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Fatty liver index and progression to type 2 diabetes in people with prediabetes

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ABSTRACT

Objective: The main aim of the study was to evaluate the association between Non-alcoholic fatty liver disease (NAFLD), estimated by fatty liver index (FLI), and the development of type 2 diabetes (T2D) in a large cohort of adult workers with prediabetes.

Design: Prospective cohort study.

Setting: Occupational health services from Spain.

Participants: 16,648 adult workers (aged 20 to 65 years) with prediabetes (fasting plasma glucose (FPG) of 100-125 mg/dl).

Outcome and measures: FLI was calculated based on measurements of triglycerides, body mass index, waist circumference and γ-glutamyltransferase. The population was classified into three categories: FLI <30 (no hepatic steatosis), FLI 30–59 (intermediate status), and FLI >60 (hepatic steatosis). Sociodemographic, anthropometric, dietary habits, physical activity and clinical data were collected from all subjects. The incidence rate of T2D was determined after 5 years of follow-up.

Results: After 5 years of follow-up, 3,706 of the 16,648 participants (22.2%) were diagnosed with T2D, corresponding to an annual rate of progression of 4.5%. FLI was strongly associated with T2D conversion. The incidence rates of T2D in the FLI<30, FLI 30-59 and FLI>60 groups after 5 years of follow-up were 19/6,421 (0.3%), 338/4,318 (7.8%) and 3,349/5,909 (56.7%), respectively. This association remained significant (OR = 6.16; 95% CI 5.22 to 7.26) for FLI>60 after adjustment for sex, age, diet, lifestyle and blood pressure.

Conclusion: NAFLD assessed by FLI independently predicted the risk of conversion to T2D among people with prediabetes. FLI may be an easily determined and valuable early predictor for T2D in people with prediabetes. FLI-based assessment of NAFLD in subjects with prediabetes in routine clinical practice could allow the adoption of effective measures to prevent and reduce their progression to T2D.

Strengths and limitations of this study

- This is a prospective study, with large sample size and 5-years follow-up.
- Study participants had multiple occupations and were from several geographical locations.
- Fatty liver index used as a surrogate of fatty liver does not detect progression of fatty liver disease.
- Lifestyle modifications of study participants were not evaluated throughout the 5-year follow-up

INTRODUCTION

Type 2 Diabetes (T2D) is closely associated with a constellation of metabolic comorbidities, including obesity, hypertension, hypercholesterolemia, dyslipidemia and non-alcoholic fatty liver disease (NAFLD).[1] The main characteristic of NAFLD is the infiltration of hepatocytes by free fatty acids and triglycerides not related to significant alcohol intake. NAFLD is an entity that encompasses a wide spectrum of lesions ranging from indolent liver fat storage followed by lipotoxicity,[2] to hepatic inflammation, also known as non-alcoholic steatohepatitis (NASH). NAFLD is the most common chronic liver disease worldwide that is associated with excess health-related expenditures, making it a community health problem.[3] The estimated overall worldwide prevalence of NAFLD in the general adult population is about 25–30%,[3,4] but ranges from 40%–70% in subjects with established T2D.[5,6] In fact, NAFLD and T2D are conditions that frequently coexist and can act synergistically to drive adverse outcomes.[7] NAFLD is considered the hepatic manifestation of metabolic syndrome (MetS)[8] because epidemiological studies have consistently shown that NAFLD is strongly linked to obesity, dyslipidemia, and insulin resistance.[9,10] Therefore, NAFLD is thought to be an independent risk factor for incident T2D[7] and cardiovascular disease.[11]

Liver biopsy is currently the gold standard for diagnosing progressive NAFLD.[12] Biopsies are invasive procedures with several drawbacks, including sampling error, interobserver variability, high cost, patient discomfort and risk of complications.[5] Moreover, obtaining liver biopsies from all patients with NAFLD is unrealistic. Abdominal ultrasonography is a simple, inexpensive, widely available and minimally invasive technique that is used to diagnose fatty liver in most subjects. However, its sensitivity is low in subjects with fatty retention less than 20%–30% and it does not provide information on the degree of fibrosis.[13] Consequently, attempts have been made to diagnose NAFLD/NASH using clinical and laboratory-based biomarkers and scoring systems that can predict fatty changes in the liver. These indices for the diagnosis of NAFLD/NASH include the fatty liver index (FLI),[14] NAFLD liver fat score,[15] the hepatic steatosis index (HSI),[16] the ALD/NAFLD index (ANI),[17] the lipid accumulation product (LAP)[18] and the SteatoTest (ST).[19] These indices require the measurement of patient characteristics, including concentrations of triglycerides (TG), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine transaminase (ALT), insulin, body mass index (BMI), waist circumference (WC), gender,

mean corpuscular value and presence or absence of T2D or metabolic syndrome.[20] The FLI is a simple and accurate algorithm that combines routine measurements of TG and GGT concentrations, WC and BMI, showing an excellent discriminative ability to predict ultrasonographic NAFLD and hepatic steatosis in the general population.[14,21]

The FLI has been reported to correlate with: 1) insulin resistance; 2) risk of coronary heart disease; 3) MetS; 4) early atherosclerosis; and 5) rates of non-hepatic-related morbidity and mortality in nondiabetic subjects.[22] Thus, FLI-diagnosed NAFLD may be an indicator of incident T2D.[10] Nonetheless, the risk of progression to T2D determined by FLI in patients with prediabetes remains poorly understood. Determining FLI in subjects with prediabetes may be highly relevant, as both epidemiological and clinical evidence have shown that primary health care prevention programs should target people at greater risk of developing T2D. The present study was therefore designed to evaluate the association between NAFLD, as estimated by FLI, and the development of T2D in a large cohort of South-European Mediterranean workers with prediabetes.

Methods

Study population and design

This cohort study included 16,648 Spanish working adults with prediabetes who worked in public administration, construction, health departments or post offices. The study methods have been described in detail previously.[23] Briefly, participants were carefully chosen from 234,995 potentially eligible individuals who underwent periodic occupational health assessments between 2012 and 2013. Participants were included if they were aged 20–65 years and had an FPG of 100–125 mg/dL.[24] Subjects were excluded if they had a history of physician-diagnosed diabetes, had been treated with an oral antidiabetic agent or a systemic glucocorticoid, had an FPG ≥126 mg/dL or an HbA1c ≥6.5% at baseline, had received cancer treatment during the preceding 5 years, had anemia (hematocrit <36% in men and <33% in women) or were pregnant. All subjects underwent standard health examinations, anthropometric measurements, and metabolic tests at baseline and were followed-up 5 years later, in 2017 and 2018.

All the procedures in the study protocol were in accordance with the Declaration of Helsinki for research on human participants and were approved by the Balearic Islands Ethical Committee of Clinical Research (Ref. No: CEI-IB-1887). All participants were carefully informed of the purpose and demands of the study. Informed consent was obtained from all participants included in the study.

Patient and public involvement

People were not involved in setting the research question nor in the study design. Participants were interviewed face to face by trained researchers for a detailed explanation of the purpose of this research and informed consent at the beginning. Results of the research will be disseminated to the participants.

Data collection

At baseline, anthropometric measurements and fasting blood sample were taken from all subjects during occupational health examinations. A questionnaire was administered to collect data on sociodemographic characteristics, dietary habits, physical activity (PA) and clinical data. Participants were asked to report if they performed moderate and/or vigorous exercise (at least 150 min/week, according to World Health Organization [WHO] recommendations) and if they consumed fruits and vegetables daily. Each individual was also categorized as a smoker, former smoker, or never smoker. Social class was defined using the Spanish Epidemiology Society classification, which is based on occupation and it has shown high correlation with level of education.[25] Class I (upper class) includes executives, managers, and university professionals; Class II (middle class) includes intermediate occupations and employees; and Class III (lower class) includes manual workers.

All anthropometric measurements were made in the morning, after an overnight fast, at the same time and according to the guidelines and recommendations in the International Standards for Anthropometric Assessment (ISAK) manual.[26] All measurements were performed by well trained technicians or researchers to minimize coefficients of variation. Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca 700 scale, Hamburg); height was measured to the nearest 0.5 cm using a stadiometer (Seca 220) Telescopic Height Rod for Column Scales, Hamburg); and BMI was calculated as weight (kg) divided by height (m) squared (kg/m^2). Obesity

was defined as BMI ≥30.0 kg/m^2, in agreement with WHO guidelines. Blood pressure was measured after a resting period of 10 minutes, with the subject in the supine position, using an electric and calibrated sphygmomanometer (OMRON M3, Healthcare Europe, Spain). Blood pressure in each subject was measured three times with a one-minute gap between measurements and their average was calculated.

Venous blood samples were taken from the antecubital vein of each subject in a sitting position, in the morning after a 12 h overnight fast. Blood samples were collected in suitable vacutainers without anticoagulant to obtain serum. Serum concentrations of glucose, TG and cholesterol were measured by standard procedures using a Beckman Coulter SYNCHRON CX® 9 PRO clinical system (La Brea, CA, USA).

Incident T2D was defined as FPG ≥126 mg/dl, or the initiation of anti-hyperglycemic medications for diabetes control during the follow-up period.

FLI as a surrogate measure of fatty liver

The FLI was calculated based on measurements of TG, GGT, BMI and WC, using the formula[14]:

$$Fatty\ Liver\ Index\ (FLI) = e^y / (1 + e^y) \times 100$$

$$Where\ y = 0.953 \times ln(TG) + 0.139 \times BMI + 0.718 \times ln(GGT) + 0.053 \times WC - 15.745$$

Here, TG indicates triglyceride concentration, measured as mg/dl; BMI indicates body mass index, measured as kg/m^2; GGT indicates γ-glutamyl transpeptidase, measured as U/l; and WC indicates waist circumference, measured as cm.

FLI, which ranges from 0 to 100, has shown good diagnostic accuracy in detecting fatty liver, with an Area Under the Curve (AUC) of 0.85 and a 95% confidence interval (CI) of 0.81–0.88.[10,14] FLI <30 was found to rule out steatosis with a sensitivity of 87% and a specificity of 64%, whereas FLI >60 was indicative of the presence of steatosis with a sensitivity of 61% and specificity of 86%.[14] FLI scores have been validated by comparison with the results of liver ultrasound and nuclear magnetic resonance spectroscopy. An FLI of 30–60 indicated indeterminate risk, in which fatty liver could not be ruled in or out.

Statistical analyses

Continuous variables were expressed in means (±SDs) and compared by Student's t-test, whereas categorical variables were expressed as n (%) and compared by chi-square (χ^2) tests. Multivariate logistic regression analyses were performed to calculate odds ratios (ORs) for the development of diabetes, adjusting for potential confounders that showed significant association in univariate analysis. For this analysis participants were classified into two categories: those with FLI ≥60 and FLI <60. The statistical method of receiver operating characteristic (ROC) curves was used to determine the FLI breakpoint. The optimal cut-off scores and the values of sensitivity and specificity for maximum accuracy were calculated according to Youden index.[27]

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Company, New York, NY, USA) for Windows. All statistical tests were two-sided, and p values <0.05 were considered statistically significant.

RESULTS

Baseline demographic and anthropometric characteristics of the study subjects by NAFLD are shown in Table 1. The sample included 16,648 individuals with prediabetes, comprised of 12,080 (72.6%) men and 4,568 (27.4%) women, of mean age 44.81± 9.91 years. The prevalence of obesity in the entire sample was 26.9%. The percentage of men was significantly higher among subjects with than without NAFLD. There were also significant differences in all anthropometrical and biochemical parameters analyzed, with BMI, WC, TG, fasting plasma glucose (FPG), cholesterol, GGT and SBP and DBP being significantly higher in subjects with than without NAFLD. The percentages of subjects who performed at least 150 min per week of PA (4.3% vs. 61.8%; p<0.001) and who did not consume fruits and vegetables every day (12.0% vs. 56.4%; p<0.001) were significantly lower in subjects with than without NAFLD.

Table 1 Anthropometric characteristics and biochemical parameters of subjects with and without NAFLD at baseline

Characteristics	Total (n = 16,648)	FLI ≥60 (n = 5,909)	FLI <60 (n = 10,739)	*P* value
Age (years)	44.8 ± 9.91	43.5 ± 10.3	46.3 ± 8.9	< 0.001
Sex (male)	12,080 (72.6%)	4,917 (83.2%)	713 (66.7%)	< 0.001
Social class				0.074
I	741 (4.5%)	239 (4.0%)	502 (4.7%)	
II	2,779 (16.7%)	961 (16.3%)	1,818 (16.9%)	
III	13,128 (78.9%)	4,709 (79.7%)	8,419 (78.4%)	
BMI (kg/m^2)	27.9 ± 4.88	32.0 ± 4.4	25.3 ± 3.0	< 0.001
BMI categories				< 0.001
Normal weight	5,049 (30.3%)	83 (1.4%)	4,966 (46.2%)	
Overweight	7,120 (42.8%)	1,888 (32.0%)	5,232 (48.7%)	
Obese	4,479 (26.9%)	3,938 (66.6%)	541 (5.0%)	
WC (cm)	87.3 ± 10.58	95.1 ± 7.3	82.5 ± 8.2	< 0.001
Triglycerides (mg/dL)	139.8 ± 110.67	197.8 ± 146.2	104.6 ± 52.0	< 0.001
Glucose (mg/dL)	108.4 ± 8.51	112.9 ±12.4	100.8 ± 7.6	< 0.001
Cholesterol (mg/dL)	203.9 ± 38.61	212.0 ± 38.7	197.1 ± 36.7	< 0.001
GGT (UI/l)	45.6 ± 54.5	72.7 ± 76.3	28.5 ± 24.0	< 0.001
SBP (mmHg)	128.1 ± 17.3	134.2 ± 16.9	124.4 ± 15.6	< 0.001
DBP (mmHg)	78.4 ± 11.2	82.5 ± 10.9	76.0 ± 10.3	< 0.001
PA (≥150 min/week)	6,892 (41.4%)	256 (4.3%)	6,636 (61.8%)	< 0.001
Diet (daily fruits and vegetables)	6,771 (40.7%)	709 (12.0%)	6,060 (56.4%)	< 0.001
Smoking habit		1,791 (30.3%)	3,663 (34.1%)	< 0.001
Never	7,645 (45.9%)	2,599 (44.0%)	5,046 (47.0%)	
Former	3,549 (21.3%)	1,519 (25.2%)	2,030 (18.9%)	
Current	5,454 (32.8%)	1,791 (30.3%)	3,663 (34.1%)	

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity.

