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Association between type two diabetes and non-alcoholic fatty liver disease in youth

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Abstract

In the last three decades prevalence of insulin related diseases has been growing worldwide with epidemic obesity, type 2 diabetes mellitus and non alcoholic fatty liver disease. In children such epidemics are particularly worrisome, since metabolic abnormalities track to the adulthood with significant implications for the health care system. Epidemiological studies support a close association between type 2 diabetes and fatty liver disease. We review the most recent epidemiological data on prevalence of both diseases in youth and their association.

Key words: Aminotransferases, glucose, insulin resistance, non alcoholic steatohepatitis, obesity.

Introduction

Estimates from population- and hospital-based studies indicate that the number of children and adolescents with type two diabetes mellitus (T2DM) has been increasing in the last decades.[1] This is likely to occur since the number of adolescents and young adults affected by insulin resistance-associated morbidities is increasing.[2] In fact, T2DM is characterized by the simultaneous occurrence of both insulin-resistance and relative insulin-deficiency.[3] During puberty, insulin resistance represents a physiological condition aimed to favor growth. Obesity may exacerbate this condition. It has been shown that up to one in third obese adolescents with insulin resistance will keep this condition until the adulthood, whilst the remaining two will recover normal insulin sensitivity as it happens for all the normal weight adolescents.[4] Subjects with severe insulin resistance which persists over the puberty are candidate to develop type 2 diabetes mellitus, when their beta cell activity fails to compensate for increased and long term insulin resistance.[5]

T2DM in youth is usually not an isolated condition, but it is often accompanied by other metabolic abnormalities which represent cardiovascular risk factors and cluster together in the metabolic syndrome (MetS),[6] i.e. obesity, dyslipidemia, hypertension and low-grade inflammation.[2,7] Recent evidence suggests a close association also between T2DM and two other condition of insulin resistance, namely the polycystic ovary syndrome and the non-alcoholic fatty liver disease (NAFLD).[8,9]

NAFLD has a broad spectrum of manifestations, ranging from simple steatosis to its inflammatory representation of nonalcoholic steatohepatitis (NASH). The boundaries between NAFLD and NASH are defined only by liver biopsy and prediction is difficult using any single or combined clinical or laboratory test.[10] A small proportion of patients with NAFLD progresses to cirrhosis, hepatocellular carcinoma and liver failure.[11,12]

Pediatric NAFLD is becoming the leading cause for referral to liver clinics especially in overweigh/obese children from Western country.[13] The occurrence of NAFLD in these children who are already at cardiovascular risk, is particularly worrisome, since the disease appears to be a *per se* independent cardiovascular risk.[9] Patients with NAFLD seem also prone to develop more frequently an impairment of the carbohydrate metabolism varying from a condition of pre-diabetes to overt T2DM. In our series of children and adolescents with biopsy proven NAFLD, we observed a prevalence of pre-diabetes and diabetes as high as 10-12%,[14,15] and, in most of them the impaired glucose metabolism will likely track to the adulthood.

The meaning of this association remains unclear. The question is whether NAFLD is *per sè* a determinant of di-

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Abbreviations:
Type two diabetes mellitus (T2DM), metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), National Health and Nutrition Examination Survey (NHANES), alanine aminotransferases (ALT), aspartate aminotransferase (AST), free fatty acid (FFA), Gamma-Glutamil-Transpeptidasi (GGT).

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abetes or its effect is mediated by more severe obesity and insulin resistance.

In the present review, we will try to verify the occurrence of this association in youth by resuming the most recent epidemiological data on prevalence/incidence of both diseases, thus highlighting their common trends; and considering pathogenetic mechanisms which may underlie both. The epidemic of T2DM and NAFLD will bear fruit in forthcoming decades, putting further stress on the healthcare system and, probably, leading to increased morbidity and a shorter lifespan for future generations.

Epidemiology of T2DM

Prediabetes and T2DM are serious adverse consequences of obesity and pediatric metabolic syndrome, more likely to manifest in adolescence and early adulthood than clinical atherosclerotic diseases.[16]

The American Diabetes Association defines diabetes as occurring if one of three criteria are present: 1) a casual plasma glucose of > 200 mg/dL in some one with symptoms of diabetes; fasting plasma glucose of ≥ 126 mg/dL; 3) 2 hr plasma glucose of ≥ 200 as part of an oral glucose tolerance test.[17] The dose of glucose used as load is of 1.75 g/kg to a maximum of 75 g, although the precise pediatric dose is not well validated.[18] Impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) characterize prediabetes.[19]

