Received: 21 March 2023 | Accepted: 24 May 2023

DOI: 10.1111/eci.14036

ORIGINAL ARTICLE

WILEY

# Estimation of fatty liver disease clinical role on glucose metabolic remodelling phenotypes and T2DM onset

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## Abstract

**Introduction:** Metabolic syndrome (MetS), prediabetes (PreDM) and Fatty Liver Disease (FLD) share pathophysiological pathways concerning type 2 diabetes mellitus (T2DM) onset. The non-invasive assessment of fatty liver combined with PreDM and MetS features screening might provide further accuracy in predicting hyperglycemic status in the clinical setting with the putative description of singular phenotypes. The objective of the study is to evaluate and describe the links of a widely available FLD surrogate -the non-invasive serological biomarker Hepatic Steatosis Index (HSI)- with previously described T2DM risk predictors, such as preDM and MetS in forecasting T2DM onset.

**Patients and methods:** A retrospective ancillary cohort study was performed on 2799 patients recruited in the Vascular-Metabolic CUN cohort. The main outcome was the incidence of T2DM according to ADA criteria. MetS and PreDM were defined according to ATP III and ADA criteria, respectively. Hepatic steatosis index (HSI) with standardized thresholds was used to discriminate patients with FLD, which was referred as estimated FLD (eFLD).

**Results:** MetS and PreDM were more common in patients with eFLD as compared to those with an HSI < 36 points (35% vs 8% and 34% vs. 18%, respectively). Interestingly, eFLD showed clinical effect modification with MetS and PreDM in the prediction of T2DM [eFLD-MetS interaction HR = 4.48 (3.37-5.97) and eFLD-PreDM interaction HR = 6.34 (4.67-8.62)]. These findings supported the

**Abbreviations:** ALAT, Alanine aminotransferase; ASAT, Aspartate aminotransferase; BMI, Body Mass Index; CI, Confidence interval; DBP, Diastolic blood pressure; eFLD, Estimated fatty liver disease; FLD, Fatty liver disease; HDL, High density lipoprotein; HSI, Hepatic steatosis index; LDL, Low density lipoprotein; MAFLD, Metabolic associated fatty liver disease; MetS, Metabolic syndrome; MHO, Metabolically healthy obesity; MUO, Metabolically unhealthy obesity (MUO); NAFLD, Nonalcoholic fatty liver disease; PreDM, Prediabetes mellitus Type 2; SBP, Systolic blood pressure; SD, Standard deviation; T2DM, Type 2 diabetes mellitus; WHO, World Health Organization.

Diego Martinez-Urbistondo and Ana Huerta equally contributed in the study performance.

Reporting of the study conforms to broad EQUATOR guidelines [Simera et al. A catalogue of reporting guidelines for health research. Eur J Clin Invest. 2010 Jan;40 (1):35–53 through the STROBE Checklist].

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description of 5 different liver status-linked phenotypes with increasing risk of T2DM: Control group (1,5% of T2DM incidence), eFLD patients (4,4% of T2DM incidence), eFLD and MetS patients (10,6% of T2DM incidence), PreDM patients (11,1% of T2DM incidence) and eFLD and PreDM patients (28,2% of T2DM incidence). These phenotypes provided independent capacity of prediction of T2DM incidence after adjustment for age, sex, tobacco and alcohol consumption, obesity and number of SMet features with a c-Harrell=0.84.

**Conclusion:** Estimated Fatty Liver Disease using HSI criteria (eFLD) interplay with MetS features and PreDM might help to discriminate patient risk of T2DM in the clinical setting through the description of independent metabolic risk phenotypes.

[Correction added on 15 June 2023, after first online publication: The abstract section was updated in this current version.]

**KEYWORDS**
clinical assessment, fatty liver disease, metabolic syndrome, prediabetes, risk factors

## 1 | INTRODUCTION

The World Health Organization (WHO) stated that the prevention of acquired metabolic disorders is a priority in public health.[1] In this context, different metabolic disorders were reunited in the so-called metabolic syndrome (MetS) to identify individuals at higher risk of disease in the clinical setting.[2] This condition was defined by the presence of 3 or more of the following characteristics: Abdominal obesity, hypertension, low HDL, high triglycerides and T2DM diagnosis, impaired glucose tolerance or fasting elevated glucose in plasma.[3,4] The MetS is a cornerstone of cardiovascular risk factor assessment which is attributable to the epidemiological association of MetS components among individuals.[5,6] Besides, MetS has demonstrated to be a predictor of Type 2 diabetes mellitus (T2DM) and cardiovascular disease.[7–9]