Baseline FLI showed a significant correlation with FPG concentration at 5 years' follow-up with a Pearson's correlation coefficient of 0.528 ($p < 0.0001$) (Figure 1).

Figure 1 Correlation of Baseline FLI and FPG after 5 years of follow-up.

Of the 16,648 subjects with prediabetes, 3,706 (22.2%) progressed to T2D at 5 years, corresponding to an annual rate of 4.5%. Rates of T2D development according to the three baseline FLI categories are shown in Figure 2. The incidence of T2D after 5 years was 19/6,421 (0.30%) in the low risk group (FLI <30), corresponding to an annual rate of 0.05%. In the intermediate risk group (FLI 30–59), the incidence of T2D after 5 years was 338/4,318 (7.83%), corresponding to an annual rate of 1.57%. The incidence of T2D in the high-risk group (FLI >60), was 3,349/5,909 (56.7%), corresponding to an annual rate of 11.3% (Figure 2).

Figure 2 Incidence of T2D after 5 years of follow-up based on baseline FLI classification.

In bivariate analysis (Table 2), high FLI (>60) was strongly associated with progression to T2D (OR = 38.04; 95% CI 33.83 to 42.78), as were age, BMI, smoking habits and SBP. An adjusted binomial logistic regression model showed that high FLI (>60) remained independently associated with conversion to T2D (adjusted OR = 6.05; 95% CI 5.12 to 7.15). Most of the evaluated factors also remained significant after adjustment. Performing at least 150 min/week of physical activity (aOR = 0.17; 95% CI 0.12 to 0.23) and daily consumption of fruits and vegetables (aOR = 0.73; 95% CI 0.61 to 0.86) were significantly protective against conversion to T2D. Current smokers were also less likely to convert to T2D (aOR = 0.85; 95% CI 0.74 to 0.97).

Table 2 Odds ratio for conversion from prediabetes to T2D

Variables	OR $_{crude}$ (95% CI)	OR $_{adjusted}$ (95% CI)
Age	1.06 (1.05 - 1.06)	1.10 (1.09 - 1.11)
Men (Ref: women)	1.03 (0.95 -1.11)	1.75 (1.49 – 2,06)
Social class (Ref: I)		
II	0.86 (0.75 - 1.03)	0.77 (0.56 - 1.05)
III	0.96 (0.87 - 1.06)	0.79 (0.59 - 1.05)
PA (≥150 min/week)	0.01 (0.01 - 0.02)	0.17 (0.12 - 0.23)
Diet (daily fruits and vegetables)	0.13 (0.11 - 0.14)	0.73 (0.61 - 0.86)
Smoking habits (Ref: never smoker)		
Former	1.42 (1.30 - 1.55)	0.99 (0.85 - 1.15)
Current	0.72 (0.66 - 0.79)	0.85 (0.74 - 0.97)
BMI	1.72 (1.69 - 1.75)	1.55 (1.51 - 1.59)
SBP	1.03 (1.02 - 1.04)	1.00 (0.99 - 1.00)
FPG	1.08 (1.07 – 1.09)	1.09 (1.08 – 1.10)
FLI>60	38.04 (33.83 - 42.78)	6.05 (5.12 - 7.15)

PA, physical activity; BMI, body mass index; SBP, systolic blood pressure; FLI, fatty liver index; FPG, fasting plasma glucose.

Our results indicated the FLI was the strongest predictor of progression to T2D. In particular, participants who progressed to T2D had significantly higher scores of FLI than those who did not (Figure 3). ROC curves showed that FLI level had better predictive performance (AUC = 0.922 [95% CI 0.918 to 0.926]) for T2D progression than FPG (AUC = 0.629 [95% CI 0.618 to 0.639]). The optimal cut-off score for maximum accuracy for FLI was 59.5 and provides a sensitivity of 90.4% (95% CI 89.4 to 91.3) and a specificity of 80.2% (95% CI 79.5 to 80.9).

Figure 3 ROC curves for the prognostic value of FLI and FPG in predicting progression from prediabetes to diabetes after 5 years.

DISCUSSION

The present study evaluated the association of hepatic steatosis with progression to T2D in a large and representative sample of South-European Mediterranean workers with prediabetes. The study found that FLI-diagnosed NAFLD was strongly associated with conversion to T2D and that FLI, as a simple surrogate indicator of hepatic steatosis, could identify subjects at high risk for T2D conversion. The risk factors for progression to T2D in subjects with prediabetes include hepatic steatosis and less than 150 min/week of physical activity. Identification of subjects who could benefit from preventive strategies represents an opportunity to assist vulnerable individuals to understand their risk to progression to T2D and encourage them to take steps to reduce this risk. This prospective study of workers with prediabetes showed that FLI-diagnosed hepatic steatosis increased the risk of developing T2D after 5 years of follow-up. Certainly, in terms of FLI values, we found a cut-off for T2D conversion of 59.5, which is very similar to the cut-off of 60 for predicting NAFLD. Furthermore, the sensibility and specificity for progression to T2D with both cutoffs are exactly the same (data not shown), and showed a high accuracy (sensitivity 90.4% and specificity 80.2%). Furthermore, this study found that older age, male sex, higher BMI, physical inactivity and low-quality diet were independent risk factors for progression to diabetes.

FLI may be an appropriate indicator of NAFLD in clinical practice, as it includes simple anthropometric (BMI and WC) and biochemical (TG and GGT) measurements. The results of the present study are in accordance with previous studies that assessed the incidence of T2D in smaller populations of individuals from Japan[28] and Spain[5] with prediabetes and FLI-diagnosed NAFLD. Those studies reported that NAFLD is a strong predictor of T2D in subjects with prediabetes. Similarly, the present study found that FLI was the strongest predictor of T2D progression among people with prediabetes.

The baseline prevalence of hepatic steatosis (i.e. FLI>60) in our study population was 35.4%, higher than the 19.3% reported in a Japanese study,[28] but closer to the approximately 22–40% reported by studies using ultrasonography-diagnosed NAFLD.[29,30] However, the prevalence that we observed was lower than the 55.7% observed in the PREDAPS study.[5] Notably, 66.6% of people with NAFLD in our population were obese, whereas 32.0% were overweight. It has been previously described

a comparison of NAFLD patients with and without diabetes found that the components of MetS, including central obesity, high TG levels and hypertension, were more frequent in subjects with diabetes.[31]

A study performed in France reported that FLI >70 was associated with the development of T2D.[32] Similarly, we found that FLI >60 could predict the development of T2D in subjects with impaired glucose metabolism. Metabolic profiles also differed in subjects with FLI>60 and FLI<30, with components of the MetS and other metabolic parameters, such as BMI, WC, TG, glucose, cholesterol, GGT and SBP, being higher in subjects with FLI>60. These results are also in good agreement with those of studies in patients with prediabetes[28] and previously established T2D.[33] The degree of liver fat content was found to correlate with all components of the MetS.[34] This correlation may be due to NAFLD and T2D sharing a series of common physiopathological functions. Although the mechanisms leading to NAFLD and its progression to NASH and liver injury remain incompletely understood, they include alterations in glucose and lipid metabolism, insulin resistance, insulin secretion and a genetic predisposition. Environmental factors are also involved, including exposure to endocrine disruptors, epigenetic factors, and lifestyle alterations. The same mechanisms are involved in the development of T2D.[33,35]

Nearly one in four individuals (22.2%) with prediabetes in the present study progressed to T2D after 5 years of follow-up, resulting in an annual rate of 4.5%. In comparison, the French IT-DIAB study,[36] a 5-year, prospective, observational study in subjects with impaired FPG, defined by a higher cut off point (>110 and <126 mg/dL), reported an annual conversion rate to diabetes of 7.1%. The IT-DIAB study also reported that FLI could stratify the risk of conversion to T2D or the possibility of prediabetes reversion in clinical practice, independent of classical glucose parameters. That study reported that the probability of prediabetes reversion was higher in subjects with FLI <30 than in those with FLI 30-59 and FLI>60. We found that the incidence of T2D was higher in our study than in previous studies.[37–39] These differences could have resulted from differences in sociodemographic characteristics in study populations. The ARIC study,[37] which reported an annual conversion rate to T2D of 2.3%, included a higher percentage of women than in our cohort, whereas the ELSA-Brasil study,[39] which found that the annual conversion rate to T2D was 3.5%, included a higher percentage of subjects with high educational level. The PREDAPS study[40] showed a similar annual

conversion rate (4.2%). The incidence of T2D incidence in our sample was lower than that (5.8%) in a Korean population[41] of 7,680 subjects who had undergone general routine health evaluations. Similar to our study, 65.5% of the Korean subjects were men, with male sex being a risk factor for development of T2D in patients with prediabetes.

The FLI should be utilized as a practical tool in primary care for the early detection of NAFLD in subjects with prediabetes and to analyze the risk of developing T2D.[42] This would benefit patients at greater risk for T2D, allowing more careful monitoring and providing an opportunity for early interventions to prevent and reduce both the progression of hepatic disease. The present study also highlighted the importance of controlling BMI and promote PA and consumption of fruits and vegetables in preventing progression to T2D. Determining lifestyle-related factors, particularly PA, together with repeated anthropometrical measurements in subjects with prediabetes may be crucial in properly assessing the risks of progression to T2D and of cardiovascular events.[43]

Strengths and limitations

This study had some limitations. First, it incorporated data from periodic health assessments performed in the workplace. None of these subjects underwent oral glucose tolerance tests (OGTT), which is considered more sensitive but less specific than FPG for identifying people at risk of developing T2D. Secondly, lifestyle modifications (e.g. diet, PA) of study participants were not evaluated throughout the 5-year follow-up, which may have resulted in misclassification bias. The main strengths of this study were the large sample size (16,648 subjects) and the relatively long follow-up period. Study participants had multiple occupations and were from several geographical locations, suggesting that the study population was representative of Spanish workforce. Finally, the different statistical analyses performed point out analogous results evidencing the ability of FLI values to predict conversion to T2D in 5 years.

Clinical implications

This study highlights the importance of FLI as an easily calculated and valuable early indicator for high risk of T2D in subjects with prediabetes. FLI-based screening could allow the adoption of effective measures to prevent and reduce the progression of NAFLD. The workplace could be a feasible setting for implementing diabetes prevention programs based on early detection and lifestyle changes.

CONCLUSION

Because of the progressive nature of NAFLD and the risk of serious consequences, health care providers should be strongly advised to screen routinely for NAFLD in all subjects with prediabetes or at risk of T2D. Fatty liver indices are simple clinical tools for evaluating the extent of liver fat and are predictive of incident diabetes. Concretely, the FLI is a simple, effective and practical method of stratifying the risk of conversion to T2D based on the degree of hepatic steatosis. FLI may be useful in routine clinical practice as an additional screening tool to identify subjects with prediabetes who are at high risk of progression and could benefit from early interventions. The workplace may be a feasible setting for the assessment of risk factors, allowing early detection of NAFLD in younger subjects with prediabetes who are likely to progress to T2D and the implementation of T2D prevention programs.

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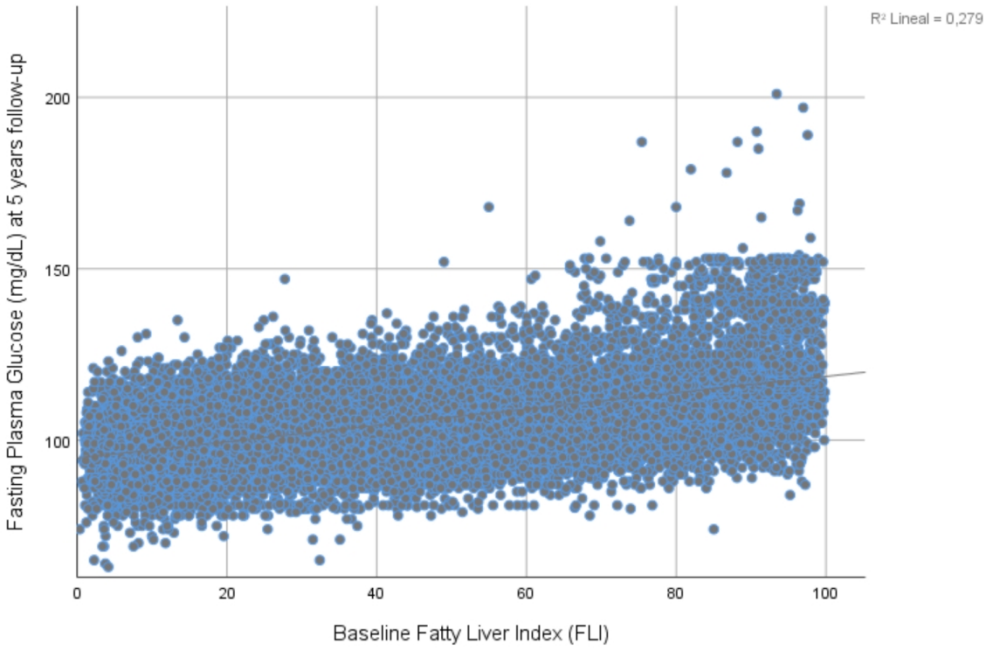

