Nutrition, Metabolism & Cardiovascular Diseases (2012) 22, 120—126

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/nmcd

Nutrition, Metabolism & Cardiovascular Diseases

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

G. Bedogni [a,b,1], A. Gastaldelli [c,*,1], M. Manco [d], A. De Col [e], F. Agosti [e], C. Tiribelli [a], A. Sartorio [e,f]

[a] *Centro Studi Fegato, Basovizza e Dipartimento ACADEM, Università di Trieste, Trieste, Italy*
[b] *Dipartimento di Scienze Materne e Pediatriche, Università di Milano, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milano, Italy*
[c] *Istituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche, Pisa, Italy*
[d] *Direzione Scientifica, Ospedale Pediatrico 'Bambino Gesù', IRCCS, Roma, Italy*
[e] *Istituto Auxologico Italiano, IRCCS, Laboratorio Sperimentale Ricerche Auxo-endocrinologiche, Milano and Piancavallo, Verbania, Italy*
[f] *Istituto Auxologico Italiano, IRCCS, Divisione di Auxologia, Piancavallo, Verbania, Italy*

Received 11 September 2009; received in revised form 19 April 2010; accepted 7 May 2010

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 ($p \leq 0.002$). 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

* Corresponding author. Istituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche, Via Moruzzi 1, 56100 Pisa, Italy. Tel.: +39 050 31 52 67; fax: +39 050 31 52 166.
E-mail address: amalia@ifc.cnr.it (A. Gastaldelli).
[1] These authors contributed equally to this article.

doi:10.1016/j.numecd.2010.05.003

were obtained using continuous liver steatosis as the predictor, instead of dichotomous NAFLD. *Conclusion:* NAFLD was present in 41% of our obese children and was associated with higher insulin resistance, but not with impaired beta-cell function.

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 $>100\ mg\ dl^{-1}$ 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 $\leq$18 years and (2) body mass index (BMI) $\geq$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.

Clinical examination

Pubertal status was classified as pre-pubertal (stage 1), early pubertal (stages 2 and 3) or late pubertal (stages 4 and 5), according to Tanner [14]. Alcohol consumption was determined by interview with the children and parents. Weight and stature were measured following standard procedures [15]. BMI was calculated as weight (kg)/stature $(m)^2$. Standard deviation scores (SDS) of weight, stature and BMI were calculated using Italian reference data [16].

Laboratory assessment

HBV surface antigen and antibodies against HCV were measured to exclude hepatitis B and C [17]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides were measured using standard laboratory methods. Glucose tolerance was assessed by means of an OGTT with 1.75 g of glucose kg^{-1} of weight (up to 75 g) [18]. Glucose and insulin were measured at 0, 30, 60, 90 and 120 min during OGTT. Glucose was measured using standard laboratory methods and insulin using a chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). T2DM was defined as fasting glucose $\geq 126\ mg\ dl^{-1}$ or 120-min OGTT glucose $\geq 200\ mg\ dl^{-1}$; IFG as fasting glucose between 100 and 126 $mg\ dl^{-1}$; and IGT as 120-min OGTT glucose between 140 and 200 $mg\ dl^{-1}$ [18]. The insulin sensitivity index (ISI) was calculated from OGTT as described by Matsuda and DeFronzo [19]. The ratio between the incremental AUCs of insulin and glucose (incAUCins/incAUCglu) was used as surrogate index of beta-cell function [8]. The quantitative insulin-sensitivity check index (QUICKI) was also calculated to allow comparisons with other studies [20].

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

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, inconstant 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.

Table 1 Continuous measurements of the children with and without non-alcoholic fatty liver disease.