Meanwhile, in the past years, some debate has been risen about MetS in the pathophysiological arena. Critics state that insulin resistance is not the unique mechanism to explain the full complexity of such metabolic risk.[10] In fact, the release of unspecific and specific adipocitary and hepatic cytokines are also relevant in metabolic disease development while not directly linked to insulin resistance.[11–14] Besides, in the clinical scenario, the MetS risk is not different from the added risk of the MetS components in the T2DM and cardiovascular disease prediction.[15,16] Thus, the concepts of metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) might be more accurate concepts to express metabolic risk than MetS.[17] In the diabetes onset setting, the definition of prediabetes (PreDM) had a better performance in T2DM onset prediction than MetS.[18–20] Thus, the MetS usefulness in metabolic risk prediction has become controversial.

Recently, the MetS features have returned to the pipeline with the increasing prevalence and mortality impact of fatty liver disease (FLD).[21] FLD is related to important metabolic pathways of the MetS, such as insulin resistance,[22] lipid regulation and lipoprotein synthesis, inflammatory system dysregulation and specific cytokine release, encompassing hepatic metabolic remodelling.[23] Also, other pathophysiological processes such as mitochondrial dysfunction, oxidative stress and disrupted gut-liver axis have been linked to FLD and MetS.[24] Thus, FLD is often described as the MetS reflection in the liver.[25] However, the finding of lean patients with FLD at a higher risk of T2DM and cardiovascular disease,[26] the potential metabolic protection of patients with genetic origin of FLD[27] as well as the description of subgroups of patients meeting MetS criteria without FLD[28] might point out a specific role of liver steatosis in the characterization of metabolic risks.[29]

To be clinically relevant, FLD should be easy to be detected and clearly defined. In the FLD detection, biopsy is still considered the gold standard for diagnosis.[30] Nevertheless, cost-effectiveness and risk–benefit balances may rise some objections to this approach in the clinical setting, particularly in patients at early stages of the disease.[30] Other non-invasive methods of detection of FLD such as echography or controlled attenuation parameter (CAP©) have been examined with interesting results.[31] However, these tests are not available enough for a widespread disease as liver steatosis. For this reason, simple

serum-based indexes such as Hepatic Steatosis Index (HSI) have been validated to detect and stratify patients at risk of FLD.[32,33]

In this regard, clinical definitions of fatty liver disease such as nonalcoholic fatty liver disease (NAFLD) and metabolic associated fatty liver disease (MAFLD) are useful to identify patients at hepatic risk, but have some practical flaws. NAFLD is restricted to patients with no excessive alcohol consumption, which is often an underrecognized condition.[34] The relationship between alcohol and liver injury depends on several cofactors, such as type of alcoholic beverage, drinking patterns, duration of exposure and individual susceptibility, rendering quantitative thresholds at least partially arbitrary.[34] Thus, some pathophysiological overlap with alcoholic fatty liver disease in those patients consuming alcohol should be expected, making the concept unclear.[35] To avoid this controversy, the MAFLD definition was proposed to detect patients with fatty liver disease of metabolic origin.[36] Although useful in the detection of patients at hepatic risk, the combination of metabolic risk factors in the definition complicates the independent evaluation of fatty liver in the development of metabolic disease.

The non-invasive estimation of fatty liver disease with available and reproducible methods such as HSI—estimated fatty liver disease (eFLD)—could be useful when added to previously proven risk factors. This approach may enable to predict the incidence of FLD in the clinical context, avoiding availability and conceptual dilemmas.[32] The objective of this study is to evaluate and describe the interplay of eFLD—assessed by HSI—with MetS features and PreDM in the prediction of T2DM onset.

## 2 | PATIENTS AND METHODS

### 2.1 | Population

The vascular metabolic CUN cohort is a population-based, observational retrospective study designed to examine the incidence of cardiovascular and metabolic diseases including type 2 diabetes, hypertension, obesity, stroke or coronary heart disease in a large Southern European population, recording patients from the 1 February 1997 to the 31 December 2002, and subsequently followed up until 31 December 2012. The cohort has been described elsewhere.[37]

### 2.2 | Inclusion and exclusion criteria

The vascular metabolic CUN cohort included patients who were evaluated for a medical check-up by specialists in Internal Medicine. Patients were excluded from the general cohort according to the following criteria: Age <18 or >90 years old, history of type 1 diabetes or latent autoimmune diabetes of the adult, cancer in the palliative phase, previously diagnosed liver disease different from fatty liver disease, excessive alcohol consumption according to the hazardous drinking criteria,[38] familial lipid disorders, extreme (>45 kg/m$^2$) body mass index (BMI), as well as an inherited and clinically relevant hypercoagulable state. All analysis included patients with an initial 8 h-fasting blood test evaluation.

