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Nonalcoholic fatty liver disease and the heart in children and adolescents

Lucia Pacifico, Claudio Chiesa, Caterina Anania, Antonio De Merulis, John Frederick Osborn, Sara Romaggioli, Eugenio Gaudio

Lucia Pacifico, Caterina Anania, Antonio De Merulis, Sara Romaggioli, Department of Pediatrics and Child Neuropsychiatry, Sapienza University of Rome, 00161 Rome, Italy
Claudio Chiesa, Institute of Translational Pharmacology, National Research Council, 00133 Rome, Italy
John Frederick Osborn, Department of Public Health and Infectious Diseases, Sapienza University of Rome, 00161 Rome, Italy
Eugenio Gaudio, Department of Human Anatomy, Histology, Forensic Medicine and Orthopedics Sciences, Sapienza University of Rome, 00161 Rome, Italy
Author contributions: Pacifico L, Chiesa C, Osborn JF and Gaudio E designed the study, analyzed the data and wrote the manuscript; Anania C, De Merulis A and Romaggioli S collected the data; all the authors participated in the critical review and in the final approval of the manuscript.
Correspondence to: Claudio Chiesa, MD, Institute of Translational Pharmacology, National Research Council, Via Fosso del Cavaliere, 100, 00133-Rome, Italy. claudio.chiesa@ift.cnr.it
Telephone: +39-6-49979215   Fax: +39-6-49979216
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Abstract

Over the last two decades, the rise in the prevalence rates of overweight and obesity explains the emergence of nonalcoholic fatty liver disease (NAFLD) as the leading cause of chronic liver disease worldwide. As described in adults, children and adolescents with fatty liver display insulin resistance, glucose intolerance, and dyslipidemia. Thus NAFLD has emerged as the hepatic component of the metabolic syndrome (MetS) and a strong cardiovascular risk factor even at a very early age. Several studies, including pediatric populations, have reported independent associations between NAFLD and markers of subclinical atherosclerosis including impaired flow-mediated vasodilation, increased carotid artery intima-media thickness, and arterial stiffness, after adjusting for cardiovascular risk factors and MetS. Also, it has been shown that NAFLD is associated with cardiac alterations, including abnormal left ventricular structure and impaired diastolic function. The duration of these subclinical abnormalities may be important, because treatment to reverse the process is most likely to be effective earlier in the disease. In the present review, we examine the current evidence on the association between NAFLD and atherosclerosis as well as between NAFLD and cardiac dysfunction in the pediatric population, and discuss briefly the possible biological mechanisms linking NAFLD and cardiovascular changes. We also address the approach to treatment for this increasingly prevalent disease, which is likely to have an important future global impact on the burden of ill health, to prevent not only end-stage liver disease but also cardiovascular disease.

Core tip: Nonalcoholic fatty liver disease (NAFLD) is an important and emerging health problem in childhood. It is recognized as part of the metabolic syndrome and especially the necroinflammatory form is associated with a high risk for the development of functional and structural vascular changes as well as left ventricular dysfunction at an early age. In addition, there seems to be a complex bidirectional relationship between the progression to nonalcoholic steatohepatitis (NASH) and the development of insulin resistance and cardiovascular abnormalities. Early intervention during childhood to recognize NAFLD, as well as to prevent its progression to NASH, may be a crucial step in averting an unfavorable cardiac phenotype.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of fat-associated liver conditions that can result in end-stage liver disease and the need for liver transplantation[1]. Simple steatosis, or fatty liver, occurs early in NAFLD and may progress to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis with increased risk of hepatocellular carcinoma[1]. Over the last two decades, the rise in the prevalence rates of overweight and obesity explains the emergence of NAFLD as the leading cause of chronic liver disease in pediatric populations worldwide[2,3]. The liver is one of the main ectopic sites where lipids may accumulate in obese subjects. Ectopic fat disposition occurs particularly when the energy storage capacity of the adipose tissue is exceeded, leading to increased net lipid flux to non-adipose organs, thereby causing lipotoxiciy and insulin resistance[4,5]. As described in adults, children and adolescents with fatty liver display insulin resistance, glucose intolerance, and dyslipidemia[6,7]. Thus NAFLD has emerged as the hepatic component of the metabolic syndrome (MetS)[8] and a strong cardiovascular risk factor even at a very early age[9,10].

Several studies (including pediatric populations) have reported independent associations between NAFLD and markers of subclinical atherosclerosis such as impaired flow-mediated vasodilation (FMD), increased carotid artery intima-media thickness (cIMT) and arterial stiffness, after adjusting for cardiovascular risk factors and MetS[9-14]. Also, it has been shown that NAFLD is associated with cardiac alterations, including myocardial insulin resistance[15], altered cardiac energy metabolism[16], abnormal left ventricular (LV) structure and impaired diastolic function[17,18]. The duration of these subclinical abnormalities may be important, because treatment to reverse the process is most likely to be effective earlier in the disease. In the present review, we examine the current evidence on the association between NAFLD and subclinical atherosclerosis as well as between NAFLD and cardiac dysfunction in the pediatric population, and discuss briefly the possible biological mechanisms linking NAFLD and cardiovascular changes. We also address the approach to treatment for this increasingly prevalent disease, which is likely to have an important future global impact on the burden of ill health, to prevent not only end-stage liver disease but also cardiovascular disease (CVD).

This is a clinical, narrative review and not a systematic review and meta-analysis. PubMed was extensively searched for articles using keywords and mesh terms: "nonalcoholic fatty liver disease", "fatty liver", "cardiovascular risk", "atherosclerosis", "endothelial dysfunction", arterial stiffness", "cardiac structure", "cardiac dysfunction", and "children".

NAFLD AND MARKERS OF SUBCLINICAL ATHEROSCLEROSIS

Pathologic studies have shown that atherosclerosis is an early process beginning in childhood, with fatty streaks observed in the aorta and the coronary and carotid arteries of children and adolescents[19,20]. Early assessment of the arterial damage is therefore important to prevent future vascular risk since subclinical atherosclerosis can be reversible if detected early and intervention is provided[10].

Several studies have focused on the relation between NAFLD and atherosclerosis in the pediatric population (Table 1)[9,14,21-36]. In the earliest study, involving 817 children (aged 2 to 19 years) who died of external causes (accident, homicide, suicide) from 1993 to 2003, Schwimmer *et al*[21] showed that the prevalence of atherosclerosis was increased by a factor of 2 among those with NAFLD. Fatty liver was present in 15% of the children. Mild atherosclerosis was present in 21% of the children, and moderate to severe atherosclerosis in 2%. Atherosclerosis was significantly more common in children with fatty liver than those without. Body mass index (BMI) was not independently associated with the presence of atherosclerosis, but fatty liver status and BMI did interact significantly ($P < 0.01$). Consequently, for obese subjects, the odds of having atherosclerosis was more than 6 times higher in children with fatty liver than those without[21]. Despite this, there are few data regarding the possible association between liver histopathologic changes and atherogenic risk in children.

