only test that can reliably differentiate steatosis from NASH and exclude potentially treatable liver disease.

Histologically, NAFLD is characterised by steatosis affecting more than 5% of hepatocytes - a definition developed from adult data[17]. Steatosis with mild inflammation can progress to NASH, with increasing inflammation, hepatocyte injury and fibrosis. Worryingly, in children and adolescents, NASH rather than steatosis is more commonly identified by the time of liver biopsy[18].

The histological pattern of NASH differs depending on age, gender and ethnicity[19]. Two types of paediatric NASH have been described, although features may overlap. Type 1 or 'adult type' NASH is characterised by steatosis, inflammation and hepatocyte injury, demonstrated by ballooning and the variable presence of Mallory-Denk bodies, predominantly affecting 'zone 3' hepatocytes surrounding central veins[20]. In Type 2 NASH, steatosis is often more pronounced in 'zone 1' hepatocytes, which surround portal tracts[20]. Inflammation is generally mild and evidence of hepatocyte injury is difficult to identify[20].

An estimated 30 – 40% of adults with NAFLD develop NASH and 40 – 50% of these develop hepatic fibrosis, which can progress to cirrhosis and end-stage liver failure, an increasingly common indication for transplantation[1]. Although the natural history of paediatric NAFLD remains largely unknown, available evidence indicates that severe fibrosis and cirrhosis are potential consequences of disease starting in childhood and worryingly, disease progression can be rapid[2]. In adults, NASH is also a well-documented risk factor for hepatocellular carcinoma[1]. Although rare, cases of hepatocellular carcinoma have been reported in paediatric NAFLD[2].

Despite the risk posed by NASH, a presumptive diagnosis of paediatric NAFLD is often made in the absence of other causes of fatty liver, largely due to the limitations of liver biopsy – specifically the invasive nature of the procedure, the risk of sampling error and the high cost

involved[20]. There is no current consensus on the indications for liver biopsy in children and adolescents[6,7].

Non-invasive evaluation of hepatic fibrosis

There is therefore a need for accurate non-invasive tests for liver fibrosis in children and young people. Certain hepatic fibrosis scores (aspartate aminotransferase (AST)/ALT ratio, AST/ platelet ratio index (APRI), NAFLD fibrosis score (NFS) and FIB-4 index), developed for the adult population, have not been validated in paediatric studies of biopsy-proven NAFLD[21].

The enhanced liver fibrosis (ELF) test has been shown to reliably reflect liver fibrosis in paediatric NAFLD[22]. However, this test is reported to be expensive and not widely available outside the United Kingdom[23]. One of the biomarkers used in the ELF test, P3NP, has recently been shown to accurately predict liver fibrosis severity in children[23]. This test, together with promising paediatric fibrosis prediction scores (PNFI and PNFS), await external validation[22,24]. ^{13}C-liver function breath tests represent a novel non-invasive approach for monitoring NAFLD progression in adults, although their application in children with NAFLD is yet to be ascertained[25,26].

In terms of imaging techniques, ultrasound is unable to differentiate between steatosis and NASH[9]. Transient elastography and magnetic resonance elastography (MRE) are both used for the non-invasive assessment of liver fibrosis in the adult population, but their usefulness in children and adolescents with fatty liver disease remains under evaluation[27-29].

Other tests

NAFLD is increasingly considered to be an additional component of metabolic syndrome, although the diagnostic criteria for metabolic syndrome in children and adolescents remain inconsistently defined[30]. Metabolic investigations, including fasting plasma glucose, glycosylated haemoglobin A1c (HbA1c) and lipid profile (total cholesterol, high-density

lipoprotein, low-density lipoprotein, triglycerides), are therefore indicated to screen for co-morbidities associated with NAFLD such as impaired fasting glycaemia, type 2 diabetes mellitus and dyslipidaemia[6,7].

c) Risk factors and pathogenesis of NAFLD in childhood

i) Risk factors (Table 2)

Age and sex:

Males have a higher prevalence of NAFLD than females, both before and during puberty[31]. Hormonal changes accompanying puberty contribute to NAFLD pathogenesis. Increases in insulin-like growth factor 1 (IGF-1) protect against hepatic steatosis[32]. Similarly, oestrogens are thought to have an anti-inflammatory and insulin sensitising role, as well as influencing body composition, favouring non-visceral rather than visceral adipose tissue distribution[31,32]. However, the persistence of pre-pubertal sex differences in NAFLD prevalence through adolescence, highlights the influence of other factors, not just pubertal factors, on pathophysiology.