The present study enrolled patients from this cohort excluding those with prevalent T2DM diagnosed by ADA criteria[20] or under glucose lowering treatment, cardiovascular or any renal impairment. Patients with missing variables concerning age, sex and parameters concerning cardiovascular risk factors to evaluate MetS and HSI were also excluded. The research was conducted according to the standards of the Declaration of Helsinki on medical research and was approved by the Ethics Committee of the University of Navarra, weaving the need of informed consent (CEI 30/2015).

### 2.3 | Main variables

Patients were categorized into estimated fatty liver disease (eFLD) when scoring HSI > 36 points as calculated according to the following formula[32]:

$$\mathrm{HSI} = 8 \times (\mathrm{ALT}/\mathrm{AST}) + \mathrm{BMI}\ (+2, \text{if female};\ +2, \text{if diabetes mellitus}).$$

Prediabetes was defined according to published criteria, using a fasting glucose >100 mg/dL as cut-off.[20] HbA1c was not used to select patients with prediabetes due to the small quantity of patients ($n = 16$) with HbA1c values at baseline, which is consistent with the availability and validation of the technique at the recruitment dates.

The diagnosis of T2 DM was based on ADA criteria Standards of Medical Care in Diabetes: (i) A1C ≥ 6.5 in a NGSP certified laboratory, (ii) fasting plasma glucose ≥126 mg/dL, (iii) 2 h plasma glucose ≥200 during an OGTT or (iv) hyperglycaemic symptoms with a random plasma glucose >200 mg/dL.[20]

## 2.4 | Covariables

Data regarding medical history, health-related behaviours and blood biochemical measurements were retrieved at each patients' visit. Health-related behaviours including cigarette smoking (none, former smoker or current smoker) and daily alcohol intake (yes/no) were obtained by physicians at the consultation, as reported elsewhere.[37] Metabolic syndrome features were recorded as described in the ATP III criteria except for waist circumference, which was substituted by BMI and included as overweight according to WHO criteria due to the lack of waist circumference measurements. Anthropometric measurements and determinations of biochemical parameters including fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) and liver enzymes (ASAT and ALAT) were obtained and analysed as described elsewhere.[37] No precise measure of alcohol consumption was registered in the medical records. Nevertheless, due to the importance of alcohol consumption in fatty liver evolution and known impact on T2DM development we distributed patients into 2 main groups according to alcohol consumption: (i) Daily or almost daily consumers of some amount of alcohol but not fulfilling the criteria of hazardous drinking ('Daily alcohol consumption') and (ii) sporadic or no alcohol consumers for those who did not declare alcohol consumption in a daily basis. Patients under blood pressure control and triglyceride control drugs were recorded.

Regarding the retrospective condition of the analysis and the heterogeneity of the population, there was no defined follow-up strategy. Nevertheless, patients were encouraged to repeat a medical check-up at least every year.

## 2.5 | Statistical analyses

Categorical variables were reported as percentages. Student's *t*-test, one-way ANOVA or chi-square test was implemented to compare the baseline characteristics of study participants. Different Cox proportional-hazard analysis was carried out to estimate the univariate hazard ratio (HR) and their 95% confidence interval (CI) of T2DM of different variables. A comprehensive clinical and statistical analysis of these results was performed to generate clinically relevant interactions between variables by using Cox Regression analyses, to screen for independent risk phenotypes as described: (i) control group (No eFLD, no MetS and no PreDM), (ii) patients with eFLD (eFLD with no MetS and no PreDM), (iii) patients with eFLD and MetS (eFLD and 3 MetS features with no PreDM), and (iv) patients with PreDM and no eFLD and (v).

Finally, a multivariate Cox regression model was fitted to test the prediction capacity of incident T2DM of these phenotypes after adjustment for relevant variables. Harrel C test was applied to explore the discrimination capacity of survival models. All statistical analyses were performed with SPSS version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0: IBM Corp), whose manuals were used for statistical test guidance. All *p* values are two-tailed, and statistical significance was set at the conventional cut-off of $p < 0.05$.