	NAFLD ($n = 234$)				No NAFLD ($n = 337$)				WMW test
	Median	IQR	Min	Max	Median	IQR	Min	Max	p-value
Age (years)	15	4	8	18	15	3	8	18	0.986
Weight (kg)	103.2	31.6	54.3	177.0	91.1	23.5	45.2	155.3	<0.001
Weight (SDS)	2.74	0.72	1.46	4.19	2.34	0.60	0.61	4.02	<0.001
Height (m)	1.63	0.14	1.27	1.89	1.61	0.12	1.33	1.97	<0.010
Height (SDS)	0.15	1.46	−2.63	3.09	0.10	1.43	−3.05	3.34	0.700
BMI (kg/m^2)	38.1	8.5	27.8	62.0	34.7	7.1	24.4	58.6	<0.001
BMI (SDS)	3.23	0.85	1.92	5.13	2.84	0.80	1.68	4.46	<0.001
Fasting glucose (mg/dL)	78	11	54	120	76	9	51	97	0.002
120-min OGTT glucose (mg/dL)	122	33	80	304	111	24	55	204	<0.001
Fasting insulin (μU/mL)	15	11	3	82	11	7	2	73	<0.001
QUICKI	0.14	0.01	0.12	0.18	0.15	0.02	0.11	0.21	<0.001
ISI[a]	8	5	2	28	12	7	3	39	<0.001
incAUCins/incAUCglu[a]	196	181	4	747	183	134	18	971	<0.001
Cholesterol (mg/dL)	165	37	75	258	162	43	74	287	0.688
HDL-cholesterol (mg/dL)	43	12	24	80	48	14	24	113	<0.001
LDL-cholesterol (mg/dL)	109	35	21	207	102	41	19	222	0.119
Triglycerides (mg/dL)	98	56	40	284	82	42	21	279	<0.001
ALT (U/L)	37	30	7	245	20	12	6	327	<0.001
AST (U/L)	26	14	9	92	19	6	10	112	<0.001
GGT (U/L)	21	13	6	133	15	7	2	166	<0.001

Abbreviations: NAFLD, non-alcoholic fatty liver disease; WMW test, Wilcoxon-Mann-Whitney test; IQR, interquartile range; Min, minimum value; Max, maximum value; SDS, standard deviation score; BMI, body mass index; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin-sensitivity check index; ISI, insulin sensitivity index; incAUCins/incAUCglu, ratio between the incremental areas under the curve of insulin and of glucose; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl-transferase.
[a] Calculated using SI Units for insulin (pmol/L) and glucose (mmol/L).

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

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

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.

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, fasting insulin, incAUCins/incAUCglu, triglycerides, ALT, AST, GGT and lower values of QUICKI, ISI and HDL-cholesterol ($p \leq 0.002$ 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 (NGT; $n = 487$) or IGT/DM ($n = 79/5$) 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 incAUCins/incAUCglu according to NAFLD and glucose tolerance status. We tested whether NAFLD contributed to ISI and 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% CI -4 to -2, $p < 0.001$) units independently of IGT/DM (-2, 95% CI -3 to -1 units, $p < 0.001$). NAFLD had, however, no effect on incAUCins/incAUCglu (median effect = 16, 95% CI -9 to 41 units, $p = 0.201$), contrary to IGT/DM (median effect = -58, 95% CI -83 to -33 units, $p < 0.001$).

