The Liver in Children With Metabolic Syndrome

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Non-alcoholic fatty liver disease (NAFLD) is recognized as an emerging health risk in obese children and adolescents. NAFLD represents a wide spectrum of liver conditions, ranging from asymptomatic steatosis to steatohepatitis. The growing prevalence of fatty liver disease in children is associated with an increased risk of metabolic and cardiovascular complications. NAFLD is considered the hepatic manifestation of Metabolic Syndrome (MetS) and several lines of evidence have reported that children with NAFLD present one or more features of MetS. The pathogenetic mechanisms explaining the interrelationships between fatty liver disease and MetS are not clearly understood. Altough central obesity and insulin resistance seem to represent the core of the pathophysiology in both diseases, genetic susceptibility and enviromental triggers are emerging as crucial components promoting the development of NAFLD and MetS in children. In the present review we have identified and summarized studies discussing current pathogenetic data of the association between NAFLD and MetS in children.

Keywords: non-alcoholic fatty liver disease, metabolic syndrome, insulin resistance, childhood obesity, children

INTRODUCTION

In parallel with increasing prevalence of childhood obesity, non-alcoholic fatty liver disease (NAFLD) is becoming one of the most common health problems in obese children and adolescents (1). Hepatic steatosis is a clinical condition characterized by fat infiltration in more than 5% of hepatocytes in liver biopsy which is not related to excessive alcohol intake, autoimmune disease, viral infections or the use of steatogenic drugs (2). NAFLD encompasses a spectrum of histological hepatic alterations, ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, end-stage liver disease, and hepatocellular carcinoma (3).

Available data are strongly suggestive that morbidity of NAFLD extends beyond the liver and is associated with the major components of metabolic syndrome (MetS) already in pediatric age (4, 5). First described in 1988 (6), MetS has been defined as a constellation of metabolic abnormalities and cardiovascular risk factors. Although MetS has received several definitions in pediatric population, there is agreement with respect to consider central obesity, impaired glucose tolerance, dyslipidemia, and hypertension as essential features (7) and, more importantly, NAFLD as the hepatic manifestation of this dysmetabolic state (8).

The molecular mechanisms linking NAFLD and MetS are controversial and the association of NAFLD with the main diagnostic criteria of MetS opens the debate as to whether hepatic steatosis is a consequence or a cause of this disease (9).

In the present review, we provide an overview of the most recent epidemiological and pathophysiological data of NAFLD and of the most common pathogenetic mechanisms linking NAFLD and MetS in childhood.
RESEARCH STRATEGY

This systematic review is based on articles published over the past 10 years. A PubMed/MEDLINE search of literature was performed using the following key words: "Non-alcoholic fatty liver disease," "Metabolic syndrome," "Insulin resistance," "Central Obesity," "Ectopic fat deposition," "Dyslipidemia," "Genetic predisposition to metabolic diseases," "Pathogenesis of NAFLD and MetS," and "children." During the research process bibliographic updates were performed.

EPIDEMIOLOGY

In the last 20 years the prevalence of NAFLD has more than doubled in childhood. This escalation is partly due to the strong contribution of the growing rate of obesity in children, making NAFLD the most common cause of hepatic disease.

In the United States, 7 million children and adolescents are affected by hepatic steatosis (10) with a prevalence of 3–10% in the American population (11). Different prevalence data have been reported in Europe, ranging from 2.5% in UK (12) to 12.5% in Italy (13).

It is known that the presence of NAFLD increases with increasing body mass index (BMI) category. In an autopic study conducted in California, the prevalence of histologically defined NAFLD was 38% in obese children (10). Accordingly, in a large community-based autopic study, the prevalence of fatty liver disease was 26% in pediatric obese subjects (14). Furthermore, in a recent systematic review, Anderson et al. (15) have reported a prevalence rate of pediatric NAFLD ranging from 8% in non-obese children to 34% in obese children.

Different epidemiological studies (15, 16) reported that NAFLD appears twice as often in boys than in girls. In particular, Sartorio et al. (17) observed an higher prevalence of NAFLD in males than in females. Similar results have also been obtained in Asian and American obese children where hepatic steatosis was documented more frequently in boys than in girls (18–22). In the study by Gupta et al. (23), 22% of males were affected by NAFLD compared to 9.8% of girls. In accordance with the latest studies, Yu et al. (14) have recently observed an estimated NAFLD prevalence of 29.4% in males and 22.6% in females. Of note, in accordance with previous study (24), El-Karaksy et al. (25) and Di Bonito et al. (26) observed higher prevalence of abnormal serum alanineaminotransferase (ALT) levels in girls (55%) than in boys (36%). Also in chinese obese children, it was identified NAFLD more often in female than in male (27). The significant variance documented across the reported studies is partly explicated by the technique used to diagnosed NAFLD and probably by the influence of the pubertal development in which sex hormones could influence the onset of NAFLD (15, 28).

It is important to highlight that the prevalence of fatty liver disease varies widely also depending region and ethnic descent. NAFLD is more frequent in Asian population than in Europe, Middle East/North Africa, and North America (15). South-American data have reported a prevalence of NAFLD of 2.3% in obese Brazilian children (29) and of 16% in Chilean pediatric population with obesity (30). Furthermore, NAFLD has been observed in 62.5% of obese Indian adolescents (31) and in 45% of obese Chinese children and adolescents (32).

Based on ethnicity, several studies observed that African Americans are less and Hispanics are more predisposed to develop fatty liver disease than Caucasians (33, 34). In accordance with evidence in adults (35, 36), in the prospective cohort study performed by Trícó et al. (37), 59.6% of Hispanic, 42.9% of white, and 15.7% of black obese adolescents were affected by NAFLD.

Of note, as suggested by Lomonaco et al. (38) in many epidemiological reports the ethnic groups were not matched for the major unfavorable metabolic factors, usually observed in Hispanics patients. By recruiting 152 Hispanics and Caucasian patients with biopsy-proven NASH matching for age, sex, and total body fat, the authors (38) observed a higher but not significant trend of liver fat content in Hispanic than Caucasian patients, without differences in the severity of NASH.

Interestingly, when Hispanic and Caucasian subjects are matched for obesity, NASH ethnic differences between the two groups disappear, underlining how it’s important to take into account the metabolic profile of patients.

Another potential factors explaining the wide range of steatosis prevalence is the test utilized for the diagnosis, based on liver histology, imaging studies, and evaluation of liver enzymes.

