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One-hour post-load glucose levels are associated with hepatic steatosis assessed by transient elastography

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Abstract

Aim: To examine the association between 1-hour plasma glucose (PG) concentration and markers of non-alcoholic fatty liver disease (NAFLD) assessed by transient elastography (TE).

Methods: We performed TE in 107 metabolically well-characterized non-diabetic White individuals. Controlled attenuation parameter (CAP) was used to quantify liver steatosis, while liver stiffness marker (LS) was used to evaluate fibrosis.

Results: Controlled attenuation parameter correlated significantly with 1-hour PG ($r = 0.301$, $P < 0.01$), fasting insulin ($r = 0.285$, $P < 0.01$), 2-hour insulin ($r = 0.257$, $P < 0.02$), homeostasis model assessment index of insulin resistance ($r = 0.252$, $P < 0.01$), high-density lipoprotein cholesterol ($r = -0.252$, $P < 0.02$), body mass index (BMI; $r = 0.248$, $P < 0.02$) and age ($r = 0.212$, $P < 0.03$), after correction for age, sex and BMI. In a multivariable linear regression analysis, 1-hour PG ($\beta = 0.274$, $P = 0.008$) and fasting insulin levels ($\beta = 0.225$, $P = 0.029$) were found to be independent predictors of CAP. After excluding subjects with prediabetes, 1-hour PG was the sole predictor of CAP variation ($\beta = 0.442$, $P < 0.001$). In a logistic regression model, we observed that the group with 1-hour PG ≥ 8.6 mmol/L (155 mg/dL) had a significantly higher risk of steatosis (odds ratio 3.98, 95% confidence interval 1.43-11.13; $P = 0.008$) than individuals with 1-hour PG < 8.6 mmol/L, after correction for potential confounders. No association was observed between 1-hour PG and LS.

Conclusion: Our data confirm that 1-hour PG ≥ 8.6 mmol/L is associated with higher signs of NAFLD, even among individuals with normal glucose tolerance, categorized as low risk by canonical diagnostic standards. TE is a safe low-impact approach that could be employed for stratifying the risk profile in these patients, with a high level of accuracy.

KEYWORDS
cohort study, fatty liver disease, observational study, population study, type 2 diabetes

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) prevalence is reaching alarming proportions worldwide in both adults and adolescents, due to a combination of genetic and environmental factors.[1] This presents a tremendous burden on healthcare systems because diabetes is accompanied by an increased risk of premature complications. The prevalence of prediabetes, a condition of dysglycaemia defined by impaired fasting glucose, impaired glucose tolerance (IGT),[2] or glycated haemoglobin (HbA1c) between 39 and 46 mmol/mol (5.7% and 6.4%),[3] is increasing alongside the diabetes epidemic.[4] It is now widely recognized that the organ damage typically associated with T2DM is already present in the early stages of the disease and, in some instances, even before diagnosis.[5] Indeed, associations of prediabetes with non-alcoholic fatty liver disease (NAFLD), nephropathy, retinopathy, neuropathy, and cardiovascular disease have been reported.[6–9] Recently, a large body of evidence has shown that higher plasma glucose (PG) concentrations at 1 hour during an oral glucose tolerance test (OGTT) are associated with an increased risk of T2DM incidence.[10] In this regard, a 1-hour PG value of ≥8.6 mmol/L (155 mg/dL) has emerged as a reliable glycaemic marker capable of discriminating individuals with increased risk for future T2DM.[11–13] Evidence has accumulated indicating that subjects with 1-hour PG ≥ 8.6 mmol/L (1-hour high) are at increased risk of progressing to overt diabetes even if they have normal glucose tolerance (NGT).[14] However, interestingly, individuals characterized as having NGT and also 1-hour high have been found to exhibit increased risk of hepatic steatosis.[15,16] NAFLD is the most common chronic liver disease, affecting up to 46% of the general population.[17,18] 'NAFLD' is an umbrella definition that encompasses a spectrum of conditions ranging from the relatively benign simple steatosis to inflammatory non-alcoholic steatohepatitis, which may progress to cirrhosis and, ultimately, to hepatocellular carcinoma.[19,20] The association between the conditions of prediabetes and NAFLD is clinically and pathophysiologically relevant.[21] Indeed, the liver plays an important role in the maintenance of glucose homeostasis;[22] a diagnosis of NAFLD is not only cross-sectionally associated with altered glucose tolerance and T2DM[7,23,24] but it may also predict their development[25–27] and the onset of severe complications.[28,29]

