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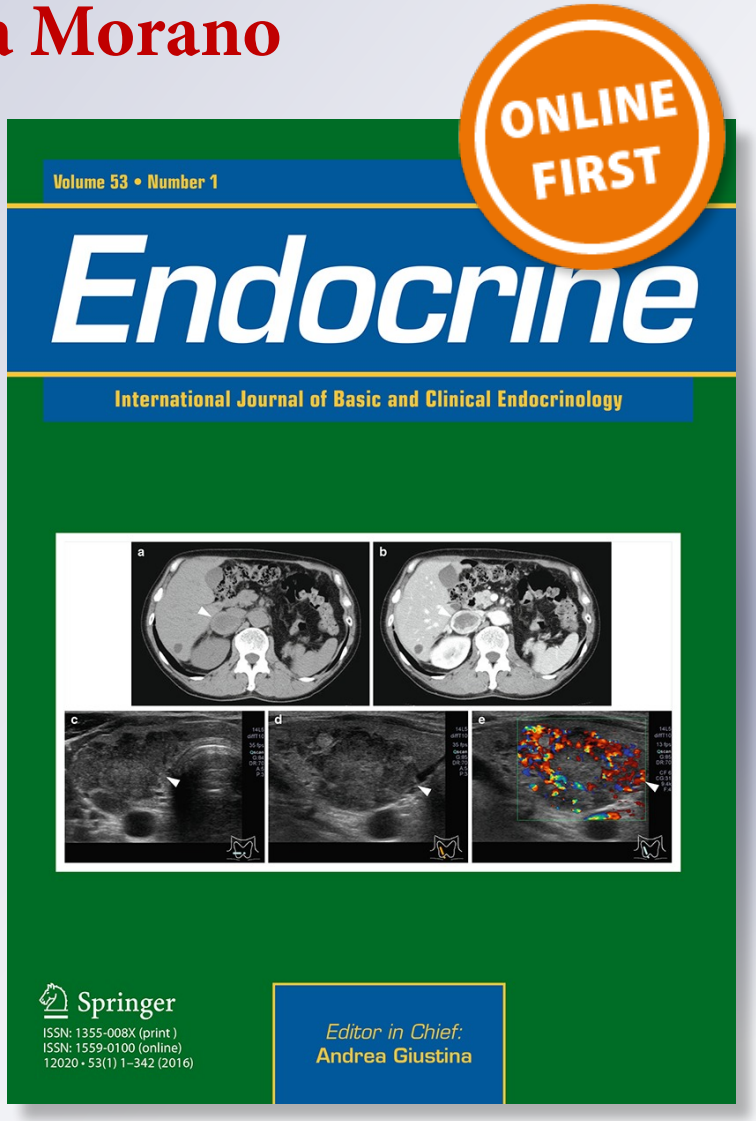

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RESEARCH LETTER

Two-hour postload glycemia is associated to an increased risk of NAFLD in healthy subjects with family history of type 2 diabetes: a case control study

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Introduction

Nonalcoholic fatty liver disease (NAFLD) includes steatosis and nonalcoholic steatohepatitis (NASH), which can be complicated by cirrhosis and hepatocellular carcinoma [1]. NAFLD affects over 30 % of the general population and is associated with type 2 diabetes mellitus (T2DM), obesity and metabolic syndrome [2, 3]. NAFLD prevalence in T2DM patients is about 70 % using ultrasonography (US) [4]. NAFLD and T2DM share insulin-resistance, which in the liver increases gluconeogenesis and glycogenolysis, resulting in hyperglycemia. The pancreatic beta islet cells adapt to hyperglycemia by increasing insulin secretion. Hyperinsulinemia up-regulates several lipogenic transcription factors, promoting hepatic lipid synthesis [5]. The association between NAFLD and T2DM seems to be the result of a "common soil" [3]. Several studies showed that NAFLD predicts T2DM and vice versa, and that each condition may act as a progression factor for the other [4]. There is evidence of a high risk of NASH and its progression to hepatocellular carcinoma in T2DM patients [6]. Conversely, recent studies showed that NAFLD not only predicts diabetes [7], but also contributes to poor glycemic control and chronic complications [8]. Despite its clear link with T2DM, the association of NAFLD with family history of diabetes has been poorly investigated. A recent cross-sectional study in nondiabetic individuals with NAFLD demonstrated that family history of diabetes increased the risk of NASH and fibrosis [9].