Atherogenic dyslipidemia

In the Bogalusa Heart study in children, investigators found that the extent to which the intimal surface was covered with atherosclerotic lesions was significantly associated with elevation of concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), and lower concentration of high-density lipoprotein cholesterol (HDL-c)[37]. In particular, the TC/HDL-c, LDL-c/HDL-c, and TG/HDL-c ratios have been reported as useful markers of atherogenic lipid abnormalities, as well as of insulin resistance, MetS, and high cardiovascular risk[38,39]. Schwimmer *et al*[9] in a case-control study involving a large clinical sample of overweight and obese children and adolescents, showed that children with a biopsy-proven NAFLD had a significantly higher fasting glucose, insulin, TC, LDL-c, TG, systolic and diastolic blood pressure than age-, gender-, and BMI-matched peers without NAFLD. Thus, obese children and adolescents with a definitive diagnosis of NAFLD had a more severe cardiovascular risk profile. Nobili *et al*[27], in a large group of consecutively recruited

Table 1 Studies of the association between nonalcoholic fatty liver disease and markers of atherosclerosis in children and adolescents

Ref.	Study population and sample size	Diagnosis	Outcome	Main results
Schwimmer *et al*[21]	Children (n = 817) who died of external causes from 1993 to 2003; 15% with NAFLD	Autoptic liver biopsy	Atherosclerosis was assessed as absent, mild (aorta only), moderate (coronary artery streaks/plaques), or severe (coronary artery narrowing)	For the entire cohort, mild atherosclerosis was present in 21% and moderate to severe in 2%. Atherosclerosis was significantly more common in children with fatty liver than those without (30% *vs* 19%, $P < 0.001$)
Schwimmer *et al*[9]	Overweight children with (n = 150), and without (n = 150) NAFLD, matched for gender, age, and severity of obesity	Liver biopsy	Prevalence of cardiovascular risk factors (abdominal obesity, dyslipidemia, hypertension, IR, and glucose abnormalities)	NAFLD was strongly associated with multiple cardiovascular risk factors independently of both BMI and hyperinsulinemia
Pacifico *et al*[22]	Obese children with (n = 29), and without NAFLD (n = 33); healthy lean controls (n = 30)	Liver ultrasound	cIMT, mean (95%CI)	NAFLD *vs* no NAFLD and controls: 0.58 (0.54-0.62) mm *vs* 0.49 (0.46-0.52) mm and 0.40 (0.36-0.43) mm; $P < 0.01$ and $P < 0.0005$, respectively Log cIMT was associated with NAFLD severity in a multiple linear regression analysis adjusted for age, gender, Tanner stage, and cardiovascular risk factors Coefficient *b*, 0.08; $P < 0.0005$
Demircioğlu *et al*[23]	Obese children with mild (n = 32), moderate-severe NAFLD (n = 22), and without NAFLD (n = 26); healthy lean controls (n = 30) matched for age and gender	Liver ultrasound	cIMT, mean ± SD	All obese *vs* controls: Left CCA, 0.414 ± 0.071 mm *vs* 0.352 ± 0.054 mm, $P < 0.0001$ Left CB, 0.412 ± 0.067 mm *vs* 0.350 ± 0.058 mm, $P < 0.0001$ Left ICA, 0.324 ± 0.068 mm *vs* 0.266 ± 0.056 mm, $P < 0.0001$ NAFLD was significantly associated with left CCA, CB, ICA in multiple regression linear analyses adjusted for age, gender, weight, mean ALT level, TC, obesity, and grade of hepatosteatosis CCA = standardized β, 0.451; $P = 0.01$ CB = standardized β, 0.627; $P < 0.0001$ ICA = standardized β, 0.501; $P = 0.020$
Kelishadi *et al*[24]	Obese adolescents with (n = 25), and without (n = 25) components of MetS; normal weight adolescents with (n = 25) and without (n = 25) components of MetS	Liver ultrasound and elevated ALT	cIMT, mean ± SD	NWMN *vs* NWMA *vs* POMN *vs* POMA: 0.29 ± 0.02 mm *vs* 0.37 ± 0.04 mm *vs* 0.41 ± 0.05 mm; the differences were significant between groups with the exception of NWMA *vs* POMN cIMT was significantly associated with NAFLD in a logistic regression analysis after adjustment for age, gender and pubertal status Odds ratio, 1.2 (95%CI: 1.03-2.1)
Manco *et al*[25]	Overweight and obese children with (n = 31), and without (n = 49) NAFLD, matched for age, gender, and BMI	Liver biopsy	cIMT, median (IQR)	NAFLD *vs* no NAFLD: Right cIMT, 0.47 (0.07) mm *vs* 0.48 (0.05) mm, $P = 0.659$ Left cIMT, 0.49 (0.12) mm *vs* 0.47 (0.05) mm, $P = 0.039$ NAFLD was not associated with cIMT in a multivariate analysis
Caserta *et al*[26]	Randomly selected adolescents (n = 642) of whom 30.5% and 13.5% were, respectively, overweight and obese. Overall prevalence of NAFLD, 12.5%	Liver ultrasound	cIMT, mean (95%CI)	NAFLD *vs* no NAFLD: 0.417 (0.409-0.425) mm *vs* 0.395 (0.392-0.397) mm, $P < 0.001$ NAFLD was significantly associated with cIMT in a multivariate analysis after adjustment for age, BP, BMI, TG, c-HDL, TC, IR, MetS, grade of steatosis Standardized β, 0.0147 (95%CI: 0.0054-0.0240); $P = 0.002$
Pacifico *et al*[14]	Obese children with (n = 100), and without (n = 150) NAFLD; healthy lean controls (n = 150)	Liver ultrasound and elevated ALT	cIMT and FMD, mean (95%CI)	Controls and no NAFLD *vs* NAFLD: cIMT, 0.47 (0.46-0.48) mm and 0.52 (0.50-0.54) mm *vs* 0.55 (0.53-0.54) mm, $P < 0.0001$ and $P < 0.01$, respectively FMD, 15.0 (13.9-17.3) and 11.8 (10.1-13.7) *vs* 6.7 (5.0-8.6) %, $P < 0.01$ and $P < 0.001$ respectively NAFLD was associated with low FMD and increased cIMT in a multiple logistic regression analysis after adjustment for age, gender, Tanner stage, and MetS Odds ratio, 2.31 (95%CI: 1.35-3.97); $P = 0.002$ and 1.99 (95%CI: 1.18-3.38); $P = 0.010$, respectively
Nobili *et al*[27]	Children with NAFLD (n = 118)	Liver biopsy	Atherogenic lipid profile (TG/HDL-c, TC/HDL-c and LDL-c/HDL-c ratios)	The severity of liver injury was strongly associated with a more atherogenic profile, independently of BMI, insulin resistance, and the presence of MetS