In the autopsy study conducted by Schwimmer et al. (10) the prevalence of pediatric NAFLD ranged from 0.7% in 2- to 4-year-old to 17.3% in 15- to 19-year-old subjects, with an increase to 38% in obese children.
By using imaging methods, Franzese et al. (39) have conducted the first study on the incidence of fatty liver disease as assessed by ultrasound. Liver steatosis was detected in 52% of obese Italian children. In accordance with this previous study, in recent evidence NAFLD was detected in 41% of obese pediatric population with liver ultrasonography (40) and 41.6% (37) with liver magnetic resonance imaging (MRI).

Different results have been reported measuring ALT levels, with a NAFLD prevalence ranging from 8 to 42% (41).

Although liver biopsy remains the gold standard method to diagnose NAFLD, it’s invasive in children and not feasible in population study (2).

The currently recommended first-line screening test for fatty liver disease in children is ALT (42). ALT is an inexpensive, minimally invasive and universally available blood test with an acceptable sensitivity (41). Several studies in children have evaluated the upper limits of normal ALT (43–45). In the US, the ALT cutoffs are 22 mg/dl for girls and 26 mg/dl for boys (41). In a Canadian study the upper normal limit of normal ALT is 30 mg/dl in children between 1 and 12 years of age and 24 mg/dl in those between 13 and 19 years (44). For the diagnosis of NAFLD in obese pediatric population with >10 years of age, the threshold for ALT is ≥50 for boys and ≥44 for girls, while NASH has been observed in children with ALT ≥80 (45).

Recently, Bussler et al. (46) have provided new age- and sex-related percentiles for ALT in pediatric population. During early adolescence, the median of ALT serum concentration varies between 14.0 and 20.3 U/L in girls and between 17.1 and 21.1 U/L in boys. The 97th percentile spans from 24.2 to 31.7 U/L in girls and from 29.9 to 38.0 U/L in boys and children with ALT values >97th percentile present high risk of NAFLD.

Interestingly, the authors documented a significant positive correlation between BMI-SD score (BMI-SDS) and ALT serum concentrations. A rise of the BMI-SDS of around +1 results in an ALT serum level increase of around 2.21 U/L in boys and around 1.11 U/L in girls.

**PATHOGENESIS OF NAFLD**

Despite the advances in understanding the pathogenesis of NAFLD, the exact mechanisms underlying the development of fatty liver disease are still unclear.

The first and the most accepted hypothesis to describe the pathophysiology of NAFLD is the "two hits" theory (2) according to which liver damage is developed through two main components. In the "first hit" obesity, high-fat diet and insulin resistance (IR) seem to be responsible for the deposition of triglycerides (TG) in the hepatocytes, a pre-requisite for hepatocyte injury (2, 47). In the "second hit" oxidative stress, inflammatory cytokines, adipokines, mitochondrial dysfunction, and endoplasmic reticulum stress trigger the progression to NASH (2, 47).

During the last few years, this traditional model has been modified by the "multiple parallel hits" hypothesis (48), according to which NAFLD is defined as an epiphenomenon of several metabolic mechanisms involving genetic and environmental factors as well as an inter-organ crosstalk between liver, adipose tissue, pancreas, and gut (47).

The pathogenesis of NAFLD seems to start in the intrauterine life, during which maternal BMI, MetS, gestational diabetes and low birth weight during pregnancy have been identified as prenatal risk factors (49, 50).

Furthermore, a genetic background characterized by genes involved in the main metabolic processes has been shown to predispose to NAFLD development (48, 51).

After birth, obesity and IR still represent the main factors involved in the first accumulation of fat in the liver.

It is well-established that higher BMI values are associated with the development of fatty liver disease in children and that most obese children are not adherent to lifestyle modifications and hypocaloric diets (52).

As shown by Mager et al. (53) children with hepatic steatosis spent more than 65% of their leisure time in sedentary. Nobili et al. (54) have reported that a 2-year lifestyle intervention, including monthly dietary counseling and physical activity, is correlated with weight loss, reduced dyslipidemia and ALT levels and improvement in liver histology in children with biopsy proven NAFLD. In accordance with the latest study (54), Pozzato et al. (55) have evaluated the effect of a 1-year lifestyle intervention, including regular physical activity (30–45 min-day⁻¹, aerobic exercise) with a normocaloric balanced diet. The authors observed that the prevalence of liver steatosis decreased significantly from 34.6 to 7.7% as well as the obesity degree, the triglycerides, and total cholesterol levels (55). Of note, physically active lifestyle improves insulin sensitivity through decreasing fasting insulin and increasing lean mass (56).

Thus, the adoption of a physically active lifestyle and gradual weight loss should be considered in the prevention and treatment of the development of NAFLD in obese children.

As recently illustrated by several authors (47–49), not only the degree of obesity but also fat distribution and the lipodystrophic state in obese patients are associated with an increased efflux of free fatty acids (FFA) to the liver that contribute to the development of IR (47). The “insulin resistance state” is a general concept that should be refined to systemic IR, characterized by altered blood glucose concentrations, adipose tissue IR, characterized by the inability of insulin to suppress lipolysis, and hepatic IR, in which there is an impairment of hepatic glucose production (48). Furthermore, in a state of IR there is an over-expression of the sterol regulatory element binding protein-1c (SREBP-1c) with a consequent upregulation of de novo lipogenesis, an impairment of FFA β-oxidation and of very low-density lipoprotein (VLDL) secretion, with a further increase of hepatic lipid accumulation (2, 47). The FFA overload in the hepatocytes together with the release of adipokines and proinflammatory cytokines coming from the adipose tissue cause lipotoxicity, with a consequent mitochondrial dysfunction, increase of cytotoxic reactive oxygen species (ROS) production and endoplasmic reticulum stress (2, 57).

