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Nonalcoholic Fatty Liver Is Not Associated with the Relationship between Insulin Secretion and Insulin Sensitivity in Obese Children: Matched Case–Control Study

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Abstract

Background: No study so far has evaluated the relationship between insulin secretion (ISEC) and insulin sensitivity (ISEN) in pediatric nonalcoholic fatty liver disease (NAFLD). We evaluated the relationship between ISEC and ISEN in young obese patients with and without NAFLD.

Methods: We matched 401 $NAFLD^+$ and 595 $NAFLD^-$ children by sex (same), age (≤1 year), pubertal status (same), and body mass index (BMI; ≤0.05 standard deviation scores) using coarsened exact matching. The insulinogenic index and the ratio between the incremental areas under the curve of insulin and glucose were used as indices of ISEC. The quantitative ISEN check index, the oral glucose ISEN index, the Stumvoll index, and the Matsuda ISEN index were used as indices of ISEN. The association of NAFLD with the relationship between ISEC (response) and ISEN (predictor) was evaluated using median regression.

Results: The NAFLD·ISEN interaction was not significant in any regression model, implying commons slopes for $NAFLD^+$ and $NAFLD^-$ children. When such interaction was removed from the models, the NAFLD term was not significant, implying commons intercepts for $NAFLD^+$ and $NAFLD^-$ children.

Conclusion: NAFLD is not associated with the relationship between ISEN and ISEC in young obese children strictly matched for sex, age, pubertal status, and BMI.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in Western countries.[1] The current increase in the prevalence of pediatric NAFLD is strictly linked to the ongoing epidemic of pediatric obesity.[2]

NAFLD is presently regarded as an independent risk factor for type 2 diabetes mellitus (T2DM) in adults[1,3,4] and is likely to contribute to glucose dysregulation in young obese patients.[5,6] Both insulin resistance and beta-cell dysfunction play a role in the pathogenesis of T2DM and their joint evaluation in NAFLD may allow to better understand the NAFLD-associated glucose dysregulation.[4]

Many studies of pediatric NAFLD have shown that fatty liver is associated with increased insulin resistance.[5,7–9] Few studies have, however, evaluated beta-cell function in pediatric and adult NAFLD.[7,9,10] Moreover, no adult or

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pediatric study has been performed so far to evaluate the relationship between insulin secretion (ISEC) and insulin sensitivity (ISEN) in NAFLD, which is important to assess whether the insulin response is truly altered.[11]

Because sex, age, pubertal status, and body mass index (BMI) strongly affect glucose metabolism,[12] an accurate quantification of the effect of NAFLD on the relationship between ISEC and ISEN in obese children would require strict matching for these covariates.[13,14] In a previous study, we compared the beta-cell function of NAFLD$^+$ vs. NAFLD$^-$ children using regression modeling to control for potential confounders.[7] Even though matching would be more suitable for this task, one has to recruit a very large starting population to obtain a reasonable number of strictly matched NAFLD$^+$ and NAFLD$^-$ patients.[13] We are not aware of studies that have used strict matching rules to control for the confounding effects of sex, age, pubertal status, and BMI on the glucose metabolism of young NAFLD patients.

The aim of the present study was therefore to compare the relationship between ISEC and ISEN in NAFLD$^+$ and NAFLD$^-$ children and adolescents strictly matched by sex, age, pubertal status, and BMI.

Subjects and Methods

Study Design

We performed a matched case–control study. The starting population was 1666 young obese patients consecutively followed at our Pediatric Obesity Clinic between January 2009 and March 2014. All the patients had been admitted to the clinic to undergo a short-term structured multidisciplinary weight-loss program. The inclusion criteria were as follows: (1) age ≤18 years; (2) BMI ≥95th percentile for age and sex according to Italian reference charts[15]; (3) availability of oral glucose tolerance testing (OGTT); and (4) availability of liver ultrasonography (LUS). The exclusion criteria were as follows: (1) genetic or syndromic obesity; (2) treatment with any drug; (3) alcohol consumption (any quantity); and (4) hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. One thousand four hundred seventy-three of the 1666 children were eligible for the present study. Using coarsened exact matching (CEM),[16] we were able to match 401 NAFLD$^+$ (''cases'') to 595 NAFLD$^-$ patients (''controls'') on the basis of sex (same), age (≤1 year), pubertal status (same of five stages), and BMI (≤0.05 standard deviation scores [SDS]). The study was approved by the local ethics committee and was conducted in accordance with the 1975 Declaration of Helsinki as revised in 2008.

