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Pediatric Nonalcoholic Fatty Liver Disease

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1. Introduction

Paralleling the burgeoning epidemics of childhood obesity, nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common cause of chronic liver disease in children [1,2].

NAFLD is a clinic-pathological condition defined by the accumulation of intrahepatic triglyceride fat (IHTF) content in the absence of alcohol consumption [1,2].

NAFLD encompasses a wide spectrum of liver damage, ranging from asymptomatic steatosis with elevated or normal aminotransferases to steatosis with inflammation, ballooning degeneration and pericellular fibrosis (Nonalcoholic Steatohepatitis, NASH) to cirrhosis [2,3].

Although there are limited long-term data on the natural history of NAFLD in children, NASH is increasingly diagnosed in obese children [4] and it may progress to cirrhosis even in this age group [2,5]. In addition, children with NAFLD have a 13.6-fold higher risk of mortality or requiring a liver transplant as compared to age/sex matched controls [6].

During the last years there has been a growing interest in the relationship between NAFLD and the development of metabolic and cardiovascular diseases [7-9].

Several lines of evidence have shown that in obese children and adolescents excessive accumulation of IHTF is associated with important alterations in glucose, fatty acid (FA), lipoprotein metabolism and inflammation, suggesting that IHTF represents a strong risk factor for the development of the Metabolic Syndrome (MetS) and type 2 diabetes mellitus (T2D) [7-10].

Although a multifactorial pathogenesis of NAFLD has been postulated [12-14], obesity and insulin resistance represent two important players in the development of the early stages of the disease [2,14]. Interestingly, although the insulin resistance state could explain the relationship between NAFLD and the development of metabolic alterations [10,15], the presence of liver steatosis is also an important marker of multiorgan insulin resistance [16], opening a debate as to whether hepatic steatosis is a consequence or cause of insulin resistance.

Therefore, it would be of paramount importance to identify children affected by NAFLD and to better understand the pathogenesis of this condition in order to prevent the development of the associated metabolic complications early in life.

2. Definition and prevalence of NAFLD

Despite fatty liver is becoming one of the most common hepatic alterations in obese children [1,2], the prevalence of pediatric NAFLD is uncertain, mainly due to the methods used to assess fatty liver.

NAFLD is defined as an IHTF content > 5% of liver volume or weight and as a presence \geq 5% of hepatocytes containing intracellular triglycerides, in the absence of alcohol consumption [17,18].

The gold standard for diagnosing NAFLD is liver biopsy [19,20]. Liver biopsy allows an accurate assessment of histopathological findings, providing information on the type of NAFLD (simple steatosis or steatohepatitis) and the various degrees of hepatic fibrosis [20]. However, the main limitation of the application of liver biopsy in the pediatric age group is due to the fact that it is an invasive procedure; thus, it is not considered as first line to screen the presence of liver disease [19,20].

Although non-invasive methods, such as computer tomography, MRI, or ultrasonography are unable to distinguish between NASH and other forms of NAFLD [19,21], they have an acceptable sensitivity and specificity for the diagnosis of increased fat accumulation in the liver [21]. Furthermore, ultrasound has been shown to have a good correlation with the histological findings of liver biopsy, particularly macrovesicular steatosis [22].

In clinical practice, combining liver function tests, such as serum aminotransferases [18], with liver ultrasound represents a useful way of identifying the presence of liver steatosis in obese children.

In spite of the method used, it is clear that the prevalence of NAFLD is increasing in children and adolescence. NAFLD affects 2.6% of normal children [23] and up to 77% of obese individuals [24,25]. Pediatric NAFLD extends beyond North America according to centers in Europe, Asia, South America and Australia [2,3]. The prevalence of fatty liver in obese children in China, Italy, Japan, and the United States has been reported to be between 10% and 77% [2,3]. Data derived from the National Health and Nutrition Examination Survey III (1988-1994) suggest that approximately 3% of adolescents present abnormal serum amino-

transferase values [26]. Moreover, studies from autopsies of 742 children (ages 2–19 years) reported fatty liver prevalence at 9.6%, and in obese children this rate increased to an alarming 38% [25].

NAFLD has been also described in obese prepubertal children. In the study by Manco et al. [27] NAFLD was detected in children as young as 3 years old.

The prevalence of NAFLD is around 30% in children with a Tanner pubertal stage I, significantly lower when compared to that found in the pubertal age [28].

Alarming data come from our study population of prepubertal Caucasian obese children [29]. Out of 100 severely obese prepubertal children, liver steatosis was found in 52% and was equally distributed between the two sexes [29].