The important role of oxidative stress in the progression of NAFLD has been clearly demonstrated by several studies.
which determine the subsequent synthesis of tumor necrosis factor-α (TNF-α), reactive carbonyl species, catalase, and anti-oxidants compounds such as glutathione peroxidase (61). Pirgon et al. (62) performed a cross-sectional study in pediatric population and observed that obese adolescents with NAFLD reported increased oxidative stress indeces compared to adolescents without NAFLD. The pathogenetic mechanism linking NAFLD and oxidative stress is characterized by the storage of lipids in hepatocytes that induces β-oxidation of fatty acids, which promotes ROS production leading to the oxidative damage of mitochondrial membrane (63). This further worsens oxidative damage conducing to hepatocellular death and NASH progression (64). The oxidative stress caused by fatty acid overload in hepatocytes result from mitochondria, peroxisomes and microsomes. IR significantly increases peroxisomal oxidation because insulin is the pivotal inhibitor of cytochrome P450 4A (CYP4A), a key enzyme in this pathway (65). This inhibition amplifies cytotoxic ROS and lipid peroxidation. These products can diffuse into the extracellular space, influence Kupffer cells and hepatic stellate cells (HSCs) and active the nuclear factor-κB (NF-κB) pathway, which determine the subsequent synthesis of tumor necrosis factor-α (TNF-α) and several other pro-inflammatory and fibrogenic cytokines (66). Among this inter-organ crosstalk, human gut microbiota has been recently identified as a crucial player in the pathogenesis of NAFLD (67–69). Intestinal dysbiosis, bacterial overgrowth, and impairment of mucosal permeability lead to an increased bacterial flow into the liver thus exposing the hepatic tissue to pathogen associated molecular patterns (PAMP) (48, 67). By linking specific pattern recognition receptors (PRRs) on hepatic immune cells, PAMP activate a pro-inflammatory cascade with a consequent increase of hepatic inflammation (67). Lipopolysaccharide (LPS), highly immunogenic component of gram-negative cell walls, is the most studied PAMP (2). Raised LPS and LPS-binding protein levels have been observed in children with NASH (70, 71).

Moreover, bacterial fermentation of food substrate may contribute to the severity of NAFLD by increasing endogenous alcohol production and short chain fatty acids (SCFA) and decreasing choline metabolism (67, 72, 73). In accordance with previous study (74), Pierri et al. (75) observed that children with NAFLD showed more fermenting bacteria, particularly Escherichia species, and enhanced serum alcohol levels. Thus, although several potential pathogenetic mechanisms of NAFLD are emerging, it must be acknowledged that one factor is insufficient to drive liver disease progression, confirming its multifactorial nature.

COMMON PATHOGENETIC MECHANISMS LINKING NAFLD AND METS

Despite the correlation between hepatic steatosis and MetS is often reported, this relationship is complex and not completely understood, due to the multifacted pathogenesis of both diseases (7, 76).

Several studies in children support that NAFLD is associated with several components of MetS, such as IR, central obesity and dyslipidemia (7). Furthermore, emerging data underline a pivotal role of genetic predisposition (50, 77) and intrauterine events (50, 77) in the pathogenesis of the metabolic derangements in the pediatric age group.

The common pathogenetic linking NAFLD and Mets are summarized in the Figure 1.

Insulin Resistance (IR)

IR represents the critical connector linking NAFLD and MetS in obese children and adolescents (76).

As shown in the Table 1, this relationship has been observed in several studies (17–19, 21–27).

The strict connection between fatty liver disease and IR has been clearly documented by Caprio et al. (78). To evaluate the independent contribution of the liver in the alteration of insulin sensitivity, the authors have studied obese adolescents with similar overall degree of obesity and intramyocellular lipid content (IMCL) and different hepatic fat fraction. To note, hepatic steatosis was related to impaired insulin sensitivity and secretion (48), leading to the development of metabolic alterations. By recruiting 254 children and adolescents aged 6–17 years, Patton et al. (79) have demonstrated that the severity of IR was significantly correlated with histological features of NAFLD and the risk of MetS was greater among those with severe steatosis. In accordance with previous studies (40, 80), Prokopowicz et al. (81) observed an impaired metabolic profile, characterized by greater waist circumference, IR, glucose dysregulation, and dyslipidemia in 45% of overweight adolescents with hepatic steatosis than children without. Moreover, 40.8% of children with NAFLD presented MetS. These results have been further confirmed in the prospective study conducted by Tricó et al. (37) in a multiethnic cohort of obese adolescents. Particularly, authors underlined the association between high hepatic fat fraction, impaired insulin sensitivity and the development of metabolic alterations in children. The association between IR, NAFLD, and MetS has been also confirmed by using ALT levels as marker of hepatic steatosis. In a recent cross-sectional study of 77 obese non-diabetic children, Hampe et al. (82) have demonstrated that the frequency of high ALT levels increased parallelising to the increased number of MetS diagnostic criteria.

To note, the influence of IR on the metabolic parameters persists over time. By following for an average of 1.9 years 76 obese youths, Kim et al. (83) observed a persistence of IR state in children with increased hepatic fat fraction and an improvement of insulin sensitivity index and glucose metabolism in children without fatty liver.

Thus, independently of the diagnostic tool and the age of the patients, IR represents a key factor linking the development of MetS features in children with liver steatosis.
Ectopic Fat Accumulation

During the last few years, several studies have shown that altered partitioning of fat plays a pivotal role in the development of IR, NAFLD, and MetS in obese children and adolescents (84).

As clearly demonstrated by Caprio et al. (85), increased visceral adipose tissue (VAT) and a thin superficial layer of abdominal subcutaneous fat (SAT) are associated with the propensity to store fat in the liver and muscle leading to IR and the MetS (86). By stratifying a multiethnic cohort of obese adolescents into tertiles based on the proportion of abdominal fat, the authors have demonstrated that the increased proportion of VAT and the decreased percentage of SAT were associated with muscle and hepatic steatosis, IR, and a higher risk of MetS in obese adolescents with this phenotype than those without (87).

The association between higher VAT proportion, ectopic liver fat accumulation, and metabolic alterations seem to persist over time. In a longitudinal evaluation of 151 obese adolescents, Umano et al. (88) have recently demonstrated that baseline VAT/(VAT + SAT) ratio represents a predictor of changes in intrahepatic fat content, degree of IR, and impaired glucose metabolisms at follow-up among girls. Moreover, the prevalence of MetS at follow-up was higher in obese girls with high proportion of VAT to abdominal SAT than those with a low ratio.

Based on this “lipodistrofy model,” the poor expandability of SAT might lead to an excess accumulation of lipids in the VAT, which represents a major source of proinflammatory mediators and FFA, the two major players in the development of fatty liver and IR (88).

