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ORIGINAL ARTICLE

Relationship between glucose metabolism and non-alcoholic fatty liver disease severity in morbidly obese women

Giorgio Bedogni · Amalia Gastaldelli · Claudio Tiribelli · Fiorenza Agosti · Alessandra De Col · Rezene Fessehatsion · Alessandro Sartorio

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Abstract
Background Non-alcoholic fatty liver disease (NAFLD) is an independent predictor of type 2 diabetes mellitus (T2DM). Insulin resistance and beta-cell dysfunction are involved in the pathogenesis of T2DM. Insulin resistance is associated with NAFLD but little is known about beta-cell dysfunction and NAFLD.
Aim We tested whether NAFLD severity is associated with insulin sensitivity and beta-cell function in morbidly obese women.
Subjects and methods We studied 61 Caucasian women aged 18–60 years without T2DM and with a body mass index ranging from 35.3 to 48.8 kg/m^2. The insulin sensitivity index (ISI) and the disposition index (DI) from oral glucose tolerance testing were used as measures of insulin sensitivity and beta-cell function, respectively. Fat was measured by dual-energy X-ray absorptiometry. Fatty liver was diagnosed by ultrasonography and ordinally coded as 0 = none, 1 = light, 2 = moderate, 3 = severe. Proportional-odds logistic regression was used to evaluate the association of NAFLD severity with $\log_e$ISI and $\log_e$DI with and without correction for total and truncal fat.
Results The odds of more severe vs. less severe NAFLD decreased for increasing $\log_e$ISI [odds ratio (OR) 0.40, 95 % CI 0.19–0.84, $p < 0.05$] and $\log_e$DI (OR 0.80, 95 % CI 0.69–0.92, $p < 0.01$). Neither total nor truncal fat had any effect on these associations.
Conclusion In morbidly obese women, NAFLD severity is inversely associated with insulin sensitivity and beta-cell function. The association of NAFLD severity with beta-cell dysfunction is stronger than that with insulin resistance.

Keywords Non-alcoholic fatty liver disease · Obesity · Body composition · Body fat · Dual-energy X-ray absorptiometry · Oral glucose tolerance testing · Insulin resistance · Beta-cell function

G. Bedogni (✉) · C. Tiribelli
Liver Research Center, AREA Science Park, Building Q, Strada Statale 14 km 163.5, Basovizza, 34012 Trieste, Italy
e-mail: giorgiobedogni@gmail.com

G. Bedogni
International Center for the Assessment of Nutritional Status (ICANS), University of Milano, Milan, Italy

A. Gastaldelli
Istituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche (CNR), Pisa, Italy

F. Agosti · A. De Col · A. Sartorio
Istituto Auxologico Italiano, IRCCS, Experimental Laboratory for Auxo-Endocrinological Research, Milan, Italy

R. Fessehatsion · A. Sartorio
Istituto Auxologico Italiano, IRCCS, Division of Metabolic Diseases, Verbania, Italy

Introduction

Non-alcoholic fatty liver disease (NAFLD), the most common liver disease in Western countries, is being increasingly recognized as an independent predictor of type 2 diabetes mellitus (T2DM) [1–3].

Insulin resistance and beta-cell dysfunction play a major role in the pathogenesis of T2DM and are detected many years before its onset [4–6]. Insulin resistance promotes NAFLD by suppressing the antilipolytic effect of insulin [7] and even a surrogate marker of insulin resistance such as fasting insulin is an excellent predictor of fatty liver in the general population [8]. Much less is known about

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NAFLD and beta-cell function, especially in non-diabetic subjects [9, 10].

Percent body fat (PBF) is inversely associated with insulin sensitivity [11] and body fat (BF) is distributed preferentially at the abdominal level in subjects with NAFLD [12, 13]. Thus, when investigating the NAFLD-glucose metabolism association, one should take into account the potential confounding effect of total and truncal fat [11, 14]. However, most of the available data about body composition and NAFLD were obtained with indirect methods such as anthropometry and bioelectrical impedance analysis [11–13] and there is a need of investigating such relationship using more accurate methods such as dual-energy X-ray absorptiometry (DXA) [15].