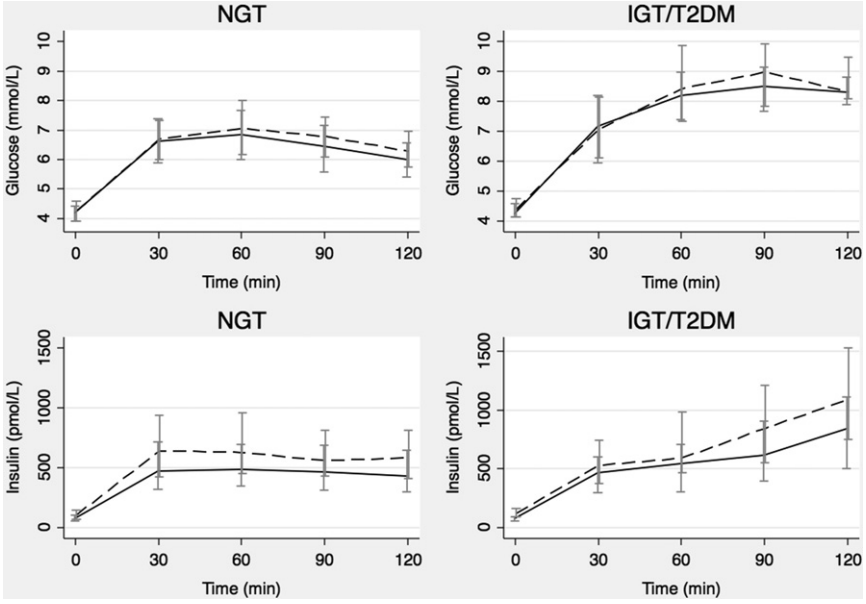

Figure 1 Changes of glucose and insulin during oral glucose tolerance testing according to the absence (continuous line) or presence (broken line) of non-alcoholic fatty liver disease and the absence or presence of impaired glucose tolerance or diabetes mellitus. Values are medians and interquartile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

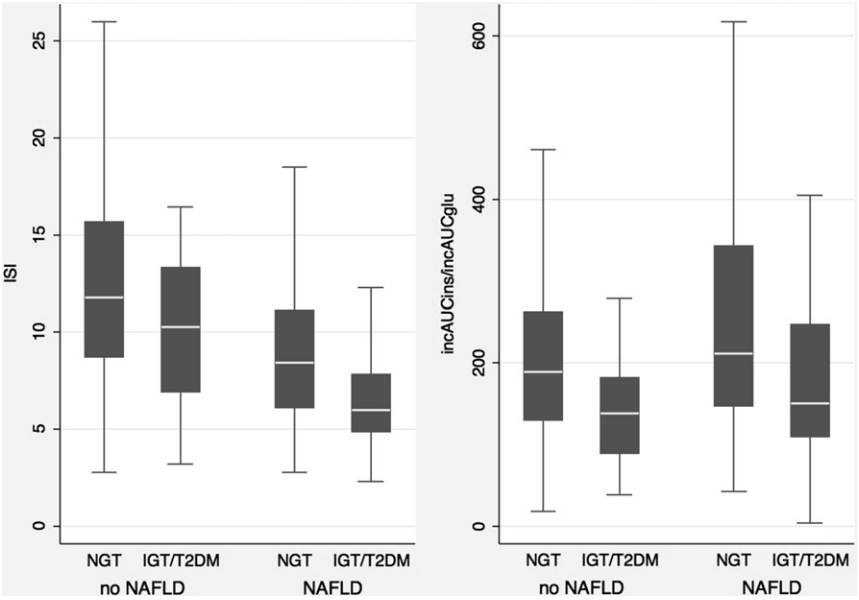

Figure 2 Insulin sensitivity index and ratio between the incremental areas under the curve of insulin and glucose in children with and without non-alcoholic fatty liver disease according to the absence or presence of impaired glucose tolerance or type 2 diabetes mellitus. Box-plots give the median value (white), 25th and 75th percentiles (lower and upper limits of the box) and lower and upper adjacent values (whiskers). Abbreviations: NGT, normal glucose tolerance; IGT/T2DM, impaired glucose tolerance/type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; ISI,insulin sensitivity index; incAUCins/incAUCglu, ratio between the incremental areas under the curve of insulin and glucose. At multivariable bootstrapped median regression, NAFLD contributed to ISI but no to incAUCins/incAUCglu independently of glucose tolerance status (see text for measures of effect size).

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 (-3.11 vs. -1.90 units, $p < 0.001$ for both). Importantly, Model 3 did not confirm the association of incAUCins/incAUCglu with NAFLD.