Our research group has above all demonstrated that patients with 1-hour high have a higher prevalence of hepatic steatosis (assessed by ultrasonography) than individuals with prediabetes according to the current criteria (HbA1c between 5.7% and 6.4%)[30] and increased levels of circulating markers of hepatic alterations.[31]

Currently, the diagnosis of NAFLD and particularly the assessment of the presence of fibrosis depends on invasive methods such as liver biopsy, which is the 'gold standard', or on expensive and time-consuming techniques such as proton magnetic resonance spectroscopy. Since liver biopsy or magnetic resonance are not recommended to all patients with NAFLD, researchers have been evaluating the use of non-invasive biological markers, scoring systems, and other convenient methods to evaluate fibrosis stage in patients with NAFLD.[16,32]

Among the non-invasive approaches, conventional ultrasonography is quite common. Nevertheless it is an operator-dependent examination and present multiple uncontrolled variables, which can affect the outcome of the examination.[33] Transient elastography (TE; Fibroscan, Echosens, Paris, France) allows for the measurement of controlled attenuation parameter (CAP) and liver stiffness (LS) as a viable alternative to ultrasonography for the initial and follow-up assessment of patients with NAFLD.[34] This method is fast, reliable and reproducible, with good intra- and inter-observer levels of agreement.[33]

In order to verify the hypothesis that prediabetic individuals with 1-hour high have a higher risk of liver steatosis or fibrosis than individuals with 1-hour PG < 8.6 mmol/L (1-hour low), we performed TE in a metabolically well-characterized cohort of Italian adults recruited for the CATAnzaro MEtabolic RIsk factors Study (CATAMERIS).

2 | MATERIALS AND METHODS

2.1 | Study population

The present study was cross-sectional and consisted of 107 non-diabetic White subjects (43 men and 64 women) enrolled in CATAMERIS, an observational study whose aim is to assess cardiometabolic risk in individuals carrying at least one risk factor including overweight/obesity, hypertension, dyslipidaemia, dysglycaemia, and family history of T2DM.[12,30,35,36]

Eligible patients, aged 18 years or older, self-reported non-diabetic, were consecutively recruited at the Department of Medical and Surgical Sciences of the University 'Magna Graecia' of Catanzaro. Exclusion criteria were the following: history of type 1 diabetes or T2DM, heart failure, end-stage renal disease, anaemia or haemoglobinopathies; history of malignant disease, gastrointestinal diseases

associated with bleeding or malabsorption, autoimmune diseases, acute or chronic infections; positivity for hepatitis C virus (HCV) or hepatitis B surface antigen (HBsAg) antibodies; accumulation diseases such as amyloidosis and haemochromatosis; history of drug abuse; self-reporting alcohol consumption of >20 g/d; and history of use of drugs known to cause liver injury including tamoxifen, glucocorticoids, tetracycline, oestrogens, methotrexate and amiodarone.

After a 12-hour fast, all individuals underwent anthropometrical evaluation including measurements of body mass index (BMI), waist circumference, body composition assessed by bioelectrical impedance and readings of clinic blood pressure. A 75-g OGTT was administered, with sampling for PG and insulin assays. The day after, TE was performed by an expert radiologist using a TOUCH502 Fibre Scanner. Patients who were newly diagnosed with diabetes during the study were excluded from it. The protocol was approved by the hospital ethics committee (*Comitato Etico Azienda Ospedaliera 'Mater Domini'*) and written informed consent was obtained from all participants in the study in accordance with the principles of the Declaration of Helsinki.

2.2 | Analytical determinations

Blood glucose, triglycerides, total and high-density lipoprotein (HDL) cholesterol concentrations were determined by enzymatic methods on Roche/Hitachi cobas c systems (Roche, Basel, Switzerland). The enzymatic reference method with hexokinase was used specifically for glucose measurement. Plasma insulin concentration was measured with a chemiluminescence-based assay (Immulite; Siemens Healthcare GmbH, Erlangen, Germany). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using the α-ketoglutarate reaction, and gamma-glutamyltransferase (γGT) with the L-gamma-glutamyl-3-carboxy4-nitroanilide rate method (Roche, Basel, Switzerland).