The aim of this study was to evaluate the prevalence of NAFLD in healthy first degree relatives of T2DM patients (T2DM-rel) and in healthy subjects without family history of T2DM and to assess the risk factors associated with NAFLD development.

Patients and methods

One hundred twenty-five healthy subjects were enrolled. A medical history was obtained, and vital and anthropometric parameters were assessed. The exclusion criteria were: known diabetes mellitus, impaired fasting glucose and dyslipidemia; body mass index (BMI) ≥ 30 kg/m^2; alcohol consumption >20 g/d for women and >30 g/d for men; drug abuse; history of liver diseases; current use of hepatotoxic drugs; psychiatric and neoplastic diseases. A 75 g-oral glucose tolerance test (OGTT) with assessment of fasting and 2-h plasma glucose (2-h PG) was performed. Basal insulinemia, homeostasis model assessment index (HOMA index), lipid profile, renal and liver function markers, and inflammation indices were assessed.

Ninety four healthy subjects were eligible, 60 T2DM-rel (37 females, 23 males, median age 35.5 years, range

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Table 1 Clinical and biochemical parameters of T2DM-rel and control subjects

Variable (mean ± SD or median range)	T2DM-rel	Control group	*p*
Age (years)	35.5 (range 22–65)	27.5 (range 20–60)	0.005
BMI (kg/m^2)	22.72 ± 2.38	21.62 ± 1.77	0.010
Waist (cm)	78.85 ± 7.89	73.82 ± 5.47	0.003
FPG (mmol/L)	4.65 ± 0.49	4.46 ± 0.30	ns
PPG (mmol/L)	4.88 ± 1.07	4.35 ± 0.87	ns
Insulinemia (μU/mL)	11.56 ± 5.77	13.11 ± 5.02	ns
HOMA index	2.36 ± 1.12	2.59 ± 1.02	ns
Total cholesterol (mmol/L)	4.93 ± 0.84	4.47 ± 0.83	0.016
LDL-cholesterol (mmol/L)	2.91 ± 0.81	2.41 ± 0.74	0.015
HDL-cholesterol (mmol/L)	1.64 ± 0.4	1.68 ± 0.3	ns
Triglycerides (mmol/L)	0.91 ± 0.32	0.71 ± 0.29	ns
GPT (UI/L)	17.5 ± 9.7	13.6 ± 4.9	ns
Gamma-GT (UI/L)	14.3 ± 7.7	12.0 ± 6.0	ns
CRP (μg/L)	1250 ± 1271	901 ± 1582	ns

FPG fasting plasma glucose, *PPG* 2-h plasma glucose at OGTT, *GPT* glutamic pyruvate transaminase, *CRP* C-reactive protein

22–65) and 34 control subjects without family history of T2DM (26 females, 8 males, median age 27.5 years, range 20–60).

After an overnight fasting, all elegible subjects underwent transabdominal US with a 3.5 MHz convex (Toshiba Tosbee Tokyo, Japan), performed by two gastroenterologists with experience of more than 5000 abdominal US examinations (>1500 scans/year). Presence and severity of liver steatosis was established according to a previously published method [10]. After having rehearsed together several times and after agreement on the modality to identify the presence and severity of fatty liver, the two ultrasonographers independently performed US and reported their results in the standardized form. The interobserver agreement in detecting presence and severity of fatty liver was evaluated using the concordance index by kappa statistics [11]. In case of conflict, the examinations were reviewed together to provide the best interobserver agreement. The operators were blinded of the laboratory data.

All subjects gave written informed consent. The protocol was approved by the hospital ethics committee.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) or median and range. Differences between groups were evaluated using Wilcoxon rank sum test for unpaired samples, and by Fisher's exact test for categorical variables. The independent variables assessed were: family history, gender, age, BMI, waist circumference, smoking habits, physical activity, triglycerides, HDL-cholesterol and LDL-cholesterol, fasting and 2-h PG at OGTT. The association between steatosis and each variable was assessed fitting a logistic model after adjustment for gender, age, and BMI. Values of $p < 0.05$ were considered significant. All analyzes were performed with the software R v3.13.