Weghuber *et al*[28]	Obese children with (*n* = 14), and without (*n* = 14) NAFLD	Proton MR spectroscopy	FMD, mean ± SD	NAFLD *vs* no NAFLD: 108.6% ± 11.8% *vs* 110.7% ± 9.0%; *P* = 0.41
El-Koofy *et al*[29]	Overweight/obese children (*n* = 33)	Liver biopsy	Atherogenic lipid profile (TC, LDL-c, HDL-c, TG)	Children with NAFLD had significantly higher TC, LDL-c, TG and lower HDL-c compared to patients with normal liver histology (*P* < 0.05)
Sert *et al*[30]	Obese children with (*n* = 44), and without (*n* = 36) NAFLD; lean subjects (*n* = 37)	Liver ultrasound and elevated ALT	cIMT, mean ± SD	Lean and no NAFLD *vs* NAFLD: 0.0359 ± 0.012 mm *vs* 0.378 ± 0.017 mm *vs* 0.440 ± 0.026 mm, *P* < 0.05 and *P* < 0.05, respectively
Akın *et al*[31]	Obese children with (*n* = 56), and without (*n* = 101) NAFLD	Liver ultrasound	cIMT, mean (95%CI)	NAFLD *vs* no NAFLD: 0.48 (0.47-0.49) mm *vs* 0.45 (0.44-0.45) mm, *P* < 0.001 NAFLD was the only variable associated with increased cIMT in a multiple regression adjusted for age and gender β, 0.031 [SE (β) = 0.008]; *P* < 0.001
Gökçe *et al*[32]	Obese children with (*n* = 50), and without (*n* = 30) NAFLD; healthy lean controls (*n* = 30)	Liver ultrasound	cIMT, mean ± SD	NAFLD *vs* no NAFLD *vs* control group: Right cIMT, 0.46 ± 0.21 mm *vs* 0.35 ± 0.09 mm *vs* 0.30 ± 0.13 mm, *P* < 0.01 Left cIMT, 0.44 ± 0.09 mm *vs* 0.35 ± 0.08 mm *vs* 0.27 ± 0.04 mm, *P* < 0.01 NAFLD was the only variable associated with increased cIMT in a multiple regression adjusted for age, gender, BMI, BP, TG, HDL-c, IR and MetS Right cIMT = β, 0.241; *P* < 0.05 Left cIMT = β, 0.425; *P* < 0.01
Sert *et al*[33]	Obese children with (*n* = 97), and without (*n* = 83) NAFLD; lean subjects (*n* = 68)	Liver ultrasound and elevated ALT	cIMT, mean ± SD	Lean and no NAFLD *vs* NAFLD: 0.354 ± 0.009 mm *vs* 0.383 ± 0.019 mm *vs* 0.437 ± 0.028 mm; *P* < 0.05 and *P* < 0.05, respectively
Alp *et al*[34]	Obese children with (*n* = 93), and without (*n* = 307) NAFLD; healthy lean controls (*n* = 150)	Liver ultrasound	cIMT, mean ± SD	Severe NAFLD *vs* mild NAFLD *vs* no NAFLD *vs* controls: 0.09 ± 0.01 cm *vs* 0.10 ± 0.01 cm *vs* 0.09 ± 0.01 cm *vs* 0.06 ± 0.01 cm, *P* < 0.001
Huang *et al*[35]	Adolescents (*n* = 964)	Liver ultrasound	PWV, mean ± SD	No NAFLD, low metabolic risk *vs* NAFLD, low metabolic risk *vs* no NAFLD, high metabolic risk *vs* NAFLD, high metabolic risk: males, 6.6 ± 0.7 m/s *vs* 6.7 ± 0.6 m/s *vs* 6.9 ± 1.0 m/s; females, 6.2 ± 0.7 m/s *vs* 6.3 ± 0.7 m/s *vs* 6.5 ± 0.7 m/s *vs* 6.4 ± 0.6 m/s Males and females who had NAFLD in the presence of the metabolic cluster had greater PWV b, 0.20 (95%CI: 0.01-0.38); *P* = 0.037
Jin *et al*[36]	Obese children (*n* = 71), and healthy controls (*n* = 47)	Liver ultrasound	PWV, mean ± SD	Obese *vs* controls: 4.54 ± 0.66 m/s *vs* 3.70 ± 0.66 m/s, *P* < 0.001 Fatty liver was positively correlated with PWV (*P* < 0.01)

NAFLD: Nonalcoholic fatty liver disease; IR: Insulin resistance; BMI: Body mass index; cIMT: Carotid intima media thickness; CCA: Common carotid artery; CB: Carotid bulbus; ICA: Internal carotid artery; ALT: Alanine aminotransferase; TC: Total cholesterol; MetS: Metabolic syndrome; NWMN: Normal weight metabolically normal; NWMA: Normal weight metabolically abnormal; POMN: Phenotipically obese metabolically normal; POMA: Phenotipically obese metabolically abnormal; IQR: Interquartile range; BP: Blood pressure; TG: Triglycerides; FMD: Flow-mediated dilation; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; MR: Magnetic resonance; PWV: Pulse wave velocity.

children with liver biopsy-proven NAFLD, found that the NAFLD activity and fibrosis scores had significant positive correlations with TG/HDL-c, TC/HDL-c, and LDL-c/HDL-c ratios. After adjusting for potential confounders including BMI, insulin resistance, impaired glucose tolerance, and presence of MetS, both NAFLD activity score and stage of fibrosis remained independent predictors of an atherogenic lipid profile. The lipid ratios were found to be markedly higher in children with established NASH compared with those with simple steatosis or borderline disease, indicating that severity of liver injury in children with NAFLD is strongly associated with increased atherogenic risk. Very recently, El-Koofy *et al*[29] studied the prevalence of MetS, insulin resistance, and NAFLD in a small group of overweight/obese children presenting with hepatomegaly and/or raised alanine aminotransferase. Laboratory analysis included fasting blood glucose, serum insulin, TG, HDL-c, LDL-c and liver biochemical profile in addition to liver biopsy. They found a close association between obesity, MetS, insulin resistance and NAFLD. Children with NAFLD had significantly higher TC, LDL-c, TG, fasting insulin, and lower HDL-c

compared to patients with normal liver histology.

Vascular imaging

Recent improvements in imaging technology have identified early vascular changes that can be assessed noninvasively using ultrasonography[40-42]. These early changes include impairment of FMD, arterial stiffness, and increased cIMT. Measurement of these changes have been shown to be good surrogate markers for atherosclerosis disease identification and progression as well as of future clinical cardiovascular events[43,44].

Carotid intima-media thickness and endothelial dysfunction

Pacifico *et al*[22] first showed that severity of ultrasonographycally diagnosed NAFLD in obese children was significantly associated with cIMT, independently of anthropometric and metabolic features. Similarly, Demircioğlu *et al*[23], in a subsequent study, observed an association between ultrasonographically detected NAFLD and cIMT measured at the common artery, carotid bulb and internal carotid artery. Moreover, there was an increase in cIMT values of each segment with the increase in grades of hepatosteatosis. Kelishadi *et al*[24] reported a significant association between cIMT and NAFLD in children and adolescents, suggesting that the liver and the vessels share common mediators. In a case-control study involving a mixed population of 80 overweight and mildly obese children of whom 31 had biopsy-proven NAFLD, and 49 had no ultrasound evidence of NAFLD as well as no abnormal levels of aminotransferases, Manco *et al*[25] found that cIMT were significantly higher on the left side in NAFLD cases, though there was a substantial overlap of cIMT values between cases and controls. No association was found between cIMT and histologic severity of steatosis, NAFLD activity score, and fibrosis.

The association between NAFLD and carotid atherosclerosis has also been determined in a large, randomly selected adolescent population from Reggio Calabria, a town in southern Italy[26]. The authors found that NAFLD, as well as BMI, waist circumference, and systolic blood pressure were independent markers of increased cIMT. In a study involving a large sample size, Pacifico *et al*[14] showed that obese children with ultrasound-diagnosed NAFLD have a significantly lower FMD response and increased cIMT compared to obese children without NAFLD independently of other cardiovascular risk factors and MetS, and that obese children exibit more functional and morphologic vascular changes than healthy lean controls, regardless of liver involvement. The large number of subjects in that study may in part account for the associations the authors were able to identify between NAFLD and functional vascular changes, in contrast to the study by Weghuber *et al*[28] in which a very small sample of obese children with NAFLD [diagnosed by nuclear magnetic resonance spectroscopy (MRS)] had a FMD response similar to those without NAFLD. In a recent study, Akın *et al*[31] demonstrated that obese children and adolescents

with ultrasonographycally detected NAFLD had higher cIMT than those without NAFLD regardless of association with elevated liver enzymes. Moreover, there was a statistically significant correlation between cIMT values and the grade of NAFLD. Similar results were obtained by Gökçe *et al*[32] and by Sert *et al*[30,33] in obese children with ultrasound diagnosed NAFLD. Gökçe *et al*[32] found that cIMT was significantly higher in obese with NAFLD than in obese children without NAFLD and in a control group. After adjusting for potential confounders (age, blood pressure, BMI, TG, HDL-c, insulin resistance, and MetS), NAFLD was observed to be strongly correlated with cIMT. Sert and colleagues[30,33] confirmed that children with NAFLD have significantly higher cIMT values than those without NAFLD and the lean group. Components of MetS, such as dyslipidemia, elevated fasting glucose levels and insulin resistance did not show a significant association with increased cIMT. Finally, in a very recent study, Alp *et al*[34] demonstrated that cIMT was significantly higher in children with ultrasonographycally detected NAFLD than the control subjects. Additionally, there was an increase in cIMT values with the increase in grades of liver steatosis.