To understand the mechanisms responsible for the inefficient storage of fat in the abdominal SAT, Kursaw et al. (89) have evaluated the fat cell size in a group of obese adolescents with similar degree of obesity and different VAT/SAT ratio. Obese subjects with high VAT/(VAT/SAT) ratio were characterized by a low fraction of large adipocytes showing an increased diameter and a greater proportion of the smaller adipose cells. In contrast, a more homogenous cellularity has been observed in the obese adolescents with a low VAT/SAT ratio. Thus, the hypertrophy of the largest cells and the reduced capacity of the smaller adipocytes to store fat might explain the high plasma levels of FFA and the associated fatty liver disease in obese adolescents.

Moreover, to evaluate the relationship between altered fat partitioning and the development of impaired glucose metabolism and IR, the authors have investigated the expression of lipogenic genes in abdominal subcutaneous adipose and liver tissue of 53 adolescents with similar degree of obesity across the spectrum of glucose tolerance (90). The expression of carbohydrate-responsive element binding protein (ChREBP) was decreased in the SAT of adolescents with impaired glucose tolerance and increased in the liver, explaining the association between upregulation of lipogenesis and NAFLD (90).

Thus, independent of the overall body fat mass, an impaired abdominal fat distribution represents a key factor in the pathogenesis of IR, fatty liver disease and MetS in the pediatric age.
Dyslipidemia

There is a growing body of evidence suggesting an association between NAFLD and atherogenic lipid profile (91). Studies in adults (92, 93) have shown that increasing grades of NAFLD are significantly associated with increasing levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL) and VLDL levels and with decreasing concentration of high-density lipoprotein cholesterol (HDL) (93).

Recently Zheng et al. (94) performed a cross-sectional study confirming that NAFLD is positively correlated with atherogenic lipid profile and patients enrolled with NAFLD shown carotid intima-media thickness (CIMT) and elevated brachial-ankle pulse wave velocity (ba-PWV) for stiffening.

Over the last decade, an increased incidence of alterations in lipid profile has also been documented in obese youth (95). In American population study, the prevalence of high total cholesterol among children and adolescents has been estimated to be 7.4%, with a higher rate among subjects aged 16–19 (8.9%) than in children aged 6–8 (6.0%) (96). In Italy, it was reported that 15% of children present a lipid profile classifiable as atherogenic dyslipidemia (97).

Dyslipidemia is a crucial diagnostic criterion of MetS but there is increasing evidence proving a close association between impaired lipid profile and NAFLD in children and adolescents.

In 2010, for the first time, Nobili et al. (98) have shown that in children with NAFLD the severity of liver injury was strongly associated with the presence of a more atherogenic

Table 1: Studies evaluating the association between insulin resistance, non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MetS) in children.

| References | Study design | Sample size and Gender-NAFLD prevalence | Ethnic group | IR indexes | Findings |
|------------|--------------|----------------------------------------|--------------|------------|----------|
| Sartorio et al. (17) | Cross sectional study | 268 obese children (58% M/42% F) | Caucasian | OGTT, HOMA-IR | NAFLD is associated with impaired glucose profile and MetS criteria (systolic blood pressure, lipid profile, BMI). |
| Yoo et al. (18) | Cross-sectional study | 909 obese children (78% M/22% F) | Asian | HOMA-IR, Fasting insulin | Significant association between NAFLD and components of MetS (IR, dyslipidemia, hypertension) |
| Love-Osborne et al. (24) | Cross-sectional study | 85 obese children (42% M/58% F) | Hispanic Black White American-Indian Asian | OGTT, HOMA-IR, Fasting insulin | Impaired glucose regulation and NAFLD in subjects meeting 3 or more criteria of MetS. Fasting insulin and TG were significantly higher in subjects with steatosis |
| Di Bonito et al. (26) | Cross-sectional study | 584 obese and lean children (67% M/33% F) | Caucasian | HOMA-IR, Fasting insulin Fasting glucose | A positive correlation between ALT levels, IR, and dyslipidemia |
| Shi et al. (27) | Cross-sectional study | 308 obese children (48% M/52% F) | Asian | HOMA-IR, WBISI | High prevalence of NAFLD in children with MetS. NAFLD and MetS shared the common mechanism of IR. |
| Fu et al. (19) | Cross-sectional study | 861 obese children (70% M/30% F) | Asian | OGTT, HOMA-IR, Fasting insulin Fasting glucose HbA1c WBISI | Association between NAFLD, hypertension, dyslipidemia, impaired fasting glucose, and MetS. The state of insulin resistance deteriorated as the degree of fatty infiltration increased. |
| Boyraz et al. (22) | Cross-sectional study | 451 obese children (65% M/35% F) | Caucasian | OGTT, Fasting glucose Hyperinsulinemia | Association between NAFLD, MetS criteria, and IR. |
| Mager et al. (20) | Cross-sectional study | 46 obese and lean children (94% M/6% F) | Canadian | HOMA-IR | Metabolic dysregulation (insulin resistance, obesity, elevated TG, and lower HDL cholesterol) in children with NAFLD. |
| El-Karaky et al. (25) | Cross-sectional study | 76 children overweight/obese (31% M/69% F) | Egyptians | QUICKI, HOMA-IR | 36.8% of children with NAFLD presented MetS. IR was higher in children with NAFLD than controls. |
| Lee et al. (21) | Cross-sectional study | 12 obese children (58% M/42% F) | Black White | 3-h hyperinsulinemic-euglycemic clamp | Association between NAFLD, IR, and adverse cardiometabolic profile. |

NAFLD, non-alcoholic fatty liver disease; MetS, metabolic syndrome; BMI, body mass index; ALT, alanine aminotransferase; IR, insulin resistance; OGTT, oral glucose tolerance test; HOMA-IR, homeostatic model assessment of insulin resistance; WBISI, whole body insulin sensitivity; QUICKI, quantitative insulin-sensitivity check index; HbA1c, hemoglobin A1c; TG, triglycerides; HDL, high-density lipoprotein; M, males; F, females.
lipid profile measured as TG/HDL-C, total cholesterol/HDL-C, and LDL/HDL-C ratios. TG/HDL-C ratio is a powerful marker of atherogenesis induced by IR and is associated with a more atherogenic lipid profile and early cardiovascular events (99, 100).

In 2015, Corey et al. (101) obtained similar results observing that dyslipidemia, characterized by elevated non-HDL-C levels and increased TG/HDL-C, is frequent in children and adolescents with NAFLD. Furthermore, the authors observed that both resolution and histologic improvement of NASH were associated with the improvement of non-HDL-C, LDL, and total cholesterol levels.