Relationship between 120-min glucose, insulin sensitivity, beta-cell function and liver steatosis

We refitted the Models in Table 3, replacing dichotomous NAFLD with categorically coded steatosis (1 = none, $n = 337$; 2 = light or moderate, $n = 198$; 3 = severe, $n = 36$). The results of these analyses were coherent with those shown in Table 3. In the new Model 1A, only liver steatosis (regression coefficient [β] = 0.40, 95% CI 0.20—0.60, $p < 0.001$ for 1-level increase) and fasting glucose ($\beta = 0.70$, 95% CI 0.40—0.99, $p < 0.001$ for 1 mmol^{-1} increase) were confirmed as predictors. Likewise, liver steatosis ($\beta = 0.39$, 95% CI 0.19—0.60, $p < 0.001$) and fasting glucose ($\beta = 0.67$, 95% CI 0.36—0.97, $p < 0.001$) were confirmed as predictors in the new Model 1B. Moreover, liver steatosis ($\beta = -2.19$, 95% CI -3.09 to -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

Table 3 Multivariable prediction of glucose metabolism.

	Model 1A 120-min OGTT glucose (mmol/l)	Model 1B 120-min OGTT glucose (mmol/l)	Model 2 ISI[a]	Model 3 incAUCins/incAUCglu[a]
NAFLD	0.54 [0.24 to 0.84] $p < 0.001$	0.57 [0.29 to 0.85] $p < 0.001$	−3.11 [−4.22 to −1.99] $p < 0.001$	−3.86 [−37.00 to 29.29] $p = 0.819$
Male	−0.07 [−0.36 to 0.21] $p = 0.605$	−0.11 [−0.40 to 0.17] $p = 0.431$	0.16 [−1.07 to 1.40] $p = 0.794$	6.90 [−23.34 to 37.13] $p = 0.654$
Age (years)	0.05 [−0.05 to 0.15] $p = 0.315$	0.06 [−0.04 to 0.16] $p = 0.210$	0.20 [−0.09 to 0.49] $p = 0.180$	−2.42 [−10.37 to 5.53] $p = 0.550$
Early pubertal[b]	0.10 [−0.49 to 0.68] $p = 0.747$	0.21 [−0.38 to 0.79] $p = 0.488$	−1.87 [−5.69 to 1.95] $p = 0.336$	−44.59 [−145.51 to 56.33] $p = 0.386$
Late pubertal[b]	0.29 [−0.51 to 1.09] $p = 0.479$	0.39 [−0.42 to 1.20] $p = 0.344$	−0.73 [−4.82 to 3.36] $p = 0.725$	−70.11 [−176.00 to 35.78] $p = 0.194$
BMI (SDS)	−0.02 [−0.23 to 0.19] $p = 0.874$	−0.12 [−0.37 to 0.12] $p = 0.319$	−1.90 [−2.95 to −0.86] $p < 0.001$	15.46 [−8.30 to 39.22] $p = 0.202$
Glucose (mmol/L)	0.66 [0.35 to 0.97] $p < 0.001$	0.62 [0.31 to 0.94] $p < 0.001$	–	–
Insulin (pmol/L)	–	0.002 [−0.001 to 0.004] $p = 0.184$	–	–
Intercept	2.48 [0.80 to 4.16] $p = 0.004$	2.50 [0.82 to 4.18] $p = 0.004$	15.13 [10.21 to 20.05] $p < 0.001$	241.95 [108.53 to 375.37] $p < 0.001$

Values are regression coefficients and 95% confidence intervals obtained at bootstrapped median regression (see text for details). Abbreviations: NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; incAUCins/incAUCglu, ratio between the incremental areas under the curve of insulin and that of glucose.

[a] Calculated from insulin in pmol/L and glucose in mmol/L.

[b] vs. pre-pubertal.

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.

Acknowledgements

We are grateful to the children and their families for their co-operation and to Dr. G. Grugni, Dr. G. Mazzilli, Ms A. Seddone and the Nursing Staff at the Division for Auxology, Istituto Auxologico Italiano, IRCCS, for their qualified assistance. The study was supported by Progetti di Ricerca Corrente, Istituto Italiano di Auxologia, IRCCS, Milano, Italy.