2.3 | Calculations

Participants were classified according to glucose tolerance status as having NGT when fasting plasma glucose (FPG) was <7.0 mmol/L (100 mg/dL) and 2-hour post-load was <7.8 mmol/L (140 mg/dL), or as having IGT when FPG was <7.0 mmol/L (126 mg/dL) and 2-hour post-load was 7.8 to 11.0 mmol/L (140-199 mg/dL). Individuals were stratified into two groups (1-hour low and 1-hour high) based on 1-hour PG concentration, below or above 8.6 mmol/L (155 mg/dL), respectively. To determine the amount of hepatic steatosis, the CAP test was used and the results were reported in decibel/metre (dB/m). CAP cutoff values <237.0 dB/m were defined as S0 (no steatosis).[33] Hepatic fibrosis values were expressed as LS and measured in kilopascal (kPa). Values lower than 5 to 6 kPa indicate absent or minimal liver fibrosis, whereas values greater than 12 to 14 kPa are highly suggestive of cirrhosis.[33] Homeostasis model assessment index of insulin resistance (HOMA-IR) was calculated as fasting insulin × fasting glucose/22.5.[37]

2.4 | Statistical analysis

Results are given as means ± SD for continuous variables or as absolute numbers and percentages for categorical variables. Metabolic variables showing a skewed distribution including triglycerides, AST, ALT, γGT, fasting insulin and HOMA-IR were natural log-transformed for statistical analyses to meet the assumption of normality for statistical purposes. Anthropometric and biochemical differences between groups were tested by applying Student's *t*-test for continuous variables or a χ^2 test for categorical variables. Adjustment for age, sex and BMI was applied by general linear model with Bonferroni post hoc correction for multiple comparisons. Pearson *r* coefficients were estimated by partial correlation (corrected for age, sex and BMI as appropriate). The variables that were found to be significantly correlated with CAP were included in a multivariable linear regression model, with CAP as dependent variable. Multivariable logistic regression analyses were undertaken to test the independent association between significant liver steatosis (defined as CAP ≥238 dB/m [using M/XL probes]) and stratification into groups 1-hour low or 1-hour high was performed, adjusted for age, sex and BMI. A *P* value <0.05 was taken to indicate statistical significance. All analyses were performed using the statistical package SPSS 22.0 for Windows (SPSS, IBM, Chicago, Illinois).

3 | RESULTS

Anthropometric and biochemical characteristics of the study cohort, stratified using a cutoff of 1-hour PG of 8.6 mmol/L (155 mg/dL), are summarized in Table 1. The resulting groups were: 1-hour low, N = 60, and 1-hour high, N = 47. The study included 107 individuals, of whom 43 were male (40.2%) and 64 were female (59.8%). The mean age of the whole study sample was 49 ± 15 years. Eighty individuals (74.8%) had NGT and 27 (25.2%) had IGT, 11 of whom were in the 1-hour low group, and the remaining 16 were classified as 1-hour high. In both groups the number of women was higher than the number of men.

No differences were found in the distribution of age, sex, systolic blood pressure, diastolic blood pressure, lipid profile parameters or hepatic enzymes. Insulin levels at 1-hour and 2-hour OGTT timepoints were not available for the entire cohort (N = 94 and N = 95, respectively).The 1-hour low cohort showed significantly lower levels of fasting glucose ($P < 0.001$), 1-hour PG ($P < 0.001$), 2-hour PG ($P < 0.001$), fasting insulin ($P = 0.044$), 1-hour insulin ($P < 0.01$), 2-hour insulin ($P < 0.001$) and HOMA-IR ($P < 0.01$) when compared with the 1-hour high group. After adjustment for age, sex and BMI the difference in fasting insulin concentration was overridden ($P = 0.125$), whereas 1-hour insulin concentration and HOMA-IR was

TABLE 1 Clinical and biochemical data of the study population.