Most data on the epidemiology of diabetes comes from national surveys. However, they provide data on incidence and prevalence of the disease based on the number of cases referred by physicians or self reported by patients. For instance, the Search for diabetes in Youth is a 6-center observational study conducting population-based ascertainment of physician diagnosed diabetes in youth.[20] The National Health and Nutrition Examination Survey (NHANES) is a stratified, multistage probability sample of the civilian non-institutionalized US population and diagnosis of diabetes was self reported. To estimate prevalence and incidence of diabetes, the adolescents of the NHANES study (N = 4,370, age 12-19 years) were simply asked about having or not the disease. Then, they underwent measurement of fasting glucose. Subjects without self reported diabetes but presenting with IFG (N = 1,496) were considered diabetic as well.[21] In a longitudinal study, performed in the United Kingdom to evaluate the incidence of T2DM in subjects < 17 years of age, the estimate of cases was based on active monthly reporting of cases by consultant pediatricians, as described above.[22] Therefore, the major limit of this survey studies is related to the fact that they do not estimate the rate of prediabetes assessed by the impaired glucose tolerance, which is, on the contrary the most frequent form of impairment in the carbohydrate metabolism in the youth. Therefore, large surveys such as the SEARCH and the NHANES are likely to see just the top of the iceberg, and to severely underestimate a more common phenomenon. On the other hand, surveys offer the opportunity to evaluate the effect of several factors, mainly age, sex and races.

Both prevalence and incidence of T2DM vary among races, with the highest prevalence and incidence in minorities.[20-22] The SEARCH found that overall prevalence ranged from 6% in non Hispanic white youth to 76% in American Indians; incidence from 17 in Hispanic to 49.9 in American-Indian per x $100{,}000^{-1}$ x year-^{1} in 15-19 year olds. In the same range of age, incidence for non-Hispanic white was 5.6.[20] Data from NHANES found an incidence of 8.1 x $100{,}000^{-1}$ in 10–14-year-olds and 11.8 x $100{,}000^{-1}$ in 15–19-year-olds in 2002–2003. The estimated incidence of T2DM in the Bostonian Youth varies from 0.79 x 1000^{-1} in children ≤ 9 years of age to 1.74 x 1000^{-1} in adolescents (age 10-19 years).[23] In the UK study, a total of 168 confirmed cases of non-type 1 diabetes were reported, resulting in a national incidence of 1.3 x $100{,}000^{-1}$ x year^{-1}. Of these, 40% were diagnosed with type 2 diabetes giving a minimum incidence of 0.53 x $100{,}000^{-1}$ x year^{-}1.[22] In New Zeeland, a 6 year study, which included 1,095.074 children of ≤ 14 years old estimated incidence of impaired glucose tolerance (IGT) was 0.72 x100000^{-1} x year^{-1} in subjects of ≤ 14 years and 2.27 x100000^{-1} in those of 10-14 years. Type 2 DM had a rate of 0.84 x $100{,}000^{-1}$ x year^{-1}.[24] In Chinese youth, a nationally representative cross-sectional survey enrolling 44 880 children aged 7–17.9 years, showed an incidence of diseases, varying from 0.2 in subjects 7-12 year old to 0.4 in 12-18 year olds.[25]

To obtain data on rates of IGT we have to look to small size studies, often performed in high risk populations, i.e. including obese individuals with co-morbidities and/or familiar history of diabetes. We resume in Table 1 the most significant among those conducted and published in the last 5 years, and using the oral glucose tolerance test to diagnose diabetes.[26-36] Of note, even in samples of individuals with severe obesity and familiarity for diabetes and cardiovascular disease, European rates were not as high as those in US studies. In Europe, T2DM remains a rarity, accounting only for 1% to 2% of all cases of diabetes mellitus. Although, differences in obesity rates between US and European youth are likely contributors, the full explanation for this discrepancies remains uncertain.

Epidemiology of NAFLD

The real prevalence of NAFLD/NASH remains unknown in the general population because prospective studies lack and the information available in a given population strictly depend on the diagnostic criteria used.[37] NAFLD, as estimated on the basis of ultrasonography and increased levels of liver enzymes, seems to be very common, occurring in persons of all ages and ethnic groups.

Table I. Prevalence/incidence of type 2 diabetes mellitus in youth.

