Relationship between fatty liver and glucose metabolism: A cross-sectional study in 571 obese children

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KEYWORDS
Children; Obesity; Oral-glucose tolerance test; Impaired glucose tolerance; Type 2 diabetes mellitus; Insulin resistance; Non-alcoholic fatty liver disease

Abstract  Background and aims: Early onset type 2 diabetes mellitus (T2DM) is associated with obesity, insulin resistance and impaired beta-cell function. Non-alcoholic fatty liver disease (NAFLD) may be an independent risk factor for T2DM. We investigated the relationship between NAFLD and glucose metabolism in a large sample of obese children.

Methods and Results: A total of 571 obese children (57% males and 43% females) aged 8–18 years were consecutively studied at a tertiary care centre specialised in paediatric obesity. Liver ultrasonography was used to diagnose NAFLD after exclusion of hepatitis B and C and alcohol consumption. Oral-glucose tolerance testing (OGTT) was performed; insulin sensitivity was evaluated by using the insulin sensitivity index (ISI) and beta-cell function by using the ratio between the incremental areas under the curve (AUC) of insulin and glucose (incAUCins/incAUCglu). A total of 41% of the obese children had NAFLD. Impaired glucose tolerance or T2DM was present in 25% of the children with NAFLD versus 8% of those without it (p < 0.001). Children with NAFLD had higher body mass index (BMI), fasting glucose, 120-min OGTT glucose, incAUCins/incAUCglu and lower ISI as compared with children without NAFLD (p < 0.002). At bootstrapped multivariable median regression analysis controlling for gender, age, pubertal status and BMI, NAFLD was an independent predictor of 120-min OGTT glucose and ISI, but not of incAUCins/incAUCglu. Similar findings...

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Introduction

Early-onset type 2 diabetes mellitus (T2DM) is characterised by marked visceral obesity, extreme insulin resistance and defective beta-cell function [1]. Non-alcoholic fatty liver disease (NAFLD) may be an independent risk factor for T2DM [2]. In the last decade, both obesity and NAFLD have reached epidemic proportions, especially among children and adolescents [3]. In adults, obesity is associated with both glucose intolerance and NAFLD, which may partly contribute to the current epidemic of cardiovascular disease [4–6]. In children, elevated levels of serum aminotransferases – employed as surrogate markers of NAFLD – are more common than in adults and cluster with T2DM, hypertension and dyslipidaemia [7]. Since glucose metabolism deteriorates more rapidly in children than in adults, the identification of early metabolic defects such as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) in children is important to prevent the development of diabetes, and possibly liver disease at a later age [7].

Both insulin resistance and beta-cell dysfunction play an important role in the progression from glucose intolerance to diabetes. In populations with high prevalence of T2DM, insulin resistance is well established long before the development of any impairment in glucose homeostasis [8,9]. In adults, beta-cell function is already decreased by 50% in normal glucose-tolerant individuals with 120-min glucose concentration >100 mg/dl during oral-glucose tolerance testing (OGTT) [8,10]. Recent paediatric studies have shown that not only obesity but also ectopic fat accumulation, especially in the liver, may further deteriorate glucose homeostasis [11,12]. We, and others, have shown that adults with NAFLD have decreased hepatic insulin clearance and increased peripheral insulin concentration in proportion to the degree of liver steatosis detected by magnetic resonance imaging (MRI) [13].

The present study aimed at evaluating the association between glucose tolerance, insulin resistance, beta-cell function and NAFLD in a large group of Caucasian obese children and adolescents.

Methods

Subjects

A total of 571 children and adolescents were consecutively enrolled into the study at the Division of Auxology, Istituto Auxologico Italiano (Piancavallo, Verbania, Italy) between February 2007 and February 2009. The entry criteria were: (1) age ≤18 years and (2) body mass index (BMI) ≥95th percentile for gender and age. The exclusion criteria were: (1) genetic or syndromic obesity; (2) treatment with any drugs; (3) alcohol consumption and; (4) presence of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. The study protocol was approved by the local Ethics Committee and parental consent was obtained.

Liver ultrasonography

Liver ultrasonography was performed by the same radiologist using the standard criteria [21,22]. Light steatosis was defined as slightly increased liver echogenicity with normal vessels and absent posterior attenuation; moderate steatosis as moderately increased liver echogenicity with partial dimming of vessels and early posterior attenuation; and severe steatosis as diffusely increased liver echogenicity with absence of visible vessels and heavy posterior attenuation. Normal liver was defined as the absence of

Conclusion: NAFLD was present in 41% of our obese children and was associated with higher insulin resistance, but not with impaired beta-cell function.

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liver steatosis or other liver abnormalities. NAFLD was operationally defined as any degree of liver steatosis in the absence of HBV and HCV infection and alcohol intake.