Variables	Whole study group	1-hour low	1-hour high	*P*	*P*[a]
Sex: male/female	43/64	23/37	20/27	0.403	-
Age, years	49 ± 15	48 ± 15	50 ± 14	0.445	0.443*
BMI, kg/m^2	30.3 ± 5.7	29.7 ± 5.8	31.2 ± 5.4	0.167	0.152**
SBP, mmHg	120.1 ± 13.8	120.1 ± 14.1	120.3 ± 13.5	0.947	0.551
DBP, mmHg	74.6 ± 10.1	75.6 ± 10.6	73.1 ± 8.7	0.137	0.019
Total cholesterol, mmol/L	4.92 ± 0.83	5.01 ± 0.86	4.82 ± 0.78	0.273	0.337
mg/dL	190.5 ± 32.3	193.7 ± 33.1	186.7 ± 30.1	0.273	0.337
LDL cholesterol, mmol/L	3.34 ± 0.85	3.41 ± 0.87	3.25 ± 0.83	0.348	0.395
mg/dL	129.2 ± 32.8	132.0 ± 33.8	125.8 ± 32.0	0.348	0.395
HDL cholesterol, mmol/L	1.31 ± 0.31	1.33 ± 0.32	1.29 ± 0.30	0.477	0.626
mg/dL	50.8 ± 12.1	51.6 ± 12.3	49.8 ± 11.7	0.477	0.626
Triglycerides, mmol/L	1.25 ± 0.61	1.22 ± 0.64	1.28 ± 0.58	0.617	0.644
mg/dL	110.7 ± 54.0	108.2 ± 56.3	113.7 ± 51.4	0.617	0.644
AST, U/L	23.0 ± 13.3	21.1 ± 10.8	25.5 ± 15.7	0.099	0.119
ALT, U/L	23.4 ± 13.1	22.6 ± 14.3	24.6 ± 11.5	0.449	0.278
γGT, U/L	18.9 ± 11.2	17.1 ± 12.2	21.3 ± 9.2	0.055	0.133
Fasting glucose, mmol/L	5.00 ± 0.57	4.83 ± 0.57	5.22 ± 0.49	<0.001	<0.001
mg/dL	90.2 ± 10.2	87.2 ± 10.3	94.1 ± 8.8	<0.001	<0.001
1-hour glucose, mmol/L	8.25 ± 1.93	6.91 ± 1.32	9.97 ± 1.02	<0.001	<0.001
mg/dL	148.8 ± 34.9	124.4 ± 23.9	179.0 ± 18.3	<0.001	<0.001
2-hour glucose, mmol/L	6.81 ± 1.60	6.26 ± 1.59	7.47 ± 1.35	<0.001	<0.001
mg/dL	122.8 ± 28.8	113.4 ± 28.9	134.5 ± 24.3	<0.001	<0.001
Fasting insulin, μU/mL	10.9 ± 6.3	10.1 ± 6.7	11.9 ± 5.6	0.044	0.125
1-hour insulin (N = 94), μU/mL	93.1 ± 78.5	75.2 ± 63.2	114.4 ± 89.5	<0.01	0.014
2-hour insulin (N = 95), μU/mL	80.8 ± 60.6	63.0 ± 42.9	102.3 ± 71.6	<0.001	<0.001
HOMA-IR	2.46 ± 1.47	2.20 ± 1.50	2.78 ± 1.39	<0.01	0.035
CAP, dB/m	252.1 ± 55.1	237.5 ± 52.4	270.6 ± 53.4	<0.001	<0.01
LS, kPa	5.63 ± 2.39	5.57 ± 2.79	5.82 ± 1.86	0.593	0.966

Note: Data are means ± SD except for sex which is reported as absolute numbers. *P* values for comparisons between two groups were obtained using unpaired Student's *t*-test or χ^2 test as appropriate.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; γGT, gamma glutamyl transferase; HDL, high density lipoprotein; HOMA-IR, the homeostasis model assessment index of insulin resistance; LDL, low density lipoprotein; LS, liver stiffness; SBP, systolic blood pressure.

[a]*P* values were obtained after adjusting for age, sex, and BMI using a general linear model with post hoc Bonferroni correction.

*P = adjusted for sex and BMI.

**P = adjusted for sex and age.

attenuated ($P = 0.014$ and $P = 0.035$, respectively) and the difference in the distribution of DBP values acquired statistical significance (higher in the 1-hour low group; $P = 0.019$).