By evaluating a cohort of children and adolescents with NAFLD, Pacifco et al. (102) have demonstrated that subjects with a high triglycerides/HDL-C ratio have an increased risk of IR and an association between this ratio and NAFLD.

In accordance with previous studies (103), Balanescu et al. (104) have recently documented that NAFLD patients presented a very high TG/HDL-C ratio and a tendency to hyperuricemia and lower HDL levels compared with non-NAFLD patients.

Thus, TG/HDL-C ratio might represent a useful cardiovascular risk marker in pediatric patients with NAFLD.

Recently, Dowla et al. (105) by evaluating 309 children with NAFLD have observed elevated levels of TG, non-HDL-C and LDL cholesterol and low serum concentrations of HDL-C in children with NAFLD compared to healthy youths. Moreover, the authors documented a correlation between systolic blood pressure (SBP), insulin, glucose, hemoglobin A1c (HbA1c), liver enzyme [aspartate transaminase (AST)] and serum TG and non-HDL-C.

In accordance with other studies (106), Lee et al. (107) enrolled 548 obese pediatric subjects with NAFLD to investigate their anthropometric parameters and correlate risk factors presenting with hepatic steatosis. The authors observed that mean TG levels were in proportion to NAFLD severity.

Thus, lipid profile is an emerging risk factor in the development of cardiovascular diseases and in the pathophysiology of pediatric NAFLD and metabolic disorders.

**New Potential Pathogenetic Triggers of NAFLD**

**Vitamin D**

In addition to the discussed pathogenetic mechanisms, Vitamin D levels have been recently identified as a risk factor for metabolic alterations in children. Poor Vitamin D status has been previously correlated with childhood obesity and insulin IR (108, 109). In a cross-sectional study Turer et al. (110) observed a significantly greater prevalence of Vitamin D deficiency in overweight, obese, and severely obese US children than controls. Furthermore, Olson et al. (111) observed a significant inverse relationship between 25(OH)D levels and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) in obese children.

A growing body of evidence has recently demonstrated an association between Vitamin D levels and pediatric NAFLD (112). Vitamin D deficiency has been correlated with a high risk of steatosis, necroinflammation, and fibrosis in adults (113, 114) as well as in children with biopsy-proven NAFLD (115). Nobili et al. (116) observed an inverse association between serum level of Vitamin D and histological liver damage in children with NAFLD, which also presented elevated BMI, dyslipidemia, impaired glucose metabolism, and hypertension. In accordance with the latest study (116), Black et al. (117) have documented a significant correlation between 25(OH) Vitamin D concentrations and hepatic fibrosis in a cohort of Australian children with hepatic steatosis. Moreover, children with NAFLD have shown more features of MetS, such as IR, impaired lipid profile, and elevated waist circumference than healthy controls.

The exact mechanisms by which Vitamin D influences the development of NAFLD is still unclear. However, current data suggest that Vitamin D modulates fibrotic mechanisms in the liver by inhibiting the expression of transforming growth factor beta (TGF-β). Therefore, Vitamin D suppresses the deposition of collagen Iα1 and activates the alpha-smooth muscle actine (α-SMA) in HSCs influencing the development of hepatic steatosis (118). Studies in rats have also documented that Vitamin D deficiency upregulates hepatic inflammatory genes (119, 120) with a consequent NAFLD exacerbation.

Moreover, many studies (121, 122) highlighted the role of gene variants in the modulation of Vitamin D concentrations. Gibson et al. (123) performed the first study showing a relationship between genetic variations of Vitamin D receptor (rs2228570 polymorphism) and the severity of liver histology in UK children with NAFLD.

To note, several authors have also proposed a close link between Vitamin D deficiency and MetS in children and adolescents (124). In 2011 Ganji et al. (125) have documented that Vitamin D levels were inversely related to the prevalence of MetS phenotype, waist circumference and systolic blood pressure in children. Moreover, the authors observed a direct association between 25(OH)D and HDL cholesterol levels. Accordingly, Lee et al. (126), reported a cross-sectional association between Vitamin D levels and MetS among 1,660 Korean children. In a Caucasian pediatric cohort, Pacifco et al. (127) concluded that central obesity, hypertension, hypertriglyceridemia, low HDL cholesterol levels, and MetS were correlated with low 25(OH)D levels.

Based on these findings, several studies (128, 129) have evaluated the possible therapeutic role of diet interventions in children with liver steatosis. In a randomized controlled trial (RCT), Della Corte et al. (130) have observed that administration of docosahexaenoic acid (DHA) and Vitamin D reduced steatosis, ballooning and lobular inflammation and improve IR in a cohort of obese Caucasian children.

Thus, Vitamin D levels should be investigated in obese children with metabolic complications.

**Obstructive Sleep Apnea (OSA)**

Recently, increasing studies suggested that nocturnal hypoxia (NH) associated with obstructive sleep apnea (OSA) may promote the progression of pediatric NAFLD (131). In 2016, Sundaram et al. (132) observed a strict correlation between OSA/hypoxia in obese adolescents and advanced NAFLD histology and an increased systemic and hepatic oxidative stress
with a reduction of circulating antioxidant agents. The study highlighted how low oxygen levels could be an important trigger of oxidative stress that promotes progression to NASH.

Interestingly, Paschetta et al. (133) reported that, during hypoxia, many transcription factors, including hypoxia-inducible factors (HIFs), modulate target genes expression that are involved in hepatic disease. In particular, the chronic intermittent cycles of hypoxia and reoxygennation (CHI) increase HIFs levels, which regulates lipogenesis and triglycerides storage with increased fatty acid synthesis in hepatocytes (134). To note, Nobili et al. (135) revealed that the presence and severity of OSA are associated with significant fibrosis and with NAFLD activity score in children, independently of body mass index, abdominal adiposity, metabolic syndrome, and IR. This relationship held also in non-obese subjects with NAFLD. Thus, in pediatric NAFLD, OSA could modulate biochemical, immunohistochemical, and histological features of NAFLD, NASH, and fibrosis.

**Uric Acid and Fructose**

Actually, there is a growing body of evidence suggesting that high serum uric acid (SUA) level is an independent risk factor of NAFLD (136, 137). The pathological mechanism linking NAFLD and hyperuricemia is not completely understood (138–140). Experimental data suggested that SUA is able to determine systemic inflammation, endothelial dysfunction and IR (141, 142) and to induce ROS (143) and activation of the NOD-like receptor family and pyrin domain containing 3 (NLRP3) inflammasome (144).