Lifestyle factors:

BMI remains an important risk factor for NAFLD in children and young people, with adipose tissue dysfunction contributing to NAFLD pathogenesis[33,34]. Unsurprisingly, greater energy intake in childhood and early adolescence is associated with an increased risk of NAFLD[8]. As well as overnutrition, the type of nutrition is important, with fructose, trans- and saturated fatty acids playing a role in disease aetiology[35]. Risk is compounded by obesity-related complications, such as obstructive sleep apnoea (OSA), where chronic intermittent hypoxia is thought to exacerbate liver oxidative stress, through reactive oxygen species (ROS) generation, thereby promoting progression of NAFLD[36]. Importantly, NAFLD does not occur in all individuals who are overweight or obese and can occur in lean individuals[37, 38].

Metabolic factors:

Insulin resistance, secondary to lifestyle, hormonal or genetic factors, is central to the development of NASH. Children with NAFLD are more likely to have biochemical evidence of insulin resistance, with higher fasting blood glucose, HbA1c, insulin and homeostatic model of assessment of insulin resistance (HOMA-IR) levels[34]. Hepatic mitochondrial dysfunction could also play a significant role in disease progression to NASH, but is yet to be confirmed in paediatric NAFLD [39,40].

Gut-liver axis:

Studies of the faecal microbiome have identified intestinal dysbiosis in children and adolescents with NAFLD[41,42]. Altered gut flora is thought to result in increased free fatty acid production and changes to small bowel permeability. This leads to greater absorption of these free fatty acids, contributing to lipotoxicity and inflammation[33,42].

Genetic factors:

Siblings and parents of children with NAFLD are more likely to have fatty livers[43]. This may represent the influence of environment, however a genetic susceptibility to NAFLD aetiology is well described[2,3,44]. Reported genetic risk factors for NAFLD include PNPLA3, TM6SF2, GCKR and MBOAT7 variants[3,45]. The PNPLA3 variant encodes the protein I148M, which is strongly associated with increased hepatic fat levels and inflammation. This predisposing variant is more commonly found in Hispanic individuals compared to other ethnic groups, explaining their increased susceptibility to NAFLD[46].

Epigenetic factors:

The maternal environment is increasingly thought to program predisposition to NAFLD in offspring through epigenetic changes[47]. A history of maternal obesity is associated with increased liver fat in adolescence, independent of adiposity[48]. Alterations to the gut microbiome of infants born to obese mothers is one of a variety of causal mechanisms

proposed[47,49,50]. Extremes of birth weight, compared to normal birth weight, are associated with more severe liver disease at biopsy, implicating maternal and uterine factors in pathogenesis[51]. Animal models also highlight the potential importance of paternally inherited epigenetic modifications in offspring susceptibility to fatty liver[52].

Environmental factors:

Animal studies suggest that environmental exposure to endocrine disrupting chemicals (EDCs) may contribute to the development and progression of NAFLD[53].

Table 2: Risk factors for paediatric NAFLD

Modifiable risk factor	**Non-modifiable risk factor**
BMI Diet Physical activity Vitamin D deficiency OSA	Age Sex Ethnicity Birth weight Genetic pre-disposition Family history of obesity or NAFLD

ii) Pathogenesis

The 'multiple hit' hypothesis describes NAFLD pathogenesis, where a number of insults are thought to act together to induce NAFLD development and progression in genetically pre-disposed or high-risk individuals (Figure 1)[32].

Figure 1: The pathogenesis of NAFLD

In obesity, pro-inflammatory cytokines released from hypertrophic adipose tissue, contribute to systemic insulin resistance[32]. Adipose tissue dysfunction also alters the balance of secreted adipokines, specifically the hormones leptin and adiponectin, which are thought to have pro- and anti-inflammatory roles respectively[33]. Uninhibited adipose tissue lipolysis, together with dietary intake and intestinal dysbiosis, leads to increased levels of free fatty acids within the circulation. This results in a hepatic influx of free fatty

acids. Hepatic de novo lipogenesis also adds to the accumulation of these free fatty acids, which undergo oxidation, storage as triglyceride or excretion via very low-density lipoprotein (VLDL)[32]. Resultant lipotoxicity occurs when hepatocellular metabolic capacity is exceeded. Mitochondrial dysfunction leads to the overproduction of ROS and endoplasmic reticulum (ER) stress results in the accumulation of unfolded proteins. The combined effect accelerates progression to NASH with hepatocyte inflammation, apoptosis and fibrosis[33].

d) The management and treatment of NAFLD in childhood.

i) Management (Table 3)

Lifestyle interventions

In children and young people, biomarkers of NAFLD have been shown to improve following weight loss secondary to lifestyle modifications[54]. However, lifestyle interventions, such as low-calorie diets or exercise programmes, that do not culminate in weight loss, also appear to be beneficial[54]. There is no compelling high-quality evidence to recommend one nutritional intervention over another. Whilst low fructose, low fat and low glycaemic index diets have shown limited results, preliminary data on the efficacy of a low free sugar diet is promising[55]. General healthy eating advice and physical activity is currently recommended to promote weight loss in paediatric NAFLD, although the amount of weight loss necessary remains unclear[54,56,57].