As shown in Table 1, CAP levels in the 1-hour low group were lower than in the 1-hour high group (237.5 ± 52.4 and 270.6 ± 53.4, respectively; $P < 0.001$); this difference remained statistically significant even after correction for age, sex and BMI ($P < 0.01$), whereas LS was similar across groups. Presence of steatosis (defined as CAP ≥ 238 dB/m) was found more frequent in the 1-hour high cohort ($P = 0.013$).

The existence of a correlation between CAP and several metabolic and anthropometric variables in the whole study population (Table 2) was tested. CAP value correlated significantly with 1-hour PG ($r = 0.301$, $P < 0.01$), fasting insulin ($r = 0.285$, $P < 0.01$), 2-hour insulin ($r = 0.257$, $P < 0.02$), HOMA-IR ($r = 0.252$, $P < 0.01$), HDL cholesterol ($r = -0.252$, $P < 0.02$), BMI ($r = 0.248$, $P < 0.02$) and age ($r = 0.212$, $P < 0.03$), after correction for age, sex and BMI, as appropriate.

All variables that were significantly correlated with CAP, plus sex as a dichotomic value, were inserted in a multivariable linear regression model to determine their contribution to CAP variability. Overall, 1-hour PG ($\beta = 0.274$, $P = 0.008$) and fasting insulin levels ($\beta = 0.225$, $P = 0.029$) were the only two parameters found to be independent predictors of CAP (Table 3).

TABLE 2 Univariate correlations between controlled attenuation parameter measurements and anthropometric and biochemical variables.

Variables	Pearson's correlation, *r*	*P*
1-hour glucose (mg/dL)	0.301	<0.01
Fasting insulin (μU/mL)	0.285	<0.01
2-hour insulin (N = 95; μU/mL)	0.257	<0.02
HOMA-IR	0.252	0.01
HDL cholesterol (mg/dL)	−0.252	<0.02
BMI (kg/m^2)	0.248*	<0.02
Age (years)	0.212**	<0.03
1-hour insulin (N = 94; μU/mL)	0.198	0.059
γGT (U/L)	0.177	0.082
2-hour glucose (mg/dL)	0.158	0.113
ALT (U/L)	0.137	0.180
Triglycerides (mg/dL)	0.107	0.287
AST (U/L)	0.093	0.360
Fasting glucose (mg/dL)	0.062	0.532
LDL cholesterol (mg/dL)	−0.035	0.734
Total cholesterol (mg/dL)	−0.043	0.676

Note: *P* values of partial correlation coefficients are adjusted for age, sex and BMI.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; γGT, gamma glutamyl transferase; HDL, high density lipoprotein; HOMA-IR, the homeostasis model assessment index of insulin resistance; LDL, low density lipoprotein; LS, liver stiffness; SBP, systolic blood pressure.
*The *P* adjusted for age and sex.
**The *P* adjusted for sex and BMI.

TABLE 3 Multivariable regression analysis with controlled attenuation parameter as dependent variables.

Variables	Standardized coefficient (β)	*P*
1-hour glucose (mg/dL)	**0.274**	**0.008**
Fasting insulin (μU/mL)	**0.225**	**0.029**
Age (years)	0.167	0.089
BMI (kg/m^2)	0.113	0.291
HOMA-IR	0.051	0.922
2-hour insulin (N = 95; μU/mL)	0.013	0.923
Gender (male/female)	−0.012	0.904
HDL cholesterol (mg/dL)	−0.138	0.172

Note: Effect sizes (β standardized coefficients) per unit increase and corresponding *P*-values are shown. Bold indicates statistical significant results.
Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment index of insulin resistance.

Because our population encompassed multiple metabolic phenotypes (74.8% NGT and 25.2% IGT), we decided to perform a sensitivity analysis in order to verify whether the presence of individuals with higher levels of glucose intolerance was affecting CAP, on top of the cofactors included in the regression model. To achieve this, we excluded from the analysis individuals with IGT (Table 4). The sensitivity model showed that 1-hour PG remained the only determinant of CAP variation ($\beta = 0.442$, $P < 0.001$).

TABLE 4 Sensitivity analysis with controlled attenuation parameter as dependent variable.