Figure 1

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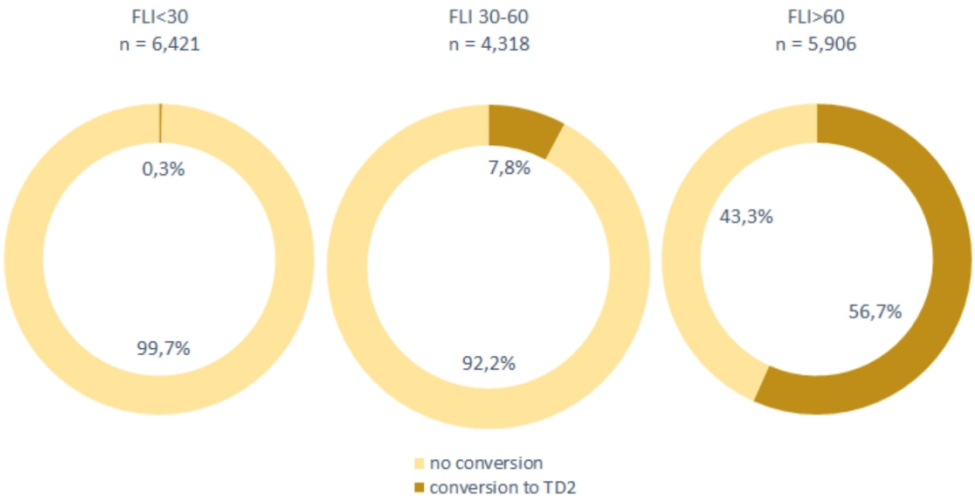

Figure 2

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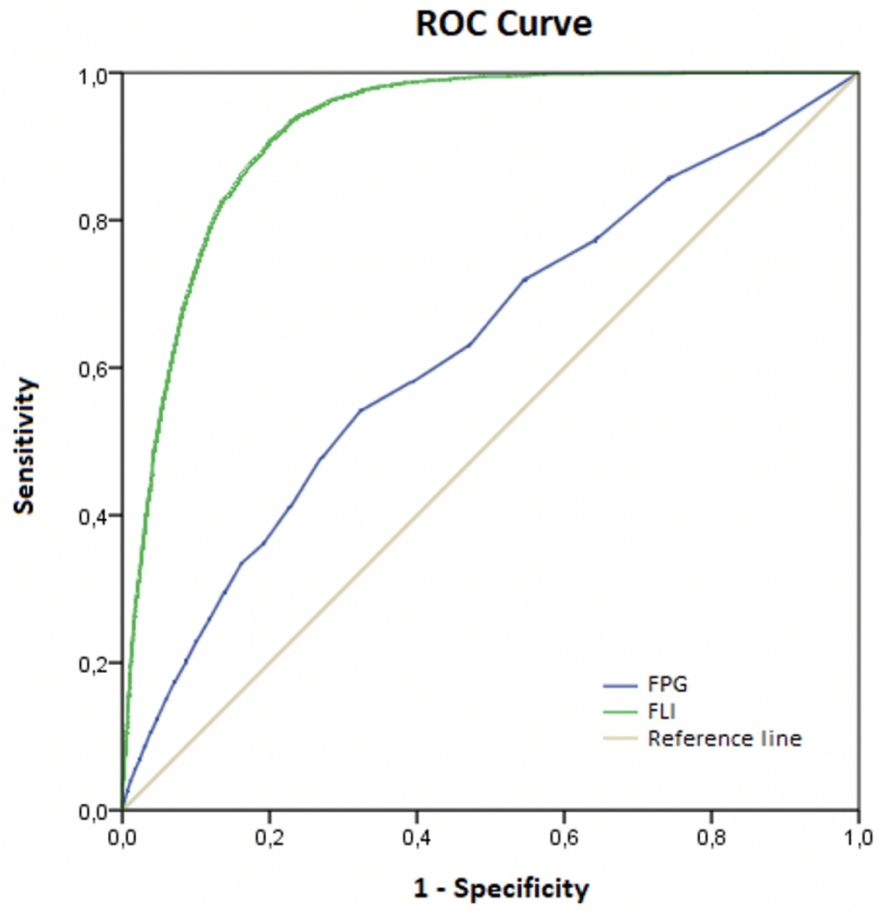

FPG: Area under the curve (AUC) = 0.629 (95% CI 0.618 to 0.639);

FLI: AUC = 0.922 (95% CI 0.918 to 0.926)

Figure 3

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	14
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	-

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Fatty liver index and progression to type 2 diabetes: a five-year longitudinal study in Spanish workers with prediabetes

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ABSTRACT

Objective: The main aim of the study was to evaluate the association between non-alcoholic fatty liver disease (NAFLD), estimated by fatty liver index (FLI), and the development of type 2 diabetes (T2D) in a large cohort of adult workers with prediabetes.

Design: Prospective cohort study.

Setting: Occupational health services from Spain.

Participants: 16,648 adult workers (aged 20 to 65 years) with prediabetes (fasting plasma glucose (FPG) of 100-125 mg/dl).

Outcome and measures: FLI was calculated based on measurements of triglycerides, body mass index, waist circumference and γ-glutamyltransferase. The population was classified into three categories: FLI <30 (no hepatic steatosis), FLI 30–60 (intermediate status), and FLI >60 (hepatic steatosis). Sociodemographic, anthropometric, dietary habits, physical activity and clinical data were collected from all subjects. The incidence rate of T2D was determined after 5 years of follow-up.

Results: After 5 years of follow-up, 3,706 of the 16,648 participants (22.2%) were diagnosed with T2D, corresponding to an annual rate of progression of 4.5%. FLI was strongly associated with T2D conversion. The incidence rates of T2D in the FLI <30, FLI 30-60 and FLI >60 groups were significantly different after 5 years of follow-up were 19/6,421 (0.3%), 338/4,318 (7.8%) and 3,349/5,909 (56.7%), respectively. This association remained significant for FLI >60 after adjustment for, age, diet, physical activity, FPG blood pressure, social class and smoking habits (adjusted HR=6.879; 95% CI 5.873 to 8.057 for men, and HR=5.806; 95% CI 4.863 to 6.932 for women).

Conclusion: NAFLD assessed by FLI independently predicted the risk of conversion to T2D among people with prediabetes. FLI may be an easily determined and valuable early predictor for T2D in people with prediabetes. FLI-based assessment of NAFLD in subjects with prediabetes in routine clinical practice could allow the adoption of effective measures to prevent and reduce their progression to T2D.

Strengths and limitations of this study

- This is a prospective study, with large sample size and 5-years follow-up.
- Study participants had multiple occupations and were from several geographical locations.
- The study sample included only adult workers therefore the results cannot be generalized to the general population.

INTRODUCTION

Type 2 Diabetes (T2D) is closely associated with a constellation of metabolic comorbidities, including obesity, hypertension, hypercholesterolemia, dyslipidemia and non-alcoholic fatty liver disease (NAFLD).[1] The main characteristic of NAFLD is the infiltration of hepatocytes by free fatty acids and triglycerides not related to significant alcohol intake. NAFLD is an entity that encompasses a wide spectrum of lesions ranging from indolent liver fat storage followed by lipotoxicity,[2] to hepatic inflammation, also known as non-alcoholic steatohepatitis (NASH). NAFLD is the most common chronic liver disease worldwide that is associated with excess health-related expenditures, making it a community health problem.[3]

Mounting evidence indicates a close association between the pathogenesis of T2D and NAFLD;[4–8] evidence suggests a complex bidirectional relationship, whereby presence of one leads to the progression of the other.[9] The presence of NAFLD increases the incidence of T2D, while diabetes might contribute to the worsening of NAFLD to more advanced stages such as steatohepatitis and even hepatocellular carcinoma.[10]

NAFLD is strongly associated with insulin resistance such that prevalence of NAFLD is 5-fold higher in patients with T2D compared to those without.[8] Recent data showed that there is a solid genetic basis that support their association, since gene variants in numerous proteins related to lipid and glucose metabolism, appear to significantly raise the risk of NAFLD and T2D.[10,11] These genetic abnormalities are directly linked to hepatic and peripheral insulin resistance, resulting in a deficient inhibition of hepatic gluconeogenesis, diminished glycogen synthesis and increased extrahepatic lipid accumulation. Other mechanisms underlying these NAFLD-T2D pathogenic duo involve excessive hepatic fat accumulation, diverse alterations in energy metabolism, altered microbiome, comorbidities, increased reactive oxygen species production and inflammatory signals derived from different cell types including immune cells, such as proinflammatory cytokines.[12]

The estimated overall worldwide prevalence of NAFLD in the general adult population is about 25–30%,[3,13] but ranges from 40%–70% in subjects with established T2D.[14,15] In fact, NAFLD and T2D are conditions that frequently coexist

and can act synergistically to drive adverse outcomes.[16] NAFLD is considered the hepatic manifestation of metabolic syndrome (MetS)[17] because epidemiological studies have consistently shown that NAFLD is strongly linked to obesity, dyslipidemia, and insulin resistance.[18,19] Therefore, NAFLD is thought to be an independent risk factor for incident T2D[16] and cardiovascular disease.[20]

Liver biopsy is currently the gold standard for diagnosing progressive NAFLD.[21] Biopsies are invasive procedures with several drawbacks, including sampling error, interobserver variability, high cost, patient discomfort and risk of complications.[14] Moreover, obtaining liver biopsies from all patients with NAFLD is unrealistic. Abdominal ultrasonography is a simple, inexpensive, widely available and minimally invasive technique that is used to diagnose fatty liver in most subjects. However, its sensitivity is low in subjects with fatty retention less than 20%–30% and it does not provide information on the degree of fibrosis.[22] Consequently, attempts have been made to diagnose NAFLD/NASH using clinical and laboratory-based biomarkers and scoring systems that can predict fatty changes in the liver. These indices for the diagnosis of NAFLD/NASH include the fatty liver index (FLI),[23] NAFLD liver fat score,[24] the hepatic steatosis index (HSI),[25] the ALD/NAFLD index (ANI),[26] the lipid accumulation product (LAP)[27] and the SteatoTest (ST).[28] These indices require the measurement of patient characteristics, including concentrations of triglycerides (TG), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine transaminase (ALT), insulin, body mass index (BMI), waist circumference (WC), gender, mean corpuscular value and presence or absence of T2D or metabolic syndrome.[29] The FLI is a simple and accurate algorithm that combines routine measurements of TG and GGT concentrations, WC and BMI, showing an excellent discriminative ability to predict ultrasonographic NAFLD and hepatic steatosis in the general population.[23,30]

The FLI has been reported to correlate with: 1) insulin resistance; 2) risk of coronary heart disease; 3) MetS; 4) early atherosclerosis; and 5) rates of non-hepatic-related morbidity and mortality in nondiabetic subjects.[31] Thus, FLI-diagnosed NAFLD may be an indicator of incident T2D.[19] Nonetheless, the risk of progression to T2D determined by FLI in patients with prediabetes remains poorly understood.

Few studies have evaluated the influence of NAFLD as a risk factor for T2D development in a cohort of workers with prediabetes. Determining FLI in subjects with

prediabetes may be highly relevant, as both epidemiological and clinical evidence have shown that primary health care prevention programs should target people at greater risk of developing T2D. The present study was therefore designed to evaluate the association between NAFLD, as estimated by FLI, and the development of T2D in a large cohort of South-European Mediterranean workers with prediabetes.

Methods

Study population and design

This cohort study included 16,648 Spanish working adults with prediabetes who worked in public administration, construction, health departments or post offices. The study methods have been described in detail previously.[32] Briefly, participants were carefully chosen from 234,995 potentially eligible individuals who underwent periodic occupational health assessments between 2012 and 2013. Participants were included if they were aged 20–65 years and had an FPG of 100–125 mg/dL.[33] Subjects were excluded if they had a history of physician-diagnosed diabetes, had been treated with an oral antidiabetic agent or a systemic glucocorticoid, had an FPG ≥126 mg/dL or an HbA1c ≥6.5% at baseline, had received cancer treatment during the preceding 5 years, had anemia (hematocrit <36% in men and <33% in women) or were pregnant. All subjects underwent standard health examinations, anthropometric measurements, and metabolic tests at baseline and were followed-up 5 years later, in 2017 and 2018.

All the procedures in the study protocol were in accordance with the Declaration of Helsinki for research on human participants and were approved by the Balearic Islands Ethical Committee of Clinical Research (Ref. No: CEI-IB-1887). All participants were carefully informed of the purpose and demands of the study. Informed consent was obtained from all participants included in the study.

Patient and public involvement

People were not involved in setting the research question nor in the study design. Participants were interviewed face to face by trained researchers for a detailed explanation of the purpose of this research and informed consent at the beginning. Results of the research will be disseminated to the participants.

Data collection

At baseline, anthropometric measurements and fasting blood sample were taken from all subjects during occupational health examinations. A questionnaire was administered to collect data on sociodemographic characteristics, dietary habits, physical activity (PA) and clinical data. Participants were asked to report if they performed moderate and/or vigorous exercise (at least 150 min/week, according to World Health Organization [WHO] recommendations) and if they consumed fruits and vegetables daily. Each individual was also categorized as a current smoker (habitual or casual), former smoker, or never smoker, according to WHO criteria. Social class was defined using the Spanish Epidemiology Society classification, which is based on occupation and it has shown high correlation with level of education.[34] Class I (upper class) includes executives, managers, and university professionals; Class II (middle class) includes intermediate occupations and employees; and Class III (lower class) includes manual workers.

All anthropometric measurements were made in the morning, after an overnight fast, at the same time and according to the guidelines and recommendations in the International Standards for Anthropometric Assessment (ISAK) manual.[35] All measurements were performed by well trained technicians or researchers to minimize coefficients of variation. Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca 700 scale, Hamburg); height was measured to the nearest 0.5 cm using a stadiometer (Seca 220) Telescopic Height Rod for Column Scales, Hamburg); and BMI was calculated as weight (kg) divided by height (m) squared (kg/m^2). Obesity was defined as BMI $\geq$30.0 kg/m^2, in agreement with WHO guidelines. Blood pressure was measured after a resting period of 10 minutes, with the subject in the supine position, using an electric and calibrated sphygmomanometer (OMRON M3, Healthcare Europe, Spain). Blood pressure in each subject was measured three times with a one-minute gap between measurements and their average was calculated.