**Statistical analysis**

Values of continuous variables are given as median, interquartile range (IQR) and minimum and maximum values because of skewed distributions. IQR was calculated as the difference between the 75th and 25th percentile. Between-group comparisons of continuous variables were performed with the Wilcoxon—Mann—Whitney test and those of categorical variables with the Fisher’s exact test. Median regression was used to define the relationship between the three outcomes of interest (120-min OGTT glucose, ISI and incAUCins/incAUCglu) and NAFLD after accounting for potential confounders (gender, age, pubertal status and BMI for all models; fasting glucose and fasting insulin for selected models) [23]. Four pre-specified regression models were evaluated: Model 1A employed 120-min OGTT glucose as the outcome and NAFLD, gender, age, pubertal status, BMI and fasting glucose as predictors; Model 1B added insulin to the predictors of Model 1A; Model 2 had ISI as outcome and the same predictors of Model 1A, except glucose; Model 3 had incAUCins/incAUCglu as outcome and the same predictors of Model 2.

Because liver steatosis is a continuous outcome whose categorisation as dichotomous variable (fatty liver ‘yes’ or ‘no’) may involve loss of information [25], we refitted the models in Table 3 replacing dichotomous NAFLD with categorically coded steatosis. We tested whether a linear trend in the severity of liver steatosis could be an adequate representation of the association with the outcomes of interest using a test for linear trend across the categorical levels of the predictor [26]. Because a linear trend was detected for all models, further analysis was performed with liver steatosis modelled as the continuous predictor. Because of heteroskedasticity, that is, constant variance of residuals, 95% confidence intervals (95%CI) of regression coefficients were calculated by bootstrapping 1000 random samples of 571 subjects [24]. All statistical tests were two-tailed and statistical significance was set to a value of $p < 0.05$. Statistical analysis was performed using STATA 11 (STATA Corporation, College Station, TX, USA).

**Results**

**Characteristics of the study children**

A total of 571 obese children (57% males and 43% females) aged 8–18 years were consecutively enrolled into the study at the Division of Auxology, Istituto Auxologico Italiano (Piancavallo, Verbania, Italy). NAFLD was detected in 41% of children ($n = 234$). Liver steatosis was light in 10% ($n = 58$), moderate in 25% ($n = 140$) and severe in 6% ($n = 36$) of the children.
Comparison of the children with and without NAFLD

The measurements of the children with and without NAFLD are given in Tables 1 and 2. NAFLD was more frequent in males \((p < 0.001)\), and there was a different distribution of pubertal stages among the children with and without NAFLD \((p = 0.003)\), even if most of the subjects were postpubertal in both groups.

As compared with children without NAFLD, those with NAFLD had higher BMI, fasting glucose, 120-min OGTT glucose, insulin, \(\text{incAUCins/incAUCglu}\), triglycerides, ALT, AST, GGT and lower values of QUICKI, ISI and HDL-cholesterol \((p < 0.002\) for all comparisons).

As detected by fasting glucose, only five children with NAFLD had IFG and none had T2DM \((p = 0.011)\). As detected by OGTT, 55 children (24%) with NAFLD and 24 children (7%) without NAFLD had IGT, while the corresponding figures for T2DM were three (1.3%) and two (0.6%) \((p < 0.001)\), respectively. Of the five children with IFG, three had IGT and two T2DM at OGTT.

Relationship between glucose intolerance and NAFLD

Fig. 1 shows the OGTT curves of glucose and insulin according to the presence of normal glucose tolerance \((\text{NGT}; \text{~}n = 487)\) or IGT/DM \((\text{~}n = 79/5)\) in subjects with and without NAFLD. No inferential analysis was performed on these curves owing to the low number of subjects with IGT/DM relative to the number of time points.

Fig. 2 shows the values of ISI and \(\text{incAUCins/incAUCglu}\) according to NAFLD and glucose tolerance status. We tested whether NAFLD contributed to ISI and \(\text{incAUCins/incAUCglu}\) independently of IGT/DM by using bootstrapped median regression with NAFLD and IGT/DM as binary (yes vs. no) covariates. NAFLD decreased ISI of \(-3\) \((95\% \text{ CI: } -4 \text{ to } -2, p < 0.001)\) units independently of IGT/DM \((-2, 95\% \text{ CI: } -3 \text{ to } -1 \text{ units, } p < 0.001)\). NAFLD had, however, no effect on \(\text{incAUCins/incAUCglu}\) (median effect \(-16, 95\% \text{ CI: } -39 \text{ to } -11 \text{ units, } p = 0.201\), contrary to IGT/DM (median effect \(-58, 95\% \text{ CI: } -83 \text{ to } -33 \text{ units, } p < 0.001)\).

Table 2  Categorical measurements of children with and without non-alcoholic fatty liver disease.

<table>
<thead>
<tr>
<th></th>
<th>NAFLD ((n = 234))</th>
<th>No NAFLD ((n = 337))</th>
<th>Fisher’s Exact test, (p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>139/95</td>
<td>105/232</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Tanner stage (pre-pub/early-pub/late-pub)</td>
<td>12/83/139</td>
<td>12/79/246</td>
<td>0.003</td>
</tr>
<tr>
<td>Normal/IFG/T2DM (with fasting glucose)</td>
<td>229/5/0</td>
<td>337/0/0</td>
<td>0.011</td>
</tr>
<tr>
<td>Normal/IGT/T2DM (with OGTT)</td>
<td>176/55/3</td>
<td>311/24/2</td>
<td>(&lt; 0.001)</td>
</tr>
</tbody>
</table>

The table gives the number of subjects with the characteristic of interest. Abbreviations: IFG, impaired fasting glucose; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test.

