

# Elevated 1 h postload plasma glucose levels identify adults with normal glucose tolerance but increased risk of non-alcoholic fatty liver disease

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## ABSTRACT

**Objective:** To determine the ability of the proposed diagnostic value of a 1-h OGTT glucose  $\geq 155\text{ mg/dL}$  to identify individuals with non-alcoholic fatty liver disease (NAFLD) diagnosed by ultrasonography in a cohort of adult white individuals.

**Design:** The study group comprised 710 white individuals participating to the CATAnzaro MEtabolic RIrisk factors (CATAMERI) Study, a cross-sectional study assessing cardio-metabolic risk factors in individuals carrying at least one risk factor including dysglycemia, overweight/obesity, hypertension, dyslipidemia. a 75 g oral Oral Glucose Tolerance Test (OGTT) was performed with 0, 30, 60, 90 and 120 min sampling for plasma glucose and insulin measurements. Cardio-metabolic risk factors including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were assessed in the whole cohort.

**Results:** Of the 710 participants examined, 295 had normal glucose tolerance (NGT) with 1-hour post-load plasma glucose  $< 155\text{ mg/dL}$  (NGT 1h-low), 109 individuals had NGT 1h-high, 104 had isolated impaired fasting glucose (IFG), and 202 had impaired glucose tolerance (IGT). As compared with NGT 1h-low, NGT 1h-high and IGT subjects exhibited significantly higher body mass index (BMI), triglycerides, high sensitivity C reactive protein, ALT, GGT, and hepatic insulin resistance (IR), assessed by the liver IR index, as well as lower high density lipoprotein, and insulin-like growth factor-1 (IGF-1) levels. In a logistic regression analysis adjusted for age, gender, and BMI, NGT 1h-high participants had a 1.5-fold increased risk of having NAFLD and an even increased risk was observed in subjects with IGT (1.8-fold), but not in the isolated IFG group (1.01-fold).

**Conclusions:** These data suggest that the value of a 1-hour OGTT glucose  $\geq 155\text{ mg/dL}$  may be helpful to identify a subset of NGT individuals at risk for NAFLD.

## Key messages

- Our data suggest that normal glucose tolerance (NGT) participants with 1 h postload glucose  $\geq 155\text{ mg/dL}$  (NGT-1 h-high) are at increased risk of having non-alcoholic fatty liver disease (NAFLD).
- In addition, NGT 1 h-high individuals have higher levels of biomarkers of NAFLD.
- It would thus be useful to perform liver ultrasonography in NGT 1 h-high individuals to delay adverse clinical outcomes.

high-risk individuals who may mostly benefit from lifestyle<sup>1–2</sup> or pharmacological interventions<sup>2–4</sup> are crucial to implement focused and cost-effective prevention plans. Participants with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) have been demonstrated to be at increased risk for the future development of type 2 diabetes as compared with individuals with normal glucose tolerance (NGT).<sup>5–6</sup> However, prospective studies have also shown the limitations of IFG and IGT as ‘prediabetes’ categories in predicting risk, as only 35–50% of individuals with IFG and/or IGT eventually develop the disease, whereas 30–40% of diabetic participants had NGT at baseline.<sup>5–6</sup> These data suggest that using exclusively IFG/IGT criteria to identify participants at increased risk to develop type 2 diabetes may cause one to miss a significant number of individuals who will actually become diabetic. Recently, it has been reported that plasma glucose levels  $\geq 155\text{ mg/dL}$  at 1 h during an oral glucose tolerance test (OGTT) can help one to recognize among NGT individuals those at increased risk for type 2 diabetes (NGT 1 h-high).<sup>7–8</sup> It is notable that NGT 1 h-high individuals present an impaired cardiometabolic profile and show a pattern of subclinical organ damage comparable to the one observed in IGT individuals.<sup>9–18</sup>



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## INTRODUCTION

The prevalence of type 2 diabetes mellitus has reached pandemic proportions, and reliable screening tools aimed at identifying