Venous blood samples were taken from the antecubital vein of each subject in a sitting position, in the morning after a 12 h overnight fast. Blood samples were collected in suitable vacutainers without anticoagulant to obtain serum. Serum concentrations of glucose, TG and cholesterol were measured by standard procedures using a Beckman Coulter SYNCHRON CX® 9 PRO clinical system (La Brea, CA, USA).

The main outcome variable of the study was the time elapsed until T2D onset, defined as FPG ≥126 mg/dl,[36] or the time until initiation of anti-hyperglycemic medications for diabetes control in people with prediabetes during the follow-up period.

FLI as a surrogate measure of fatty liver

The FLI was calculated based on measurements of TG, GGT, BMI and WC, using the formula[23]:

$$Fatty\ Liver\ Index\ (FLI) = e^{y} / (1 + e^{y}) \times 100$$

$$Where\ y = 0.953 \times ln(TG) + 0.139 \times BMI + 0.718 \times ln(GGT) + 0.053 \times WC - 15.745$$

Here, TG indicates triglyceride concentration, measured as mg/dl; BMI indicates body mass index, measured as kg/m^2; GGT indicates γ-glutamyl transpeptidase, measured as U/l; and WC indicates waist circumference, measured as cm.

FLI, which ranges from 0 to 100, has shown good diagnostic accuracy in detecting fatty liver, with an Area Under the Curve (AUC) of 0.85 and a 95% confidence interval (CI) of 0.81–0.88.[19,23] FLI <30 was found to rule out steatosis with a sensitivity of 87% and a specificity of 64%, whereas FLI >60 was indicative of the presence of steatosis with a sensitivity of 61% and specificity of 86%.[23] FLI scores have been validated by comparison with the results of liver ultrasound and nuclear magnetic resonance spectroscopy. An FLI of 30–60 indicated indeterminate risk, in which fatty liver could not be ruled in or out.

Statistical analyses

Continuous variables were expressed in means (±SDs) and compared by Student's t-test and one-way analysis of variance (ANOVA), with post-hoc Bonferroni contrast method. Categorical variables were expressed as n (%) and compared by chi-square (χ^2) tests with Bonferroni post-hoc method. Crude and multivariable Cox regression analyses were performed to calculate FLI, diet and PA hazard ratios (HR) for the development of diabetes, adjusting for potential confounders (age, social class, BMI, smoking, SBP, FPG) that showed significant association in univariate analysis. Schoenfeld residuals were used to check the proportional hazard assumption. For this analysis participants were classified into two categories: those with FLI >60 and FLI <60

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Company, New York, NY, USA) for Windows. All statistical tests were two-sided, and p values <0.05 were considered statistically significant.

RESULTS

Baseline demographic and anthropometric characteristics of the study subjects by sex are shown in Table 1. The sample included 16,648 individuals with prediabetes, comprised of 12,080 (72.6%) men and 4,568 (27.4%) women, of mean age 44.81± 9.91 years. The prevalence of obesity in the entire sample was 26.9%. The percentage of men was significantly higher among subjects with than without NAFLD. There were also significant differences in all anthropometrical and biochemical parameters analyzed, with BMI, WC, TG, fasting plasma glucose (FPG), cholesterol, GGT and SBP and DBP being significantly higher in subjects with than without NAFLD. The percentages of subjects who performed at least 150 min per week of PA (4.3% vs. 61.8%; p<0.001) and who did not consume fruits and vegetables every day (12.0% vs. 56.4%; p<0.001) were significantly lower in subjects with than without NAFLD.

Table 1 Basal anthropometric characteristics and biochemical parameters of subjects by sex

Characteristics	All (n = 16,648)	Men (n = 12,080)	Women (n= 4,568)	P value
Age (years)	44.51 ± 9.89	44.38 ± 9.87	44.84 ± 9.94	< 0.01
Social class				<0.001
I	741 (4.5%)	558 (4.6%)	183 (4.0%)	
II	2,779 (16.7%)	1,902 (15.7%)	877 (19.2%)	
III	13,128 (78.9%)	9,620 (79.6%)	3,508 (76.8%)	
BMI (kg/m2)	27.66 ± 4.81	27.76 ± 4.47	27.42 ± 5.61	< 0.001
BMI categories				< 0.001
Normal weight	5,049 (30.3%)	3,300 (27.3%)	1,749 (38.3%)	
Overweight	7,120 (42.8%)	5,596 (46.3%)	1,524 (33.4%)	
Obese	4,479 (26.9%)	3,184 (26.4%)	1,295 (28.3%)	
WC (cm)	87.00 ± 9.95	90.28 ± 8.62	78.32 ± 7.78	< 0.001
Triglycerides (mg/dL)	137.66 ± 106.39	150.08 ± 117.11	104.81 ± 59.14	< 0.001
Glucose (mg/dL)	106.22 ± 5.82	106.43 ± 5.90	105.68 ± 5.56	< 0.001
Cholesterol (mg/dL)	202.40 ± 38.09	202.49 ± 38.59	202.18 ± 36.74	0.642
GGT (UI/l)	44.20 ± 55.68	48.03 ± 59.07	34.08 ± 33.69	< 0.001
SBP (mmHg)	127.86 ± 16.74	130.16 ± 16.10	121.79 ± 16.88	< 0.001
DBP (mmHg)	78.32 ± 11.01	79.51 ± 10.94	75.18 ± 10.58	< 0.001
PA (≥150 min/week)	6,892 (41.4%)	4,787 (39.6%)	2,105 (46.1%)	< 0.001
Diet (daily fruits and vegetables)	6,771 (40.7%)	4,654 (38.5%)	2,117 (46.3%)	< 0.001
Smoking habit				< 0.001
Never	7,645 (45.9%)	5,124 (42.4%)	2,521 (55.2%)	
Former	3,549 (21.3%)	2,750 (22.8%)	799 (17.5%)	
Current	5,454 (32.8%)	4,206 (34.8%)	1,248 (27.3%)	

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by Student's t-test, whereas categorical variables were compared by χ^2 tests.

General characteristic of the study population stratified by gender and FLI categories are shown in Table 2 for men and Table 3 for women. In both men and women,

those with FLI>60 presented a significantly worse anthropometric and biochemical profile, as compared with the other two groups.

Among men, 40.7% presented a FLI>60, 29.5% a FLI 30-60, and 29.8% a FLI <30. As compared to men in the other two categories, those with FLI>60 were older, more obese, and presented higher values of WC, triglycerides, glucose, cholesterol, GGT, SBP, and DBP (all p<0.001). Men with FLI<30 consumed more fruits and vegetables daily, and dedicated more time to PA, than men with FLI>60 (all p<0.001).

Table 2. Basal anthropometric characteristics and biochemical parameters of men according to FLI categories (n = 12,080).

Men characteristics	FLI <30 n = 3,605 (29.8%) (a)	FLI 30-60 n= 3,558 (29.5%) (b)	FLI >60 n = 4,917 (40.7%) (c)	P value	Post-hoc
Age (years)	41.02 ± 10.66	45.08 ± 9.55	46.34 ± 8.81	< 0.001	
Social class				0.137	NS
I	153 (4.2%)	191 (5.4%)	214 (4.4%)		
II	559 (15.5%)	554 (15.6%)	789 (16.0%)		
III	2,893 (80.2%)	2,813 (79.1%)	3,914 (79.6%)		
BMI (kg/m2)	23.74 ± 2.24	26.78 ± 2.02	31.41 ± 4.09	< 0.001	a>b>c
BMI categories				< 0.001	
Normal weight	2,616 (72.6%)	603 (16.9%)	81 (1.6%)		a>b,c; b>c
Overweight	980 (27.2%)	2,787 (78.3%)	1,829 (37.2%)		b>a,c; c>a
Obese	9 (0.2%)	168 (4.7%)	3,007 (61.2%)		b>a; c>a,b
WC (cm)	82.67 ± 5.85	89.13 ± 6.06	96.68 ± 6.83	< 0.001	c>b>a
Triglycerides (mg/dL)	88.46 ± 37.22	130.95 ± 60.70	209.1-0 ± 153.25	< 0.001	c>b>a
Glucose (mg/dL)	105.56 ± 5.36	106.05 ± 5.63	107.34 ± 6.34	< 0.001	c>b>a
Cholesterol (mg/dL)	187.32 ± 34.39	203.72 ± 37.20	212.71 ± 38.95	< 0.001	c>b>a
GGT (UI/l)	23.02 ± 13.22	38.89 ± 31.70	72.98 ± 81.09	< 0.001	c>b>a
SBP (mmHg)	124.08 ± 14.42	129.23 ± 14.63	135.30 ± 16.60	< 0.001	c>b>a
DBP (mmHg)	74.98 ± 10.08	79.03 ± 10.02	83.18 ± 10.86	< 0.001	c>b>a
PA (≥150 min/week)	3,108 (86.2%)	1,425 (40.1%)	254 (5.2%)	< 0.001	a>b,c; b>c
Diet (daily fruits and vegetables)	2,693 (74.7%)	1,372 (38.6%)	589 (12.0%)	< 0.001	a>b,c; b>c
Smoking habit				< 0.001	
Never	1,539 (42.7%)	1,571 (44.2%)	2,014 (41.0%)		b>c
Former	620 (17.2%)	800 (22.5%)	1,330 (27.0%)		b>a; c>a,b

Current	1,446 (40.1%)	1,187 (33.4%)	1,573 (32.0%)	a>b,c

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by ANOVA, whereas categorical variables were compared by χ^2 tests.

Among women, 21.7% had a FLI>60, 16.6% a FLI 30-60, and 61.7% a FLI<30. As compared to women in the other two categories, those with FLI>60 were more obese, and had worse anthropometric and biochemical values (WC, triglycerides, glucose, cholesterol, GGT, SBP, and DBP) (all p<0.001). Women with FLI<30 also consumed more fruits and vegetables daily, and dedicated more time to PA, than women with FLI>60 (all p<0.001).

Table 3. Anthropometric characteristics and biochemical parameters of women according to FLI categories (n = 4,568).

Women characteristics	FLI <30 n = 2,816 (61.7%) (a)	FLI 30-60 n = 760 (16.6%) (b)	FLI >60 n = 992 (21.7%) (c)	P value	Post-hoc
Age (years)	43.91 ± 10.22	46.55 ± 9.31	46.20 ± 9.28	< 0.001	a<b,c
Social class				<0.01	
I	131 (4.7%)	27 (3.6%)	25 (2.5%)		a>c
II	570 (20.2%)	135 (17.8%)	172 (17.3%)		
III	2,115 (75.1%)	598 (78.7%)	795 (80.1%)		c>a
BMI (kg/m2)	24.13 ± 3.02	29.56 ± 2.62	35.12 ± 4.49	< 0.001	c>b>a
BMI categories				< 0.001	
Normal weight	1,724 (61.2%)	23 (3.0%)	2 (0.2%)		a>b,c; b>c
Overweight	1,034 (36.7%)	431 (56.7%)	59 (5.9%)		a>c; b>a,c
Obese	58 (2.1%)	306 (40.3%)	931 (93.9%)		b>a; c>a,b
WC (cm)	74.00 ± 5.35	82.22 ± 5.70	87.63 ± 4.60	< 0.001	c>b>a
Triglycerides (mg/dL)	86.33 ± 35.15	125.00 ± 60.71	141.83 ± 84.45	< 0.001	c>b>a
Glucose (mg/dL)	105.13 ± 5.17	105.84 ± 5.73	107.12 ± 6.18	< 0.001	c>b>a
Cholesterol (mg/dL)	198.09 ± 36.29	209.10 ± 35.61	208.52 ± 37.20	< 0.001	a<b,c
GGT (UI/l)	19.21 ± 12.83	40.74 ± 29.70	71.16 ± 45.26	< 0.001	c>b>a
SBP (mmHg)	118.28 ± 15.80	125.42 ± 16.34	128.98 ± 17.42	< 0.001	c>b>a
DBP (mmHg)	73.25 ± 10.02	77.25 ± 10.50	79.08 ± 10.82	< 0.001	c>b>a

PA (≥150 min/week)	2,048 (72.7%)	55 (7.2%)	2 (0.2%)	< 0.001	a>b,c; b>c
Diet (daily fruits and vegetables)	1,809 (64.2%)	188 (24.7%)	120 (12.1%)	< 0.001	a>b,c; b>c
Smoking habit				< 0.001	
Never	1,526 (54.2%)	410 (53.9%)	585 (59.0%)		c>a
Former	457 (16.2%)	153 (20.1%)	189 (19.1%)		b>a
Current	833 (29.6%)	197 (25.9%)	218 (22.0%)		a>c

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by ANOVA, whereas categorical variables were compared by χ^2 tests.

Baseline FLI showed a significant correlation with FPG concentration at 5-year follow-up with a Pearson's correlation coefficient of 0.528 ($p < 0.001$) (Figure 1).

Figure 1 Correlation of Baseline FLI and FPG after 5 years of follow-up.

Of the 16,648 subjects with prediabetes, 3,706 (22.2%) progressed to T2D at 5 years, corresponding to an annual rate of 4.5%. The incidence of T2D after 5 years was similar between men (22.1%) and women (22.6%). When specifically looking at FLI categories, 0.2% (6/3,605) of men and 0.5% (13/2,816) of women in the low-risk group (FLI<30), progressed to T2D, corresponding to an annual rate of 0.04% for men and 0.1% for women. In the intermediate risk group (FLI 30-60), progression to T2D occurred in 4.3% (152/3,558) of men and 24.5% (186/760) in women, corresponding to an annual rate of 0.86% and 4.9%, respectively. Finally, in the high-risk group (FLI>60), incidence of T2D was 51.2% (2,516/4,917) in men and 84.0% (833/992) in women, corresponding to an annual rate of 11.34% and 16.8% respectively. Rates of progression to T2D in men and women according baseline FLI categories are shown in Figure 2.