In line with recent cross-sectional studies in adults (145, 146), Vos et al. (147) examined the relationship between SUA levels and NAFLD in the pediatric population, reporting that uric acid was significantly increased in those children with histological diagnosis of NASH compared with milder forms of NAFLD. In accordance with the latest study, Nobili et al. (148) have recently observed that hyperuricemia, coupled with elevated dietary fructose consumption, could be responsible for the different pattern of lobular disease observed in pediatric NAFLD, determining greater damage in the perportal zone rather than in perivenous zone (148). Similarly, in a pilot study by Sullivan et al. (149) children with NAFLD after fructose ingestion reported an exacerbated metabolic profile characterized by elevated serum glucose, insulin, and uric acid associated with an higher urinary levels of uric acid and a lower fructose excretion than lean subjects. The authors hypothesized that children with NAFLD may be both absorbing and metabolizing fructose more than lean subjects, which could contribute to the pathophysiology of hepatic steatosis (149). On the contrary, the restriction of fructose for 9 days determined a decrease of liver fat and de novo lipogenesis (150).

Of note, these studies suggest a correlation between high fructose consumption, SUA, and NAFLD (151). In 2017, Mosca et al. (152) reported that dietary fructose consumption was positively and independently associated with hyperuricemia and that SUA concentrations and fructose intake are independently and positively associated with NASH in a cohort of children and adolescents with a histological diagnosis of NAFLD.

Thus, many data have identified the role of hyperuricemia in NAFLD, suggesting that SUA may be a new potential pathogenetic trigger for NAFLD patients.

**Endocrine Disruptors**

Endocrine disruptors (EDs) are “exogenous substances or mixtures that modify the function of the endocrine system, determining adverse effects on the health of an organism, or its progeny, or (sub)population” (153, 154).

During the last decade, several studies (155, 156) have demonstrated the relationship between EDs and the progression of metabolic and cardiovascular diseases, including obesity, IR, type 2 diabetes, hepatic injury, and dyslipidemia (157). In these alterations, EDs interact with the peroxisome proliferator-activated receptors (PPARs), with sex steroid and with corticosteroid receptors, influencing the cortisol sex steroid metabolism or induce epigenetic alterations promoting adipogenesis and lipid accumulation (158).

Over the past four decades, several EDs have been mass produced due to their widespread use. Among them, bisphenol A (BPA) is considered an EDs that largely promote metabolic and cardiovascular dysfunction (159). It is a weakly estrogenic chemical that is largely employed in manufacturing polycarbonate plastics, in food packaging and in toys (160). Animal studies reported that the perinatal exposure to BPA predisposed to increased body weight, elevated serum insulin, and impaired glucose tolerance in adult’s offspring. Moreover, high fat-fed offspring exposed to BPA presented obesity, hyperglycemia, hyperleptinemia, hyperinsulinemia, and dyslipidemia (161). Menale et al. (162) observed a direct relationship between BPA and insulin resistance in a cohort of obese children suggesting that BPA exerts a suppression of adiponectin gene expression, leading to a reduction of adiponectin release and an increased of resistin expression.

Recently, increasing data have provided evidence that BPA exposure enhances susceptibility to NAFLD (157). As reported by Wei et al. (163), in an animal study an high-fat diet and BPA-exposition, are associated with the severity of NAFLD, increased liver triglycerides, liver free fatty acids, and serum ALT levels.

Khalil et al. (164) performed a cross sectional study in obese and overweight children and demonstrated an association between urinary BPA (creatinine), elevated liver enzyme and diastolic blood pressure even after adjusting for age and ethnicity. Interestingly, also in male children, serum fasting insulin (FI) and HOMA-IR showed a non-linear association with increasing urinary BPA concentration (164). Recently, Verstraete et al. (165) performed the first cross-sectional study in children that evaluated the association between BPA and suspected NAFLD, defined as elevated ALT levels, overweight or obesity and evidence of IR. The authors observed that the risk of NAFLD was increased in the higher quartile of BPA urinary levels (165).

Thus, bisphenol A exposure represents a potential risk factor for the initiation and progression of NAFLD.
Genetic Susceptibility

Genetic pre-disposition and epigenetic mechanisms are emerging as one of the most important risk factors in the development and progression of NAFLD and MetS.

Genetic predisposition is suggested by documented familial clustering of NAFLD, as demonstrated by the ethnic differences in the prevalence of these disorders. Furthermore, not all the obese patients develop NAFLD suggesting the complex interplay between environmental factors and genetic predisposition (51).

Recently, several genetic variants in the DNA code (single DNA base-pair or single nucleotide polymorphism-SNPs) have been identified as potential markers for either susceptibility and progression of MetS and NAFLD (see Table 2) (166–172).

As shown by Santoro et al. (166) in a cohort of obese children and adolescents the rs738409 variant of adiponutrin/patatin-like phospholipase domain-containing 3 (PNPLA3) confers susceptibility to hepatic steatosis by determining elevated serum ALT levels. In accordance with the latest study, Mangge et al. (173) have observed that the PNPLA3 rs738409 polymorphism is correlated with increased ALT levels in pediatric age group and it’s more frequent in obese children with MetS. PNPLA3 encodes for a hepatic enzyme, the adiponutrin, that is also present in adipose tissue showing both a lipogenic and lipolytic activity in vitro (174). This missense variant causes a cytosine to guanosine substitution determining an isoleucine to methionine substitution at the amino acid position 148 (I148M). Although the mechanisms underlying the association with this variant and liver steatosis is still debated, studies in vitro have shown that PNPLA3 rs738409 variant limits TG hydrolysis by inhibiting catalytic activity of the enzyme and increases the lipogenesis activity of the PNPLA3 (175). Furthermore, it has been shown that FFA potentiate the effect of PNPLA3 rs738409 variant on intra-hepatic triglycerides accumulation (176). Thus, PNPLA3 genotyping might become a test to characterize patients with NAFLD, however more studies are needed to clarify how this variant affects the development of fatty liver disease (177).