Clinical and Anthropometric Assessment

Pubertal status was classified in five stages (early pubertal to late pubertal) according to Tanner.[17] Weight and stature were measured following standard procedures.[18] BMI ($kg \cdot m^{-2}$) was calculated as weight/squared height. SDS of weight, stature, and BMI were calculated using the Italian reference data.[15]

Oral Glucose Tolerance Testing

Glucose tolerance was assessed by means of an OGTT with 1.75 g of glucose per kg of weight (up to 75 g).[19] Glucose and insulin were measured at 0, 30, 60, 90, and 120 minutes during OGTT. Glucose was measured using standard laboratory methods, and insulin was measured using a chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA). T2DM was defined as 120-minutes OGTT glucose $\geq 200\ mg \cdot dL^{-1}$ and impaired glucose tolerance (IGT) as 120-minutes OGTT glucose $\geq 140\ mg \cdot dL^{-1}$ and $< 200\ mg \cdot dL^{-1}$.[19]

Calculation of the Indices of ISEC and ISEN

From the OGTT, the insulinogenic index (IGI) was calculated as the ratio of the increments from 0 to 30 minutes of insulin and glucose and used as an index of ISEC.[7,20] The ratio between the incremental areas under the curve of insulin and glucose (incAUCR) was also used as an index of ISEC.[7,20] The quantitative insulin sensitivity check index (QUICKI),[21] the oral glucose insulin sensitivity index at 2 hours (OGIS),[22] the Stumvoll index (SI),[23] and the Matsuda insulin sensitivity index (ISI)[24] were used as indices of ISEN.[20] IGI, incAUCR, OGIS, SI, and ISI were calculated from glucose and insulin expressed as international units. The conversion of insulin from standard to international units was done using a conversion factor of $6.0\ \mu U \cdot mL^{-1}$ to $pmol \cdot L^{-1}$.

Liver Ultrasonography

LUS was performed by the same radiologist using standardized criteria.[1,25] 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 and other liver abnormalities.

Diagnosis of NAFLD

HBV surface antigen and anti-HCV antibodies were measured to exclude hepatitis B and C. Alcohol consumption was excluded by interview with the patients and their parents. NAFLD was operationally defined as any degree of liver steatosis in the absence of HBV and HCV infection and alcohol intake.[1,25]

Statistical Analysis

CEM was used to match NAFLD$^+$ (''cases'') to NAFLD$^-$ (''controls'') patients on the basis of sex (same), age (≤1 year), pubertal status (same of five stages), and BMI (≤0.05 SDS).[16] Descriptive statistics of continuous variables are reported as percentiles because most of them had non-Gaussian distributions. Between-group comparisons were performed by robust median regression using CEM-related

weights. The association of NAFLD with the relationship between ISEC and ISEN was evaluated using median regression models having $\log_e$IGI or $\log_e$incAUCR as the response variable.[26] The predictors of such models were as follows: (1) the index of ISEN (continuous; $\log_e$QUICKI, $\log_e$OGIS, $\log_e$SI, $\log_e$ISI); (2) NAFLD (discrete; 0 = no; 1 = yes); and (3) the NAFLD·ISEN index (discrete·continuous) interaction.[11] All regression analyses took CEM into account by using CEM-related weights and robust 95% confidence intervals.[16] Statistical analysis was performed using Stata 14.1 (Stata Corporation, College Station, TX) together with the user-written cem command.[27]

Results

Starting from a population of 1666 consecutive patients, we selected 1473 subjects respecting the inclusion and exclusion criteria, and used CEM to match 401 NAFLD$^+$ children to 595 NAFLD$^-$ children on the basis of sex (same), age (≤1 year), pubertal status (same of five stages), and BMI (≤0.05 SDS).