Arterial stiffness

A few pediatric studies have analyzed the relationship between NAFLD and carotid artery stiffness. In a well-defined community-based cohort of Australian adolescents, Huang *et al*[35] aimed to examine the association between NAFLD (diagnosed by ultrasound), MetS, and arterial stiffness as measured by applanation tonometry. The enrolled subjects were identified at "high metabolic risk" and at "low metabolic risk" according to systolic blood pressure, homeostasis model assessment of insulin resistance (HOMA-IR), TG, and BMI. The authors found that NAFLD is associated with increased arterial stiffness only in the presence of the "high risk" metabolic cluster. Jin *et al*[36], in an attempt to identify a marker of early vascular functional change in obese children, compared carotid artery stiffness parameters (*i.e.*, compliance coefficient, stiffness index, and pulse wave velocity, obtained with ultrasound radiofrequency technology) in obese children and healthy controls. Arterial stiffness was higher in obese children than in healthy subjects; in addition the authors demonstrated that in obese children the carotid arterial stiffness parameters, especially the pulse wave velocity, were correlated with obesity-related risk factors, the systolic blood pressure, and the presence of fatty liver.

NAFLD AND CARDIAC DYSFUNCTION

Information regarding abnormalities in cardiac function among NAFLD patients is limited in both adults and children. Moreover, the data are conflicting.

Adult studies

Table 2 summarizes the studies on the effects of NAFLD on

cardiac metabolism, structure and function in adults[15-17,45-50]. Goland *et al*[17] have shown a markedly impaired diastolic function and mild alterations in LV structure in 38 adult patients with (ultrasound-diagnosed, *n* = 27; biopsy-proven, *n* = 11) NAFLD, in the absence of diabetes, hypertension, and morbid obesity. Using multivariate analysis, early diastolic myocardial velocity on tissue Doppler imaging (TDI) was the only independent index able to characterize patients with NAFLD. Similar findings have been later reported by Fotbolcu *et al*[18] in 35 non-diabetic, normotensive adult patients with ultrasound-diagnosed NAFLD. However, independent predictors of LV impairment were not determined. Recently, in a study examining cardiac status by high resolution magnetic resonance imaging (MRI) in a clinical group of 19 adult patients with NAFLD (defined as > 5% intrahepatic lipid on MRS), Hallsworth *et al*[49] demonstrated significant changes in cardiac structure and evidence of early diastolic dysfunction in the 19 patients with NAFLD compared to the 19 age-, gender-, and BMI-matched controls, in the absence of cardiac metabolic changes or overt cardiac disease. There was no correlation between blood pressure and cardiac parameters. Finally, Karabay *et al*[50] found that patients with biopsy-proven NAFLD have evidence of subclinical myocardial dysfunction. However, no significant differences were found among NAFLD groups (*i.e.*, simple steatosis, borderline NASH, and definite NASH). The absence of differences in cardiac function between subgroup patients may be explained by similar HOMA-IR values. Conversely, Perseghin *et al*[16] showed that 21 men with higher intrahepatic fat content, as measured by MRS, had excessive fat accumulation in the epicardial area and abnormal LV energy metabolism compared to the 21 men matched for anthropometric features with lower intrahepatic fat content. These alterations were detected despite normal LV morphology and function by cardiac MRI. Similarly, in a study using cardiac MRI and involving 61 diabetic male subjects, Rijzewijk *et al*[46] found that, compared with the 29 men with lower intrahepatic fat content on MRS, the 32 patients with higher intrahepatic fat content had decreased myocardial perfusion, glucose uptake, and high-energy phosphate metabolism but similar values of LV function and morphology.

Pediatric studies

In the pediatric population, information on the relationship between NAFLD and cardiac structure and function is very scant (Table 3)[30,33,34,51-53]. In the earliest study by Sert *et al*[30], increased LV mass was found in adolescents with NAFLD compared to both lean controls and obese subjects without liver involvement. Similar results were obtained in a subsequent study by the same authors[33]. In a study including 93 obese children with ultrasound-diagnosed NAFLD, 307 obese subjects without liver involvement, and 150 age- and gender-matched healthy controls, Alp *et al*[34] showed that subclinical systolic and diastolic impairment could be detected by TDI in obese children with NAFLD. Also, cardiac dysfunction was

correlated with the increase in grades of liver steatosis. Recently, Singh *et al*[51] measured by 2-dimensional speckle tracking echocardiography myocardial function in 3 groups of age-, gender-, and Tanner stage-matched adolescents [lean (*n* = 14); obese with normal (*n* = 15) or increased (*n* = 15) intrahepatic triglyceride (IHTG) content (≥ 5.6%)]. The authors showed that obese adolescents with increased IHTG had greater impairment of systolic and diastolic function, manifested by decreased systolic and diastolic myocardial strain and strain rate than BMI-SD score matched obese adolescents with normal IHTG content. The cardiac functional abnormalities were independently associated only with HOMA-IR, after adjustment for BMI, conventional cardiovascular risk factors, and intra-abdominal, intra-cardiac, and intra-hepatic fat content. However, given the small number of adolescents included in their study, the possibility of a type 2 error raised by the authors themselves is possible. In a more recent study, Pacifico *et al*[52] showed that obese children with NAFLD have features of early LV diastolic and systolic dysfunction, as measured by two-dimensional echocardiography using TDI, compared to obese children without NAFLD and lean controls. Notably, when the group of obese subjects was divided according to the presence of NASH, it was evident that some functional cardiac differences were more pronounced in the group of NASH. A major finding of this study was that the echocardiographic features of early LV diastolic and systolic dysfunction were significantly associated with NAFLD independently of several metabolic variables.

PATHOGENESIS

The pathophysiological mechanisms of CVD in NAFLD are still poorly understood. Probably they involve insulin resistance, an abnormal ectopic fat storage as well as atherogenic dyslipidemia, and a low-grade inflammatory state in the presence of genetic susceptibility (Figure 1).

Insulin resistance

The expansion and inflammation of visceral adipose tissue mass, with consequent release of multiple molecules, is one of the earliest steps in the chain of events involved in the development of insulin resistance and NAFLD, as well as atherosclerosis, including free fatty acids (FFAs), hormones and proinflammatory adipocytokines, particularly in obese and overweight persons[54-57]. In this situation, the liver might function both as the target organ and the source of the resulting systemic abnormalities that can promote increased risk of CVD. Increased insulin resistance occurs when the advanced forms of NAFLD develop, which potentially sets up a vicious cycle of insulin resistance, increased influx of FFAs into the liver and increased hepatic steatosis[11]. In turn, hepatic steatosis (Figure 2A) leads to subacute intrahepatic inflammation through activation of nuclear factor-κB (NF-κB) pathways (Figure 2B) that exacerbate insulin resistance both locally in the liver and systematically including

Table 2 Studies of the association between nonalcoholic fatty liver disease and alterations in cardiac structure and function in the adult population