Figure 2 Incidence of T2D after 5-year follow-up according to baseline FLI classification.

In bivariate analysis (Table 4), high FLI (>60) was strongly associated with progression to T2D in both genders (HR=24.361; 95% CI 21.020 to 28.233 for men, and HR=17.816; 95% CI 15.400 to 20.611 for women), as were age, social class, BMI,

smoking habits, FPG and SBP. An adjusted cox regression model showed that high FLI scores (>60) remained independently associated with progression to T2D, (adjusted HR=6.879; 95% CI 5.873 to 8.057 for men, and HR=5.806; 95% CI 4.863 to 6.932 for women). BMI was also associated to progression to T2D in both genders after adjustment (HR=1.041; 95% CI 1.036 to 1.045 for men, and HR=1.104; 95% CI 1.036 to 1.045 for women). Some of the evaluated factors also remained significant after adjustment. Performing at least 150 min/week of physical activity (adjusted HR=0.215; 95% CI 0.173 to 0.268 for men, and HR=0.070; 95% CI 0.043 to 0.112 for women) was significantly protective against progression to T2D in both genders. Current male smokers were also less likely to progress to T2D (adjusted HR=0.909; 95% CI 0.834 to 0.991).

Table 4 Hazard Ratios for progression from prediabetes to T2D

Variables	HR_{crude} (95% CI)		$HR_{adjusted}$ (95% CI)	
	Men	Women	Men	Women
Age	**1.054 (1.051 – 1.058)**	**1.035 (1.030 – 1.041)**	**1.041 (1.036 - 1.045)**	**1.018 (1.011 – 1.026)**
Social class (Ref: I)				
II	**1.147 (0.984 – 1.338)**	**1.475 (1.102 – 1.974)**	1.009 (0.839 – 1.212)	1.216 (0.818 – 1.809)
III	1.087 (0.994 – 1.251)	**1.845 (1.402 – 2.428)**	1.005 (0.851 - 1.186)	1.170 (0.793 – 1.140)
PA (≥150 min/week)	**0.037 (0.031 – 0.046)**	**0.027 – (0.019 – 0.038)**	**0.215 (0.173 - 0.268)**	**0.070 (0.043 - 0.112)**
Diet (daily fruits and vegetables)	**0.126 (0.112 – 0.142)**	**0.141 (0.120 – 0.166)**	0.959 (0.843 – 1.091)	0.951 (0.793 – 1.140)
Smoking habits (Ref: never smoker)				
Former	**1.244 (1.153 – 1.343)**	**1.010 (0.875 – 1.165)**	0.985 (0.904 – 1.072)	1.017 (0.873 – 1.184)
Current	**0.770 (0.719 – 0.824)**	**0.714 (0.633 – 0.804)**	**0.909 (0.834 - 0.991)**	0.959 (0.830 – 1.107)
BMI	**1.174 (1.170 – 1.178)**	**1.161 (1.154 – 1.167)**	**1.041 (1.036 - 1.045)**	**1.104 (1.036 – 1.045)**
SBP	**1.023 (1.021 – 1.024)**	**1.023 (1.021 – 1.025)**	0.999 (0.996 - 1.001)	1.001 (0.997 – 1.004)
FPG	**1.037 (1.034 – 1.039)**	**1.027 (1.023 – 1.030)**	**1.021 (1.018 – 1.024)**	**1.018 (1.013 – 1.023)**
FLI (Ref: FLI < 60)				
FLI >60	**24.361 (21.020 – 28.233)**	**17.816 (15.400 – 20.611)**	**6.879 (5.873 – 8.057)**	**5.806 (4.863 – 6.932)**

PA, physical activity; BMI, body mass index; SBP, systolic blood pressure; FLI, fatty liver index; FPG, fasting plasma glucose.

DISCUSSION

The present study aimed to evaluate the possible association between hepatic steatosis, as estimated by FLI, and T2D progression in a large and representative sample of

Mediterranean workers with prediabetes. The main finding of the study was that FLI was a strong independent risk factor for the progression of T2D, in both men and women with baseline prediabetes, after a 5-year follow-up. Moreover, FLI could preventively identify subjects at high risk of progression to T2D. Other risk factor associated with progression T2D were older age, male sex, higher BMI, higher FPG, low consumption of fruits and vegetables, and performing less than 150 min/week of PA.

The results of the present study are in accordance with previous evidence reporting that NAFLD is a strong predictor of T2D in subjects with prediabetes [37,14]. The baseline prevalence of hepatic steatosis (i.e. FLI>60) in our study population was 35.4%, higher than the 19.3% reported in a Japanese study,[37] lower than the 55.7% observed in the PREDAPS study,[14] and closer to the 22–40% reported by studies using ultrasonography-diagnosed NAFLD.[38,39]

Patients with a higher FLI score, independently of gender, presented a higher BMI, a worse cardiometabolic profile and less healthy lifestyle habits. Previous studies [40] similarly observed that patients with FLI>60 were more metabolically impaired compared to patients with lower FLI, they also presented a higher risk for MetS, as well as worse lipid profile. [41] Accordingly, the degree of liver fat content correlates with MetS components,[42] and that this correlation may be due to NAFLD and T2D sharing a series of common physiopathological pathways.[43,44]

At five-year follow-up, nearly one in four individuals (22.2%) with prediabetes progressed to T2D resulting in an annual rate of progression of 4.5%. In comparison, the French IT-DIAB study,[45] a 5-year, prospective observational study reported an annual progression rate of 7.1%. The study also reported that FLI could predict the risk of progressing to T2D as well as the possibility of reverting to normoglycemia in clinical practice, independently of classical glucose parameters. Moreover, normalization of glycemia was higher in subjects with FLI <30 than in those with higher FLI scores. The incidence of T2D observed in our study was higher than in previous ones[46–48] probably due to differences in sociodemographic characteristics between study populations. The ARIC study,[46] which reported an annual progression rate to T2D of 2.3%, included a higher percentage of women than in our cohort, whereas the ELSA-Brasil study,[48] which found that the annual progression rate to T2D was 3.5%, included a higher percentage of subjects with high educational level. On the other hand, the PREDAPS study[49] showed a similar annual conversion rate (4.2%). The incidence rate of T2D in our sample was lower than that shown (5.8%) in a previous Korean study [50]

of 7,680 subjects who had undergone general routine health evaluations. Nevertheless, similar to what was observed in our study, 65.5% of the Korean subjects were men, and male sex was a risk factor for development of T2D in patients with prediabetes.

When stratifying for gender, the proportion of women in the FLI >60 category who progressed to T2D was significantly higher (80%) than the proportion of men in the same category (50%), at 5-year follow-up. Although women are generally less likely to suffer from hepatic steatosis,[51] once they do, they might present a higher risk of developing T2D than males [52]. Genetic predisposition and epigenetic mechanisms, nutritional components and lifestyle exert effects differently in both sexes. Furthermore, sexual hormones directly impact on energy metabolism, body composition, inflammatory cascades and vascular functioning. Particularly, low levels of 17β-estradiol are associated with increased risk of T2D, independently of established risk factors, including BMI and insulin resistance.[53] Thus, endocrine imbalances might relate to unfavorable cardiometabolic traits observable in female sex.[54]

Of note, results from our study show an apparently protective effect of smoking on progression to diabetes. However this could be due to the anorexigenic effect of tobacco, more than tobacco consumption itself. Smokers are generally leaner than average as nicotine may affect energy homeostasis and food consumption at brain level.[55] Accordingly, the proportion of smokers with a lower FLI was higher than that of smokers in the other two categories.

The FLI could be utilized in primary care as a practical tool for early detection of NAFLD in subjects with prediabetes, while predicting their risk of developing T2D.[56] This would benefit patients at greater risk, allowing more careful monitoring and providing an opportunity for early interventions to prevent and reduce both the progression of hepatic disease and T2D. The present study also highlights the importance of weight control, promotion of PA and of fruits and vegetables consumption in the prevention of T2D progression. Determining lifestyle-related factors, particularly PA, together with repeated anthropometrical measurements in subjects with prediabetes may be crucial in properly assessing the risks of progression to T2D and of cardiovascular events.[57]

Strengths and limitations

This study had some limitations. First, this work incorporated data from periodic health assessments performed in the workplace. None of these subjects underwent oral glucose

tolerance tests (OGTT), which is considered more sensitive but slightly less specific than FPG for identifying people at risk of developing T2D.[58] However, the low reproducibility, high cost, and prolonged time required for this test have limited its use in clinical practice.[59] Secondly, possible misclassification bias could have occurred as subjects were categorized as having prediabetes based on a single FPG sample, thus limiting the possibility to account for intra-individual variability and increasing the possibility of a regression-toward-the-mean effect, possibly affecting the progression rate. Thirdly, diet and PA were only ~~not~~ evaluated at baseline, thus lifestyles changes were not recorded during follow-up, possibly resulting in misclassification bias. Moreover, specific separate information on fruits and vegetable consumption could not be assessed, thus limiting the possibility of studying the confounding effect of excessive fruit consumption on NAFLD risk. Finally, we cannot discard the effect of job-related confounders such as job stress or the healthy worker effect. The main strengths of this study were the large sample size (16,648 subjects) and the relatively long follow-up period. Study participants had multiple occupations and were from several geographical locations, suggesting that the study population was representative of the Spanish workforce, although, our results are not applicable to the general population.

Clinical implications

This study highlights the importance of FLI as an easily calculated and valuable early indicator for high risk of T2D in subjects with prediabetes. FLI-based screening could allow the adoption of effective measures to prevent and reduce the progression of NAFLD. The workplace could be a feasible setting for implementing diabetes prevention programs based on early detection and lifestyle changes.

CONCLUSION

Because of the progressive nature of NAFLD and the risk of serious consequences, health care providers should be strongly advised to screen routinely for NAFLD in all subjects with prediabetes or at risk of T2D. Fatty liver indices are simple clinical tools for evaluating the extent of liver fat and are predictive of incident diabetes. Concretely, the FLI is a simple, effective and practical method of stratifying the risk of conversion to T2D based on the degree of hepatic steatosis. FLI may be useful in routine clinical

practice as an additional screening tool to identify subjects with prediabetes who are at high risk of progression and could benefit from early interventions. Identification of subjects who could benefit from preventive strategies represents an opportunity to assist vulnerable individuals to understand their health risks and encourage them to adopt preventive behaviors.

The workplace may be a feasible setting for the assessment of risk factors, allowing early detection of NAFLD in younger subjects with prediabetes who are likely to progress to T2D and the implementation of T2D prevention programs.

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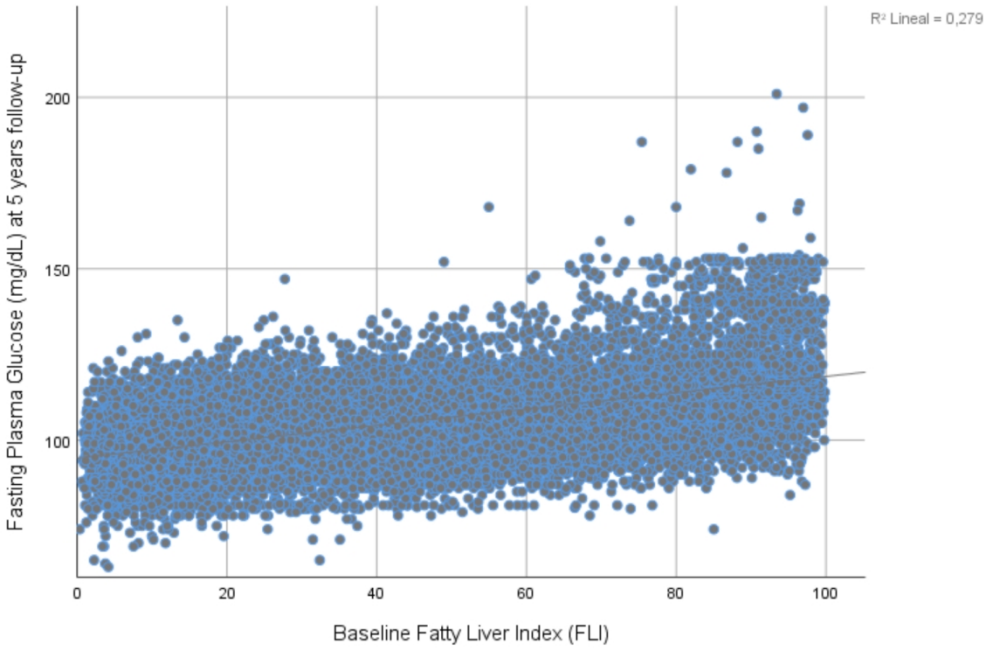

Figure 1

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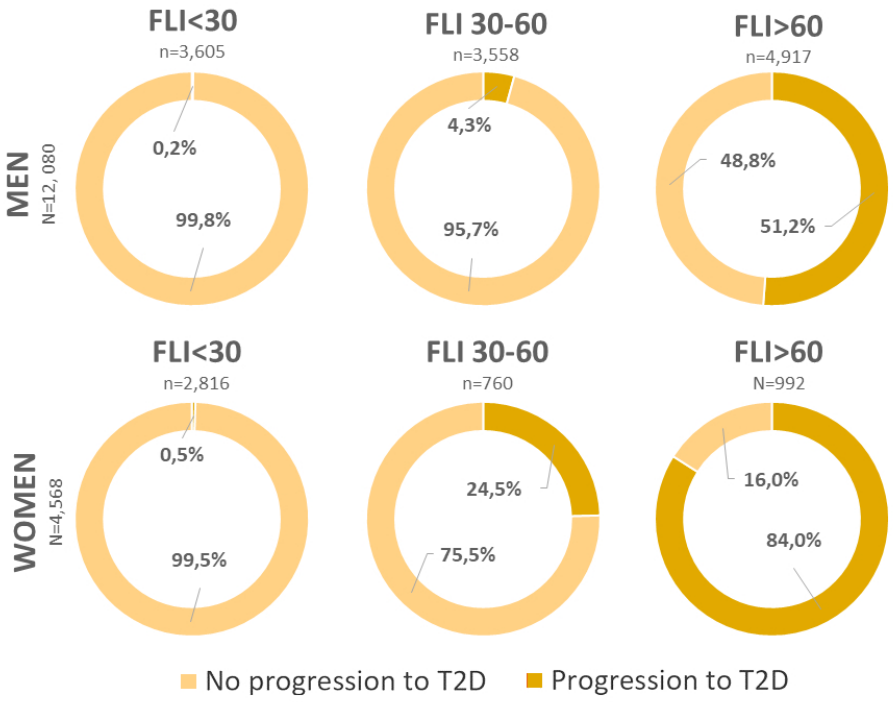

Figure 2

254x190mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	14
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	-

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Fatty liver index and progression to type 2 diabetes: a five-year longitudinal study in Spanish workers with prediabetes

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Key words: Conversion, Fatty Liver Index, Non-alcoholic Fatty Liver Disease, Fasting Plasma Glucose, Prediabetes, Type 2 Diabetes.