Variables	Standardized coefficients (β)	*P*
1-hour glucose (mg/dL)	**0.442**	**<0.001**
HOMA-IR	0.218	0.063
Fasting insulin (μU/mL)	0.213	0.079
2-hour insulin (N = 95; μU/mL)	0.206	0.074
Age (years)	0.165	0.136
HDL cholesterol (mg/dL)	−0.141	0.212
BMI (kg/m^2)	0.116	0.297
Gender (male/female)	−0.014	0.901

Note: Data represent effect sizes (β standardized coefficients) per unit increase and corresponding *P* values obtained when the analyses were limited to individuals with normal glucose tolerance. Bold indicates statistical significant results.
Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment index of insulin resistance.

Finally, logistic regression analysis was carried out to confirm the association between 1-hour PG and the prevalence of steatosis (defined as CAP ≥ 238 dB/m), adjusted for age, sex and BMI. Subjects in the 1-hour high group exhibited a 2.69-fold (95% confidence interval [CI] 1.14-6.29; $P = 0.023$) increased risk of steatosis as compared to individuals in the 1-hour low group. A logistic regression model excluding individuals with IGT showed that subjects with 1-hour PG ≥ 8.6 mmol/L still had a higher risk of having steatosis (odds ratio 3.98, 95% CI 1.43-11.13, $P = 0.008$) as compared with individuals with 1-hour PG < 8.6 mmol/L.

4 | DISCUSSION

The term NAFLD is an umbrella definition that indicates the presence of fat accumulation in hepatocytes when no primary cause such as heavy alcohol consumption is present. The term also encompasses cases of non-alcoholic steatohepatitis,[38] which is more common in patients with risk factors such as obesity, hyperlipidaemia, diabetes mellitus, pregnancy, and long-term drug use, and is associated with a higher probability of progression to cirrhosis and fibrosis.[26] An increment of circulating liver enzymes is very common in patients with T2DM and closely correlates with a higher prevalence of clinically relevant comorbidities.[39]

Consistent with this definition, NAFLD is associated with hepatic-related adverse outcomes, but it is also a marker of increased cardiometabolic risk.[17,40–46]

Most patients with NAFLD are asymptomatic and the disease may be diagnosed incidentally because of high liver enzymes levels or by imaging.[47,48] The gold standard diagnostic test of fatty liver disease is liver biopsy, an invasive technique that can cause major complications and that cannot be employed as a large-scale screening method.[49]

A potential non-invasive alternative is the performance of TE.[34,50] The diagnostic performance of Fibroscan in detecting liver steatosis is very high, with sensitivity and specificity of nearly 90%[51] and a high negative predictive value.[52] Furthermore, it reaches 83%, 88% and 99% sensitivity in measuring LS for a diagnosis of mild-to-moderate fibrosis, severe fibrosis, and cirrhosis, respectively.[33,53]

Increasing evidence suggests that normo-tolerant individuals whose 1-hour PG values reach ≥8.6 mmol/L (155 mg/dL) have increased odds of progressing to T2DM.[11,12,54,55] Moreover, these individuals have been found to exhibit increased risk of subclinical organ damage such as thickening of the common carotid artery,[56] left ventricular hypertrophy,[11] vascular stiffness,[12] and left ventricular diastolic dysfunction,[55] all of which are independent predictors of cardiovascular events. Whether individuals with prediabetes have increased risk of liver steatosis or fibrosis is less clear.[57,58] Recently, we have reported that subjects with 1-hour PG ≥ 8.6 mmol/L have increased circulating markers of hepatic alterations,[31] higher prevalence of fatty liver (assessed by abdominal ultrasonography) and higher risk of intermediate/advanced fibrosis according to FIB-4 score,[15,16] as compared with individuals with 1-hour PG < 8.6 mmol/L.

The data reported herein add to the literature and support the association between the early postprandial variability in glucose levels (ie, 1-hour PG) and hepatic steatosis evaluated by TE. These results are strongly validated by previous observations that individuals with 1-hour high may exhibit higher dietary intake of saturated fatty acids and fructose,[59] which can, in turn, directly stimulate hepatic fat accumulation.[60] Furthermore, hyperglycaemia might contribute per se to hepatic damage,[61] also because of the activation of a chronic state of low-grade inflammation.[62] Noticeably, in our study, individuals with 1-hour high had higher levels of HOMA-IR, a validated proxy of hepatic insulin resistance[37,63,64] as compared with individuals with 1-hour low. Hepatic insulin resistance is likely a core contributor to NAFLD[22] because the activation of the insulin signalling cascade leads hepatocytes to stop producing glucose[22] and an incorrect communication may foster postprandial hyperglycaemia. Consistent with this evidence, the second major determinant of CAP in our cohort after 1-hour PG was the fasting level of circulating insulin.