Thus, NAFLD is an emerging health problem even in very young age groups.

3. Pathogenesis and risk factors of NAFLD

Although the pathogenetic mechanism of NAFLD is not completely understood, in accordance with the “two hit hypothesis”, insulin resistance and oxidative stress represent two key factors for the development and progression of NAFLD/NASH [12,17,20].

3.1. Insulin resistance and central obesity

The “two-hit” model proposes that fat accumulation in the hepatocytes is a prerequisite for a second hit that induces fibrosis and inflammation [30].

Fat accumulation in the liver is likely to result from insulin resistance and concomitant impairment of fatty acid (FA) metabolism within liver, skeletal muscle and adipose tissue [31].

Insulin resistance seems to be responsible for abnormalities in lipid storage and lipolysis in insulin-sensitive tissues, leading to an increased fatty acids flux from adipose tissue to the liver and subsequent accumulation of triglycerides in the hepatocytes [31]. In particular, steatosis develops when the rate of FA input (uptake and synthesis with subsequent esterification to triglycerides (TG)) is greater than the rate of FA output (oxidation and secretion) [11]. The amount of TG in the hepatocytes represents a complex interaction among [11,31]: hepatic FA uptake, derived from plasma free fatty acid (FFA) released from hydrolysis of adipose tissue and FFA released from hydrolysis of circulating TG; *de novo* FA synthesis (*de novo* lipogenesis [DNL]); fatty acid oxidation (FAO); FA export within very low-density lipoprotein (VLDL)-TG.

Obesity is the most important cause in the development of insulin resistance and it has been demonstrated that the critical determinant of insulin sensitivity is not the degree of obesity *per se* but the distribution of fat partitioning [32,33].

Several studies [32,33] have demonstrated that obese adolescents presenting increased intramyocellular lipid content (IMCL) [32] and visceral fat and decreased subcutaneous fat deposition are more likely to develop insulin resistance.

There is extensive evidence indicating that central obesity is associated with an impaired insulin action in obese pediatric populations. Although controversy remains regarding the contribution of visceral and subcutaneous fat to the development of insulin resistance [33], a previous study by Cruz et al. [35] showed a direct impact of visceral fat accumulation on insulin sensitivity and secretion, independently of total body adiposity, in obese children with a family history of T2D. Indeed, by stratifying a multiethnic cohort of obese adolescents into tertiles based on the proportion of visceral fat in the abdomen (visceral/subcutaneous fat ratio), insulin resistance (homeostasis model assessment) significantly increased and insulin sensitivity (Matsuda index) decreased in obese adolescents with high proportion of visceral fat and relatively low abdominal subcutaneous fat [33].

These findings suggest that obese children and adolescents at risk for developing metabolic complications are not necessarily the most severely obese, but are characterized by an unfavorable lipid partitioning profile.

3.2. Insulin resistance and fatty liver disease: Which comes first?

Despite the demonstrated relationship between IMCL, visceral fat and metabolic dysfunction, the ectopic fat deposition in the liver is emerging as the most important marker of insulin resistance in adults [15] as well as in obese pediatric population [36].

In healthy nondiabetic humans the correlation between the IHTF content and peripheral insulin resistance was much stronger than the correlation with intramyocellular lipid content, visceral fat content or subcutaneous fat content [37]. The relationship between liver steatosis and insulin resistance has been clearly demonstrated in children [36,29]. In our cross sectional study, we evaluated insulin resistance indexes between obese prepubertal children with and without liver steatosis; furthermore insulin resistance indexes were compared to values of normal weight children. Our results showed that children with NAFLD not only presented severe obesity but also an increased degree of insulin resistance when compared to the sex- and age-matched normal weight children [29].

The relationship between insulin resistance and fatty liver disease is not only related to the presence of liver steatosis, but also to the degree of fatty liver. In a multiethnic cohort of obese adolescents, Calì et al. [36] clearly showed a significant decrease in insulin sensitivity and an imbalance between anti- and pro-inflammatory markers [adiponectin and interleukin 6 (IL-6)] paralleling the severity of hepatic steatosis [36]. In particular, adiponectin, the most abundant secretory protein produced by adipose tissue, is closely related with insulin action. Plasma adiponectin concentrations are inversely associated with hepatic steatosis and metabolic complications [37,38].