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ABSTRACT

Objective: The main aim of the study was to evaluate the association between non-alcoholic fatty liver disease (NAFLD), estimated by fatty liver index (FLI), and the development of type 2 diabetes (T2D) in a large cohort of adult workers with prediabetes.

Design: Prospective cohort study.

Setting: Occupational health services from Spain.

Participants: 16,648 adult workers (aged 20 to 65 years) with prediabetes (fasting plasma glucose (FPG) of 100-125 mg/dl).

Outcome and measures: FLI was calculated based on measurements of triglycerides, body mass index, waist circumference and γ-glutamyltransferase. The population was classified into three categories: FLI <30 (no hepatic steatosis), FLI 30–60 (intermediate status), and FLI >60 (hepatic steatosis). Sociodemographic, anthropometric, dietary habits, physical activity and clinical data were collected from all subjects. The incidence rate of T2D was determined after 5 years of follow-up.

Results: After 5 years of follow-up, 3,706 of the 16,648 participants (22.2%) were diagnosed with T2D, corresponding to an annual rate of progression of 4.5%. FLI was strongly associated with T2D conversion. The incidence rates of T2D in the FLI <30, FLI 30-60 and FLI >60 groups were significantly different after 5 years of follow-up were 19/6,421 (0.3%), 338/4,318 (7.8%) and 3,349/5,909 (56.7%), respectively. This association remained significant for FLI >60 after adjustment for, age, diet, physical activity, FPG, blood pressure, social class and smoking habits (adjusted HR=6.879; 95% CI 5.873 to 8.057 for men, and HR=5.806; 95% CI 4.863 to 6.932 for women).

Conclusion: NAFLD assessed by FLI independently predicted the risk of conversion to T2D among people with prediabetes. FLI may be an easily determined and valuable early predictor for T2D in people with prediabetes. FLI-based assessment of NAFLD in subjects with prediabetes in routine clinical practice could allow the adoption of effective measures to prevent and reduce their progression to T2D.

Strengths and limitations of this study

- This is a prospective study, with large sample size and 5-years follow-up.
- Study participants had multiple occupations and were from several geographical locations.
- The study sample included only adult workers therefore the results cannot be generalized to the general population.

INTRODUCTION

Type 2 Diabetes (T2D) is closely associated with a constellation of metabolic comorbidities, including obesity, hypertension, hypercholesterolemia, dyslipidemia and non-alcoholic fatty liver disease (NAFLD).[1] The main characteristic of NAFLD is the infiltration of hepatocytes by free fatty acids and triglycerides not related to significant alcohol intake. NAFLD is an entity that encompasses a wide spectrum of lesions ranging from indolent liver fat storage followed by lipotoxicity,[2] to hepatic inflammation, also known as non-alcoholic steatohepatitis (NASH). NAFLD is the most common chronic liver disease worldwide that is associated with excess health-related expenditures, making it a community health problem.[3]

Mounting evidence indicates a close association between the pathogenesis of T2D and NAFLD;[4–8] evidence suggests a complex bidirectional relationship, whereby presence of one leads to the progression of the other.[9] The presence of NAFLD increases the incidence of T2D, while diabetes might contribute to the worsening of NAFLD to more advanced stages such as steatohepatitis and even hepatocellular carcinoma.[10]

NAFLD is strongly associated with insulin resistance such that prevalence of NAFLD is 5-fold higher in patients with T2D compared to those without.[8] Recent data showed that there is a solid genetic basis that support their association, since gene variants in numerous proteins related to lipid and glucose metabolism, appear to significantly raise the risk of NAFLD and T2D.[10,11] These genetic abnormalities are directly linked to hepatic and peripheral insulin resistance, resulting in a deficient inhibition of hepatic gluconeogenesis, diminished glycogen synthesis and increased extrahepatic lipid accumulation. Other mechanisms underlying these NAFLD-T2D pathogenic duo involve excessive hepatic fat accumulation, diverse alterations in energy metabolism, altered microbiome, comorbidities, increased reactive oxygen species production and inflammatory signals derived from different cell types including immune cells, such as proinflammatory cytokines.[12]

The estimated overall worldwide prevalence of NAFLD in the general adult population is about 25–30%,[3,13] but ranges from 40%–70% in subjects with established T2D.[14,15] In fact, NAFLD and T2D are conditions that frequently coexist

and can act synergistically to drive adverse outcomes.[16] NAFLD is considered the hepatic manifestation of metabolic syndrome (MetS)[17] because epidemiological studies have consistently shown that NAFLD is strongly linked to obesity, dyslipidemia, and insulin resistance.[18,19] Therefore, NAFLD is thought to be an independent risk factor for incident T2D[16] and cardiovascular disease.[20]

Liver biopsy is currently the gold standard for diagnosing progressive NAFLD.[21] Biopsies are invasive procedures with several drawbacks, including sampling error, interobserver variability, high cost, patient discomfort and risk of complications.[14] Moreover, obtaining liver biopsies from all patients with NAFLD is unrealistic. Abdominal ultrasonography is a simple, inexpensive, widely available and minimally invasive technique that is used to diagnose fatty liver in most subjects. However, its sensitivity is low in subjects with fatty retention less than 20%–30% and it does not provide information on the degree of fibrosis.[22] Consequently, attempts have been made to diagnose NAFLD/NASH using clinical and laboratory-based biomarkers and scoring systems that can predict fatty changes in the liver. These indices for the diagnosis of NAFLD/NASH include the fatty liver index (FLI),[23] NAFLD liver fat score,[24] the hepatic steatosis index (HSI),[25] the ALD/NAFLD index (ANI),[26] the lipid accumulation product (LAP)[27] and the SteatoTest (ST).[28] These indices require the measurement of patient characteristics, including concentrations of triglycerides (TG), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine transaminase (ALT), insulin, body mass index (BMI), waist circumference (WC), gender, mean corpuscular value and presence or absence of T2D or metabolic syndrome.[29] The FLI is a simple and accurate algorithm that combines routine measurements of TG and GGT concentrations, WC and BMI, showing an excellent discriminative ability to predict ultrasonographic NAFLD and hepatic steatosis in the general population.[23,30]

The FLI has been reported to correlate with: 1) insulin resistance; 2) risk of coronary heart disease; 3) MetS; 4) early atherosclerosis; and 5) rates of non-hepatic-related morbidity and mortality in nondiabetic subjects.[31] Thus, FLI-diagnosed NAFLD may be an indicator of incident T2D.[19] Nonetheless, the risk of progression to T2D determined by FLI in patients with prediabetes remains poorly understood.

Few studies have evaluated the influence of NAFLD as a risk factor for T2D development in a cohort of workers with prediabetes. Determining FLI in subjects with

prediabetes may be highly relevant, as both epidemiological and clinical evidence have shown that primary health care prevention programs should target people at greater risk of developing T2D. The present study was therefore designed to evaluate the association between NAFLD, as estimated by FLI, and the development of T2D in a large cohort of South-European Mediterranean workers with prediabetes.

Methods

Study population and design

This cohort study included 16,648 Spanish working adults with prediabetes who worked in public administration, construction, health departments or post offices. The study methods have been described in detail previously.[32] Briefly, participants were carefully chosen from 234,995 potentially eligible individuals who underwent periodic occupational health assessments between 2012 and 2013. Participants were included if they were aged 20–65 years and had an FPG of 100–125 mg/dL.[33] Subjects were excluded if they had a history of physician-diagnosed diabetes, had been treated with an oral antidiabetic agent or a systemic glucocorticoid, had an FPG ≥126 mg/dL or an HbA1c ≥6.5% at baseline, had received cancer treatment during the preceding 5 years, had anemia (hematocrit <36% in men and <33% in women) or were pregnant. All subjects underwent standard health examinations, anthropometric measurements, and metabolic tests at baseline and were followed up 5 years later, in 2017 and 2018.

All the procedures in the study protocol were in accordance with the Declaration of Helsinki for research on human participants and were approved by the Balearic Islands Ethical Committee of Clinical Research (Ref. No: CEI-IB-1887). All participants were carefully informed of the purpose and demands of the study. Informed consent was obtained from all participants included in the study.

Patient and public involvement

People were not involved in setting the research question nor in the study design. Participants were interviewed face to face by trained researchers for a detailed explanation of the purpose of this research and informed consent at the beginning. Results of the research will be disseminated to the participants.

Data collection

At baseline, anthropometric measurements and fasting blood sample were taken from all subjects during occupational health examinations. A questionnaire was administered to collect data on sociodemographic characteristics, dietary habits, physical activity (PA) and clinical data. Participants were asked to report if they performed moderate and/or vigorous exercise (at least 150 min/week, according to World Health Organization [WHO] recommendations) and if they consumed fruits and vegetables daily. Each participant was also categorized as a current smoker (habitual or casual), former smoker, or never smoker, according to WHO criteria. Social class was defined using the Spanish Epidemiology Society classification, which is based on occupation, and it has shown high correlation with level of education.[34] Class I (upper class) includes executives, managers, and university professionals; Class II (middle class) includes intermediate occupations and employees; and Class III (lower class) includes manual workers.

All anthropometric measurements were made in the morning, after an overnight fast, at the same time and according to the guidelines and recommendations in the International Standards for Anthropometric Assessment (ISAK) manual.[35] All measurements were performed by well trained technicians or researchers to minimize coefficients of variation. Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca 700 scale, Hamburg); height was measured to the nearest 0.5 cm using a stadiometer (Seca 220) Telescopic Height Rod for Column Scales, Hamburg); and BMI was calculated as weight (kg) divided by height (m) squared (kg/m^2). Obesity was defined as BMI ≥30.0 kg/m^2, in agreement with WHO guidelines. Blood pressure was measured after a resting period of 10 minutes, with the subject in the supine position, using an electric and calibrated sphygmomanometer (OMRON M3, Healthcare Europe, Spain). Blood pressure in each subject was measured three times with a one-minute gap between measurements and their average was calculated.

Venous blood samples were taken from the antecubital vein of each subject in a sitting position, in the morning after a 12 h overnight fast. Blood samples were collected in suitable vacutainers without anticoagulant to obtain serum. Serum concentrations of glucose, TG and cholesterol were measured by standard procedures using a Beckman Coulter SYNCHRON CX® 9 PRO clinical system (La Brea, CA, USA).

The main outcome variable of the study was the time elapsed until T2D onset, defined as FPG ≥126 mg/dl,[36] or the time until initiation of anti-hyperglycemic medications for diabetes control in people with prediabetes during the follow-up period.

FLI as a surrogate measure of fatty liver

The FLI was calculated based on measurements of TG, GGT, BMI and WC, using the formula[23]:

$$Fatty\ Liver\ Index\ (FLI) = e^{y} / (1 + e^{y}) \times 100$$

$$Where\ y = 0.953 \times ln(TG) + 0.139 \times BMI + 0.718 \times ln(GGT) + 0.053 \times WC - 15.745$$

Here, TG indicates triglyceride concentration, measured as mg/dl; BMI indicates body mass index, measured as kg/m^2; GGT indicates γ-glutamyl transpeptidase, measured as U/l; and WC indicates waist circumference, measured as cm.

FLI, which ranges from 0 to 100, has shown good diagnostic accuracy in detecting fatty liver, with an Area Under the Curve (AUC) of 0.85 and a 95% confidence interval (CI) of 0.81–0.88.[19,23] FLI <30 was found to rule out steatosis with a sensitivity of 87% and a specificity of 64%, whereas FLI >60 was indicative of the presence of steatosis with a sensitivity of 61% and specificity of 86%.[23] FLI scores have been validated by comparison with the results of liver ultrasound and nuclear magnetic resonance spectroscopy. An FLI of 30–60 indicated indeterminate risk, in which fatty liver could not be ruled in or out.

Statistical analyses

Continuous variables were expressed in means (±SDs) and compared by Student's t-test and one-way analysis of variance (ANOVA), with post-hoc Bonferroni contrast method. Categorical variables were expressed as n (%) and compared by chi-square (χ^2) tests with Bonferroni post-hoc method. Crude and multivariable Cox regression analyses were performed to calculate FLI, diet and PA hazard ratios (HR) for the development of diabetes, adjusting for potential confounders (age, social class, BMI, smoking, SBP, FPG) that showed significant association in univariate analysis. Schoenfeld residuals were used to check the proportional hazard assumption. For this analysis participants were classified into two categories: those with FLI >60 and FLI <60

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Company, New York, NY, USA) for Windows. All statistical tests were two-sided, and p values <0.05 were considered statistically significant.