Ref.	Study population and sample size	Diagnosis	Outcomes	Main results
Lautamäki *et al*[15]	T2DM and coronary artery disease patients with (*n* = 27), and without (*n* = 28) fatty liver. The 2 groups were matched for age, BMI, and fasting plasma glucose	Hepatic MRS	Myocardial insulin resistance and perfusion (PET)	In patients with T2DM and coronary artery disease, liver fat is an indicator of myocardial insulin resistance and reduced coronary functional capacity
Goland *et al*[17]	Nondiabetic, normotensive patients with NAFLD (*n* = 38), and age and gender-matched controls (*n* = 25)	Liver ultrasound and liver biopsy in a subgroup of 11 NAFLD patients	LV structure and function (M-mode echocardiography; and pulsed Doppler echocardiography)	Patients with NAFLD had mild changes in cardiac geometry (thickening of the interventricular septum and posterior wall, and increased LV mass) as well as significant differences in parameters of diastolic function compared with the control group
Perseghin *et al*[16]	Young nondiabetic men matched for anthropometric features with (*n* = 21) or without (*n* = 21) fatty liver	MRS	LV morphology and function; Intrapericardial and extrapericardial fat content; and resting LV energy metabolism (Cardiac MRI and cardiac ^{31}P-MRS)	Newly found young individuals with fatty liver had excessive fat accumulation in the epicardial area and abnormal LV energy metabolism despite normal LV morphological features and systolic and diastolic functions
Fallo *et al*[45]	Never-treated essential hypertensive patients with (*n* = 48) or without (*n* = 38) fatty liver. The 2 groups were similar as to gender, age and blood pressure levels	Liver ultrasound	LV structure and function (M-mode echocardiography; and pulsed Doppler echocardiography)	NAFLD patients had similar prevalence of LV hypertrophy compared to subjects without NAFLD, but a higher prevalence of LV diastolic dysfunction
Rijzewijk *et al*[46]	T2DM patients with (*n* = 32) and without (*n* = 29) fatty liver	MRS	Cardiac perfusion and substrate metabolism; LV morphology and function (PET, cardiac MRI and cardiac ^{31}P-MRS)	T2DM patients with fatty liver showed decreased myocardial perfusion, glucose uptake, high-energy phosphate metabolism compared with similar patients without hepatic steatosis
Fotbolcu *et al*[18]	Nondiabetic, normotensive patients with NAFLD (*n* = 35) and control subjects (*n* = 30). The 2 groups were similar as to gender and age	Liver ultrasound	LV structure and function (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography)	Patients with NAFLD had changes in cardiac geometry (thickening of the interventricular septum and posterior wall, and increased LV mass) as well as significant differences in parameters of systolic and diastolic function compared with the control group
Bonapace *et al*[47]	T2DM patients with (n = 32) and without (n = 18) fatty liver. The 2 groups were similar as to gender, age, BMI, waist circumference, and diabetes duration	Liver ultrasound	LV structure and function (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography)	T2DM patients with fatty liver showed LV diastolic dysfunction, even if the LV morphology and systolic function were preserved
Mantovani *et al*[48]	Hypertensive T2DM patients with (*n* = 59) and without (*n* = 57) fatty liver	Liver ultrasound	LV structure (M-mode echocardiography)	Hypertensive T2DM patients with NAFLD have a remarkably higher frequency of LV hypertrophy than hypertensive diabetic patients without NAFLD
Hallsworth *et al*[49]	Adult subjects matched for anthropometric features with (*n* = 19) or without (*n* = 19) fatty liver	MRS	Cardiac structure, function, and metabolism (cardiac MRI, cardiac tagging, and cardiac ^{31}P-MRS)	The major findings in NAFLD patients compared to controls were: thickening of the cardiac wall, independent of changes in LV mass; altered myocardial strains; concentric remodeling; evidence of diastolic dysfunction; but no significant difference in cardiac energetics
Karabay *et al*[50]	NAFLD patients (*n* = 55) and healthy controls (*n* = 21; normal laboratory values and liver ultrasound)	Liver biopsy	LV structure and function (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography; and speckle tracking echocardiography)	Patients with NAFLD had changes in cardiac geometry (thickening of the interventricular septum and posterior wall, and increased LV mass) as well as significant differences in parameters of diastolic function compared with the control group LV global longitudinal strain and strain rate in systole were lower in NAFLD group as compared to controls; however no significant differences were found among NAFLD groups (*i.e.*, simple steatosis, borderline NASH, and definite NASH)

T2DM: Type 2 diabetes mellitus; BMI: Body mass index; MRS: Magnetic resonance spectroscopy; PET: Positron emission tomography; NAFLD: Nonalcoholic fatty liver disease; LV: Left ventricular; NASH: Nonalcoholic steatohepatitis.

Table 3 Studies of the association between nonalcoholic fatty liver disease and alterations in cardiac structure and function in children and adolescents

Ref.	Study population and sample size	Diagnosis	Outcomes	Main results
Sert *et al*[30]	Obese adolescents with (*n* = 44), and without (*n* = 36) NAFLD; and control subjects (*n* = 37)	Liver ultrasound and elevated serum alanine aminotransferase	LV structure (M-mode echocardiography)	Increased LV mass was found in NAFLD group compared to both lean controls and obese subjects without NAFLD
Alp *et al*[34]	Obese children and adolescents with (*n* = 93), and without (*n* = 307) NAFLD matched for gender and age; and control subjects (*n* = 150)	Liver ultrasound	LV structure and function; Epicardial fat (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography)	Increased end-systolic thickness of the interventricular septum, and larger LV mass, as well as LV systolic and diastolic dysfunction were found in NAFLD group. In addition, obese children with NAFLD had increased epicardial fat thickness
Singh *et al*[51]	Obese children and adolescents with (*n* = 15), and without (*n* = 15) NAFLD matched for age, gender, Tanner stage, and BMI z score; and control subjects (*n* = 15) matched for gender, age, and Tanner stage	Hepatic MRS	LV structure and function; Intracardiac triglyceride content (Integrated backscatter ultrasonography and speckle tracking echocardiography; cardiac MRS)	LV global longitudinal strain and early diastolic strain rates were significantly decreased in obese children with NAFLD compared to both lean controls and obese subjects without NAFLD. Intracardiac triglyceride content was not different among the 3 groups
Sert *et al*[33]	Obese adolescents with (*n* = 97), and without (*n* = 83) NAFLD; and control subjects (*n* = 68)	Liver ultrasound and elevated serum alanine aminotransferase	LV structure and function (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography)	Obese adolescents with NAFLD exhibited increased LV dimensions and mass, as well as LV diastolic dysfunction
Pacifico *et al*[52]	Obese children and adolescents with (*n* = 54), and without (*n* = 54) NAFLD matched for age, gender, pubertal status, and BMI-SD score; and healthy control subjects (*n* = 18) matched for gender, age, and pubertal status	Hepatic magnetic resonance imaging; and liver biopsy in a subgroup of 41 NAFLD patients	LV structure and function; Epicardial fat (M-mode echocardiography; Pulsed and Tissue Doppler echocardiography)	Increased interventricular septum thickness at end-diastole and at end-systole, as well as LV systolic and diastolic dysfunction were found in NAFLD group. Children with more severe liver histology had worse LV dysfunction than those with more mild liver changes. NAFLD group had also increased epicardial fat thickness
Fintini *et al*[53]	Children with biopsy-proven NAFLD (*n* = 50). No patients without NAFLD, and no healthy control children were included	Liver biopsy	LV structure and function (M-mode echocardiography; and pulsed Doppler echocardiography)	About 35% (*n* = 18) of the 50 children with NAFLD had LV hypertrophy. Children with NASH showed, almost invariably, the presence of clear cut LV hypertrophy

NAFLD: Nonalcoholic fatty liver disease; LV: Left ventricular; BMI: Body mass index; MRS: Magnetic resonance spectroscopy; NASH: Nonalcoholic steatohepatitis.

cardiac insulin resistance[55-58]. Indeed, Lautamäki *et al*[15] demonstrated that, in patients with type 2 diabetes and coronary artery disease, liver fat content was an independent indicator of myocardial insulin resistance and reduced coronary functional capacity. The consequences of insulin resistance in the heart are incompletely understood. Using genetically engineered mice with deletion of insulin receptors in cardiomyocites, Boudina *et al*[59] have shown that impaired myocardial insulin signaling leads to multiple mitochondrial defects that include reduced oxygen consumption and adenosine triphosphate synthesis, reduced levels of mitochondrial enzymes that regulate pyruvate and fatty acid metabolism, and decreased content of citric acid cycle proteins. Insulin signaling also regulates the expression of genes such as peroxisome proliferator-activated receptor-α in the heart, which controls the capacity of mitochondria to oxidize fatty acids. In addition, mitochondria from hearts with defective insulin signaling demonstrate evidence of oxidative stress. These mechanisms could potentially contribute to myocardial dysfunction when the heart becomes insulin resistant.