Recently, the important role played by fat accumulation, due to non-alcoholic causes, in the liver (non-alcoholic fatty liver disease (NAFLD)) in the pathogenesis of the metabolic syndrome and type 2 diabetes has gained attention.<sup>19–20</sup> Not only is NAFLD cross-sectionally associated with obesity, metabolic syndrome, altered glucose tolerance and type 2 diabetes,<sup>19–22</sup> but also its presence represents an independent predictor for future type 2 diabetes.<sup>23–28</sup> Moreover, metabolic abnormalities associated with type 2 diabetes, such as higher peripheral lipolysis from the adipose tissue, and elevated de novo lipogenesis induced by hyperglycemia and hyperinsulinemia, may cause fat accumulation in the liver.<sup>19–20</sup>

The question of whether NGT 1 h-high individuals are at increased risk of NAFLD is still unsettled. In an attempt to gain further information on clinical features of NGT 1 h-high individuals, we determined the ability of the proposed diagnostic value of a 1 h OGTT glucose  $\geq 155$  mg/dL to identify individuals with NAFLD diagnosed by ultrasonography in a cohort of adult white individuals.

## METHODS

### Study participants

The study group comprised 710 white individuals participating in the CATanzaro MEtabolic RIrisk factors (CATAMERI) Study, a cross-sectional study assessing cardiometabolic risk factors in individuals carrying at least one risk factor including dysglycemia, overweight/obesity, hypertension and dyslipidemia.<sup>10 29–33</sup> Exclusion criteria included: known diabetes, history of malignant disease, end-stage renal disease, chronic gastrointestinal diseases, chronic pancreatitis, positivity for antibodies to hepatitis C virus (HCV) or hepatitis B surface antigen (HBsAg), self-reporting alcohol consumption of  $<20$  g/day, and history of use of toxins or drugs known to induce liver damage such as tamoxifen, glucocorticoids, tetracycline, estrogens, methotrexate, and amiodarone. After a 12 h fast, all individuals underwent anthropometrical evaluation including assessment of body mass index (BMI), waist circumference, and body composition evaluated by bioelectrical impedance, and readings of clinic blood pressure (BP) obtained in the left arm of the supine patients, after 5 min of quiet rest, and a venous blood sample was drawn for laboratory determinations. Thereafter, a 75 g oral OGTT was performed with 0, 30, 60, 90, and 120 min sampling for plasma glucose and insulin measurements.<sup>10 29–33</sup>

Liver ultrasonography was performed in all participants by the same trained operator, who was blind to their clinical characteristics, using a Toshiba Aplio 50 ultrasound apparatus equipped with a 3.5 MHz linear transducer.<sup>29</sup> Longitudinal, subcostal, ascending, and oblique scans were performed. The ultrasonographic criteria used to diagnose fatty liver included liver and kidney echo discrepancy, the presence of an increased liver echogenicity or ‘bright liver’, poor echo penetration into the deep portion

of the liver, and vascular blurring either singly or in combination.<sup>34</sup> A semiquantitative ultrasound evaluation of the degree of steatosis was not available, and therefore participants were categorized as having (yes) or not having (no) liver steatosis.

### Analytical determinations

Glucose, triglyceride, total and high-density lipoprotein (HDL) cholesterol concentrations were determined by enzymatic methods (Roche, Basel, Switzerland). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using the  $\alpha$ -ketoglutarate reaction;  $\gamma$ -glutamyltransferase (GGT) levels with the L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide rate method, and alkaline phosphatase by colorimetric assay (Roche, Basel, Switzerland). Antibodies to HCV or HBsAg were assessed by enhanced chemiluminescence assays. High sensitivity C reactive protein (hsCRP) levels were measured by an automated instrument (CardioPhase hsCRP, Milan, Italy). Plasma insulin concentration was assessed with a chemiluminescence-based assay (Immulite, Siemens, Italy). Total serum IGF-1 concentrations were measured by a chemiluminescent immunoassay (Nichols Institute Diagnostic, San Juan Capistrano, California, USA).

### Calculations

Participants were classified as NGT (fasting plasma glucose (FPG)  $<100$  mg/dL and 2 h postload  $<140$  mg/dL), isolated IFG (FPG 100–126 mg/dL and 2 h postload  $<140$  mg/dL), and IGT (FPG  $<100$  mg/dL and 2 h postload 140–199 mg/dL).