Although these findings support the central role of insulin resistance in the development of fatty liver, several studies have demonstrated that the presence of liver steatosis is an important marker of multiorgan insulin resistance, independently of BMI, percent body fat, and

visceral fat mass [11,15,36]. In particular, NAFLD has been found to be associated with insulin resistance in liver (impaired suppression of insulin-mediated glucose production) [39,40], skeletal muscle (reduced insulin stimulated glucose uptake) [40] and adipose tissue (decrease inhibition of lipolysis by insulin) [41] in obese children and adolescents, independently of adiposity.

Recently, Caprio et al. [16] reported that obese adolescents with high liver fat content, independent of visceral and IMCL had an impaired insulin action (as assessed by the hyperinsulinemic-euglycemic clamp) in the liver and in the muscle and early defects in β -cell function [16]. These results suggest that the liver has a central role in the complex phenotype of the insulin resistance state in obese adolescents with fatty liver.

Although it is clear that there is an important correlation between insulin resistance and hepatic steatosis, the mechanisms responsible for the interrelationships between fatty liver disease and insulin resistance are not clearly understood. In fact, it remains unclear whether hepatic steatosis is a consequence or the primary event leading to hepatic and subsequently peripheral insulin resistance.

Petersen et al. [42] showed that the lack of adipose tissue in the congenital lipodystrophy is characterized by extreme insulin resistance associated with massive hepatic fat accumulation; intervention with subcutaneous leptin administration in these patients improved whole-body insulin sensitivity mainly due to the mobilization of the excessive fatty liver content.

Models of patients with liver cirrhosis in which hepatic dysfunction is known to be the primary disturbance provide strong support that insulin resistance in peripheral tissues develops secondary to liver disease [43]. 60-80% of patients with liver cirrhosis are glucose intolerant and in 10-15% diabetes occurs relatively rapidly (over a period of 5 years). Diabetes complicating liver cirrhosis, also known as hepatogenous diabetes, and the common form of T2D are the results of a marked reduction in insulin action and a β -cell secretion defect that is not able to compensate the severity of insulin resistance [43,44]. The important role of peripheral insulin resistance in the glucose tolerance of cirrhosis has been highlighted by the observation that liver transplantation, when the dosage of immunosuppressive agents was reduced and corticosteroids withdrawn, was able to restore normal insulin sensitivity not only in the liver but also at the level of the skeletal muscle and adipose tissue and normalizes glucose tolerance in most patients with diabetes [43,44].

The mechanism by which IHTF has an important systemic consequence to adversely affect insulin sensitivity is unknown. However, it has been proposed that fatty liver might interfere with insulin degradation [45]; the resultant hyperinsulinemia may potentially be able to impair insulin action in peripheral tissues, as shown in benign insulinoma induced hyperinsulinemia [43,44,46]. This hyperinsulinemia-induced mechanism may be justified also based on the finding of the reverse experiment: when the prolonged infusion of octreotide was administered to extremely insulin-resistant cirrhotic individuals, the correlation of hyperinsulinemia was paralleled by the restoration of normal insulin sensitivity [43,47].

Although these data showed a clear possibility that intrahepatic fat accumulation plays a key role in the onset of insulin resistance and insulin resistance syndrome, longitudinal data are needed in order to clarify which abnormality comes first.

3.3. Oxidative stress

In accordance with the “two hit hypothesis”, dysfunction of various oxidation pathways within the hepatocytes and subsequent overproduction of reactive oxygen species (ROS), may result in the peroxidation of accumulated lipids, inflammation, hepatocellular apoptosis and fibrogenesis [31].

Obese subjects affected by NAFLD present an impaired oxidant-antioxidant status than subjects without [12,48].

Interestingly, we recently observed [48] that obese prepubertal children affected by liver steatosis had impaired levels of receptors for advanced glycation endproducts (RAGEs), which has been demonstrated to be correlated with the progression of several metabolic and cardiovascular diseases. In particular, obese prepubertal children with liver steatosis presented decreased RAGEs levels compared with children without liver disease, underling that oxidative stress could play a role even in the early stages of the disease [48].

3.4. Genetic and environmental factors associated with fatty liver disease

Several genetic and environmental factors are likely responsible for NAFLD and its progression from simple steatosis to NASH.

In fact, although the development of NAFLD is strongly linked to obesity and insulin resistance, there are obese individuals who do not have NAFLD, and since NAFLD can occur in normal-weight individuals with normal metabolic profile, thus multiple genetic and environmental factors should be involved in its development [49].

Initial evidence for a *genetic component* of NAFLD comes from ethnic variation in NAFLD prevalence [50]. Children from certain ethnicities are predisposed to NAFLD, primarily Hispanics, Asians and Native Americans [25,50].