Management of co-morbidities and risk factors

Management of extra-hepatic co-morbidities, including insulin resistance and dyslipidaemia, and modification of risk factors known to compound paediatric NAFLD are important. Treating children with OSA has been shown to improve NAFLD severity and optimisation of Vitamin D levels may minimise fibrosis[58,59]. Given the frequent association with psychological morbidity, the provision of psychological support as part of multi-disciplinary management may contribute to improved clinical outcomes[60].

ii) Treatment

The efficacy of several medications, antioxidants, fatty acid supplements and probiotics have been investigated in children. Whilst pioglitazone has been recommended for advanced liver fibrosis in adults, there is currently no pharmacological treatment of proven benefit for paediatric NAFLD.

Metformin

Metformin improves insulin resistance and may reduce steatosis on ultrasound[54]. The TONIC trial compared placebo, metformin and Vitamin E, an antioxidant, in children with histologically confirmed NAFLD, using sustained reduction in ALT as their primary outcome[61]. Both metformin and Vitamin E were no better than placebo with regards the primary outcome, but these groups did show evidence of reduced hepatocyte injury on biopsy[61]. In addition, the role of metformin as a weight loss adjunct in these children and young people remains under evaluation, with only a very modest, short-term reduction in BMI seen on meta-analysis[62].

Anti-oxidants

Adjuvant Vitamin E therapy has been shown to be efficacious in adults with NAFLD, however a similar significant effect has not been demonstrated on meta-analysis in paediatric patients[63]. Vitamin E therefore remains unlicensed for this indication in the United Kingdom, although may be considered for children with advanced liver fibrosis in tertiary care settings[64].

Vitamin E in combination with another antioxidant, hydroxytyrosol (HXT), may have potential, improving oxidative stress parameters, insulin resistance and steatosis in children with NAFLD[65]. Cysteamine bitartrate, may also have therapeutic benefit[66].

Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs), including omega-3 fatty acids, are thought to have a beneficial effect on hepatic lipid accumulation[67,68]. However, a systematic review of trials involving PUFAs in paediatric NAFLD caveated benefit with variation in formulation and dosing between trials[54]. Further research is warranted.

Probiotics

Probiotics, such as bifidobacteria, are under investigation appearing to have a protective role in the development of NAFLD[54,69].

Surgical intervention

The number of children and young people undergoing bariatric surgery is increasing worldwide[70]. Although bariatric surgery can improve histological findings in paediatric NASH, surgical intervention remains controversial in all but exceptional clinical situations[71].

Current clinical trials

Losartan is currently being compared to placebo in 8 to 17 year olds with histological evidence of NAFLD. Losartan is thought to reduce plasminogen activator inhibitor-1 (PAI-1), which is elevated in children with NAFLD and associated with increased disease severity[72]. Another clinical trial is evaluating two doses of elafibranor (80 and 120 milligrams) in 8 to 17 year olds with histological evidence of NASH. Elafibranor improves insulin sensitivity and has been shown to induce resolution of NASH in adult patients[73].

Clinical trials involving supplementation with turmeric preparations, amino acid formulations and non-caloric sweetener and dietary interventions involving sugar reduction, a 5:2 diet and a low-carb-high-fat diet in paediatric NAFLD are also underway.

Table 3: The management of paediatric NAFLD

Non-pharmacological management	Pharmacological management
Dietary advice Increased physical activity Psychological support Respiratory support for OSA	Vitamin D Metformin Lipid lowering agents Blood pressure lowering agents

CONCLUSION

The true prevalence of paediatric NAFLD in obesity will remain unknown, until the field adopts a unified pragmatic approach to defining disease, to minimise study heterogeneity. With international disease registries such as the European Paediatric Non-Alcoholic Fatty Liver Disease Register (EU-PNAFLD), longitudinal data on disease progression will allow optimisation of screening practice and accurate estimation of the health and socioeconomic burden of NAFLD starting in childhood. The development of readily available non-invasive methods for evaluating hepatic fibrosis, will aid timely investigation and inform counselling discussions relating to hepatic and extra-hepatic risk. Growing knowledge of the complex pathophysiology of NAFLD continues to reveal potential therapeutic targets. However, whilst the hunt for therapeutic agents continues, healthy eating and physical activity, remain the only prevention and treatment strategies for paediatric NAFLD.

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