The liver insulin resistance (IR) index was calculated using the formula:  $-0.091 + (\log \text{insulin area under the curve (AUC)} 0\text{--}120 \text{ min} \times 0.400) + (\log \text{fat mass \%} \times 0.346) - (\log \text{HDL cholesterol} \times 0.408) + (\log \text{BMI} \times 0.435)$ .<sup>35</sup> The trapezoidal method was used to calculate glucose and insulin AUC during an OGTT.

### Statistical analysis

Variables with skewed distribution including triglyceride, fasting insulin, 1 h insulin, and 2 h insulin were natural log transformed for statistical analyses. Continuous data are expressed as means  $\pm$  SD. Categorical variables were compared by  $\chi^2$  test. Anthropometric and metabolic differences between groups were tested after adjusting for age and gender using a general linear model with a post hoc Bonferroni correction for multiple comparisons. Relationships between variables were determined by Pearson's correlation coefficient ( $r$ ). Partial correlation coefficients adjusted for age and gender were computed between variables. A multivariate logistic regression analysis was used to determine the association between the study groups and NAFLD. A  $p$  value  $<0.05$  was considered statistically significant. All analyses were performed using the SPSS software program V.16.0 for Windows.

## RESULTS

Of the 710 participants examined, 404 (56.9%) had NGT, 104 (14.6%) had isolated IFG, and 202 (28.5%) had IGT. Participants with NGT were divided into two groups: 295 participants with 1 h postload plasma glucose <155 mg/dL (NGT 1 h-low) and 109 individuals with 1 h postload plasma glucose ≥155 mg/dL (NGT 1 h-high). Table 1 shows the clinical characteristics and biochemical parameters of the four study groups. Significant differences between the four groups were observed with respect to gender (higher prevalence of men among NGT 1 h-high, and isolated IFG as compared with NGT 1 h-low) and age (NGT 1 h-high, isolated IFG, and IGT were older than NGT 1 h-low), and therefore all analyses were adjusted for age and gender.

NGT 1 h-high individuals had a metabolic risk profile which was intermediate between the one observed in NGT 1 h-low participants and the one of IGT individuals. As shown in table 1, NGT 1 h-high participants exhibited significantly higher BMI, 2 h postload plasma glucose, fasting, 1 h and 2 h postchallenge insulin levels, triglycerides, hsCRP, ALT, GGT, and hepatic IR, assessed by the liver IR index, as well as lower HDL and IGF-1 levels as compared with NGT 1 h-low participants.

As compared with NGT 1 h-low individuals, IGT participants exhibited significantly higher BMI, waist circumference, fat mass, fasting and 1 h postchallenge glucose levels, fasting, 1 h and 2 h postchallenge insulin levels, triglycerides, hsCRP, ALT, AST, GGT, and the hepatic IR index as well as lower HDL and IGF-1 levels. By definition, IGT participants exhibited significantly higher postchallenge glucose levels as compared with the three other groups.

As compared with NGT 1 h-low individuals, isolated IFG participants exhibited significantly higher 1 h and 2 h postload plasma glucose, 1 h and 2 h postchallenge insulin levels, triglycerides, and the hepatic IR index. By definition, isolated IFG participants exhibited significantly higher fasting glucose levels as compared with the three other groups.

As compared with NGT 1 h-low individuals, a greater proportion of individuals with NGT 1 h-high or IGT, but not of those with isolated IFG, had NAFLD diagnosed by ultrasonography (table 1).

A logistic regression model adjusted for age and gender was used to compare the risk of NGT 1 h-high, isolated IFG, and IGT to have NAFLD, as compared with the NGT 1 h-low group (the reference category; table 2). NGT 1 h-high participants had a 1.7-fold increased risk of having NAFLD; an even increased risk was observed in participants with IGT (2.3-fold), but not in the isolated IFG group (1.1-fold; table 2). Neither age nor gender was associated with increased risk of NAFLD. After adding BMI to the logistic regression model in addition to age and gender, the odds of NGT 1 h-high and IGT participants to have NAFLD were attenuated, but the results remained significant (table 2). Similar results were obtained when the logistic regression model included waist circumference in addition to age and gender (table 2).