Another gene correlated with susceptibility and progression of NAFLD is the glucokinase regulatory protein (GCKR) gene which encodes for the glucokinase regulatory protein (GCKRP). The GCKRP regulates the activity of glucokinase (GCK) by competing with the glucose which is a substrate of GCK (167). The rs1260326 variant has been studied as potential marker of hepatic steatosis and MetS in children and adolescents. This variant consists of proline-to leucine substitution in position 446 (P446L) and encodes for a protein characterized by a reduced capability to response to fructose 6 phosphate, leading to a constant increased of GCK activity (4). In accordance with studies in adults (178, 179), Santoro et al. (180) have demonstrated that the GCK rs1260326 variant is associated with NAFLD, elevated triglycerides and large VLDL levels in a cohort of obese children. In accordance with the latest study, Lin et al. (181) have been documented a significant correlation between GCKR rs78094 genotype and some components of MetS, such as elevated serum level of cholesterol and triglycerides and high serum levels of ALT. To note, Hudert et al. (182) have shown that GCKR modules the amount of hepatic fibrosis in pediatric NAFLD.

Similarly, uncoupling protein 3 (UCP3) gene could influence the occurrence of NAFLD in pediatric population. This gene is located on chromosome 11q13 and is involved in the regulation of energy and lipid metabolism (168). In 2013, Xu et al. (183) have found that children with NAFLD were characterized by a higher prevalence of rs11235972 GG genotype compared with the health control group. The authors observed that the increase risk of NAFLD is significantly associated with BMI, waist-to-hip ratio (WHR), blood pressure (BP), fasting blood glucose (FBG), IR, and dyslipidemia.

The Glu167Lys (E167K) transmembrane 6 superfamily member 2 (TM6SF2) variant is a further genetic polymorphism associated with liver fibrosis, ALT levels and reduced liver-derived TG rich lipoproteins plasma levels (169). Grandone et al. (184) by evaluating an Italian cohort of obese children and adolescents have shown an association between TM6SF2 rs58542926 variant, liver enzymes and hepatic steatosis. Of note, it seems to reduce cardiovascular risk.

Another gene recently associated with liver function in the pediatric population is the MBOAT7 gene, that encodes for a lysophosphatidylinositol acyltransferase-1, known as LPIAT1 or MBOAT7 which plays a relevant role in arachidonic acid recycling in human neutrophils (170). In accordance with a previous study in adults (185–187), Umano et al. (188) have recently documented a significant correlation between a gene variant rs641738 in the MBOAT7 gene and hepatic steatosis in a multiethnic cohort of obese children and adolescents. Interestingly, they observed an association between the rs626283 SNP in the MBOAT7 gene and impaired insulin sensitivity only in Caucasian obese youths.

Epigenetic Mechanisms

During the last decades several authors have postulated a strong association between epigenetic alterations in intrauterine life and the development of metabolic and cardiovascular complications later in life (50). These multifactorial mechanisms modulate gene expression of offspring (189) and consist in DNA methylation, remodeling of chromatin, histone modifications, and/or regulatory feedback by microRNAs. Studies in humans have demonstrated that these mechanisms contribute to the programming of NAFLD (50). Brandt et al. (190) have recently observed higher levels of miRNA-122 in obese children compared to healthy controls and a positive correlation between circulating miRNA122 levels and ALT, aspartate transaminase (AST), and gamma glutamyl transpeptidase (GGT) values in prepubertal children (190). On the contrary, in obesity mouse model inhibition of miR-122 activity results in decreased plasma cholesterol concentration with reduced expression of several lipogenic genes that significantly improve NAFLD (191). Probably, miR-122 might be useful as a new biochemical marker for assessing early hepatic steatosis (192).

Emerging evidence also supports a role of DNA methylation in the development of cardiometabolic disorders. Recently, Gerhard et al. (193) have observed significant differences in DNA methylation between adults with biopsy-proven of NAFLD and controls. Moreover, a different methylation pattern was documented in humans with advanced vs. mild grade of
NAFLD (194). It's clear as differences in methylation profiles could modulate hepatic genes expression, thus influencing NAFLD pathogenesis. AQP1 (aquaporin 1) gene, encoding a water channel, and FGF2 (fibroblast growth factor receptor 2) result overexpressed and hypomethylated in fibrosis condition (195, 196).

Alterations in DNA methylation and histone modifications have also been proposed to be important in the programming of NAFLD in offspring exposed to maternal obesity which causes altered expression of genes involved in gluconeogenesis and lipid metabolism (197). McCurdy et al. (198) have found that offspring from mothers chronically consuming a high-fat diet (HFD) had an increased liver triglycerides and an elevated expression of genes for mitochondrial oxidative stress or for fatty acid oxidation, with a premature activation of the gluconeogenic pathway that modulate the development of NAFLD. Recent studies in neonates have demonstrated a strict association between maternal BMI and intra-hepatocellular lipid levels assessed by magnetic resonance imaging (199, 200). Moreover, in the cross-sectional study conducted by Ayonrinde et al. (201) in a cohort of adolescents aged 17 years, maternal obesity and adolescent obesity increased the risk of a hepatic steatosis independent of a Western dietary pattern, while breastfeeding reduced the risk of NAFLD. Of note, in 2019 Nguyen et al. (202) have shown that reduced sirtuin (SIRT1) expression in offspring from mothers consuming HFD-dietinduces a pre-diabetic and NAFLD phenotype. SIRT1 is a potent regulator of energy metabolism and stress responses, express in the hypothalamus, white adipose tissues (WAT), and liver. A systemic overexpression of SIRT1, in the offspring from mothers consuming HFD-diet, determined a reduced body weight and adiposity in epididymal and retroperitoneal adipose tissues of offspring with normalized lipid metabolic markers and fibrogenesis in the liver (202).

Whether all these gene alterations are causative or simply a consequence of the induced disease state remains to be determined by other studies (203). However, these results indicate that maternal metabolic dysfunction influences NAFLD phenotype in offspring (204), underlining the importance to encourage the maintenance a normal body mass index and an healthy diet before and during pregnancy.

**Metabolomic**

Recent studies hypothesized that differences in serum and urinary metabolite profiles between healthy and obese children could be correlated with clinical phenotype and metabolic risk in pediatric population. Troisi et al. (205) performed a urinary metabolome analysis in 40 Italian children and adolescents to identify specific metabolic pathways that characterize pediatric obese subjects with or without NAFLD. The authors observed lower urinary levels of xylitol and phenylacetic acid (PAA) in obese children (with and without NAFLD) than in normal weight controls (205). Xylitol, a five-carbon sugar alcohol, is present in many types of fruits and vegetables and it is not endogenously produced by humans. PAA is an organic compound synthesized from the amino acid phenylalanine (PA) via phenylpyruvate. Plant secondary metabolites such as phenolic acids are generally correlated with positive effects for human health (206). Thus, urinary levels of these metabolites reflect the diet preferences and they have been considered beneficial in preventing the development of obesity and metabolic alterations in rats with diet-induced obesity (207).