# 3 | RESULTS

A total of 2799 patients fulfilled the inclusion criteria and conformed the study cohort. All patients accounted at least a follow-up of 1 month, with a median follow-up of 104 months and a 90.7% of patients followed for more than 1 year. In the analyses of this cohort, baseline characteristics are reported according to eFLD criteria (Table 1). Thus, eFLD was associated with older age, male sex, higher proportion of alcohol consumption, active or former smokers, and all the features regarding the MetS in both continuous and discrete variable evaluation ($p < 0.01$). As expected, patients with eFLD were more likely to have higher levels of liver enzymes and a higher HSI index, with a higher proportion of patients suffering MetS ($p < 0.01$). Finally, statistically significant differences were found between subgroups in the incidence of T2DM ($p < 0.01$) as shown (Table 1).

A higher proportion of eFLD patients was as well found within the group of MetS features, with a higher prevalence of fatty liver disease in patients with overweight (82% vs. 17%), hypertension (68% vs. 32%), elevated blood glucose (71% vs. 28%), low HDL (70% vs 30%) and high triglycerides (82% vs. 18%) using ATP III criteria (Figure S1). Only 49 patients were using blood pressure lowering drugs, and none of the patients reported to use triglyceride lowering drugs. No further analysis was performed concerning treatment due to the lack of sufficient statistical power to evaluate this concept. In a similar manner, an increasing higher proportion of eFLD patients were detected as MetS features were more prevalent, being the eFLD rate of 12% in those with no MetS features, 50% in those with 1 characteristic, 73% in those with 2 characteristics, 82% in those with 3 characteristics, 92% in patients with 4 characteristics and 100% in patients with all the MetS features. (Figure S2).

A univariate Cox regression evaluation of age, sex, daily alcohol consumption, smoking status, MetS features as sole variables, the total number of MetS accumulated by each patient, MetS diagnosis and eFLD was performed (Table 2). All the studied variables were predictors of T2DM, with a high statistically significant value ($p < 0.01$).

**TABLE 1** The study cohort characteristics including demographics, metabolic syndrome features, hepatic steatosis assessment and clinical outcomes.

| Variables | Total | No eFLD [HSI < 36] | eFLD [HSI ≥ 36] | *p* Value[a] |
|---|---|---|---|---|
| Demographics (mean ± SD or n, %) [Reference values] | *n* = 2799 | *n* = 1196 | *n* = 1603 | |
| Age, years ± SD | 53.3 ± 13.1 | 52.2 ± 15.1 | 54.1 ± 11.4 | <0.01 |
| Sex, female (%) | 1160 (41%) | 631 (53%) | 529 (33%) | <0.01 |
| Daily alcohol [yes/no] (%) | 1034 (37%) | 370 (31%) | 664 (41%) | <0.01 |
| Never smoker (%) | 1072 (38%) | 501 (42%) | 571 (36%) | <0.01 |
| Metabolic syndrome features | | | | |
| BMI ± SD, kg/m$^2$ [Reference range < 25 kg/m$^2$] | 26.7 ± 4.4 | 23.4 ± 2.6 | 29.1 ± 3.9 | <0.01 |
| Overweight [BMI > 25 kg/m$^2$], % | 1744 (62%) | 309 (26%) | 1435 (89%) | <0.01 |
| SBP, mmHg±SD [Reference range < 140 mmHg] | 129.4 ± 44.5 | 124 ± 35 | 132 ± 50 | <0.01 |
| DBP, mmHg±SD [Reference range < 90 mmHg] | 80.4 ± 16.7 | 77 ± 22 | 82 ± 10 | <0.01 |
| Hypertension [SBP > 135 mmHg or DBP > 85 mmHg or active treatment], % | 1223 (44%) | 398 (33%) | 825 (51%) | <0.01 |
| Fasting plasma glucose ± SD [Reference range < 100 mg/dL] | 95.4 ± 12.1 | 92 ± 11 | 97 ± 12 | <0.01 |
| Hyperglycaemia/PreDM [Fasting plasma glucose > 100 mg/dL], % | 763 (27%) | 216 (18%) | 547 (34%) | <0.01 |
| Fasting plasma triglycerides ± SD [Reference range < 150 mg/dL] | 98.3 ± 57.7 | 80 ± 48 | 111 ± 60 | <0.01 |
| Hypertriglyceridemia [p-Triglycerides > 150 mg/dL], % | 358 (13%) | 65 (5%) | 293 (18%) | <0.01 |
| Fasting plasma HDL levels±SD [Reference range < 40 mg/dL or < 50 mg/dL] | 56.0 ± 14.9 | 60 ± 15 | 53 ± 13 | <0.01 |
| Low HDL [p HDL < 40 mg/dL or < 50 mg/dL], % | 445 (16%) | 134 (11%) | 311 (19%) | <0.01 |
| Fasting LDLc, mg/dL ± SD | 151.2 ± 38.4 | 144 ± 37 | 156 ± 38 | <0.01 |
| Average number of MetS features ± SD | 1.62 ± 1.19 | 0.94 ± 0.98 | 2.13 ± 1.07 | <0.01 |
| Metabolic Syndrome (≥3 features) | 654 (23%) | 98 (8.2%) | 556 (35%) | <0.01 |
| Hepatic steatosis index (HSI) | | | | |
| ASAT (UI/L) ± SD [Reference range 1–25 UI/L] | 14.6 ± 13.2 | 13.4 ± 9.7 | 15.5 ± 15.2 | <0.01 |
| ALAT (UI/L) ± SD [Reference range 1–29 UI/L] | 19.6 ± 22.5 | 13.1 ± 9.5 | 24.5 ± 27.6 | <0.01 |
| HSI, points ± SD [Reference range < 36 points] | 37.9 ± 6.5 | 32.21 ± 2.65 | 42.09 ± 5.12 | <0.01 |
| Clinical outcomes | | | | |
| Follow-up (months) ± SD | 104 ± 59 | 108 ± 59 | 101 ± 59 | <0.01 |
| Incident T2DM, % | 249 (9%) | 39 (3%) | 210 (13%) | <0.01 |