RESULTS

Baseline demographic and anthropometric characteristics of the study subjects by sex are shown in Table 1. The sample included 16,648 individuals with prediabetes, comprised of 12,080 (72.6%) men and 4,568 (27.4%) women, of mean age 44.81± 9.91 years. The prevalence of obesity in the entire sample was 26.9%. The percentage of men was significantly higher among subjects with than without NAFLD. There were also significant differences in all anthropometrical and biochemical parameters analyzed, with BMI, WC, TG, fasting plasma glucose (FPG), cholesterol, GGT and SBP and DBP being significantly higher in subjects with than without NAFLD. The percentages of subjects who performed at least 150 min per week of PA (4.3% vs. 61.8%; p<0.001) and who did not consume fruits and vegetables every day (12.0% vs. 56.4%; p<0.001) were significantly lower in subjects with than without NAFLD.

Table 1 Basal anthropometric characteristics and biochemical parameters of subjects by sex

Characteristics	All (n = 16,648)	Men (n = 12,080)	Women (n= 4,568)	P value
Age (years)	44.51 ± 9.89	44.38 ± 9.87	44.84 ± 9.94	< 0.01
Social class				<0.001
I	741 (4.5%)	558 (4.6%)	183 (4.0%)	
II	2,779 (16.7%)	1,902 (15.7%)	877 (19.2%)	
III	13,128 (78.9%)	9,620 (79.6%)	3,508 (76.8%)	
BMI (kg/m2)	27.66 ± 4.81	27.76 ± 4.47	27.42 ± 5.61	< 0.001
BMI categories				< 0.001
Normal weight	5,049 (30.3%)	3,300 (27.3%)	1,749 (38.3%)	
Overweight	7,120 (42.8%)	5,596 (46.3%)	1,524 (33.4%)	
Obese	4,479 (26.9%)	3,184 (26.4%)	1,295 (28.3%)	
WC (cm)	87.00 ± 9.95	90.28 ± 8.62	78.32 ± 7.78	< 0.001
Triglycerides (mg/dL)	137.66 ± 106.39	150.08 ± 117.11	104.81 ± 59.14	< 0.001
Glucose (mg/dL)	106.22 ± 5.82	106.43 ± 5.90	105.68 ± 5.56	< 0.001
Cholesterol (mg/dL)	202.40 ± 38.09	202.49 ± 38.59	202.18 ± 36.74	0.642
GGT (UI/l)	44.20 ± 55.68	48.03 ± 59.07	34.08 ± 33.69	< 0.001
SBP (mmHg)	127.86 ± 16.74	130.16 ± 16.10	121.79 ± 16.88	< 0.001
DBP (mmHg)	78.32 ± 11.01	79.51 ± 10.94	75.18 ± 10.58	< 0.001
PA (≥150 min/week)	6,892 (41.4%)	4,787 (39.6%)	2,105 (46.1%)	< 0.001
Diet (daily fruits and vegetables)	6,771 (40.7%)	4,654 (38.5%)	2,117 (46.3%)	< 0.001
Smoking habit				< 0.001
Never	7,645 (45.9%)	5,124 (42.4%)	2,521 (55.2%)	
Former	3,549 (21.3%)	2,750 (22.8%)	799 (17.5%)	
Current	5,454 (32.8%)	4,206 (34.8%)	1,248 (27.3%)	

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by Student's t-test, whereas categorical variables were compared by χ^2 tests.

General characteristics of the study population, such as anthropometric and biochemical data, are shown in Table 2, according to FLI categories. Data stratified by gender and FLI categories are shown in Table 3 for men and Table 4 for women. In both

men and women, those with FLI>60 presented a significantly worse anthropometric and biochemical profile, as compared with the other two groups.

Among men, 40.7% presented a FLI>60, 29.5% a FLI 30-60, and 29.8% a FLI <30. As compared to men in the other two categories, those with FLI>60 were older, more obese, and presented higher values of WC, triglycerides, glucose, cholesterol, GGT, SBP, and DBP (all p<0.001). Men with FLI<30 consumed more fruits and vegetables daily, and dedicated more time to PA, than men with FLI>60 (all p<0.001).

Table 2. Basal anthropometric characteristics and biochemical parameters of men and women according to FLI categories (n = 16,648).

Characteristics	FLI <30 n = 6,421 (29.8%) (a)	FLI 30-60 n= 4,318 (29.5%) (b)	FLI >60 n = 5,909 (40.7%) (c)	P value	Post-hoc
Sex (Ref: Male)	3,605 (56.1%)	3,558 (82.4)	4,927 (83.2%)	0.008	
Age (years)	42.35 ± 10.57	45.34 ± 9.53	46.32 ± 8.89	< 0.001	a<b<c
Social class				0.107	NS
I	248 (4.4%)	218 (5.0%)	239 (4.0%)		
II	1,129 (17.6%)	689 (16.0%)	961 (16.3%)		
III	5,008 (78.0%)	3,411 (79.0%)	4,709 (79.6%)		
BMI (kg/m2)	23.91 ± 2.61	27.27 ± 2.39	32.04 ± 4.39	< 0.001	c<b<a
BMI categories				< 0.001	
Normal weight	4,340 (67.6%)	626 (14.5%)	83 (1.4%)		a>b,c; b>c
Overweight	2,014 (31.4%)	3.218 (74.5%)	1,888 (32.0%)		b>a,c; c>a
Obese	67 (1%)	474 (11.0%)	4.479 (26.9%)		b>a; c>a,b
WC (cm)	78.87 ± 7.06	87.92 ± 6.55	95.16 ± 7.34	< 0.001	c>b>a
Triglycerides (mg/dL)	87.52 ± 36.34	129.90 ± 60.74	19 197.81 ± 146.19	< 0.001	c>b>a
Glucose (mg/dL)	105.52 ± 5.28	106.01 ± 5.65	107.30 ± 6.32	< 0.001	c>b>a
Cholesterol (mg/dL)	192.04 ± 35.64	204.66 ± 36.98	212.01 ± 38.69	< 0.0	c>b>a
GGT (UI/l)	21.35 ± 13.19	39.22 ± 31.37	72.68 ± 76.26	< 0.001	c>b>a
SBP (mmHg)	121.54 ± 15.31	128.56 ± 15.01	134.24± 16.91	< 0.001	c>b>a
DBP (mmHg)	74.22 ± 10.09	78.72 ± 10.13	82.50 ± 10.96	< 0.001	c>b>a

PA (≥150 min/week)	5,156 (80.3%)	1,480 (34.3%)	256 (4.3%)	< 0.001	a>b,c; b>c
Diet (daily fruits and vegetables)	4,502 (66.5%)	1,560 (23.0%)	709 (12.0%)	< 0.001	a>b,c; b>c
Smoking habit				0.930	NS
Never	3,065 (42.7%)	1,981 (45.9%)	2,599 (44.0%)		
Former	1,077 (16.8%)	953 (22.1%)	1,519 5.7%)		
Current	2,279 (35.5%)	1,384 (32.1%)	1,791 (30.3%)		

Table 3. Basal anthropometric characteristics and biochemical parameters of men according to FLI categories (n = 12,080).

Men characteristics	FLI <30 n = 3,605 (29.8%) (a)	FLI 30-60 n= 3,558 (29.5%) (b)	FLI >60 n = 4,917 (40.7%) (c)	P value	Post-hoc
Age (years)	41.02 ± 10.66	45.08 ± 9.55	46.34 ± 8.81	< 0.001	
Social class				0.137	NS
I	153 (4.2%)	191 (5.4%)	214 (4.4%)		
II	559 (15.5%)	554 (15.6%)	789 (16.0%)		
III	2,893 (80.2%)	2,813 (79.1%)	3,914 (79.6%)		
BMI (kg/m2)	23.74 ± 2.24	26.78 ± 2.02	31.41 ± 4.09	< 0.001	c<b<a
BMI categories				< 0.001	
Normal weight	2,616 (72.6%)	603 (16.9%)	81 (1.6%)		a>b,c; b>c
Overweight	980 (27.2%)	2,787 (78.3%)	1,829 (37.2%)		b>a,c; c>a
Obese	9 (0.2%)	168 (4.7%)	3,007 (61.2%)		b>a; c>a,b
WC (cm)	82.67 ± 5.85	89.13 ± 6.06	96.68 ± 6.83	< 0.001	c>b>a
Triglycerides (mg/dL)	88.46 ± 37.22	130.95 ± 60.70	209.1-0 ± 153.25	< 0.001	c>b>a
Glucose (mg/dL)	105.56 ± 5.36	106.05 ± 5.63	107.34 ± 6.34	< 0.001	c>b>a
Cholesterol (mg/dL)	187.32 ± 34.39	203.72 ± 37.20	212.71 ± 38.95	< 0.001	c>b>a
GGT (UI/l)	23.02 ± 13.22	38.89 ± 31.70	72.98 ± 81.09	< 0.001	c>b>a
SBP (mmHg)	124.08 ± 14.42	129.23 ± 14.63	135.30 ± 16.60	< 0.001	c>b>a
DBP (mmHg)	74.98 ± 10.08	79.03 ± 10.02	83.18 ± 10.86	< 0.001	c>b>a
PA (≥150 min/week)	3,108 (86.2%)	1,425 (40.1%)	254 (5.2%)	< 0.001	a>b,c; b>c
Diet (daily fruits and vegetables)	2,693 (74.7%)	1,372 (38.6%)	589 (12.0%)	< 0.001	a>b,c; b>c

Smoking habit				< 0.001	
Never	1,539 (42.7%)	1,571 (44.2%)	2,014 (41.0%)		b>c
Former	620 (17.2%)	800 (22.5%)	1,330 (27.0%)		b>a; c>a,b
Current	1,446 (40.1%)	1,187 (33.4%)	1,573 (32.0%)		a>b,c

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by ANOVA, whereas categorical variables were compared by χ^2 tests.

Among women, 21.7% had a FLI>60, 16.6% a FLI 30-60, and 61.7% a FLI<30. As compared to women in the other two categories, those with FLI>60 were more obese, and had worse anthropometric and biochemical values (WC, triglycerides, glucose, cholesterol, GGT, SBP, and DBP) (all p<0.001). Women with FLI<30 also consumed more fruits and vegetables daily, and dedicated more time to PA, than women with FLI>60 (all p<0.001).

Table 4. Anthropometric characteristics and biochemical parameters of women according to FLI categories (n = 4,568).

Women characteristics	FLI <30 n = 2,816 (61.7%) (a)	FLI 30-60 n = 760 (16.6%) (b)	FLI >60 n = 992 (21.7%) (c)	P value	Post-hoc
Age (years)	43.91 ± 10.22	46.55 ± 9.31	46.20 ± 9.28	< 0.001	a<b,c
Social class				<0.01	
I	131 (4.7%)	27 (3.6%)	25 (2.5%)		a>c
II	570 (20.2%)	135 (17.8%)	172 (17.3%)		
III	2,115 (75.1%)	598 (78.7%)	795 (80.1%)		c>a
BMI (kg/m2)	24.13 ± 3.02	29.56 ± 2.62	35.12 ± 4.49	< 0.001	c>b>a
BMI categories				< 0.001	
Normal weight	1,724 (61.2%)	23 (3.0%)	2 (0.2%)		a>b,c; b>c
Overweight	1,034 (36.7%)	431 (56.7%)	59 (5.9%)		a>c; b>a,c
Obese	58 (2.1%)	306 (40.3%)	931 (93.9%)		b>a; c>a,b
WC (cm)	74.00 ± 5.35	82.22 ± 5.70	87.63 ± 4.60	< 0.001	c>b>a
Triglycerides (mg/dL)	86.33 ± 35.15	125.00 ± 60.71	141.83 ± 84.45	< 0.001	c>b>a
Glucose (mg/dL)	105.13 ± 5.17	105.84 ± 5.73	107.12 ± 6.18	< 0.001	c>b>a
Cholesterol (mg/dL)	198.09 ± 36.29	209.10 ± 35.61	208.52 ± 37.20	< 0.001	a<b,c

GGT (UI/l)	19.21 ± 12.83	40.74 ± 29.70	71.16 ± 45.26	< 0.001	c>b>a
SBP (mmHg)	118.28 ± 15.80	125.42 ± 16.34	128.98 ± 17.42	< 0.001	c>b>a
DBP (mmHg)	73.25 ± 10.02	77.25 ± 10.50	79.08 ± 10.82	< 0.001	c>b>a
PA (≥150 min/week)	2,048 (72.7%)	55 (7.2%)	2 (0.2%)	< 0.001	a>b,c; b>c
Diet (daily fruits and vegetables)	1,809 (64.2%)	188 (24.7%)	120 (12.1%)	< 0.001	a>b,c; b>c
Smoking habit				< 0.001	
Never	1,526 (54.2%)	410 (53.9%)	585 (59.0%)		c>a
Former	457 (16.2%)	153 (20.1%)	189 (19.1%)		b>a
Current	833 (29.6%)	197 (25.9%)	218 (22.0%)		a>c

Results are reported as mean ± SD or n (%). BMI, body mass index; WC, waist circumference; GGT, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure PA, physical activity. Continuous variables were compared by ANOVA, whereas categorical variables were compared by χ^2 tests.

Baseline FLI showed a significant correlation with FPG concentration at 5-year follow-up with a Pearson's correlation coefficient of 0.528 ($p < 0.001$) (Figure 1).

Figure 1 Correlation of Baseline FLI and FPG after 5 years of follow-up.

Of the 16,648 subjects with prediabetes, 3,706 (22.2%) progressed to T2D at 5 years, corresponding to an annual rate of 4.5%. The incidence of T2D after 5 years was similar between men (22.1%) and women (22.6%). When specifically looking at FLI categories, 0.2% (6/3,605) of men and 0.5% (13/2,816) of women in the low-risk group (FLI<30), progressed to T2D, corresponding to an annual rate of 0.04% for men and 0.1% for women. In the intermediate risk group (FLI 30-60), progression to T2D occurred in 4.3% (152/3,558) of men and 24.5% (186/760) in women, corresponding to an annual rate of 0.86% and 4.9%, respectively. Finally, in the high-risk group (FLI>60), incidence of T2D was 51.2% (2,516/4,917) in men and 84.0% (833/992) in women, corresponding to an annual rate of 11.34% and 16.8% respectively. Rates of progression to T2D in men and women according baseline FLI categories are shown in Figure 2.

