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Original article

An extended fatty liver index to predict non-alcoholic fatty liver disease

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Abstract

Background. – In clinical practice, there is a strong interest in non-invasive markers of non-alcoholic fatty liver disease (NAFLD). Our hypothesis was that the fold-change in plasma triglycerides (TG) during a 2-h oral glucose tolerance test (fold-change TG_{OGTT}) in concert with blood glucose and lipid parameters, and the rs738409 C>G single nucleotide polymorphism (SNP) in *PNPLA3* might improve the power of the widely used fatty liver index (FLI) to predict NAFLD.

Methods. – The liver fat content of 330 subjects was quantified by ^{1}H-magnetic resonance spectroscopy. Blood parameters were measured during fasting and after a 2-h OGTT. A subgroup of 213 subjects underwent these measurements before and after 9 months of a lifestyle intervention.

Results. – The fold-change TG_{OGTT} was closely associated with liver fat content ($r = 0.51$, $P < 0.0001$), but had less power to predict NAFLD (AUROC = 0.75) than the FLI (AUROC = 0.79). Not only was the fold-change TG_{OGTT} independently associated with liver fat content and NAFLD, but so also were the 2-h blood glucose level and rs738409 C>G SNP in *PNPLA3*. In fact, a novel index (extended FLI) generated from these and the usual FLI parameters considerably increased its power to predict NAFLD (AUROC = 0.79–0.86). The extended FLI also increased the power to predict changes in liver fat content with a lifestyle intervention ($n = 213$; standardized beta coefficient: 0.23–0.29).

Conclusion. – This study has provided novel data confirming that the OGTT-derived fold-change TG_{OGTT} and 2-h glucose level, together with the rs738409 C>G SNP in *PNPLA3*, allow calculation of an extended FLI that considerably improves its power to predict NAFLD.

Keywords: Fatty liver; Lifestyle intervention; NAFLD; OGTT; Prediction; Triglycerides

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has gained much attention in recent years because of its high prevalence, amounting to > 30% in the general population and to > 70% in certain high-risk groups, such as morbidly obese individuals and patients with type 2 diabetes (T2D) [1]. NAFLD is strongly associated not only with progressive hepatic disease, but also with cardiometabolic disorders, as it is also thought to be involved in the pathogenesis of cardiometabolic diseases, although the causal relationships are still not fully understood [2–12].

Diagnosis of NAFLD by the gold-standard method, liver biopsy, is invasive and, therefore, not feasible in routine clinical practice [13,14]. Proton magnetic resonance spectroscopy

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(^{1}H-MRS) is considered the most accurate non-invasive method for measuring liver fat content [15,16]. However, in addition to the high costs that limit its use, the infrastructure and knowledge needed to implement the technique are only available in a limited number of institutions. Therefore, ^{1}H-MRS is currently applied mostly for research purposes. Routine ultrasound is also being used to diagnose NAFLD, but the technique has only moderate sensitivity when liver fat content exceeds 20–30% [17].

Consequently, there has been intense interest in blood markers that, alone or in combination with clinical parameters, might be able to identify patients with NAFLD. Accordingly, NAFLD or liver fat indexes have been developed. However, some of them have only moderate predictive power and/or cannot be easily or widely used in routine clinical practice because they involve several parameters, such as insulin, that may either not be readily measurable or display wide variability in their measurement, depending on the method used [18–21]. Furthermore, as there is such wide variability in the decrease of liver fat content with lifestyle interventions [22,23], it is important to investigate whether such indexes can predict such decreases in those situations.

It is therefore of considerable interest to identify readily measurable blood parameters that can either autonomously predict NAFLD with relatively high sensitivity and specificity, or improve the predictive power of the established indexes. For this reason, only blood parameters that are commonly measured, such as serum liver enzymes, lipids and lipoproteins, or show no, or very little, variability between different laboratories were tested in the present study. In addition, the predictive power of the rs738409 C>G single nucleotide polymorphism (SNP) in *PNPLA3*, the strongest genetic determinant of NAFLD, was also studied [24]. As it was recently shown that plasma triglycerides (TGs) measured during an oral glucose tolerance test (OGTT) are closely related to abdominal obesity and insulin resistance [25], which themselves strongly correlate with liver fat content, circulating TGs were tested not only in the fasting state, but also after a standard 2-h 75-g OGTT.

2. Methods

2.1. Subjects

Data from 330 Caucasians, 130 men and 200 women, from the southern part of Germany were analyzed. These individuals had participated in the Tübingen Lifestyle Intervention Program (TULIP) [23,26]. Subjects were included in that study when they fulfilled at least one of the following criteria: family history of T2D; body mass index (BMI) > 27 kg/m^2; and previous diagnosis of impaired glucose tolerance and/or gestational diabetes. All were considered healthy according to physical examination and routine laboratory tests. If T2D had been newly diagnosed based on data from the screening visit, these subjects were also included in the study. In addition, they had no history of liver disease and did not consume more than two alcoholic drinks per day. Serum aminotransferase levels were less than two times the upper limit of normal.

Of the 330 subjects who met the above-mentioned requirements, a subgroup of 213 (127 women and 86 men) who, for mostly technical reasons, had a complete dataset of body fat distribution and liver fat content measurements, using magnetic resonance techniques, at both baseline and follow-up were also included in the longitudinal analyses.

Informed written consent was obtained from all participants, and the Ethics Committee of the University of Tübingen approved the protocol. All methods were carried out in accordance with the approved guidelines.

2.2. Lifestyle intervention

During this intervention, subjects underwent individual dietary counselling and had up to 10 sessions with a dietitian. The aim was to reduce their body weight and intake of calories, particularly their intake of calories from fat, to < 30% (< 10% from saturated fat) of total energy consumed, while increasing their intake of fibre to at least 15 g/1000 kcal. During each visit, participants brought along a three-day food diary and discussed the results with the dietitian. These subjects were also asked to perform at least 3 h of moderate sports activity per week. Aerobic endurance exercise (such as walking and swimming) causing only a moderate increase of heart rate was encouraged. Participants were seen by staff members on a regular basis to ensure that these recommendations were followed.

2.3. Total body fat mass and distribution

BMI was calculated as weight divided by the square of height (kg/m^2). Waist circumference was measured at the midpoint between the lateral iliac crest and lowest rib. Total body and visceral fat mass were measured by magnetic resonance imaging (MRI), using an axial T1-weighted fast spin-echo technique and a 1.5-T whole-body imager (MAGNETOM Sonata, Siemens Healthineers, Erlangen, Germany) [16,27].

2.4. Liver fat content

This was measured by localized ^{1}H-MRS [15,26], and NAFLD was defined as a liver fat content > 5.56% [15]. Measurement by this method correlates well with histomorphometry findings [28,29].

2.5. Oral glucose tolerance test

All participants underwent a 2-h 75-g OGTT. Venous plasma samples were obtained at 0, 30, 60, 90 and 120 min for determination of plasma glucose and insulin levels. Blood glucose was determined using a bedside glucose analyzer (glucose-oxidase method; YSI Incorporated, Yellow Springs, CO, USA). Plasma insulin was determined using the ADVIA Centaur XP immunoassay system (Siemens Healthineers). Insulin sensitivity based on the OGTT was calculated as proposed by Matsuda and DeFronzo [30]. In addition, homoeostasis model

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