Abbreviations: ALAT, Alanine aminotransferase; ASAT, Aspartate aminotransferase; BMI, Body Mass Index; DBP, Diastolic blood pressure; eFLD, estimated fatty liver disease; HDL, High density lipoprotein; HSI, Hepatic steatosis index; LDL, Low density lipoprotein; MetS, Metabolic syndrome; PreDM, Prediabetes mellitus Type 2; SBP, Systolic blood pressure; SD, Standard deviation; T2DM Type 2 diabetes mellitus.

[a]*p* value of chi-square evaluation for qualitative variables or Student's *t*-test for quantitative variables.

Then, the analysis of the most discriminative characteristics, accounting for PreDM, number of MetS features, MetS prevalence and eFLD according to HSI were evaluated in the prediction of T2DM incidence. As previously mentioned, a higher number of MetS characteristics was related to a proportionally higher risk of T2DM development. However, patients with elevated fasting glucose showed a different risk of T2DM incidence than the rest of the population. Among them, patients with eFLD showed a higher risk than those with PreDM alone, suggesting an effect modification association between both characteristics. Furthermore, patients with normal fasting plasma glucose had different risk depending on their eFLD status, increasing proportionally to MetS features accumulation. Interestingly, patients with eFLD appeared to induce a potential risk increase in comparison with those with apparently healthy liver in patients with MetS, suggesting another potential interplay. Noteworthily, both interaction models, eFLD – PreDM and eFLD – MetS were found statistically significant (Figure 1).

According to these results, five clinically relevant phenotypes were derived in the prediction of T2DM incidence as follows: (i) control group (No eFLD, no MetS and no PreDM), (ii) patients with eFLD (eFLD with no MetS and no PreDM), (iii) patients with eFLD and MetS (eFLD and 3 MetS features with no PreDM), (iv) patients with PreDM

**TABLE 2** Univariate Cox regression analysis in the prediction of incident T2DM concerning lifestyle, metabolic syndrome signatures and eFLD ($n = 2799$).

| Variables | HR of T2DM (CI 95%) | *p* Value | c- Harrell |
|---|---|---|---|
| Smoking status [Never smoker yes/no] | 0.73 (0.56–0.95) | 0.02 | 0.54 |
| Daily alcohol consumption [yes/no] | 1.66 (1.30–2.13) | <0.01 | 0.57 |
| Overweight [BMI > 25 kg/m$^2$] | 4.19 (2.92–6.01) | < 0.01 | 0.63 |
| Obesity [BMI > 30 kg/m$^2$] | 3.60 (2.80–4.63) | < 0.01 | 0.64 |
| Hypertension [SBP > 135 or DBP > 85 mmHg or active treatment] | 2.17 (1.68–2.80) | < 0.01 | 0.61 |
| Hyperglycaemia/PreDM [Fasting plasma glucose >100 mg/dL] | 7.97 (6.05–10.51) | <0.01 | 0.76 |
| Hypertriglyceridemia [Plasma triglycerides >150 mg/dL] | 3.15 (2.39–4.14) | < 0.01 | 0.59 |
| Low HDL [Plasma HDL < 40 mg/dL or < 50 mg/dL] | 1.67 (1.24–2.25) | <0.01 | 0.54 |
| Estimated fatty liver disease (eFLD) [HSI > 36 points] | 4.31 (3.06–6.07) | <0.01 | 0.65 |
| Number of MetS features [Sum up of MetS features] | 2.25 (2.03–2.49) | <0.01 | 0.78 |
| Metabolic syndrome [MetS features ≥3] | 5.98 (4.64–7.72) | <0.01 | 0.72 |