Age and gender adjusted univariate correlations between ALT, AST, and GGT, the three more commonly used biomarkers of fatty liver, and anthropometric and metabolic variables in the whole study group showed that ALT, AST, and GGT were all significantly correlated with BMI, waist circumference, fat mass, triglycerides, circulating IGF-1, hsCRP, fasting, 1 h and 2 h postchallenge glucose and insulin levels, and the liver IR index (see online supplementary table S1). Total cholesterol showed a statistically significant correlation with ALT and GGT, but not with AST, whereas HDL was negatively correlated with ALT and AST (see online supplementary table S1).

## DISCUSSION

In the Western world, NAFLD constitutes the most common chronic liver disease, and its prevalence is further rising in parallel with one of the pandemic metabolic disorders such as obesity, metabolic syndrome, abnormal glucose tolerance, and type 2 diabetes.<sup>19–20</sup> There is compelling evidence that abnormal glucose tolerance, type 2 diabetes, and NAFLD originate from shared pathophysiological mechanisms. In fact, a continuous increase of hepatic fat accumulation has been reported in parallel with the deterioration of glucose tolerance from NGT to isolated IFG, isolated IGT, and combined IFG/IGT.<sup>36</sup> In addition, it has been suggested by a number of studies that future type 2 diabetes may be predicted from the presence of NAFLD, or on the basis of liver biomarkers and hepatic proinflammatory and anti-inflammatory molecule levels.<sup>23–28 37–39</sup> Moreover, in prospective studies, a significant percentage of NGT participants has been shown to be at increased risk for type 2 diabetes.<sup>5 6</sup> In particular, a cutoff point of 155 mg/dL for 1 h postload plasma glucose concentration during the OGTT has been suggested to be able to identify a subgroup of individuals with NGT (NGT 1 h-high) who are at risk to develop the disease.<sup>7 8</sup> These observations, along with the possibility to analyze a accurately characterized cohort of non-diabetic individuals, have provided the motivation for investigating whether NGT 1 h-high individuals also show an increased prevalence of NAFLD. In the current cross-sectional study, we observe that individuals with NGT, whose 1 h postload plasma glucose is ≥155 mg/dL, have an increased risk to have NAFLD assessed by ultrasonography as compared with NGT individuals with 1 h postload plasma <155 mg/dL. In addition, NGT 1 h-high individuals have higher levels of biomarkers of NAFLD such as ALT, GGT, and hsCRP, and lower levels of circulating IGF-1, whose hepatic expression is reduced in participants with NAFLD.<sup>36</sup> Nonetheless, NGT 1 h-high participants show an intermediate metabolic risk profile between the one observed in NGT 1 h-low individuals and the one of IGT participants; these data are in keeping with the relatively higher risk of developing type 2 diabetes observed in the latter