The sample sizes of the two groups allow for a power of around 0.8 with $\alpha = 0.05$, when a *t*-test for unmatched samples and test for proportions are being considered with medium effect sizes (e.g., equal to 60 % of the common SD for the *t*-test). Analogous power can be provided using a χ^2 test.

Results

None of the subjects had OGTT alterations. T2DM-rel and control groups were different for age ($p = 0.005$) and BMI ($p = 0.010$). T2DM-rel had higher values of total cholesterol ($p = 0.016$) and LDL-cholesterol ($p = 0.015$), after adjustment for age and BMI, although in the normal range (Table 1). CRP levels were in the normal range and were not different between T2DM-rel and control groups. NAFLD was present in 70 % of T2DM-rel and 47 % of control subjects. There was an association between presence of steatosis and T2DM-rel ($p = 0.046$), not confirmed after adjustment for age, gender, and BMI ($p = 0.118$), in the logistic model.

In T2DM-rel, and not in control subjects, the odds of having steatosis, after adjustment for age, gender, and BMI, increased by 6 % for each unit of 2-h PG (mg/dl) ($p = 0.027$), although in the normal range. No differences were found in the severity of steatosis and no association was observed between grades of NAFLD and CRP levels in all subjects. The agreement between the two ultrasonographers was almost perfect with a *k* value of 0.93.

Discussion

In this study, a frequent occurrence of steatosis was observed. Moreover, T2DM-rel showed a higher prevalence of steatosis, not confirmed after adjustment for age and BMI. Since T2DM-rel had higher BMI and age, the prevalence of NAFLD in this population was not independently associated with T2DM family history.

In T2DM-rel the odds of having steatosis increased by 6 % for each unit of 2-h PG, at OGTT, after adjustment for age and BMI, showing a strong effect of higher values of 2-h PG, although within the normal range. Moreover, T2DM-rel had a worse lipid profile after adjustment for age and BMI.

Previous studies demonstrated an increased prevalence of cardiovascular disease and metabolic syndrome with increasing fasting plasma glucose levels, both in normoglycemic adults and in children/adolescents [12–14]. Elevated 1-h postload plasma glucose levels, in subjects with normal glucose tolerance, are also predictive of T2DM and 1-h OGTT glucose $\geq$ 155 mg/dL is associated with 1.5-fold increased risk of having NAFLD [15, 16]. Elevated 1-h OGTT glucose correlates with an unfavorable inflammatory profile, predictor of cardiovascular diseases [17]. Although other studies showed an association between grades of NAFLD and CRP levels, in this study this correlation was not found [18, 19]. Moreover, 2-h postload plasma glucose levels, within normal glucose tolerance, and LDL-cholesterol are associated with carotid intima-media thickness, a marker of early atherosclerosis [20, 21].

In this view, in the presence of family history of T2DM, high values of 2-h PG and a worse lipid profile may be not only predictive of steatosis but also of T2DM and cardiovascular disease.

In nondiabetic individuals with NAFLD, family history of diabetes increases the risk of NASH and fibrosis [9]. Considering the higher risk of steatosis and its progression to steatohepatitis and fibrosis, it could be appropriate to search for steatosis in T2DM-rel.

NAFLD worsens glycemic control and contributes to chronic complications in people with T2DM [8]. Thus, T2DM-rel with steatosis may have not only a further increase in the risk of developing T2DM but also a higher risk of chronic complications.

Given the high prevalence of NAFLD in T2DM-rel and the higher risk of development of T2DM and its complications in patients with NAFLD, it could be of importance to screen this population for NAFLD and for T2DM early, to provide an appropriate therapy (i.e., lifestyle intervention) to reduce the development of diabetes and liver complications.

In conclusion, first-degree family history of T2DM is associated with high prevalence of NAFLD in young healthy subjects. Moreover, in the same population, there is a strong effect of 2-h postload plasma glucose levels in the risk of development of steatosis.

Author contributions S.M. and N.P. contributed to the conception and the design of the study. A.C. and T.F. contributed to the acquisition and the collection of data. P.M. performed laboratory assays. N. P. and G.V. performed abdominal ultrasound. L.N. performed the statistical analysis. S.M., N.P., T.F., and L.N. have been involved in the interpretation of data and in drafting the manuscript. A.L. critically revised the manuscript for important intellectual content. All authors approved the version to be submitted.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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