Abbreviations: BMI, Body Mass Index; DBP, Diastolic blood pressure; eFLD, estimated fatty liver disease; HDL, High density lipoprotein; HR, Hazard ratio; HSI, Hepatic steatosis index; MetS, Metabolic syndrome; PreDM, Prediabetes mellitus Type 2; SBP, Systolic blood pressure; T2DM Type 2 diabetes mellitus.

and no eFLD and (v) patients with PreDM and eFLD. The proportion of each phenotype in the study population as well as the increasing incidence of T2DM among subgroups is shown (Table S1. Figure S3).

These phenotypes were evaluated in a multivariate Cox Regression model, which showed independent risk of T2DM when adjusted by age, sex, smoking and drinking status, obesity assessed by BMI with an increasing risk of T2DM depending on the accumulation of MetS features, with a c-Harrel test result of 0.84 (Figure 2).

Finally, a clinical evaluation of metabolic risk was proposed for the evaluation of patients at risk of T2DM according to the results of the present study (Figure S4).

## 4 | DISCUSSION

The current study supports that the assessment of estimated fatty liver disease might predispose to T2DM. Specifically, eFLD, using an easy to perform index such as HSI, in combination with previously described risk factors, as PreDM and MetS features, might provide the description of 5 clinical phenotypes of T2DM risk. These clusters of patients were independent from age, sex, MetS features, daily alcohol consumption and smoking status and provided a c-Harrell discrimination capacity of 0.84.

The description of these risk subgroups could have an impact in the clinical setting by adding the fatty liver concept in a feasible and controversy-free manner to the metabolic risk stratification, while providing a cost-effective tool to precision medicine. Besides, these findings support an independent added risk of FLD in patients at metabolic risk. Furthermore, the interplay between eFLD in and MetS or PreDM in the prediction of T2DM onset highlights the importance of understanding the bioenergetic efficiency and metabolic flexibility mechanisms in the liver to provide further understanding and to facilitate intervention in the clinical setting.[14]

The distribution of metabolic risk in our cohort is comparable to previously published populations in the MetS scenario.[39] Metabolic risk factors were more prevalent in patients with MetS than in those without MetS while patients with eFLD had a higher proportion of MetS features. The interactive role of eFLD in MetS consequences has been proven in different situations. In the epidemiological field, NAFLD has demonstrated to be related to T2DM development.[39] Furthermore, a bi-directional relationship between MetS features and NAFLD has been proposed in terms of T2DM incidence.[40] This interplay is

| Interaction | HR of T2DM (95 % CI) | *p* value* |
|---|---|---|
| **Interaction eFLD – MetS** [Adjusted by eFLD] | 4.48 (3.37-5.97) | $p < 0.01$ |
| **Interaction eFLD – PreDM** [Adjusted by eFLD] | 6.34 (4.67-8.62) | $p < 0.01$ |

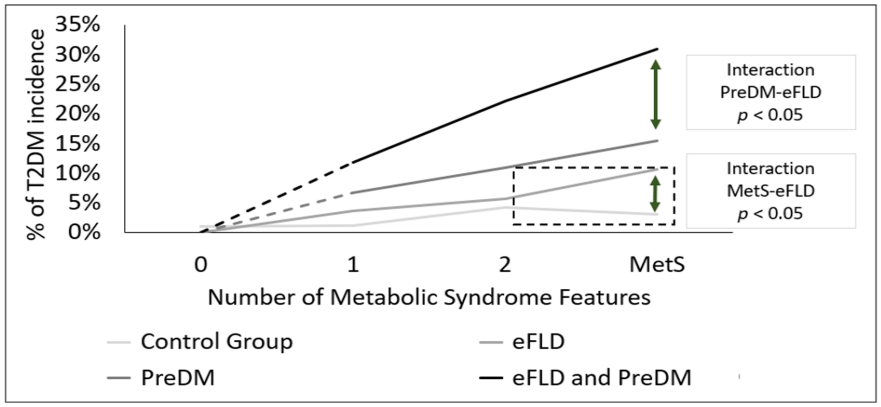


**FIGURE 1** Analysis of the interplay between baseline eFLD, PreDM and MetS in the prediction of incident T2DM. *p value for 2 way ANOVA evaluation.