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**TABLE 2 | Genes implicated in the development of NAFLD and MetS in children.**

| Gene       | Protein                                      | Polymorphism | Function                                                                 |
|------------|----------------------------------------------|--------------|--------------------------------------------------------------------------|
| PNPLA3     | Adiponutrin/patatin-like phospholipase       | rs738409     | Enzyme (lipase) that mediates triacylglycerol hydrolysis in adipocytes. The protein may be involved in the balance of energy usage/storage in adipocytes. |
| GCKR       | Glucokinase Regulatory Protein               | rs1260326    | Protein produced in hepatocytes. GCKR binds and moves glucokinase (GK), thereby controlling both activity and intracellular location of this key glucose metabolism enzyme. |
| UCP3       | Uncoupling protein 3                         | rs11235972   | The uncoupling protein is involved in the transferring of anions from inner mitochondrial membrane to outer mitochondrial membrane, thus influencing triglyceride secretion and hepatic lipid droplet content. |
| TM6SF2     | Transmembrane 6 superfamily member protein 2 | rs58542026   | The physio-pathological function is not yet completely known. May function as sterol isomerase and as regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. |
| MBOAT7     | Membrane bound O-acyltransferase domain     | rs626283     | Enzyme that have acyltransferase activity. This protein is involved in the re-acylation of phospholipids and it is implicated in arachidonic acid recycling in human neutrophils. |
| PPARC1A    | Peroxisome proliferator-activated receptor-γ| rs8192678    | Master transcriptional coactivator that module multiple key genes that play a role in energy homeostasis, mostly through the control of mitochondrial function and biogenesis. |
| MTTP       | Microsomal triglyceride transfer protein     | rs2306986    | Carrier-protein that is involved in lipid transfer function. It plays a pivotal role critical in the assembly and secretion of very-low-density lipoprotein (VLDL) removing lipid from liver. |

PNPLA3, adiponutrin/patatin-like phospholipase domain-containing 3; GCKR, glucokinase regulatory protein gene; UCP3, uncoupling protein 3; TM6SF2, transmembrane 6 superfamily member 2; MBOAT7, membrane bound O-acyltransferase domain containing protein 7; PPARC1A, peroxisome proliferator-activated receptor-γ coactivator-1α protein; MTTP, microsomal triglyceride transfer protein.
Interestingly, also high urinary concentration of methylhistidine, which derives from meat intake, have been observed in patients with NAFLD (205). This substance has been correlated with BMI in obese subjects, reflecting its association with muscle mass and dietary meat intake (208). 1-methylhistidinuria could originate also from increased oxidation in skeletal muscle, probably eased by a reduction in the body’s antioxidants pool, e.g., by alpha-tocopherol deficiency, a condition largely documented in pediatric obesity-related NAFLD (209).

Other specific metabolic pathways have been associated with gut-liver axis malfunctioning which was frequently detected in obese children with NAFLD. Indeed, urinary p-cresyl sulphate (PCS) level, an intestinal microbial metabolite, resulted increased in obese children without NAFLD and correlated negatively with the presence of small intestinal bacterial overgrowth (SIBO) (205). Branched chain amino acids (BCAAs) and/or their metabolites were associated with excess of visceral fat, elevated intestinal permeability (IP) and SIBO (205).

Recently, metabolic salivary markers of hepato-comorbidity have been identified in pediatric obese subjects (210). Troisi et al. (210) performed a preliminary study that demonstrated high serum and salivary glucose and insulin levels in obese children with or without MetS.

In this study, it was also reported that salivary insulin concentration tended to be higher in obese patients with steatosis than in those without and that increased in parallel with the number of MetS elements (211).

Further studies have recently confirmed as different salivary metabolic profiles characterized pediatric obesity, liver disease, and MetS (212, 213). Troisi et al. (213) observed high levels of two saturated fatty acids, palmitic, and myristic acid, in children with steatosis. Wasilewska et al. (214) demonstrated a positive correlation between total ceramide concentration and IR markers in obese children with NAFLD. Moreover, Qi et al. (215) observed high levels of salivary pyroglutamic acid in patient with NAFLD, suggesting that it could be a potential diagnostic biomarker of enhanced oxidative stress in liver cells.

Thus, distinct metabolomic signatures have been identified to detect fatty liver and MetS in childhood. Metabolic could represent a useful tool for identifying children at risk of obesity hepato/metabolic complications in epidemiologic evaluations.

CONCLUSIVE REMARKS AND FUTURE PERSPECTIVES

The present review confirms that the prevalence of pediatric NAFLD is increasing worldwide and there is emerging evidence supporting the relationship between fatty liver disease and MetS in children and adolescents.

The strict link between NAFLD and MetS in pediatric age group is becoming increasingly clear because these diseases are both associated with a high risk to develop cardiovascular and diabetic complications early in life.

Although central obesity and IR are still considered as the key factors linking MetS and NAFLD, a multifactorial pathogenesis characterized by the interaction between genetic background and environmental factors is increasingly recognized as a key player in the development of both metabolic disorders.

Actually, the mainstay of NAFLD therapy is characterized by lifestyle interventions on obesogenic environment and sedentary life, which the objective to reduce obesity-related hepatic and metabolic abnormalities. Unfortunately, this target is often difficult to be obtained and the results are not always satisfied. Based on the new identified potential pathogenetic triggers, novel pharmacologic therapies have been proposed such as probiotics that now seem the most remarkable and reasonable approach for their safety and acceptability.

In conclusion, children and adolescents with NAFLD should be screened for MetS as well as those with MetS for fatty liver disease.

Future perspectives must focus on understanding the natural history and etiology of NAFLD in order to identify early prevention strategies and efficient therapeutic approaches to improve the quality of life of these children.

AUTHOR CONTRIBUTIONS

All the authors contributed to the research of the articles and to the writing of the manuscript. VN revised it critically.

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The handling Editor declared a shared affiliation, though no other collaboration, with several of the authors EDA and VC.

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