**Table 1** Anthropometric and metabolic characteristics of study subjects stratified according to glucose tolerance

Variables	Whole study group	NGT with 1 h glucose	NGT with 1 h glucose	Isolated IFG (95% CI) (3)	IGT (95% CI) (4)	p Value	p Value		
		<155 mg/dL (95% CI) (1)	≥155 mg/dL (95% CI) (2)				1 vs 2	1 vs 3	1 vs 4
N (male/female)	710 (345/365)	295 (118/177)	109 (64/45)	104 (62/42)	202 (101/101)	<0.0001	0.001	0.0008	0.03
Age (year)	50±13 (49 to 51)	44±13 (42 to 46)	51±12## (48 to 53)	56±10 (54 to 58)	54±12 (52 to 56)	<0.0001*	<0.0001*	<0.0001*	<0.0001*
BMI (kg/m <sup>2</sup> )	30.5±6.2 (30.0 to 30.9)	29.3±6.1 (28.6 to 30.1)	31.0±6.1# (29.8 to 32.2)	30.5±5.3 (29.1 to 31.1)	32.1±6.4 (31.0 to 32.9)	<0.0001	0.02	0.30	<0.0001
Waist circumference (cm)	102±14 (101 to 104)	99±13 (97 to 101)	103±12 (100 to 105)	103±13 (100 to 106)	106±14 (104 to 108)	<0.0001	0.17	0.15	<0.0001
Fat mass (%)	33±9 (32 to 34)	31±8 (30 to 32)	33±9 (30 to 34)	33±8 (31 to 34)	35±8 (33 to 36)	<0.0001	0.29	0.09	<0.0001
Current smokers (n)	145 (20.4%)	67 (22.7%)	25 (22.9%)	17 (16.3%)	36 (17.8%)	0.35	0.92	0.21	0.22
SBP (mm Hg)	131±17 (130 to 133)	128±17 (126 to 130)	132±16 (128 to 135)	136±15 (133 to 139)	134±16 (131 to 136)	0.61	0.92	0.97	0.90
DBP (mm Hg)	81±11 (80 to 82)	80±11 (79 to 82)	81±10 (80 to 83)	82±10 (80 to 84)	81±10 (79 to 82)	0.73	0.97	0.94	0.95
Fasting glucose (mg/dL)	93±11 (93 to 94)	87±7 (86 to 88)	90±7### §§§ (89 to 91)	107±6 §§§ (105 to 109)	98±11 (97 to 100)	<0.0001	0.31	<0.0001	<0.0001
1 h glucose (mg/dL)	153±44 (150 to 157)	116±24 (113 to 119)	180±21##§ (176 to 184)	157±36§§§ (150 to 164)	191±35 (186 to 196)	<0.0001	<0.0001	<0.0001	<0.0001
2 h glucose (mg/dL)	123±34 (121 to 126)	101±18 (99 to 103)	113±20§§§ (109 to 117)	111±21§§§ (106 to 115)	168±21 (165 to 171)	<0.0001	<0.0001	0.01	<0.0001
Fasting insulin (μU/mL)	13±8 (12 to 14)	12±8 (11 to 13)	13±8§ (12 to 14)	13±6 (12 to 14)	14±8 (13 to 16)	<0.0001	0.05	0.001	<0.0001
1 h insulin (μU/mL)	110±76 (103 to 116)	93±72 (84 to 102)	145±92#§ (126 to 164)	111±70 (96 to 127)	114±70 (103 to 125)	<0.0001	<0.0001	0.006	<0.0001
2 h insulin (μU/mL)	95±81 (89 to 102)	68±57 (61 to 75)	96±69§§§ (81 to 110)	83±62§§§ (70 to 97)	142±102 (127 to 158)	<0.0001	<0.0001	0.003	<0.0001
Total cholesterol (mg/dL)	203±39 (200 to 206)	200±37 (196 to 204)	205±30 (200 to 211)	212±40 (204 to 220)	203±43 (197 to 209)	0.88	0.99	0.99	0.99
HDL (mg/dL)	51±14 (49 to 52)	52±14 (50 to 54)	47±12 (45 to 50)	52±13§ (50 to 55)	48±14 (47 to 51)	0.004	0.02	0.65	0.006
Triglycerides (mg/dL)	132±6 (127 to 138)	120±63 (112 to 127)	134±64 (122 to 147)	140±63 127 to 152)	147±78 (136 to 158)	0.002	0.05	0.02	<0.0001
IGF-1 (ng/mL)	158±61 (153 to 163)	178±67 (170 to 186)	152±54 (142 to 165)	156±51 (143 to 168)	138±50 (130 to 146)	0.001	0.02	0.07	<0.0001
hsCRP (mg/L)	3.7±3.3 (3.2 to 4.1)	2.9±2.5 (2.5 to 3.5)	4.1±3.4## (3.3 to 4.9)	2.7±2.5 §§ (2.0 to 3.5)	4.0±3.0 (3.5 to 4.5)	<0.0001	<0.0001	0.61	<0.0001
ALT (UI/L)	25±14 (24 to 26)	22±13 (21 to 24)	27±13 (23 to 39)	25±11 (22 to 27)	28±16 (26 to 31)	<0.0001	0.03	0.08	<0.0001
AST (UI/L)	22±8 (21 to 23)	20±7 (19 to 21)	21±6 (20 to 22)	22±7 (21 to 24)	24±9 (23 to 25)	0.001	0.83	0.12	0.001
GGT (UI/L)	26±18 (25 to 28)	23±15 (21 to 25)	31±24 (26 to 34)	28±16 (24 to 32)	33±31 (26 to 32)	0.001	0.04	0.36	<0.0001
ALK (UI/L)	69±20 (67 to 71)	66±19 (63 to 68)	72±18 (68 to 76)	71±20 (67 to 76)	71±21 (68 to 74)	0.17	0.18	0.35	0.21
Liver IR index	2.73±0.37 (2.69 to 2.76)	2.63±0.38 (2.58 to 2.68)	2.82±0.36# (2.74 to 2.89)	2.69±0.31 (2.62 to 2.76)	2.84±0.34 (2.79 to 2.89)	<0.0001	<0.0001	0.01	<0.0001
NAFLD (n)	147 (49.8%)	147 (49.8%)	68 (62.4%)	52 (50%)	135 (66.8%)	0.001	0.03	0.92	0.0002