Moreover, our sensitivity analysis showed that even when common risk factors are removed from the equation, 1-hour PG is the strongest contributor to the variation of CAP. The linear association between CAP and 1-hour PG was reinforced when we considered steatosis in individuals classified as having NGT ($\beta = 0.274$ and $\beta = 0.442$, respectively), as shown by the odds ratios for the prevalence of steatosis in the 1-hour low versus 1-hour high groups (2.69 and 3.98, respectively). These observations suggest that stratification into 1-hour high and 1-hour low groups is suitable to identify individuals with a higher risk profile and to potentially reduce clinical inertia in a population that is usually considered low risk.

Overall, our results support the notion that a 1-hour PG value of 8.6 mmol/L may be a useful glycaemic threshold for identifying individuals at risk of developing hepatic steatosis who may benefit most from lifestyle or pharmacological interventions.[65,66] In fact, taking into account the link between high glucose concentrations and hepatic accumulation of fatty acids, it is possible to hypothesize that early intervention to correct postload hyperglycaemia may counteract the increased synthesis of fatty acids in hepatocytes and consequently prevent the development of NAFLD.

The present study has some strengths including the broad range of clinical data gathered according to a standardized protocol by trained investigators, the assessment of glucose tolerance by OGTT, the centralized assay of biochemical parameters in fresh blood samples rather than in stored samples, the evaluation of NAFLD via TE performed by a qualified investigator blind to the participants, and the exclusion of numerous conditions known to negatively impact liver function and morphology, such as drugs or alcohol abuse or positivity to HCV or HBsAg.

A number of limitations of this study should also be considered, however, in interpreting its results. First, a limited number of subjects were enrolled in the study and all clinical parameters, including OGTT, were measured only once. Although such an approach is common in both clinical practice and epidemiological studies, it means that we cannot account for day-to day individual variability. In addition to this, residual confounding by unmeasured factors is always a possibility in observational research. As our study population was composed of White individuals with at least one cardiovascular risk factor from a referral university hospital, our observations may not be extendable to the general population and it remains to be addressed whether the current findings can be extended to other ethnic groups. Additionally, the cross-sectional design of the study enables us only to examine the association with prevalent steatosis or fibrosis, thereby restricting the chance of establishing a longitudinal relationship between glucose levels at 1 hour and steatosis and fibrosis progression in time. Finally, the data collected for the present study did not allow for the evaluation of other metrics of glycaemic variability. Our hypothesis is that an investigation of OGTT glucose and insulin area under the curve or the interrogation of modern technology, such as intermittently scanned glucose monitoring and continuous glucose monitoring,[67] might show significant associations with NAFLD measured through TE.

We do not claim to draw any conclusions on hepatic fibrosis from our data because the lack of association might be a consequence of inadequate sampling and should not be interpreted in any way as a reflection of the pathophysiology. In future, the recruitment of a study population more suited to the evaluation of LS might be able to reveal the real influence of 1-hour PG on this variable.

In conclusion, the present study shows that the subgroup of individuals with 1 h glucose level ≥ 8.6 mmol/L have significantly greater markers of NAFLD, even among those who could be categorized as having low-risk NGT by canonical diagnostic standards.

AUTHOR CONTRIBUTIONS

Conceptualization: Giorgio Sesti and Francesco Andreozzi. Formal analysis: Gaia Chiara Mannino and Francesco Andreozzi. Investigation and data curation: Elettra Mancuso, Elisa Mazza, Teresa Vanessa Fiorentino, Franco Arturi, Angela Sciacqua, Elena Succurro, Maria Perticone, Tiziana Montalcini and Arturo Pujia; Writing—original draft preparation: Francesco Andreozzi and Gaia Chiara Mannino. Writing—review and editing: Gaia Chiara Mannino and Francesco Andreozzi. Supervision: Francesco Andreozzi and Giorgio Sesti.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest to disclose with regard to the present manuscript. No finding was received for conducting this study.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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