The aim of the present study was to evaluate the association between insulin resistance and beta-cell dysfunction with NAFLD severity in morbidly obese women taking into account the potential confounding effect of total and segmental body composition.

Materials and methods

Subjects

Sixty-one Caucasian obese women were consecutively enrolled into the study at the Division of Metabolic Diseases of the Istituto Auxologico Italiano (Piancavallo, Verbania, Italy). Inclusion criteria were: (1) age $\geq$18 years, (2) body mass index (BMI) $\geq$30 kg/m^2 and, (3) body weight $\leq$130 kg (as the employed DXA scanner could not accommodate heavier subjects). Exclusion criteria were: (1) overt endocrine disease including T2DM and use of glucose-lowering drugs and, 2) use of drugs able to induce or reduce liver steatosis (e.g., *n*-3-polyunsaturated fatty acids). The study protocol was approved by the local Ethical Committee and all women provided written informed consent.

Anthropometry

Weight and height were measured following international guidelines [16]. BMI was calculated as weight (kg)/height (m)^2 and obesity was classified according to the World Health Organization (WHO) [17].

Dual-energy X-ray absorptiometry

DXA was performed using a GE-Lunar Prodigy scanner (GE Medical Systems, Milwaukee, WI, USA) as described in detail elsewhere [15]. The three-compartment DXA model separates body mass (BM) into fat mass (FM), lean tissue mass and bone mineral content. DXA scans were analyzed using GE Encore software version 8.80 (GE Medical Systems, Milwaukee, WI, USA). PBF was calculated as FM (kg)/BM (kg) and percent trunk fat (PTF) as trunk FM (kg)/FM (kg).

Oral glucose tolerance testing

Glucose tolerance was evaluated by OGTT using 1.75 g of glucose per kg/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 or 120-min OGTT glucose $\geq$200 mg/dl; IFG as fasting glucose between 100 and 126 mg/dl; and IGT as 120-min OGTT glucose between 140 and 200 mg/dl [18]. The insulin sensitivity index (ISI) was calculated as described by Matsuda and DeFronzo [19] and used as marker of insulin sensitivity. The disposition index (DI), i.e., the product of ISI and the ratio between the incremental areas under the curve of insulin and glucose (dAUCr), was used as measure of beta-cell function [5, 6, 20].

Liver ultrasonography

Liver ultrasonography was performed by the same radiologist using standardized criteria [21]. Normal liver was defined as the absence of liver steatosis or other liver abnormalities. Light steatosis was defined as the presence of slight "bright liver" or hepatorenal echo contrast without intrahepatic vessels blurring and deep attenuation; moderate steatosis as the presence of mild "bright liver" or hepatorenal echo contrast without intrahepatic vessel blurring and with deep attenuation; and severe steatosis as diffusely severe "bright liver" or hepatorenal echo contrast, with intrahepatic vessels blurring (no visible borders) and deep attenuation without visibility of the diaphragm. The intra-rater agreement, as evaluated by repeated measures of fatty liver severity of the same subjects on different days, was good (Cohen's $k = 0.8$) and coherent with available data [22]. NAFLD was defined as any degree of liver steatosis in the absence of HBV and HCV infection and alcohol intake [23].

Statistical analysis

Most continuous variables did not follow a Gaussian distribution and all are reported as 50th, 25th and 75th percentiles. Discrete variables are reported as counts and percentages. The association between NAFLD severity and ISI, dAUCr and DI was evaluated using

proportional-odds ordinal logistic regression [21, 24, 25]. NAFLD was ordinally coded (0 = none, 1 = light, 2 = moderate, 3 = severe) and its association with $\log_e$-transformed ($\log_e$) ISI, $\log_e$dAUCr and $\log_e$DI was evaluated by means of three models. Model 1 included only the continuous predictor of interest, i.e., $\log_e$ISI, $\log_e$dAUCr or $\log_e$DI; Model 2 added continuous PBF as covariate to Model 1; and Model 3 added continuous PTF as covariate to Model 1. Collinearity among predictors was excluded using the Belsey–Kuh–Welsh test [26]. All the outcome–predictors relationships were linear as detected also using fractional polynomials [27]. The proportional-odds assumption made by the ordinal logistic regression model was checked using the Brant test [21, 28]. Akaike information criterion (AIC) was used to evaluate model accuracy [28]. Statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, TX, USA). All statistical tests were two-tailed and statistical significance was set to a p value <0.05.