Data are means±SD. Fasting, 1 h and 2 h insulin, and triglycerides were log transformed for statistical analysis, but values in the table represent a back transformation to the original scale. Categorical variables were compared by  $\chi^2$  test. Comparisons between the four groups were performed using a general linear model with a post hoc Bonferroni correction for multiple comparisons. p Values refer to results after analyses with adjustment for age, gender, and BMI.

§p<0.05 vs IGT; §§p<0.001 vs IGT; §§§p<0.0001 vs IGT.

#p<0.05 vs IFG; ##p<0.001 vs IFG; ###p<0.0001 vs IFG.

\*p Values refer to results after analyses with adjustment for gender.

ALK, alkaline phosphatase; ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; GGT,  $\gamma$ -glutamyltransferase; HDL, high-density lipoprotein; hsCRP, high sensitivity C reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; NGT, normal glucose tolerance; SBP, systolic blood pressure.

**Table 2** OR (95% CI) by multiple logistic regression models for NAFLD in relation to glucose tolerance groups

Study group	NAFLD		
	OR	95% CI	p Value
<b>Model 1</b>			
NGT 1 h-low (reference category)	1	–	–
NGT 1 h-high	1.71	1.07 to 2.71	0.02
Isolated IFG	1.11	0.69 to 1.80	0.64
IGT	2.31	1.55 to 3.46	<0.0001
<b>Model 2</b>			
NGT 1 h-low (reference category)	1	–	–
NGT 1 h-high	1.50	1.00 to 2.63	0.049
Isolated IFG	1.01	0.59 to 1.70	0.64
IGT	1.81	1.18 to 2.72	0.01
<b>Model 3</b>			
NGT 1 h-low (reference category)	1	–	–
NGT 1 h-high	1.62	1.01 to 2.64	0.049
Isolated IFG	1.02	0.62 to 1.69	0.90
IGT	1.84	1.21 to 2.79	0.004

Model 1, adjusted for age and gender; model 2, model 1+BMI; model 3, model 1+waist circumference.

BMI, body mass index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; NGT, normal glucose tolerance.

group of prediabetic individuals.<sup>7</sup> It is notable that individuals with IGT, but not those with IFG, exhibit an increased risk to have NAFLD. These results substantiate those of a previous study demonstrating that IFG individuals have lower ectopic fat deposition in the liver, measured by MR tomography, than IGT participants, likely due to differences in insulin sensitivity as well as adiponectin and fetuin-A levels.<sup>36</sup>

The pathophysiological mechanisms underlying the associations between NAFLD and 1 h postload hyperglycemia are unsettled. A greater degree of IR in NGT 1 h-high individuals as compared with NGT 1 h-low participants is one of the possible explanations for the development of NAFLD. Hepatic IR is associated with a specific reduction of insulin's ability to suppress glucose production while its capability to promote lipogenesis is conserved; this ultimately results in increased synthesis of fatty acids and triglycerides.<sup>40</sup> Accordingly, we observed that NGT 1 h-high individuals exhibit a higher value of the liver IR index, a surrogate index of hepatic IR<sup>34</sup> that has a good accuracy at identifying NAFLD.<sup>41</sup>

An additional common mechanism may be represented by chronic inflammation, a condition that has been associated with NAFLD,<sup>20 21 27 37</sup> and which plays a key role in the pathogenesis of type 2 diabetes.<sup>38</sup> Fat accumulation in the liver might induce an inflammatory response, and elevated inflammatory biomarkers might be an indication of local and systemic inflammation associated with low levels of anti-inflammatory molecules

including IGF-1.<sup>37</sup> We observed that NGT 1 h-high individuals have higher levels of hsCRP and lower levels of circulating IGF-1 as compared with NGT 1 h-low individuals; on the basis of this observation it is possible to hypothesize that an altered equilibrium between proinflammatory and anti-inflammatory molecules contributes to the pathogenesis of NAFLD.