Country	Study and population	Prevalence/incidence%	Ref.
Argentina	Cross-sectional study		
	N = 427 obese/overweight subjects, Age 10.7 ± 3.5	IGT: 7%, T2DM: 1.6%	26
Costa Rica	Cross-sectional study		
	N = 214 obese and normal weight subjects, Age 8-10	IGT : 6.5%, T2DM 0.5%	27
Germany	Cross-sectional study		
	N = 520 obese/overweight subjects, Age 8.9-20.4	IGT: 5.2%, T2DM: 1.5%	28
Germany	Cross-sectional study		
	N = 102 with Metabolic syndrome, Age 7-18	IGT: 36%, T2DM: 6%	29
Germany	Cross-sectional study	IFG 0.41%, T2DM 0.83% (lean individuals)	
	N = 721 school-leaving boys, mean age 15.5 ± 0.7	‡IGT, T2DM 2.5% (obese/overweight)	30
Hungary	Cross-sectional study		
	N = 250 obese/overweight subjects, Age not provided	‡IGT 13.6%, T2DM 1.2%	31
Israel	Cross-sectional study		
	N = 234 obese/overweight subjects, Age 5-22	IGT, T2DM: 13.5%	32
Italy	Retrospective study		
	N = 514 obese/overweight subjects, Age 13.6-14	IGT 5.4%, T2DM 0.5%	33
Turkey	Cross-sectional study		
	N = 105 obese and normal weight subjects, Age 10-18	IGT 15.2%	34
Turkey	Cross-sectional study		
	N = 196 obese subjects, Age 7-18	IGT 18%, T2DM 3%	35
Turkey	Cross-sectional study	Prepubertal IGT 19%, T2DM 2%	
	N = 169 obese subjects, Age 7-18	Pubertal IGT 27.5%, T2DM 4.3%	36

We included exclusively data from studies published in the list five years and using standard glucose tolerance test to assess impaired glucose tolerance (IGT) and type 2 diabetes mellitus according to the ADA criteria. In few studies diagnosis was made according to the WHO criteria. (‡). Some articles were not accessible through the website, therefore the abstract alone was available.

Population-based studies suggest that, as in the adults, its prevalence has been increasing over the past three decades also in children and adolescents, and that the disease represents a worldwide problem with case series described in North and South America, Europe, Australia and Asia.[13,37] These studies indicate that prevalence increases with age, ranging from 0.7% for ages 2 to 4 up to 17.3% for ages 15 to 19 years, but these rates are likely to be underestimated and all these reports do not discriminate between simple steatosis, necro-inflammation and fibrosis.[13,37,38]

The most prominent risk factor for fatty liver is obesity and the disease is most common in males adolescents.[39-41] In a recent study on 909 Korean obese children (boys 613, girls 296) the prevalence of NAFLD, measured as surrogate of alanine aminotransferases (ALT), was 33.4% in boys, and 19.6% in girls respectively.[42] Race, ethnicity and degree of obesity significantly predicted the presence of fatty liver, with Hispanics having the highest and African Americans the lowest figure.[43] One study with 181 consecutive asymptomatic obese children demonstrated that 8% had an elevated ALT suggestive of NAFLD, but the prevalence decreased in black individuals.[44]

As shown in *table II*, studies on prevalence of NAFLD in overweigh/obese children report values ranging from 8% to 80% (USA), depending upon the methods used for the diagnosis.[45-50] Unfortunately, most studies have been limited to the use of indirect measures such as elevated serum (ALT) and ultrasound to predict histological outcome,[51] but up to a 20% of young patients have normal values of liver enzyme at the time of biopsy, despite having histological proven NASH and/or fibrosis.[52] Data

Table II. Data of prevalence of NAFLD/NASH in obese children from different countries.

Country	Number of children§	NAFLD/NASH prevalence	Ref.
USA	181	8%	44
USA	315	16%	53
USA	127	23%	43
USA	320	81%	49
México	80	42%	55
Japan	299	12%	45
Japan	228	24.1%	46
Japan	310	25%	47
Cina	113	55.7 %	48
Cina	84	77% (24%)*	40
Cina	123	80.5% (43.9%)*	59
Korea	909	19.6%° and 33.4%^	42
Italy	375	42%	57
Italy	268	44%	58
Italy	72	53% (25%)*	41
Italy	175	55(%) (15%)*	60
Turkey	101	52.4%	61

§Number of overweight/obese children included in the study.
* Percentage of presumed NASH.
° Girls ^ Boys

from NHANES in 2450 adolescents found elevated ALT in 6% of overweight and 10% of obese subjects.[53] Similarly, the 1998 Korean National Health and Nutrition Examination Survey found a prevalence of elevated ALT as high as 3.2%.[54] In a sample of Mexican obese/overweight children from an elementary school, elevated ALT were observed in 42% of subjects.[55]