Furthermore, a familial aggregation study of fatty liver in overweight children with and without NAFLD found that fatty liver is a highly heritable trait. Family members of children with biopsy-proven NAFLD and overweight children without NAFLD were evaluated by magnetic resonance imaging (MRI). Fatty liver was identified in 17% of siblings and 37% of parents of overweight children without NAFLD and in 59% of siblings and 78% of parents of children with NAFLD [51].

Interestingly, Romeo et al. [52] conducted the first genome-wide association scan conducted in a large multiethnic population. The authors demonstrated that the patatin-like phospholipase domain containing protein 3 (also known as adiponutrin) gene was strongly associated with IHTF content in adults [52].

These findings have been recently supported by Santoro et al. [53]. By genotyping the *PNPLA3* SNP in a multiethnic group of 85 obese youths, the authors found that the *PNPLA3* rs738409 SNP gene confers susceptibility to hepatic steatosis.

Nutrition and physical activity are important *environmental factors* that determine risk in NAFLD.

Excess food intake and lack of exercise contribute to weight gain, which has been shown to contribute to the progression of liver fibrosis in patients with NAFLD [54]. Specific dietary factors may also play either protective or antagonistic roles in the development and progression of NAFLD. An increased consumption of meat and soft drinks and low consumption of fish were found to be associated with NAFLD cases compared with controls [49]. Furthermore, low intakes of polyunsaturated fatty acid (PUFA) and high intakes of saturated fat and cholesterol were also shown to be associated with NAFLD [49]. Other studies have shown higher-carbohydrate and lower-fat diets to be associated with more progressive disease [49,55]. Notably, very recent animal data have shown that in both mice [49,56] and non-human primates [49] exposure to a maternal high-fat diet leads to a disturbing development and progression of NAFLD in the offspring.

It has been proposed that increase consumption of fructose in soft drinks and fruit drinks may have a role in the pathogenesis of NAFLD [2]. In one study, children with biopsy-proven NAFLD were shown to have significantly elevated plasma TG levels and oxidative stress levels after consumption of fructose as compared with glucose [57]. However, children without NAFLD were found to have no differences in TG or oxidative stress levels following the consumption of glucose compared with fructose [2].

Small intestinal bacterial overgrowth may be an additional environmental factor involved in NAFLD pathogenesis, and dietary supplements such as probiotics could have a beneficial effect [49]. Evidences from animal studies have shown that small intestinal bacterial overgrowth increases gut permeability leading to portal endotoxaemia and increased circulating inflammatory cytokines, both of which have been implicated in the progression of NAFLD [58,59].

4. NAFLD, metabolic and cardiovascular complications in obese children and adolescents

4.1. NAFLD and metabolic complications

NAFLD is nowadays considered the hepatic manifestation of the MetS in adults as well as in children [60]. This is not surprising since NAFLD is closely associated with obesity, insulin resistance, and alterations in glucose and lipid metabolism [44].

The association between NAFLD and MetS has been clearly demonstrated by Burgert et al. [61,62]. In 392 obese adolescents, elevated alanine aminotransferases (ALT) (>35 U/L) levels were found in 14% of participants, with a predominance of White/Hispanic. After adjusting

for potential confounders, rising ALT levels were associated with deterioration in insulin sensitivity and glucose tolerance, as well as increasing FFA and TG levels. Furthermore, increased hepatic fat accumulation was found in 32% of obese adolescents and was associated with decreased insulin sensitivity and increased lipid levels and visceral fat [61]. These results demonstrate that in obese children and adolescents, hepatic fat accumulation is associated with insulin resistance, dyslipidemia and altered glucose metabolism.

In addition, the Korean National Health and Nutrition Examination Survey found participants aged 10 – 19 years with three or more risk factors for MetS had an odds ratio that of 6.2 (95 % CI 2.3 – 16.8) for an elevated serum ALT, which they used as an indicator of fatty liver [62]. Furthermore, a case – control study of overweight children with biopsy-proven NAFLD and age-, sex-, and obesity-matched controls found that children with NAFLD were significantly more likely to have MetS than obese controls without evidence of fatty liver disease [9].

More recently, in a large histology-based study conducted in children with NAFLD [63], MetS was diagnosed in 25.6 % of the subjects, with central obesity and hypertension being the most common of the MetS features observed. In addition, a diagnosis of MetS was predictive of steatosis severity, NASH, hepatocellular ballooning and NAFLD pattern [63].