The present findings that demonstrate a correlation between postload hyperglycemia in NGT and NAFLD may have clinical repercussions. Indeed, it has been shown that programs aimed at modifying lifestyle and pharmacological interventions are extremely effective in preventing or delaying type 2 diabetes onset in participants at high risk for the disease.<sup>1-4</sup> It is notable that these treatments are also capable of reducing fat liver content and improving biomarkers of NAFLD.<sup>42 43</sup> The observation that NGT 1 h-high individuals are at increased risk for type 2 diabetes<sup>7 8</sup> and NAFLD suggests that the value of a 1 hour OGTT glucose  $\geq 155$  mg/dL may be suitable for identifying a subset of NGT individuals potentially harboring an increased risk of developing type 2 diabetes in whom it would be helpful to perform liver ultrasonography, not routinely carried out, because they could be targeted by these effective prevention programs.

The current study has several strengths including the homogeneity of the study group with accurate demographic, clinical, and biochemical characterization carried out by qualified personnel, the comparatively large size of the cohort, the inclusion of both sexes, the use of a centralized laboratory for analyses, and the exclusion of confounding conditions characterized by elevation in liver enzymes such as heavy drinking or positivity for antibodies to HCV or HBsAg.

However, the present study has potential limitations that must be considered. First, the diagnosis of NAFLD was made by ultrasound scanning rather than by invasive methods such as liver biopsy or expensive and time-consuming non-invasive methods such as proton magnetic resonance spectroscopy. Although ultrasonography is the most common method of diagnosing in clinical practice, it has a suboptimal sensitivity when hepatic fat infiltration is < 20%.<sup>34</sup> A second important limitation is that a semiquantitative ultrasound assessment using a three ('mild', 'moderate', and 'severe'), or more, point scoring system for the degree of liver steatosis was not available. The inability to assess the extent of liver steatosis prevents us from reaching any solid conclusion on the role of 1 h postload plasma glucose in the progression to nonalcoholic steatohepatitis (NASH), and the present results have to be considered as hypothesis generating. Additionally, data on the genotypes of the common patatin-like phospholipase domain containing the three gene (*PNPLA3*, also known as adiponutrin) rs738409 variant, which has been associated with liver fat accumulation with the G homozygous carriers having as much as double liver fat values than non-carriers, are not available for our cohort.<sup>44</sup> For these reasons, we

## Pathophysiology/Complications

cannot exclude the possibility that adjustment for the G-genotype of PNPLA3 may attenuate or abolish the risk of NGT 1 h-high participants of having NAFLD. Moreover, participants underwent a single 75 g OGTT to assess glucose levels. Although this approach is ordinarily employed in clinical practice and in the majority of the large epidemiological studies, these measures present an intraindividual variability, and thus the classification of participants into glucose tolerance groups may have been slightly inaccurate in a few cases. In addition, a surrogate measure of hepatic IR was employed because clamp studies combined with tracer techniques are not implementable in large-scale studies. However, the surrogate index employed in this study has been previously validated against hepatic glucose measurement using tracers in a large sample.<sup>34</sup> Furthermore, the information on alcohol intake was assessed by a self-reported questionnaire; this may have led to an underestimation of the actual daily alcohol consumption. Additionally, it is possible that some cases of positivity to HCV or HBsAg have been misclassified due to low blood title of antibodies. Next, this is an observational study based on individuals at risk for cardiometabolic disease, recruited at a referral university hospital, and therefore may not be extendible to the general population. Finally, as a consequence of its cross-sectional design, the study findings reflect only an association with prevalent and not incident NAFLD, and therefore it is not possible to conclude that a cause and effect relationship exists.

**Contributors** GS designed the study, analyzed and interpreted the data, and wrote the manuscript. TVF and AS acquired, analyzed, and interpreted the data, and also critically reviewed the manuscript. MLH analyzed and interpreted the data, and also wrote the manuscript. FP analyzed and interpreted the data, contributed to discussions, and reviewed the manuscript.

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**Data sharing statement** All available data have been included in the study. Raw clinical data are available on request.

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