Table 1 reports the anthropometric and laboratory measurements of the NAFLD$^+$ and NAFLD$^-$ patients. Because of the strict matching on sex, age, pubertal status, and BMI, the anthropometric measurements of the NAFLD$^+$ and

Table 1. Measurements of the Children with and without Nonalcoholic Fatty Liver Disease

	NAFLD^{-a} ($n = 595$)			NAFLD^{+a} ($n = 401$)		
	P_{50}	P_{25}	P_{75}	P_{50}	P_{25}	P_{75}
Age (years)	15.1	12.9	16.6	15.0	12.8	16.6
Weight (kg)	99.5	84.8	114.9	98.0	84.5	114.4
Weight (SDS)	3.16	2.66	3.74	3.12	2.63	3.59
Height (m)	1.63	1.56	1.71	1.62	1.56	1.69
Height (SDS)	0.25	–0.37	1.10	0.22	–0.49	0.98
BMI ($kg \cdot m^{-2}$)	36.8	33.5	40.3	37.0	33.5	40.2
BMI (SDS)	3.09	2.69	3.45	3.09	2.69	3.45
Glucose 0 minute ($mg \cdot dL^{-1}$)	78	73	82	79	74	83
Glucose 30 minutes ($mg \cdot dL^{-1}$)	116	104	129	118	101	130
Glucose 60 minutes ($mg \cdot dL^{-1}$)	122	108	139	126	111	144
Glucose 90 minutes ($mg \cdot dL^{-1}$)	119	105	131	122	109	139
Glucose 120 minutes ($mg \cdot dL^{-1}$)	110	99	123	117***	105	134
Insulin 0 minute ($\mu U \cdot mL^{-1}$)	12	8	16	14***	10	19
Insulin 30 minutes ($\mu U \cdot mL^{-1}$)	57	38	94	65*	41	98
Insulin 60 minutes ($\mu U \cdot mL^{-1}$)	64	46	93	75***	52	109
Insulin 90 minutes ($\mu U \cdot mL^{-1}$)	68	48	93	78**	52	108
Insulin 120 minutes ($\mu U \cdot mL^{-1}$)	64	45	93	81***	56	119
IGI ($pmol \cdot mmol^{-1}$)	137	99	224	149	98	228
incAUCR ($pmol \cdot mmol^{-1}$)	152	108	219	160	111	228
QUICKI (dimensionless)	1.87	1.80	1.96	1.86	1.79	1.94
OGIS ($mL \cdot min^{-1} \cdot m^{-2}$)	438	403	476	425*	391	463
SI ($\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot L^{-1}$)	0.08	0.07	0.09	0.07***	0.05	0.09
ISI ($\mu mol \cdot kg^{-1} \cdot pmol^{-1}$)	13	10	18	11***	8	14

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. NAFLD$^-$ (robust median regression with CEM-related weights).

[a]NAFLD$^+$ and NAFLD$^-$ children were matched by sex (same), age (≤1 year), pubertal status (same of five stages), and BMI (≤0.05 SDS) using CEM.

BMI, body mass index; CEM, coarsened exact matching; IGI, insulinogenic index; incAUCR, ratio between the incremental area under the curve of insulin and the incremental area under the curve of glucose; ISI, insulin sensitivity index; NAFLD, nonalcoholic fatty liver disease; *n*, number of subjects; OGIS, oral glucose insulin sensitivity index at 2 hours; P_X, X^{th} percentile; QUICKI, quantitative insulin sensitivity check index; SDS, standard deviation scores (Italian growth charts); SI, Stumvoll index.

Table 2. Association of Nonalcoholic Fatty Liver Disease with the Relationship between Insulin Secretion and Insulin Sensitivity in Obese Children