Results

Table 1 gives the measurements of the 61 women who were consecutively enrolled into the study. They were aged 18–60 years and had a BMI ranging from 35.3 to 48.8 kg/m^2 and a PBF ranging from 41 to 59 %.

Table 2 reports WHO obesity class, glucose tolerance status, and NAFLD severity. Although just one woman had a fasting glucose >100 mg/dl (102 mg/dl), 16 women were found to have IGT at OGTT.

Table 3 reports the proportional-odds logistic regression models used to evaluate the association between NAFLD severity, $\log_e$ISI, $\log_e$dAUCr, and $\log_e$DI.

The odds of more severe vs. less severe NAFLD decreased for increasing values of $\log_e$ISI (OR 0.40, 95 % CI 0.19–0.84, $p < 0.05$, Model A1) without any influence from PBF (Model A2) or TBF (Model A3). There was no association between NAFLD severity and $\log_e$dAUCr (Model B1), with virtually no influence from PBF (Model B2) or TBF (Model B3). However, $\log_e$DI was inversely associated with NAFLD severity (OR 0.80, 95 % CI 0.69–0.92, $p < 0.01$, Model C1) without any influence from PBF (Model C2) or TBF (Model C3) and was a better predictor of NAFLD severity than $\log_e$ISI (AIC 162 vs. 168).

Figure 1 plots the probability of NAFLD severity as a function of $\log_e$DI as obtained from Model C1 of Table 3.

A change in $\log_e$DI from 1.0 to 2.5 was associated with a change in the probability of no NAFLD from 0.12 (95 % CI 0.02–0.23) to 0.57 (0.36–0.77), with corresponding values of 0.20 (95 % CI 0.08–0.32) to 0.25 (0.12–0.38) for light NAFLD, 0.31 (95 % CI 0.17–0.45) to 0.12 (0.03–0.22) for moderate NAFLD, and 0.37 (95 % CI 0.16–0.56) to 0.06 (0.01–0.12) for severe NAFLD.

The odds of more severe vs. less severe NAFLD increased with increasing age (OR 1.63, 95 % CI 1.08–2.46 for each decade of age, $p = 0.019$, univariable proportional-odds logistic regression). However, age was not associated with NAFLD independently from $\log_e$ISI, $\log_e$dAUCr or $\log_e$DI (odds ratios not shown, $p > 0.05$, multivariable proportional-odds logistic regression). The logits of $\log_e$ISI, $\log_e$dAUCr and $\log_e$DI changed between 8 and 10 % when age was included in the models showing the absence of confounding from age [25].

Table 1 Measurements of the 61 women

	P_{50}	P_{25}	P_{75}
Age (years)	37	28	46
Weight (kg)	108.2	100.6	114.9
Height (m)	1.62	1.57	1.65
BMI (kg/m^2)	40.7	39.5	43.2
Fat mass (kg)	53.3	48.3	57.0
Fat mass (% body mass)	53.4	50.9	54.9
Fat mass–trunk (% fat mass)	50.8	47.1	53.4
OGTT glucose 0 min (mg/dl)	83	77	88
OGTT glucose 120 min (mg/dl)	122	101	140
OGTT insulin 0 min (μU/ml)	7	4	10
OGTT Insulin 120 min (μU/ml)	56	38	86
ISI	5.38	3.91	10.14
dAUCr	1.06	0.70	1.47
DI	5.74	3.70	7.58

P_X Xth percentile, *BMI* body mass index, *OGTT* oral glucose tolerance test, *ISI* insulin sensitivity index, *dAUCr* ratio between the incremental areas under the curve of insulin and glucose, *DI* disposition index (ISI dAUCr)