Based on ultrasonography evidence of fatty liver, NAFLD was diagnosed in 2.6% of Japanese children and occurrence of disease correlated significantly with indices of obesity such as the body mass index;[56] 42% of 375, and 44% of 268 morbidly obese Italian children had hepatic steatosis;[57,58] among 123 obese Chinese children, 99 subjects showed abnormal hepatic sonograms and 54 were diagnosed as NASH.[59] Some studies combine data from ultrasonography and elevated ALT; for instance a study demonstrating that 52.4% of obese Turkish children had fatty liver by ultrasonography and 13.8% had high ALT levels.[60] According to an Italian survey in 195 obese children, 55% had liver steatosis by ultrasonography, 20% had elevated ALT and AST levels, and 15% had both.[61]

Type 2 diabetes: pathogenetic mechanisms

T2DM is caused by a combination of increased insulin resistance and decreased insulin secretion. Peripheral insulin-resistance is tightly coupled with obesity in children and seems to be the major driving force of deteriorating glucose metabolism, and is also associated with lipid partitioning in specific compartments (i.e., viscera, muscle and liver). On the other hand, the reduction of insulin secretion is probably a secondary event evolving gradually.[62]

Insulin resistance is an impairment of the physiologic effects of insulin on glucose.[63] Normal glycemic control requires the pancreatic β-cell sensing of glucose concentration, synthesis and release of insulin, binding of insulin to receptors with a consequent activation of several signaling proteins. The activation of multiple signaling cascade causes increased glucose uptake by muscles, fat, and liver and decreased glucose production by the liver.[64] These molecular mechanisms are all altered in T2DM, causing insulin resistance in muscle tissue, decreased pancreatic insulin secretion, and increased hepatic glucose output.[65]

In children and adolescents with T2DM, defects of glucose metabolism are characterized by a decline in the first phase sensitivity of the β-cell coupled with the decline of both first and second phase sensitivity.[66] The dynamics of the impaired glucose metabolism in childhood seem to be faster than in adults, representing a limited window of opportunity for successful preventive intervention. Early identification of children with altered glucose metabolism is important in order to quantify public health needs and to allocate resources for appropriate prevention programs.

The risk factors for developing type 2 diabetes in youth include a genetic predisposition and certain environmental characteristics.[67] The majority of diabetes in both adults and children is polygenic.[68] Thus, the family history of diabetes represents the most important risk factor of developing T2DM with respect to the general population.[69]

Ethnicity is another important factor predisposing to T2DM development. The increased incidence of T2DM in youth of color was identified first in the Pima Indians of the southwest. Children of Pima Indian were found to have high rates of morbid obesity and from 1980 the research has focused on this group as well as other ethnic and racial groups with high rates of diabetes.[70] Low birthweight, maternal diabetes and the intrauterine environment also are important areas to consider in risk for the development of T2DM in youth.[71] Puberty coupled with insulin resistance provides a strong basis for the development of pre-diabetes and T2DM in youth with overweight or frank obesity.[72]

The changing environment during the past several decades provided a further contribution of the dramatic increase in T2DM in youth. There has been a movement toward a positive energy balance due to diet intake, decreased physical activity and increased sedentary activity. Fast food consumption and portion sizes have increased from 30 years ago, leading to caloric intake in excess of metabolic need, and youth are the major consumers of fast food meals. Moreover, youth are increasingly inactive, reducing their physical activity and using sedentary screen activities, such as television viewing and playing video games, for an average of 5.5 hours daily.[67]

All these evidence demonstrate that development of T2DM in youth is complex and requires astute health care providers who understand the pathophysiology, patient history, family history, and genetic predisposition, coupled with environmental factors, to manage each youth at the appropriate intervention level.

NAFLD: pathogenetic mechanisms

The pathogenesis of NAFLD is not yet completely understood, however a currently favored hypothesis is that «two hits» are required for a subject to develop the disease.[73] A first hit that provokes steatosis (i.e. fat accumulation in liver and/or insulin resistance) and predisposes the liver to a second hit which leads to necro-inflammation and fibrosis. This second hit includes the alteration of several signaling pathways regulating oxidative stress, mitochondrial dysfunctions and production of pro-inflammatory and pro-fibrotic cytokines and their signaling. Recent advances demonstrate that fatty liver and its progressive development in NASH is a more complex phenomenon which originates by multiple hits.[74,75] In fact, the subsequent development of fibrosis requires probably the coexistence of multiple factors, including

host (genes) and environmental factors as well as those related to lifestyles and behaviors.[76,77]