	Model A1 $\log_e$IGI	Model A2 $\log_e$IGI	Model B1 $\log_e$IGI	Model B2 $\log_e$IGI	Model C1 $\log_e$IGI	Model C2 $\log_e$IGI	Model D1 $\log_e$IGI	Model D2 $\log_e$IGI
$\log_e$QUICKI	−1.76* [−2.43 to −1.09]	−1.76* [−2.23 to −1.28]	—	—	—	—	—	—
NAFLD	0.03 [−0.62 to 0.68]	**0.06 [−0.05 to 0.17]**	−1.26 [−5.83 to 3.32]	**0.03 [−0.07 to 0.13]**	0.31 [−0.13 to 0.74]	**0.01 [−0.08 to 0.11]**	−0.02 [−0.42 to 0.38]	**−0.03 [−0.12 to 0.07]**
NAFLD·$\log_e$QUICKI	**0.05 [−0.91 to 1.00]**	—	—	—	—	—	—	—
$\log_e$OGIS	—	—	−1.26* [−1.76 to −0.75]	−1.20* [−1.58 to −0.82]	—	—	—	—
NAFLD·$\log_e$OGIS	—	—	**0.21 [−0.54 to 0.96]**		—	—	—	—
$\log_e$SI	—	—	—	—	−0.36* [−0.44 to −0.27]	−0.30* [−0.36 to −0.24]	—	—
NAFLD·$\log_e$SI	—	—	—	—	**0.10 [−0.05 to 0.25]**		—	—
$\log_e$ISI	—	—	—	—	—	—	−0.60* [−0.71 to −0.48]	−0.60* [−0.68 to −0.51]
NAFLD*$\log_e$ISI	—	—	—	—	—	—	**−0.00 [−0.15 to 0.14]**	
Constant	6.06* [5.60 to 6.53]	6.06* [5.72 to 6.40]	12.63* [9.54 to 15.72]	12.27* [9.93 to 14.61]	4.01* [3.75 to 4.26]	4.17* [3.99 to 4.35]	6.47* [6.16 to 6.77]	6.47* [6.23 to 6.72]
n	996	996	996	996	996	996	996	996

*$p < 0.001$.

Children with and without NAFLD were matched on sex (same), age (≤1 year), pubertal status (same of five stages), and BMI (≤0.05 SDS) using CEM.

Values are regression coefficients and robust 95% confidence intervals obtained from median regression with CEM-related weights. The NAFLD·insulin sensitivity interaction is not significant in any model (A1, B1, C1, and D1) indicating common slopes. After removal of the NAFLD*insulin sensitivity interaction, NAFLD is not associated with the outcome in any model (A2, B2, C2, and D2) indicating common intercepts. The coefficients of interest are given in bold.

$\log_e$, natural logarithm.

BMI, body mass index; CEM, coarsened exact matching; IGI, insulinogenic index; incAUCR, ratio between the incremental area under the curve of insulin and the incremental area under the curve of glucose; ISI, insulin sensitivity index; NAFLD, nonalcoholic fatty liver disease; *n*, number of subjects; OGIS, oral glucose insulin sensitivity index at 2 hours; P_X, X^{th} percentile; QUICKI, quantitative insulin sensitivity check index; SDS, standard deviation scores (Italian growth charts); SI, Stumvoll index.

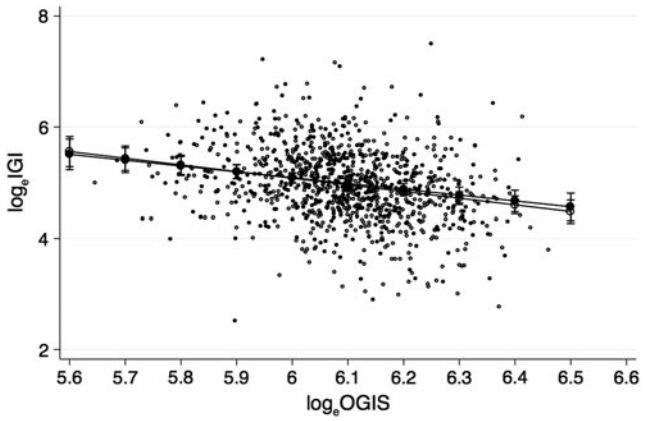

Figure 1. Relationship between log$_e$-transformed IGI and log$_e$-transformed OGIS. Values are point estimates and 95% robust confidence intervals from median regression with coarsened exact matching. The regression lines of NAFLD$^+$ (black circles) and NAFLD$^-$ (white circles) children are the same. IGI, insulinogenic index; OGIS, oral glucose insulin sensitivity index at 2 hours.