| Variables | HR of T2DM onset (CI 95 %) | *p* Value |
|---|---|---|
| Age, years | 1.02 (1.01–1.03) | < 0.01 |
| Sex, male | 1.02 (0.74–1.40) | 0.92 |
| Daily alcohol consumption | 1.37 (1.04–1.81) | 0.03 |
| Never smoker | 0.92 (0.69–1.22) | 0.55 |
| Obesity, BMI > 30 kg/m$^2$ | 1.84 (1.39–2.43) | < 0.01 |
| Number of MetS features, per feature | 1.35 (1.15–1.58) | < 0.01 |
| **Phenotype 1** – Control group (neither eFLD, MetS or PreDM) | Ref | - |
| **Phenotype 2** – eFLD | 1.82 (0.99–3.37) | 0.05 |
| **Phenotype 3** – eFLD and MetS | 2.61 (1.18–5.75) | 0.02 |
| **Phenotype 4** – PreDM | 4.60 (2.31–9.16) | < 0.01 |
| **Phenotype 5** – eFLD and PreDM | 8.07 (4.25–15.32) | < 0.01 |

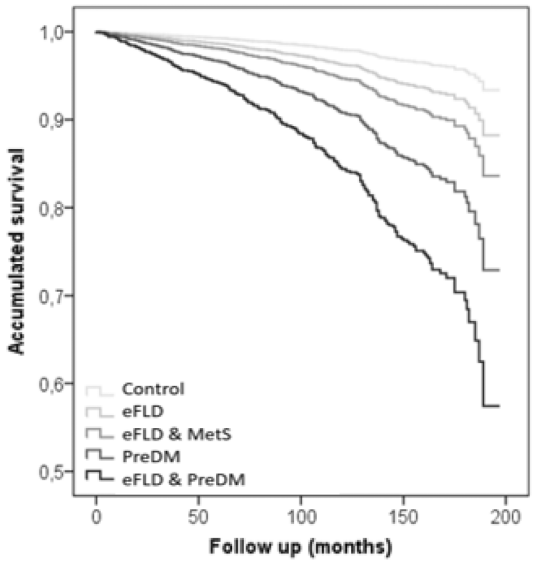


**FIGURE 2** Cox regression multivariate model in the prediction of incident T2DM and Kaplan–Meier plot for adjusted clinical phenotypes.

described in our cohort as an independent risk factor for patients without MetS. In patients with more than two metabolic risk factors, a potential modification of effect of eFLD was found, providing a potential risk excess of eFLD in patients with metabolic syndrome.[40]

The pathophysiological role of NAFLD as an enhancer of the metabolic disease burden is plausible due to the key role of the liver in both insulin resistance and dyslipidaemia through different mechanisms.[24] In fact, hepatic insulin resistance, where insulin activation of glycogen synthase is impaired,[41] would also be expected to redirect glucose into lipogenic pathways and further promote MetS and T2DM incidence. In this context, elevated diacylglycerol in the liver was related PKCε activity in the liver of obese patients with NAFLD, linking hypertriglyceridemia or dyslipidaemia, obesity and insulin resistance to fatty liver dysfunction.[42] Furthermore, the contribution to plasma glucose of gluconeogenesis which is impaired in NAFLD[43] and is not directly dependent of insulin resistance[44] might provide pathophysiological explanation to the special impact of NAFLD in patients with prediabetes. A potential role of metabolic protection could be referred to the genetic predisposition to NAFLD[27] due to the dual role of genetics in FLD related metabolic risk.[27] Thus, the proposal of a phenotypical approach to metabolic disease including FLD could help to shed light to this concept if further research is conducted.

The evaluation of an easy to perform and validated test such as HSI, which only includes ASAT, ALAT, BMI and sex, in the liver status stratification of patients makes our results highly reproducible and of a clinical impact[32,33] in contrast to other less accessible methods of liver assessment such as echography, transient liver elastography or liver biopsy which might neither efficient nor justifiable in this population.[30] Although limited, as it was established based on the suboptimal reference standard of echographic findings,[45] HSI demonstrated discrimination capacity of different phenotypes of patients in terms of T2DM development risk. In fact, previous studies had demonstrated the HSI capacity to stratify MetS patients in terms of quality of life (QoL) response to lifestyle modulation.[33] Nevertheless, the present results might have further implications in the clinical setting due to the use of a stronger outcome such as T2DM, which leads to a recognized cardiovascular disease burden.[39] Besides, some queries could be mentioned due to the dependence of HSI to adiposity and T2DM diagnosis. In this context, the development of the final Cox regression model included obesity as an adjustment criterion. In the same manner, although HSI has been linked to T2DM including the baseline diagnosis of this entity as part of the final score, patients with T2DM at baseline were excluded to avoid bias. Finally, the use of an estimation of FLD in a clinically based stratification of risk, might provide further applicability of the results in order to standardize NAFLD assessment in the metabolic disease prevention arena with impact on glucose remodelling.