Figure 1  Changes of glucose and insulin during oral glucose tolerance testing according to the absence (continuous line) or presence (broken line) of non-alcoholic fatty liver disease and the absence or presence of impaired glucose tolerance or diabetes mellitus. Values are medians and interquartile ranges. Abbreviations: NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.
Relationship between 120-min glucose, insulin sensitivity, beta-cell function and NAFLD

Although the dichotomisation of glucose status as NGT versus IGT/DM is clinically important [18], there is substantial loss of power in modelling glucose status as binary so that we performed further modelling using indicators of glucose metabolism as continuous outcomes [25].

Table 3 reports the four models used to study the relationship between NAFLD and the outcomes of interest. Model 1A evaluated which variables among NAFLD, gender, age, pubertal status, BMI and fasting glucose were associated with 120-min OGTT glucose. Only NAFLD and fasting glucose were predictors of 120-min OGTT glucose and the presence of NAFLD increased 120-min OGTT of a median value of 0.54 mmol l\(^{-1}\) (\(p<0.001\)). The addition of fasting insulin to the above predictors (Model 1B) did not virtually change these associations. Model 2 showed NAFLD to be inversely associated with ISI with a median effect greater than that of 1 SDS of BMI (\(-2.19\) vs. \(-1.29\), \(p<0.001\)) and BMI (\(\beta = -1.85\), 95% CI \(-2.80\) to \(-0.89\), \(p<0.001\) for 1 SDS increase) were confirmed as predictors for the New Model 2. On the contrary, none of the tested predictors was associated to the outcome in the New Model 3.

Discussion

Obese children are at greater risk of developing early onset T2DM and cardiovascular disease [7,27]. Insulin resistance plays a central role in glucose metabolism, but it is not until deterioration of beta-cell function that glucose intolerance and T2DM develop [10]. In adults, we have shown that an impairment of beta-cell function is already present in NGT subjects, and at the stage of IGT they have lost already 80% of their beta-cell function [8]. Because obesity is often associated with ectopic fat deposition [28], we studied the association of NAFLD with glucose metabolism in a large sample of Caucasian obese children.

Glucose metabolism and its association with fatty liver were recently evaluated in 118 (37 males and 81 females) obese children of different ethnic origins [11]. In this study, insulin sensitivity was markedly reduced in obese children with severe fatty liver, while the insulinogenic index — used...
as surrogate marker of beta-cell function — tended to increase with the severity of liver steatosis but only to a modest degree \( (p = 0.05) \) [11].

In the present study, performed on a larger sample \( (n = 571) \) of children of the same ethnicity (Caucasians) and studied at a single centre, we found that insulin sensitivity was lower in children with NAFLD. However, the apparent association of NAFLD with incAUCins/incAUCglu — used as marker of beta-cell function — disappeared at multivariable analysis after correction for potential confounders. Only fasting glucose and NAFLD were predictors of 120-min OGTT glucose, and this relationship was not affected by fasting insulin. ISI was associated not only to NAFLD but also to BMI, as we have recently reported for children with histologically proven NAFLD [29]. These findings were confirmed by modelling continuous liver steatosis, instead of dichotomous NAFLD, as predictor. Thus, in our study, the 120-min OGTT glucose concentration of children with NAFLD was explained by greater insulin resistance.

The main limitation of the present study, as well as of the presently available paediatric studies, is that insulin sensitivity and beta-cell function were obtained indirectly from OGTT and not from reference methods such as the euglycaemic hyperinsulinaemic clamp and the deconvolution of C-peptide. Although these surrogate indexes are being increasingly used in children [11,12], they were developed and validated in adults, and it is possible that their paediatric application may have some limitations. On the other hand, given the number and the age of the subjects involved in the present study, it was neither ethically possible nor logistically feasible to perform the euglycaemic hyperinsulinaemic clamp to assess insulin sensitivity. A second limitation is that ultrasonography is known to underestimate the prevalence of fatty liver and, more importantly, does not offer any information on the presence of non-alcoholic steatohepatitis and liver fibrosis [17]. A third limitation of this study is the lack of body composition measurements, including measurements of visceral fat. The degree of obesity of our children also impeded us to use waist circumference as a surrogate measure of visceral fat [17]. Further studies are clearly needed to disentangle the association of body composition and insulin resistance in children with NAFLD [11,30].

In conclusion, 41% of our obese children had NAFLD and its presence was associated with higher insulin resistance but not with impaired beta-cell function. Our findings suggest that obese children may need to be considered for an evaluation of glucose metabolism. Further studies, ideally performed with reference methods, are needed to better define the status of beta-cell function in obese children with NAFLD.

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