Table 2 Obesity class, glucose tolerance and non-alcoholic fatty liver severity

	n	%
WHO obesity class		
Class 2	20	32.8
Class 3	41	67.2
Glucose tolerance (OGTT)		
NGT	45	73.8
IGT	16	26.2
Fatty liver (ultrasonography)		
None	20	32.8
Light	16	26.2
Moderate	14	23.0
Severe	11	18.0

WHO World Health Organization, *OGTT* oral glucose tolerance test, *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance

Table 3 Association between non-alcoholic fatty liver disease severity, insulin sensitivity and beta-cell function

	A1	A2	A3	B1	B2	B3	C1	C2	C3
log_eISI	0.40* (0.19–0.84)	0.40* (0.19–0.84)	0.41* (0.19–0.86)	–	–	–	–	–	–
Fat mass (% body fat)	–	0.99 (0.88–1.12)	–	–	0.98 (0.87–1.10)	–	–	1.00 (0.89–1.13)	–
Trunk fat mass (% fat mass)	–	–	1.05 (0.97–1.14)	–	–	1.06 (0.98–1.15)	–	–	1.05 (0.97–1.14)
log_edAUCr	–	–	–	0.92 (0.40–2.11)	0.91 (0.40–2.09)	0.94 (0.41–2.15)	–	–	–
log_eDI	–	–	–	–	–	–	0.80** (0.69–0.92)	0.80** (0.69–0.92)	0.80** (0.70–0.93)
AIC	168	170	169	174	176	174	162	164	163

Values are odds ratios and 95 % confidence intervals from a proportional-odds ordinal logistic regression model

log_e natural logarithm, *ISI* insulin sensitivity index, *dAUCr* ratio between the incremental areas under the curve of insulin and glucose, *DI* disposition index, *AIC* Akaike information criterion