Abnormal ectopic fat storage and atherogenic dyslipidemia

In patients with NAFLD, the increased FFAs may induce myocardial lipid accumulation, which is detrimental to LV function[60-63]. In fact, myocardial steatosis may cause alterations in myocardial substrate metabolism and efficiency (cardiac work/myocardial oxygen consumption) that occur early in the cascade of events leading to impaired LV contractility. Rijzewijk *et al*[46] showed that intramyocardial fat content, as detected by ^{1}H-MRS was significantly higher in uncomplicated type 2 diabetic men than in nondiabetic control subjects and was associated with impaired cardiac metabolism[46]. Moreover, using cardiac MRI and ^{31}P-MRS, Perseghin *et al*[16] demonstrated that individuals with fatty liver had an increased amount of fat in the epicardial area and displayed abnormal cardiac metabolism. Epicardial fat is a metabolically active organ that generates proatherogenic, proinflammatory and prothrombotic adipo-cytokines[64-67]. Its anatomic location, without any barrier to the adjacent myocardium, enables local paracrine interaction between epicardial fat and the myocardium[64]. Thus, epicardial
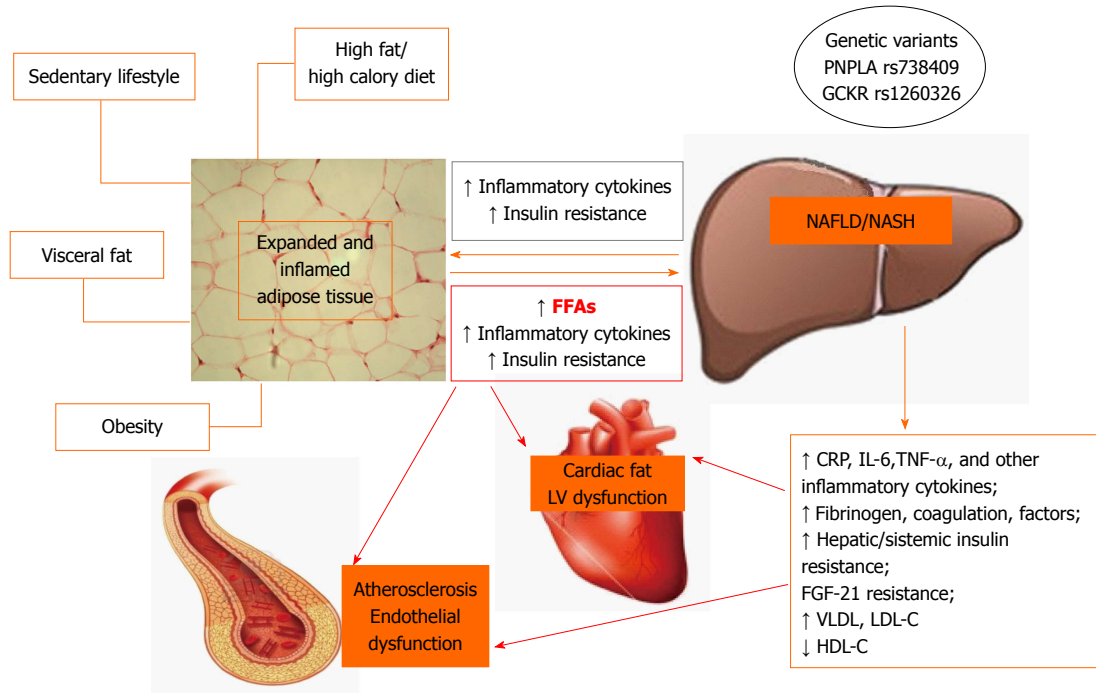
Figure 1 Suggested pathophysiological mechanisms linking nonalcoholic fatty liver disease to atherosclerosis and cardiac abnormalities in obese subjects. FFAs: Fatty free acids; CRP: C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; FGF-21: Fibroblast growth factor-21; VLDL: Very low density lipoprotein; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

and myocardial fat represent abnormal ectopic fat storage and may be a marker of the cumulative effects of NAFLD and insulin resistance in the setting of pathological adiposity, with consequent adverse associated cardiovascular outcome[65,68].

Cardiac lipotoxicity is a well-described phenomenon in insulin resistance, and is generally attributed to products of FFA excess metabolism[62,69]. Ceramide is a sphingolipid that is a key mediator of cellular stress pathways that induce apoptosis and mitochondrial dysfunction. In normal physiology, ceramide is derived from *de novo* synthesis or can be derived from sphingomyelin hydrolysis. Ceramide acts as a lipotoxic intermediate when it builds up as a result of elevated circulating FFAs. Structural alterations in mitochondria can reduce cardiac function by providing an insufficient supply of ATP to cardiac myocytes or by increasing reactive oxygen species production, which has been associated with increased apoptosis, DNA damage, and DNA repair[70]. Recently, it has been shown that ceramide also plays an important role in the pathogenesis of obesity-mediated vascular dysfunction *via* a mechanism that involves protein phosphatase 2A-mediated dephosphorylation of nitric oxide synthase III[71]. In addition, the treatment of mice with lipotoxic cardiomyopathy with the inhibitor of ceramide synthesis myriocin reversed contractile dysfunction in a mouse model of lipotoxic cardiomyopathy[72]. Taken together, it is reasonable that ceramide accumulation may contribute to the pathogenesis of cardiac and vascular dysfunction in insulin-resistant states.

NAFLD is also characterized by an atherogenic lipid profile, consisting of high TG levels, low HDL-c, an increase in small, dense LDL-c particles, increased very low-density lipoprotein (VLDL) cholesterol levels and elevated apolipoprotein B100 concentration[58]. This type of atherogenic dyslipidemia is strongly linked to adverse cardiovascular outcome[37,58]. The increased hepatic production of TG-rich VLDL provides a limited compensatory mechanism for IHTG[58,73]. However, this also results in abnormal HDL-c metabolism causing HDL-c reduction as well as compositional alterations. In fact, the amount of liver fat has a significant negative correlation with subfractions of HDL-c known to be antiatherogenic, which are reduced in NAFLD independently of peripheral insulin sensitivity[74].