In a recent study by our group [64], we assessed the role of liver steatosis in defining MetS in prepubertal children. The prevalence of the MetS was around 14% and increased to 20% when liver steatosis was included as an additional diagnostic criterion. These findings underline not only the relevance of the MetS even among prepubertal children but also emphasize the potential importance of testing for fatty liver as a component of the MetS already in this age group [64] (figure 1).

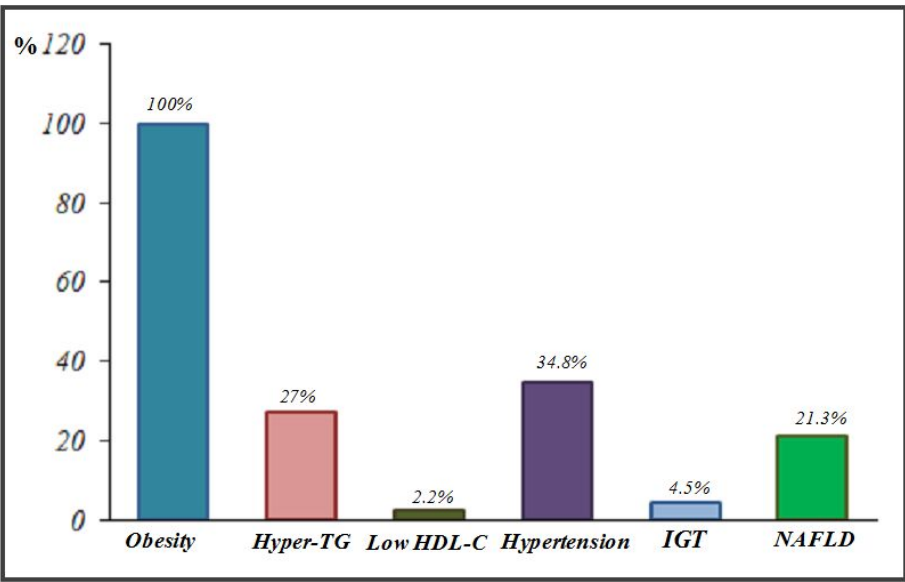


Figure 1. Prevalence of components of the MetS among obese prepubertal children [64].(TG, triglycerides; HDL-C, high density lipoprotein cholesterol; IGT, impaired glucose tolerance; NAFLD, non alcoholic fatty liver disease)

NAFLD in youth may be considered not only a strong risk factor for MetS, but also for T2D [36]. In a cohort of 118 obese adolescents [36] stratified according to tertiles of hepatic fat content (as assessed by fat gradient MRI), independently of obesity, the severity of fatty liver was associated with the presence of prediabetes [impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)/IGT]. In fact, paralleling the severity of hepatic steatosis, there was a significant decrease in insulin sensitivity and impairment in β -cell function, as indicated by the fall in the disposition index (DI). Furthermore, paralleling the severity of fatty liver, there was a significant increase in the prevalence of MetS, suggesting that hepatic steatosis may probably be a predictive factor of MetS in children [36].

The important role of intrahepatic fat content in the development of metabolic complications in obese subject has been recently underlined by Fabbrini et al. [15]. The authors showed that in adults with high IHTF insulin action in liver, skeletal muscle and adipose tissue was impaired and hepatic VLDL-TG secretion rate was increased. In contrast, they were not able to observe these metabolic alterations in subjects with high visceral fat volume and matched for IHTF. Therefore, the authors demonstrated that IHTF and not visceral fat is a better marker of metabolic derangements associated with obesity [15,16].

4.2. NAFLD and cardiovascular disease

Recent evidences suggests that individuals with NAFLD are also at high risk for coronary heart disease [3,43]. In adults, elevated serum ALT have been associated with increased risk of cardiovascular and all cause mortality (in addition to liver mortality) [3,65].

A study in Turkish children [66] showed that carotid artery intima-media thickness is significantly higher in obese children with fatty liver than in obese children without fatty liver or normal weight control.

In addition, Pacifico et al. [67] reported that carotid artery intima-media thickness was highest in obese children with echogenic liver and severity of liver fat was an independent predictor of intima-media thickness after adjustment for known cardiovascular factors.

However, given the lack of long-term longitudinal cohort studies in pediatric fatty liver disease, the relationship between the natural history of the disease and the actual risk for future cardiac events is unclear.

5. Conclusions

The prevalence of fatty liver disease is increasing in obese children and adolescence.

Although the exact pathogenetic mechanism is still unclear, there is an urgent need to screen obese children for this pathology. A misdiagnosis of fatty liver could represent a serious risk factor for the development of its associated metabolic and cardiovascular complications during childhood and for exacerbated metabolic abnormalities later in life.

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