NAFLD$^-$ patients were comparable. NAFLD$^+$ patients had higher values of OGTT insulin at all time points and higher values of OGTT glucose at 120 minutes ($p < 0.05$ for all). IGT was detected in 63 NAFLD$^+$ (16%) and 43 NAFLD$^-$ (8%) patients. T2DM was detected in two NAFLD$^+$ (0.5%) patients. The ISEC indices IGI and incAUCR were similar in NAFLD$^+$ and NAFLD$^-$ patients. ISEN evaluated from fasting values (QUICKI) was also similar in NAFLD$^+$ and NAFLD$^-$ patients, while ISEN evaluated by OGTT (OGIS, SI, and ISI) was lower in NAFLD$^+$ than in NAFLD$^-$ patients ($p < 0.05$).

Table 2 reports the median regression models used to evaluate the association of NAFLD with the relationship between ISEC (log$_e$IGI) and ISEN (log$_e$QUICKI, log$_e$OGIS, log$_e$SI, and log$_e$ISI). The NAFLD·ISEN interaction was not significant in any model, implying common slopes for NAFLD$^+$ and NAFLD$^-$ patients. When the nonsignificant interaction was removed from the models, the NAFLD term was not significant, implying common intercepts for NAFLD$^+$ and NAFLD$^-$ patients. In short, the median regression lines of NAFLD$^+$ and NAFLD$^-$ patients are superimposable. The same findings were obtained using log$_e$iAUCR in place of log$_e$IGI as the response variable of the median regression models (data not shown).

Figure 1 plots the log$_e$IGI vs. log$_e$OGIS relationship.

Discussion

In this case–control study of young obese children strictly matched for sex, age, pubertal status, and BMI, we found that NAFLD was not associated with the relationship between ISEC and ISEN. This finding was consistent across different indices of ISEC (IGI and incAUCR) and ISEN (QUICKI, OGIS, SI, and ISI).

This study confirms that pediatric NAFLD is associated with decreased ISEN,[5,7–9] although after strict matching, such impairment was small and detectable only with the OGTT indices. Although the small decrease in ISEN of NAFLD$^+$ patients produced an increase in IGI, which did not reach either statistical or biological significance, insulin concentration was higher at all time points in NAFLD$^+$ patients. This can be explained by an increase of fasting ISEC, which is also known to be upregulated by insulin resistance.[11]

Compared to previous studies, the present study has several novelties and strengths. First, we matched very strictly NAFLD$^+$ and NAFLD$^-$ patients on the basis of sex, age, pubertal status, and BMI. As these factors are physiologically important regulators of glucose metabolism,[12] such strict matching is expected to control for their confounding effect. Second, all measurements (anthropometry, OGTT, and LUS) were performed by the same operators within a single center, eliminating the effect of interoperator variability, which complicates the interpretation of multicenter studies. Third, we specifically assessed ISEC in relation to ISEN using the most reliable indices computable from OGTT. We also validated our findings by using multiple indices, which provided consistent results.

Our study has nonetheless some limitations. First, although we used multiple indices of ISEC and sensitivity, which yielded consistent results, ISEN was evaluated using OGTT-based surrogate indices and C-peptide measurements were not available to assess ISEC. In particular, the unavailability of C-peptide measurements did not allow us to take into account the role of insulin clearance on glucose metabolism.[11] In this respect, a study of NAFLD adults without T2DM has shown that the contribution of impaired insulin clearance to fasting insulin increases with increasing degrees of fatty liver.[28] Thus, the insulin-based secretion indices could give a somewhat biased estimate of the true ISEC. Second, there were too few cases of IGT in our study population to model the separate effects of NAFLD and IGT on the relationship between ISEC and insulin resistance.[7] Third, liver steatosis was diagnosed by LUS. Although LUS quantifies liver fat acceptably well compared to liver biopsy[1] and this is true also in young obese patients,[29] a strictly quantitative method such as MRI does provide more information on hepatic fat.[1,5]

In conclusion, the association between ISEC and ISEN proved to be the same in young obese patients with and without NAFLD. Such findings, obtained in long-standing obese Italian children and adolescents aged 9–18 years, mirror those obtained in Italian preschool subjects at the onset of their obesity, where glucose dysregulation was not associated with NAFLD.[30] If confirmed by further studies in other populations, this would imply that the beta-cell of young obese children and adolescents is able to counteract insulin resistance independently of NAFLD.

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Author Disclosure Statement

No competing financial interests exist.

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