Inflammation

In the presence of increased FFA flux and chronic, low grade inflammation, the liver is again both the target of and a contributor to systemic inflammatory changes. The steatotic and inflamed liver releases several mediators including C reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and other inflammatory cytokines that amplify the systemic, low-grade inflammation[11,55-57]. Increased intrahepatic cytokine expression results from local activation of the NF-κB pathway (Figure 2B), as mediated by hepatocellular damage and fat-derived factors, and is likely to play a key role in the progression of NAFLD and CVD[9,55-57]. Several studies have shown that a number of genes involved in fatty acid metabolism, lipolysis, monocyte and macrophage recruitment, coagulation, and inflammation are overexpressed in pa-
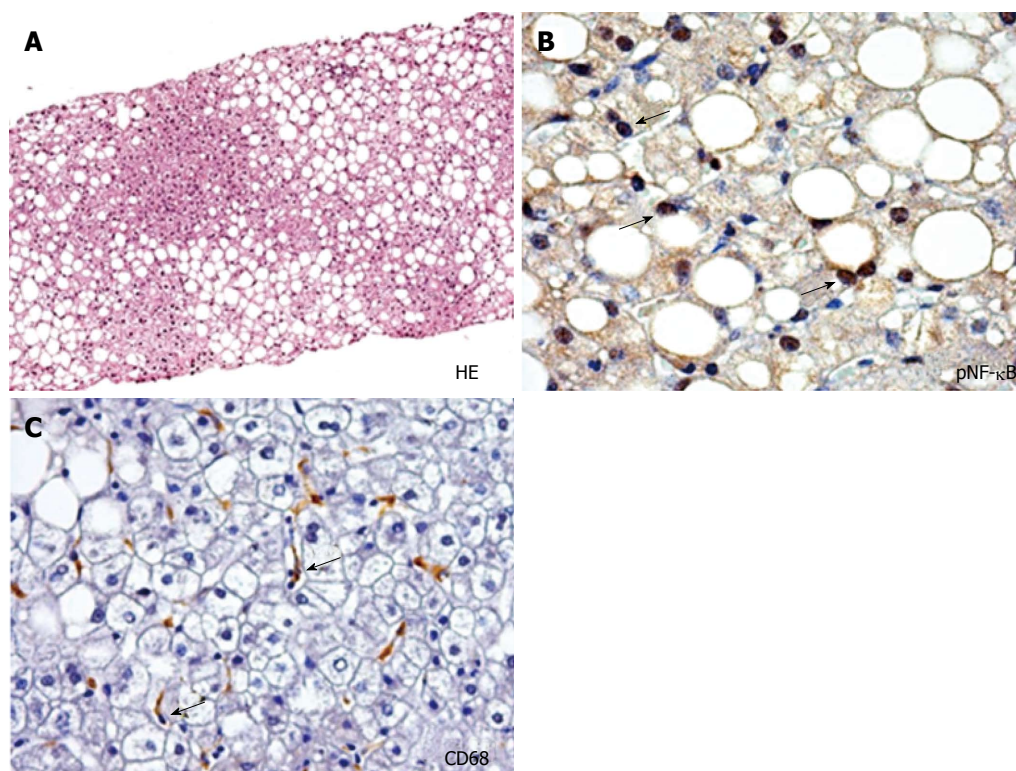
Figure 2 Histological and immunohistochemical features of nonalcoholic fatty liver disease. A: Hematoxylin-Eosin (HE) in nonalcoholic fatty liver disease (NAFLD) biopsy shows hepatic steatosis (fatty liver). Original Magnification: 10 ×; B: Immunohistochemistry for phosphorylated (p) nuclear factor κB shows the nuclear expression by hepatocytes in NAFLD (arrows). Original Magnification: 40 ×; C: Macrophages in NAFLD. Immunohistochemistry for CD68 shows the presence of macrophages in nonalcoholic steatohepatitis (yellow arrows). Original Magnification: 40 ×. Photos were obtained from a liver biopsy of a 60-year-old male affected by NAFLD. Photos are original and taken in Prof. Gaudio's Laboratory.

tients with NAFLD[75,76]. In particular, NASH presents a distinct panel of regulatory genes which are dysregulated compared to controls and subjects with simple steatosis. Indeed, circulating levels of several inflammatory markers (CRP, IL-6, monocyte chemotactic protein 1, and TNF-α), procoagulant factors (plasminogen activator inhibitor 1, fibrinogen, and factor VII), and oxidative stress markers are highest in patients with NASH, intermediate in those with simple steatosis, and lowest in control subjects without steatosis, and the differences are independent of obesity and other potentially confounding factors[76,77].

There is much evidence to suggest that macrophage infiltration (Figure 2C) could play an essential role in the pathogenesis of NAFLD and atherosclerosis by communicating inflammatory signals by scavenging modified lipids[78]. In this light, the systemic inflammation, which is exacerbated by steatohepatitis, could have a dual role on the progression of atherosclerotic plaque and steatohepatitis. Macrophages were the first inflammatory cells to be associated with atherosclerosis[79]; recently, the process of macrophage polarization has been a subject of interest[80,81]. Two distinct modes of macrophage activation were proposed to differentiate between inflammatory M1 and anti-inflammatory M2 macrophages[82]. M1-macrophages exert definitive pro-inflammatory roles and M1-derived cytokines may be involved in further activating myofibroblasts and fibrogenetic cells; M2-macrophages have been described as wound-healing, based on their ability to promote wound healing through matrix remodeling and the recruitment of fibroblasts[83]. The process of macrophage polarization during atherogenesis has been a subject of interest as macrophage subsets have been demonstrated to display some degree of plasticity and heterogeneity within atherosclerotic lesions[80,81]. In parallel, in NAFLD, the exacerbated release of M1 macrophages derived mediators contributes to the pathogenesis of several liver lesions, namely hepatocyte steatosis and apoptosis, inflammatory cell recruitment, and activation of fibrogenesis[84,85].

Adipocyte-derived hormones

The term "adipokines" (adipose tissue cytokines) comprises polypeptide factors which are expressed significantly, although not exclusively, by adipose tissue in a regulated manner. Besides adipocytes, accounting for one-third of the cells, adipose tissue is composed of stromal cells, including macrophages, fibroblasts, and infiltrating monocytes, all of which contribute to adipokine production[78,86]. The major adipokines (leptin, adiponectin, resistin) exert several metabolic actions and have a role in cellular and animal models of liver injury[87]. Leptin has several immune and metabolic functions. Obesity is associated with high circulating leptin levels and leptin resistance in the central nervous system as leptin fails to correct hyperglycemia in patients with obesity[86]. Several

in vitro and *in vivo* studies have identified a close connection between leptin and liver fibrosis. These show that leptin modulates the biology of different cell types participating in the response to liver injury, such as Kupffer cells, sinusoidal endothelial cells, and myofibroblast-like cells[88]. Leptin is also a potential mediator of cardiac hypertrophy in obesity, possibly by causing an increase in sympathetic vasoconstrictor tone and arterial blood pressure, or through direct stimulation of protein synthesis in cardiomyocytes[89].

Adiponectin exerts insulin-sensitizing effects in the liver, skeletal muscle, and adipose tissue. Adiponectin improves insulin signaling and profoundly affects glucose metabolism. Adiponectin and leptin have divergent effects on inflammation; adiponectin reduces inflammation, stimulating secretion of anti-inflammatory cytokines (*i.e.*, IL-10), and inhibiting release of TNF-α, IL-6, and chemokines[86,87]. Resistin may represent a link between obesity and insulin resistance; its action determinates reduction of peripheral insulin sensitivity, increase in endogenous glucose production by the liver, induction of insulin resistance and stimulation of proinflammatory cytokines (*i.e.*, IL-6 and TNF-α)[87].

Recently, hepatocytes and hepatic stem/progenitor cells (HPC) have been indicated as a source of adiponectin and resistin in the course of NAFLD[90,91]. In NASH, the expression of adiponectin by liver parenchymal cells (hepatocytes and HPCs) was down-regulated and it was inversely correlated with steatohepatitis grade. This is in agreement with the current understanding of this adipokine. In fact, adiponectin has anti-inflammatory and anti-fibrogenic properties and, in steatotic liver, has been shown to ameliorate necroinflammation and steatosis when administered in experimental NASH[87,92]. On the other hand, HPCs up-regulated their expression of resistin in correlation with progression towards NASH and fibrosis[93]. Several lines of evidence link the biology of resistin with hepatic inflammation, fibrogenesis and macrophage polarization. In rats, resistin administration significantly worsens inflammation after lipopolysaccharide injection[94], and activates fibrogenetic cells through the activation of NF-κB pathway[93,94]. Moreover, hepatic resistin expression increases in NASH; is correlated with inflammatory cell infiltration, and has been associated with macrophage recruitment within the liver[91].