* $p < 0.05$; ** $p < 0.01$

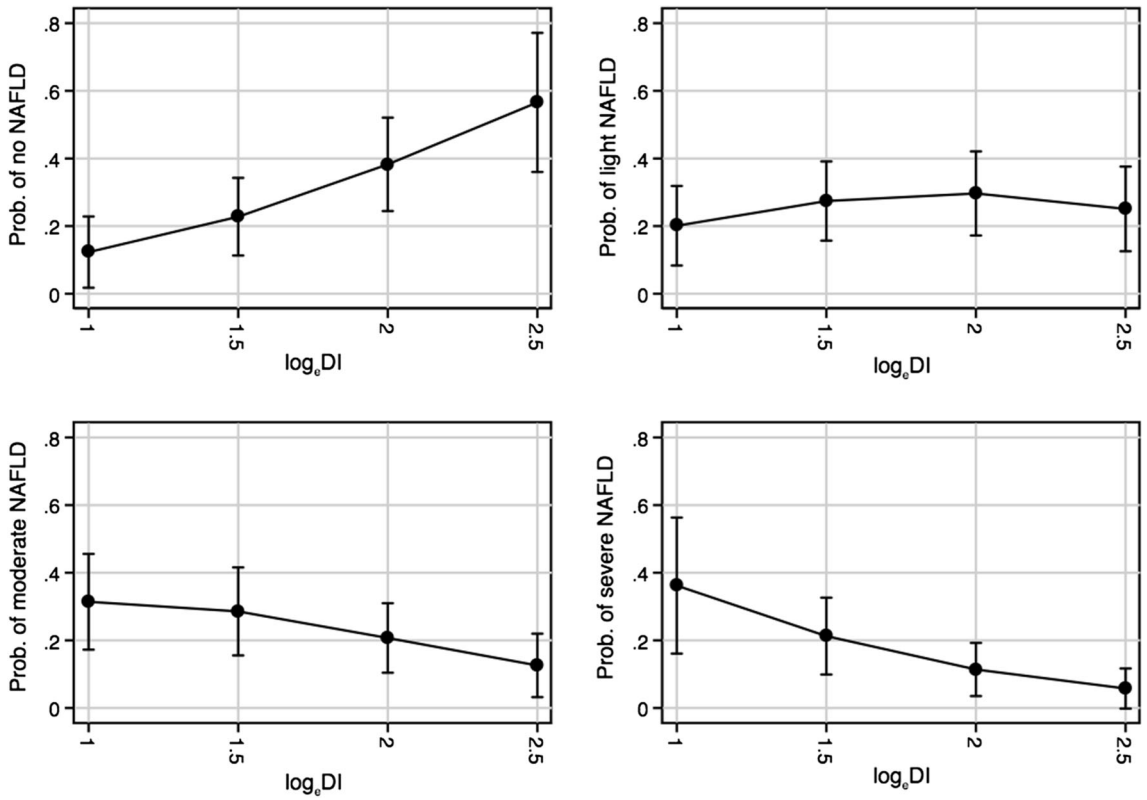

Fig. 1 Fatty liver severity and beta-cell function. *NAFLD* non-alcoholic fatty liver disease, *log_eDI* natural logarithm of the disposition index

Discussion

In the present study, we evaluated whether NAFLD severity is associated with reduced insulin sensitivity and beta-cell dysfunction in morbidly obese women. We found that NAFLD severity was inversely associated with insulin sensitivity as detected by ISI and beta-cell function as detected by DI. Even if dAUCR was not associated with NALFD severity, the ISI dAUCr product, i.e., DI, was associated more strongly than ISI with NAFLD severity.

To gain a better understanding of the NAFLD-glucose metabolism relationship, we modeled ultrasonographically

detected NAFLD as ordinal measure [21, 29]. Ultrasonography is the method most commonly employed to assess fatty liver in the general population [8, 22]. As compared to liver biopsy, ultrasonography has a sensitivity of 84.8 %, a specificity of 93.6 %, a positive likelihood ratio of 13.3, and a negative likelihood ratio of 0.16 for the detection of moderate to severe fatty liver [22]. As shown in Fig. 1, the progressive decrease of the probability of having fatty liver (57–12 %) observed with $\log_e$DI increasing from 1.0 to 2.5 was attributable mostly to a decreasing probability of moderate (31–12 %) and severe (37–6 %) fatty liver. Similar probabilities were found for $\log_e$ISI (not shown). Our findings suggest therefore a dose–effect relationship between liver fat, ISI, and DI in morbidly obese women and are in agreement with those of a previous study where we found that ISI was inversely associated and dAUCr was not associated with fatty liver in morbidly obese children [20].

Although ISI was associated and dAUCr was not associated with NAFLD severity as in our previous study of obese children [20] and in another study of non-obese adults [10], the ISI dAUCr product, i.e., DI, was a better predictor of NAFLD severity than ISI alone. This is clearly shown by the higher precision of the estimate of the effect size (OR 0.80, 95 % CI 0.69–0.92 vs. 0.40, 95 % CI 0.19–0.84) and the lower AIC (162 vs. 168) of $\log_e$DI vs. $\log_e$ISI. Thus, despite the fact that DI incorporates ISI which is associated with NAFLD severity and dAUCR which is not, a measure of beta-cell function is associated more strongly than a measure of insulin sensitivity with NAFLD severity.

This study has some limitations. First, we studied morbidly obese Caucasian women and our findings may not generalize to non-obese individuals, non-Caucasians and men. Studying normal weight and overweight men and women will be central to disentangling the relationship between beta-cell function and NAFLD. Second, we used OGTT to obtain surrogate measures of insulin sensitivity and beta-cell function. Even though direct measures are certainly preferable, ISI and DI have been extensively validated and were instrumental to the current understanding of the pathophysiology of T2DM [5, 6, 20]. Third, we used ultrasonography to evaluate fatty liver. Although ultrasonography is an indirect method, it offers an accurate assessment of FL starting from an intrahepatic triglyceride content as low as 10 % [22]. Moreover, liver ultrasonography was performed by the same trained radiologist with good reproducibility [22].

In conclusion, in morbidly obese women without T2DM, NAFLD severity is inversely associated with insulin sensitivity and beta-cell function. The association of NAFLD severity with beta-cell function is stronger than that with insulin sensitivity and none of these associations is affected by total or truncal fat.

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Conflict of interest None.

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