All today theories consider insulin resistance as an important driving force, which promotes lipolysis of peripheral adipose tissue which, in turn, increases free fatty acid (FFA) influx into the liver.[78] Hyperinsulinemia and hyperglycemia promote *de novo* lipogenesis and inhibit simultaneously FFA oxidation.[79] Fatty deposition at the liver side is also favored by defective incorporation of triglyceride into apolipoprotein carrier proteins and lipid export.[80]

Fatty loaded hepatocytes are susceptible of additional insults, which may lead to hepatocyte injury, inflammation, and fibrosis. Also the role of oxidative stress is well documented in NASH., There is accumulating evidence that oxidative stress and mitochondrial dysfunction play a key role in the physiopathology of NAFLD/NASH whatever its initial cause.[81] Moreover, there is a close interaction between development of mitochondrial dysfunction, insulin resistance and cytokines in many liver diseases.[82] Conversely, many forms of oxidative stress lead to antioxidant depletion, which then further enhances oxidative stress and cytokine-mediated hepatoxicity.[83] In addition, although the exact mechanisms promoting progressive liver injury are not well defined, also substrates derived from adipose tissue such as FFA, tumor necrosis factor alpha, leptin, and adiponectin have been implicated.[84,85]

Taken together, all discussed evidences highlight the complicated network of interactions existing among the several molecules and signaling pathways which contribute do development of NAFLD/NASH. Thus, today it is impossible to distinguish between causes or effects during NAFLD/NASH development and progression.

More than a clinical association

The NHANES found NAFLD to be more prevalent in obese race minorities, with T2DM, hypertension and hyperlipidemia.[86] These associations have led to the hypothesis that NAFLD may precede the onset of type 2 diabetes in some individuals. But, why should NAFLD associate with diabetes? The links between the two diseases reflect processes related to insulin action or resistance which may be mediated through the location and function of fat, excess total body fatness or hepatic fat. Otherwise, the risk for new onset diabetes may be mediated by components of the MetS which occur very frequently in NAFLD.

NAFLD, as estimated by elevated ALT levels, and prediabetes or T2DM were found to be associated independently of confounders, including obesity in adults.[87] The West of Scotland Coronary Prevention Study, consistently with a number of other studies, found that compared with men with values for baseline ALT in the bottom quartile (< 17 U/L), those with levels in the top quartile (> 29 U/L) had an adjusted odds ratio of 2.04 (95% CI 1.16–3.58) for incident diabetes.[88] In the British Hearth Regional Study, the risk of type 2 diabetes increased significantly with increasing levels of ALT and g**amma-**g**lutamil-**transpeptidase (GGT), even after adjustment for a range of confounders, again including BMI (top *vs* bottom quartile, ALT: RR 2.72, 95% CI 1.47–5.02; GGT: RR 3.68, 95% CI 1.68–8.04) or with further additional adjustment for insulin resistance.[89]

Data on the association between T2DM and NAFLD in paediatric settings are poor, but nevertheless very strong. In the San Diego series of biopsy proven NAFLD, 6 out of 43 children had type 2 diabetes mellitus (14%).[49,90] In our series of 120 babies,[14] all subjects underwent oral glucose tolerance test and diagnosis of impaired glucose tolerance or T2DM was done according to the criteria of the American diabetes Association. We observed a prevalence of IGT as high as 9% and T2DM of 2%. The degree of insulin resistance was not correlated with liver histology in terms of grade of steatosis, inflammation or fibrosis. Subjects with IGT/T2DM did differ from normo-tolerant individuals neither in anthropometrics or liver histology. Conversely the prevalence of overt MetS was higher in subjects presenting with fibrosis and/or NASH.[14] The limited number of subjects with impaired carbohydrate metabolism did not allow excluding that visceral adiposity (i.e. through reduced levels of adiponectin or increased pro-inflammatory adipocytokines) mediates both derangements in liver histology and carbohydrate metabolism.

Conclusion

Also in youth, T2DM and NAFLD seem to be significantly associated. The meaning and the causative relation of this finding is still unclear. It is likely that the insulin resistant phenotype, charactering non alcoholic fatty liver disease, contributes to anticipate significantly the onset of type 2 diabetes from mature adulthood to youth. This observation may translate into the worrisome anticipation of all cardiovascular abnormalities linked to T2DM. In addiction, NAFLD may represent an independent risk factor which augments further the total cardiovascular risk. Therefore, both NAFLD and T2DM embody a growing healthcare burden which will boost health related costs in the next future.

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