Widespread research has been conducted on the relationship of adiponectin, as well as of resistin, with cardiovascular risk. Adiponectin exerts a protective effect against endothelial dysfunction induced by advanced glycation end-products[95]. This process is, in part, mediated by a decrease in the expression of adhesion molecules, and provides evidence of the protective role of adiponectin in the pathogenesis of the vascular complications of obesity/MetS. Low adiponectin levels may impair the ability of the heart to adapt to acute and chronic stress, as suggested by studies of adiponectin deficiency in mice[61]. On the other hand, resistin can act as an effector molecule that leads to an atherosclerotic state, possibly through several mechanisms. It has been shown that resistin has direct effects on endothelial cell activation by inducing the expression of cell adhesion molecules, thereby enhancing leukocyte adhesion[96,97]. Previous "*in vitro*" experimental studies on endothelial cells and atherosclerotic plaque progression also showed that resistin can impair endothelium-dependent relaxation, promote angiogenesis[98], and induce vascular inflammation[99]. An increase in resistin concentration significantly decreases endothelial nitric oxide synthase expression and nitric oxide production through oxidative stress in cultured human coronary artery endothelial cells[100], suggesting that the effects of resistin can be mediated by oxidative stress. However, the precise role of resistin in the clinical scenario remains to be fully elucidated.

Fibroblast growth factor-21 (FGF-21) has emerged as important endocrine factor involved in glucose and lipid metabolism and energy regulation[101]. FGF-21 is primarily expressed by liver, adipose tissue, and pancreas. FGF-21 stimulates glucose uptake in adipocytes and regulates energy metabolism and enhanced mitochondrial oxidative function through the activation of AMP-activated protein kinase and sirtuin 1[101]. FGF-21 has a hepato-protective action, but subjects with NAFLD show a condition of "FGF-21 resistance", which worsens in subjects with NASH[102]. A recent study has demonstrated that FGF-21 knockout mice exhibit an increased relative heart weight and develop enhanced signs of dilation and cardiac dysfunction[103]. In addition, *in vitro* treatment of cardiomyocytes with FGF-21 reverses these cardiac alterations[103]. Thus, FGF-21 resistance or reduced levels observed in subjects with NAFLD/NASH, might play a role in cardiac anatomic and functional abnormalities.

Genetic factors

In recent years, genetic studies have highlighted several single nucleotide polymorphisms (SNPs) that may characterize children with a high risk for NAFLD development and progression[104-110]. In particular, a common missense variant (rs738409), characterized by a C-to-G substitution encoding an isoleucine-to-methionine substitution at amino acid position 148 (I148M), in the patatin-like phospholipase 3 (*PNPLA3*) gene has been associated not only with hepatic fat content and increased serum liver enzymes but also with increased risk of NASH and fibrosis progression[104,111-114]. The I148M PNPLA3 variant influences liver triglyceride content without apparently affecting body mass, serum lipid levels and systemic insulin resistance[113,115]. The association between I148M variant and both liver enzymes and steatosis has been confirmed in obese children of different ethnicities[111,112,116,117]. More recently, a SNP (rs1260326) in the glucokinase regulatory protein (*GCKR*) gene has been associated with fatty liver and with higher serum triglycerides and large VLDL levels in obese children and adolescents[109]. This association was independent of ethnicity, age, gender, z-score BMI,

and glucose tolerance[109].

PREVENTION AND TREATMENT

Because of the limited knowledge of the molecular pathogenesis of NAFLD, the current therapies consist of strategies aimed at decreasing the incidence of the known risk factors. Prevention and control of modifiable risk factors such as overweight and unhealthy lifestyle can have an impact on the overall health of children and adolescents as well as on the prevention and control of pediatric NAFLD and the related MetS[2,3,10]. Lifestyle changes and pharmacological treatment of pediatric NAFLD have extensively been discussed elsewhere[2,3,10]. However, it is not known how treatment of NAFLD modulates the risk of CVD.

It has been established that preclinical atherosclerosis is not an irreversible but rather a dynamic process. Different interventions on cardiovascular risk factors (dyslipidemia, hypertension, diabetes mellitus, and obesity) have been shown to slow or even regress the progression of atherosclerosis[118-123]. However, data on the reversibility of subclinical atherosclerotic markers in children with NAFLD are scant. In a study evaluating a 1-year intervention program with diet and physical exercise in children and adolescents with NAFLD, Pacifico *et al*[124] showed favorable changes in vascular function as estimated by FMD. In the same study, the authors failed to demonstrate a significant regression of cIMT, though there was a trend after the lifestyle intervention. Studies in adults and children have shown that lifestyle interventions can halt the progression of cIMT[118-120,122,123], but others have shown no such effect[125,126]. Possible reasons for such conflicting results include the age of population (prepubertal children, adolescents, or adults), the type of population (healthy, otherwise healthy obese, or obese subjects with obesity related comorbidities), the length of intervention, the type and intensity of lifestyle intervention, the degree of weight and visceral fat loss, and different analyses of cIMT measurements (*i.e.*, maximum or mean value of cIMT). In this context, of great interest is the study by Koskinen *et al*[127] who showed that in young adults recovery from the MetS was associated with reduced cIMT progression during a 6-year follow-up period. Thus, it is possible that a longer lifestyle intervention may be necessary to regress cIMT in children with NAFLD. Of note, Pacifico *et al*[124] demonstrated that higher cIMT values in obese children with fatty liver as well as in those with MetS were related to impaired brachial FMD, supporting the idea that endothelial dysfunction is a necessary step before the development of structural arterial disease. The restoration of FMD observed in such patients might be the initial step to halt the progression of the atherosclerotic disease.

In a recent systematic review on the effect of current non-surgical treatments on liver disease and cardio-metabolic risk in NAFLD, Musso *et al*[128] found that weight loss is safe and may ameliorate both liver and cardio-

metabolic disease in NAFLD. Although a ≥ 5% weight loss improves steatosis and cardio-metabolic variables, a ≥ 7% weight loss improves also histological disease activity in NASH; however, the latter goal was achieved by < 50% individuals even in randomized controlled studies adopting intensive multidisciplinary lifestyle interventions, making patient compliance a concern. No studies have yet examined the effect of reducing liver fat and inflammation on cardiac function and geometry both in children and adults.

CONCLUSION

Although cross-sectional studies have shown that children with NAFLD are at risk for early atherosclerotic changes and cardiac abnormalities, long-term longitudinal studies are required to determine more definitively the extent to which pediatric NAFLD and its severity influence long-term cardiovascular outcomes in the general population. In particular, follow-up studies may clarify whether the increased risk of atherosclerotic changes and cardiac alterations might reflect the clustering of underlying metabolic risk factors, or NAFLD *per se*, especially NASH, might confer a risk of adverse cardiovascular outcome above and beyond that associated with the individual components of MetS. In children, the cardiovascular system remains plastic and damage-reversible if early and appropriate interventions are established effectively. Therapeutic goals for NAFLD should address nutrition, physical activity and avoidance of smoking to prevent not only end-stage liver disease but also CVD. Future studies should also examine the long-term effect of reducing liver fat and inflammation on vascular functional and structural changes as well as on cardiac function and geometry in children.

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