References

[1] Burns N, Finucane FM, Hatunic M, Gilman M, Murphy M, Gasparro D, et al. Early-onset type 2 diabetes in obese white subjects is characterised by a marked defect in beta cell insulin secretion, severe insulin resistance and a lack of response to aerobic exercise training. Diabetologia 2007;50:1500—8.

[2] Moscatiello S, Manini R, Marchesini G. Diabetes and liver disease: an ominous association. Nutr Metab Cardiovasc Dis 2007;17:63—70.

[3] Dunn W, Schwimmer JB. The obesity epidemic and nonalcoholic fatty liver disease in children. Curr Gastroenterol Rep 2008;10:67—72.

[4] Gastaldelli A, Kozakova M, Højlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. Hepatology 2009;49:1537—44.

[5] Loria P, Lonardo A, Bellentani S, Day CP, Marchesini G, Carulli N. Non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease: an open question. Nutr Metab Cardiovasc Dis 2007;17:684—98.

[6] Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? Diabetologia 2008;51:1947—53.

[7] Manco M, Bottazzo G, DeVito R, Marcellini M, Mingrone G, Nobili V. Nonalcoholic fatty liver disease in children. J Am Coll Nutr 2008;27:667—76.

[8] Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, DeFronzo RA. San Antonio metabolism study. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. Diabetologia 2004;47:31—9.

[9] Gastaldelli A, Miyazaki Y, Pettiti M, Buzzigoli E, Mahankali S, Ferrannini E, et al. Separate contribution of diabetes, total fat mass, and fat topography to glucose production, gluconeogenesis, and glycogenolysis. J Clin Endocrinol Metab 2004; 89:3914—21.

[10] DeFronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 2009;58:773—95.

[11] Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Magical M, Escalera S, et al. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? Hepatology 2009;49: 1896—903.

[12] Yeckel CW, Taksali SE, Dziura J, Weiss R, Burgert TS, Sherwin RS, et al. The normal glucose tolerance continuum in obese youth: evidence for impairment in beta-cell function independent of insulin resistance. J Clin Endocrinol Metab 2005;90:747—54.

[13] Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology 2007;133:496—506.

[14] Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170—9.

[15] Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988.

[16] Cacciari E, Milani S, Balsamo A, Spada E, Bona G, Cavallo L, et al. Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). J Endocrinol Invest 2006;29:581—93.

[17] Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:44—52.

[18] Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia, Report of a WHO/IDF consultation. Geneva: World Health Organization (WHO) and International Diabetes Federation (IDF); 2006.

[19] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462—70.

[20] Katz A, Nimbi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402—10.

[21] Sartorio A, Del Col A, Agosti F, Mazzilli G, Bellentani S, Tiribelli C, et al. Predictors of non-alcoholic fatty liver disease in obese children. Eur J Clin Nutr 2007;61:877—83.

[22] Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J (Clin Res Ed) 1986;292:13—5.

[23] Ryan TP. Modern regression methods. New York: Wiley; 2009.

[24] Gould. Quantile regression with bootstrapped standard errors. STATA J; 1992:19—21.

[25] Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med 2006; 25:127—41.

[26] Vittinghoff E, Glidden D, Shiboski SC, McCulloch CE. Regression methods in biostatistics: linear, logistic, survival, and repeated measures models. New York: Springer; 2005.

[27] Bloomgarden ZT. Type 2 diabetes in the young: the evolving epidemic. Diabetes Care 2004;27:998—1010.

[28] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 2010;51:679—89.

[29] Manco M, Bedogni G, Monti L, Morino G, Natali G, Nobili V. Intima-media thickness and liver histology in obese children and adolescents with non-alcoholic fatty liver disease. Atherosclerosis 2010;209:463—8.

[30] Manco M, Bedogni G, Marcellini M, Devito R, Ciampalini P, Sartorelli MR, et al. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. Gut 2008;57:1283—7.