The current research has some limitations. The retrospective recollection of data and the absence of control on medical interventions may reduce the merit of these results. Besides, some uncontrolled facts could disturb the accuracy of the study due to the recruitment of patients in a real-life scenario. The reduced number of patients under pharmacological treatment for metabolic alterations such as lipid and blood pressure lowering drugs may introduce a further bias in the eFLD single modification of effect role, due to their potential effect on both eFLD and T2DM development. Nevertheless, the low number of patients under active treatment is plausible, as patients were recruited in the first visit to the medical centre, with few or no previous medical evaluation at all. The evaluation of the effect of interventions in metabolic control was not possible as therapeutic changes were not recorded on data curation. Nevertheless, these concepts should affect in a reduction of risk of stablished conditions and thus, to an underestimation of the effect of baseline characteristics on the further development of T2DM. Besides, the multivariate adjustments of the final model may help to reduce this bias. In this context, the inclusion of well-defined variables in the multivariate model might reinforce the utility of a phenotype-based approach to metabolic risk in the clinical setting.

The absence of waist circumference measurement may also bias our results. In fact, the use of BMI and the adjustment of the final model using obesity (BMI > 30 kg/m$^2$) could help to mitigate the potential effect of this inaccuracy in the results, while using a common adiposity marker in the clinical setting. Although some collinearity could be assumed with HSI, the fact of both being independent predictors of T2DM incidence in the final model highlights the potential capacity of discrimination of both concepts and thus the contribution of their evaluation in a precision medicine context. Furthermore, the qualitative assessment of alcohol consumption might contribute to the potential inaccuracy of the study. Nevertheless, a consideration of alcohol drinking status is necessary to provide valid investigation in the FLD setting.[30,38] The independent capacity of daily alcohol consumption (as described in the material and method setting) to predict T2DM in the final model and the exclusion of patients with hazardous alcohol consumption provide some internal validity to the results. However, further prospective analysis should be performed to describe the influence of a grams per day alcohol assessment of alcohol consumption in this setting. Some concern could be raised due to the classification of prediabetes using a unique glucose determination. Although this is consistent with the ADA criteria[20] and an 8-hour-fasting was ensured in all the patients, this issue might provide some inaccuracy. However, the differences between phenotypes according to this classification and the simplicity of assessment contribute to the reproducibility of the study. Despite these potential limitations, the

methodology of the study, with a non-negligible sample size and follow-up, a teaching hospital as a source of patients, the use of precise and previously validated tools in the adjustment of models and the plausibility of the results provide further support to our hypotheses.

To sum up, the non-invasive eFLD assessment in patients at risk of T2DM might provide a useful patient phenotyping tool in the clinical setting with implications in patient surveillance. Besides, these results could be of higher interest in the mid-term with the development of a NAFLD specific therapeutic armamentarium.[46] Thus, the evaluation of the impact in T2DM incidence of different NAFLD drug therapies in both PreDM and MetS patients using FLD estimation might provide further practical value to the current results.

## 5 | CONCLUSION

Estimated fatty liver disease using HSI might play an interactive role on PreDM and MetS features capacity to predict T2DM incidence. Indeed, five discriminating T2DM risk phenotypes were identified including eFLD. These risk subgroups showed potential value in the clinical surveillance of patients at metabolic hazard.

### AUTHOR CONTRIBUTIONS

D.M-U., A.H, D.N-G, L.S-I, A. F-M, M.F.L, J.A.M and J.C.P were involved in the conception, design, and conduct of the study and the analysis and interpretation of the results. D.M.U. wrote the first draft of the manuscript, and all authors edited, reviewed and approved the final version of the manuscript. J.C.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

### ACKNOWLEDGEMENTS

To the patients who contributed to the CUN Vascular Metabolica Cohort. To Jaime Huerta-Muñoz for his major support.

### CONFLICT OF INTEREST STATEMENT

No conflicts of interest were declared by authors.

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## SUPPORTING INFORMATION

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**How to cite this article:** Martinez-Urbistondo D, Huerta A, Navarro-González D, et al. Estimation of fatty liver disease clinical role on glucose metabolic remodelling phenotypes and T2DM onset. *Eur J Clin Invest*. 2023;53:e14036. doi:10.1111/eci.14036