Figure 2 Incidence of T2D after 5-year follow-up according to baseline FLI classification.

In bivariate analysis (Table 5), high FLI (>60) was strongly associated with progression to T2D in both genders (HR=24.361; 95% CI 21.020 to 28.233 for men, and HR=17.816; 95% CI 15.400 to 20.611 for women), as were age, social class, BMI, smoking habits, FPG and SBP. An adjusted cox regression model showed that high FLI scores (>60) remained independently associated with progression to T2D, (adjusted HR=6.879; 95% CI 5.873 to 8.057 for men, and HR=5.806; 95% CI 4.863 to 6.932 for women). BMI was also associated to progression to T2D in both genders after adjustment (HR=1.041; 95% CI 1.036 to 1.045 for men, and HR=1.104; 95% CI 1.036 to 1.045 for women). Some of the evaluated factors also remained significant after adjustment. Performing at least 150 min/week of physical activity (adjusted HR=0.215; 95% CI 0.173 to 0.268 for men, and HR=0.070; 95% CI 0.043 to 0.112 for women) was significantly protective against progression to T2D in both genders. Current male smokers were also less likely to progress to T2D (adjusted HR=0.909; 95% CI 0.834 to 0.991).

Table 5. Hazard Ratios for progression from prediabetes to T2D

Variables	HR_{crude} (95% CI)			$HR_{adjusted}$ (95% CI)		
	Men	Women	All subjects	Men	Women	All subjects
Age	**1.054 (1.051 – 1.058)**	**1.035 (1.030 – 1.050)**	**1.042 (1.040 - 1.054)**	**1.041 (1.036 - 1.045)**	**1.018 (1.011 – 1.026)**	**1.029 (1.024 - 1.036)**
Social class (Ref: I)						
II	**1.147 (0.984 – 1.338)**	**1.475 (1.102 – 1.974)**	**1.331 (1.043 - 1.656)**	1.009 (0.839 – 1.212)	1.216 (0.818 – 1.809)	1.113 (0.826 - 1.511)
III	1.087 (0.994 – 1.251)	**1.845 (1.402 – 2.428)**	**1,446 (1.198 - 1.840)**	1.005 (0.851 - 1.186)	1.170 (0.793 – 1.140)	1.088 (0.822 - 1.163)
PA (≥150 min/week)	**0.037 (0.031 – 0.046)**	**0.027 (0.019 – 0.038)**	**0.032 (0.025 - 0.042)**	**0.215 (0.173 - 0.268)**	**0.070 (0.043 - 0.112)**	**0.143 (0.108 - 0.190)**
Diet (daily fruits and vegetables)	**0.126 (0.112 – 0.142)**	**0.141 (0.120 – 0.166)**	**0.134 (0.116 - 0.154)**	0.959 (0.843 – 1.091)	0.951 (0.793 – 1.140)	0.955 (0.818 -1.116)
Smoking habits (Ref: never smoker)						
Former	**1.244 (1.153 – 1.343)**	**1.010 (0.875 – 1.165)**	**1.749 (1.014 - 1.254)**	0.985 (0.904 – 1.072)	1.017 (0.873 – 1.184)	1.046 (1.340 - 1.128)
Current	**0.770 (0.719 – 0.824)**	**0.714 (0.633 – 0.804)**	**0.742 (0.676 - 0.814)**	**0.909 (0.834 - 0.991)**	0.959 (0.830 – 1.107)	0.934 (0.832 - 1.049)
BMI	**1.174 (1.170 – 1.178)**	**1.161 (1.154 – 1.167)**	**1.168 (1.162 - 1.172)**	**1.041 (1.036 - 1.045)**	**1.104 (1.101 – 1.107)**	**1.073 (1.069 - 1.076)**
SBP	**1.023 (1.021 – 1.024)**	**1.023 (1.021 – 1.025)**	**1.023 (1.021 – 1.025)**	0.999 (0.996 - 1.001)	1.001 (0.997 – 1.004)	1.000 (0.997 - 1.003)
FPG	**1.037 (1.034 – 1.039)**	**1.027 (1.023 – 1.030)**	**1.032 (1.029 - 1.035)**	**1.021 (1.018 – 1.024)**	**1.018 (1.013 – 1.023)**	**1.020 (1.016 - 1.024)**
FLI (Ref: FLI < 60)						
FLI >60	**24.361 (21.020 – 28.233)**	**17.816 (15.400 – 20.611)**	**21.089 (18.210 - 24.422)**	**6.879 (5.873 – 8.057)**	**5.806 (4.863 – 6.932)**	**6.343 (5.368 - 7.495)**

PA, physical activity; BMI, body mass index; SBP, systolic blood pressure; FLI, fatty liver index; FPG, fasting plasma glucose.

DISCUSSION

The present study aimed to evaluate the possible association between hepatic steatosis, as estimated by FLI, and T2D progression in a large and representative sample of Mediterranean workers with prediabetes. The main finding of the study was that FLI was a strong independent risk factor for the progression of T2D, in both men and women with baseline prediabetes, after a 5-year follow-up. Moreover, FLI could preventively identify subjects at high risk of progression to T2D. Other risk factor associated with progression T2D were older age, male sex, higher BMI, higher FPG, low consumption of fruits and vegetables, and performing less than 150 min/week of PA.

The results of the present study are in accordance with previous evidence reporting that NAFLD is a strong predictor of T2D in subjects with prediabetes [37,14]. The baseline prevalence of hepatic steatosis (i.e. FLI>60) in our study population was 35.4%, higher than the 19.3% reported in a Japanese study,[37] lower than the 55.7% observed in the PREDAPS study,[14] and closer to the 22–40% reported by studies using ultrasonography-diagnosed NAFLD.[38,39]

Patients with a higher FLI score, independently of gender, presented a higher BMI, a worse cardiometabolic profile and less healthy lifestyle habits. Previous studies [40] similarly observed that patients with FLI>60 were more metabolically impaired compared to patients with lower FLI, they also presented a higher risk for MetS, as well as worse lipid profile. [41] Accordingly, the degree of liver fat content correlates with MetS components,[42] and that this correlation may be due to NAFLD and T2D sharing a series of common physiopathological pathways.[43,44]

At five-year follow-up, nearly one in four individuals (22.2%) with prediabetes progressed to T2D resulting in an annual rate of progression of 4.5%. In comparison, the French IT-DIAB study,[45] a 5-year, prospective observational study reported an annual progression rate of 7.1%. The study also reported that FLI could predict the risk of progressing to T2D as well as the possibility of reverting to normoglycemia in clinical practice, independently of classical glucose parameters.[46] Moreover, normalization of glycemia was higher in subjects with FLI <30 than in those with higher FLI scores. The incidence of T2D observed in our study was higher than in previous ones[47–49] probably due to differences in sociodemographic characteristics between study populations. The ARIC study,[47] which reported an annual progression rate to T2D of 2.3%, included a higher percentage of women than in our cohort, whereas the ELSA-

Brasil study,[49] which found that the annual progression rate to T2D was 3.5%, included a higher percentage of subjects with high educational level. On the other hand, the PREDAPS study[50] showed a similar annual conversion rate (4.2%). The incidence rate of T2D in our sample was lower than that shown (5.8%) in a previous Korean study [51] of 7,680 subjects who had undergone general routine health evaluations. Nevertheless, similar to what was observed in our study, 65.5% of the Korean subjects were men, and male sex was a risk factor for development of T2D in patients with prediabetes.

When stratifying for gender, the proportion of women in the FLI >60 category who progressed to T2D was significantly higher (80%) than the proportion of men in the same category (50%), at 5-year follow-up. Although women are generally less likely to suffer from hepatic steatosis,[52] once they do, they might present a higher risk of developing T2D than males [53]. Genetic predisposition and epigenetic mechanisms, nutritional components and lifestyle exert effects differently in both sexes. Furthermore, sexual hormones directly impact on energy metabolism, body composition, inflammatory cascades and vascular functioning. Particularly, low levels of 17β-estradiol are associated with increased risk of T2D, independently of established risk factors, including BMI and insulin resistance.[54] Thus, endocrine imbalances might relate to unfavorable cardiometabolic traits observable in female sex.[55]

Of note, results from our study show an apparently protective effect of smoking on progression to diabetes. However this could be due to the anorexigenic effect of tobacco, more than tobacco consumption itself. Smokers are generally leaner than average as nicotine may affect energy homeostasis and food consumption at brain level.[56] Accordingly, the proportion of smokers with a lower FLI was higher than that of smokers in the other two categories.

The FLI could be utilized in primary care as a practical tool for early detection of NAFLD in subjects with prediabetes, while predicting their risk of developing T2D.[57] This would benefit patients at greater risk, allowing more careful monitoring and providing an opportunity for early interventions to prevent and reduce both the progression of hepatic disease and T2D. The present study also highlights the importance of weight control, promotion of PA and of fruits and vegetables consumption in the prevention of T2D progression. Determining lifestyle-related factors, particularly PA, together with repeated anthropometrical measurements in subjects with prediabetes may be crucial in properly assessing the risks of progression to T2D and of cardiovascular events.[58]

Strengths and limitations

This study had some limitations. First, this work incorporated data from periodic health assessments performed in the workplace. None of these subjects underwent oral glucose tolerance tests (OGTT), which is considered more sensitive but slightly less specific than FPG for identifying people at risk of developing T2D.[59] However, the low reproducibility, high cost, and prolonged time required for this test have limited its use in clinical practice.[60] Secondly, possible misclassification bias could have occurred as subjects were categorized as having prediabetes based on a single FPG sample, thus limiting the possibility to account for intra-individual variability and increasing the possibility of a regression-toward-the-mean effect, possibly affecting the progression rate. Thirdly, diet and PA were only evaluated at baseline, thus lifestyles changes were not recorded during follow-up, possibly resulting in misclassification bias. Moreover, specific separate information on fruits and vegetable consumption could not be assessed, thus limiting the possibility of studying the confounding effect of excessive fruit consumption on NAFLD risk. Finally, we cannot discard the effect of job-related confounders such as job stress or the healthy worker effect. The main strengths of this study were the large sample size (16,648 subjects) and the relatively long follow-up period. Study participants had multiple occupations and were from several geographical locations, suggesting that the study population was representative of the Spanish workforce, although, our results are not applicable to the general population.

Clinical implications

This study highlights the importance of FLI as an easily calculated and valuable early indicator for high risk of T2D in subjects with prediabetes. FLI-based screening could allow the adoption of effective measures to prevent and reduce the progression of NAFLD. The workplace could be a feasible setting for implementing diabetes prevention programs based on early detection and lifestyle changes.

CONCLUSION

Because of the progressive nature of NAFLD and the risk of serious consequences, health care providers should be strongly advised to screen routinely for NAFLD in all subjects with prediabetes or at risk of T2D. Fatty liver indices are simple clinical tools for

evaluating the extent of liver fat and are predictive of incident diabetes. Concretely, the FLI is a simple, effective and practical method of stratifying the risk of conversion to T2D based on the degree of hepatic steatosis. FLI may be useful in routine clinical practice as an additional screening tool to identify subjects with prediabetes who are at high risk of progression and could benefit from early interventions. Identification of subjects who could benefit from preventive strategies represents an opportunity to assist vulnerable individuals to understand their health risks and encourage them to adopt preventive behaviors.

The workplace may be a feasible setting for the assessment of risk factors, allowing early detection of NAFLD in younger subjects with prediabetes who are likely to progress to T2D and the implementation of T2D prevention programs.

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Data sharing statement Data are available upon reasonable request. Readers may contact Dr. Arturo Lopez (angarturo@gmail.com) regarding the data. No additional data are available.

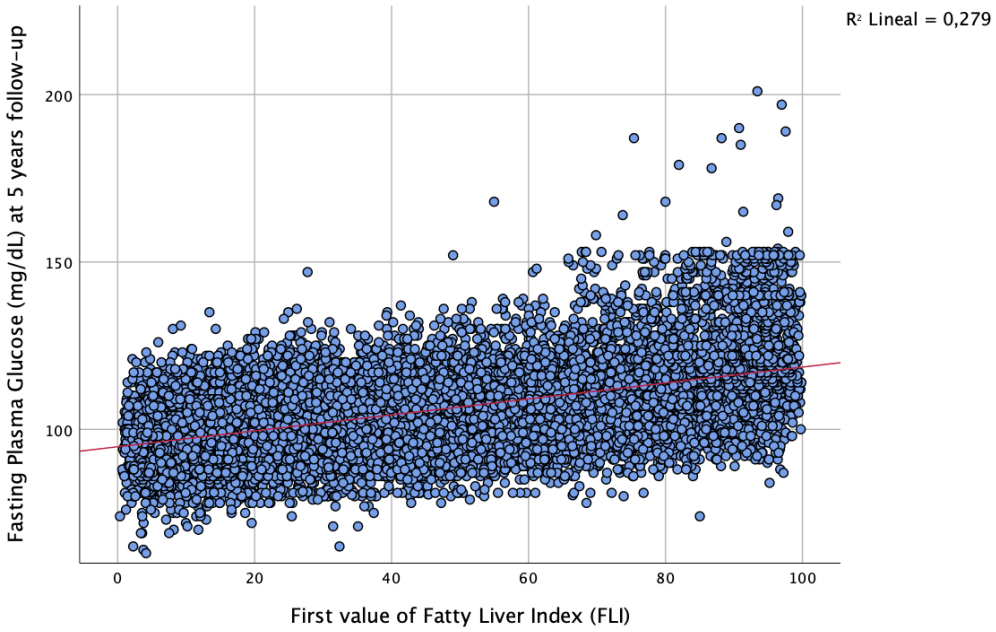

Figure 1

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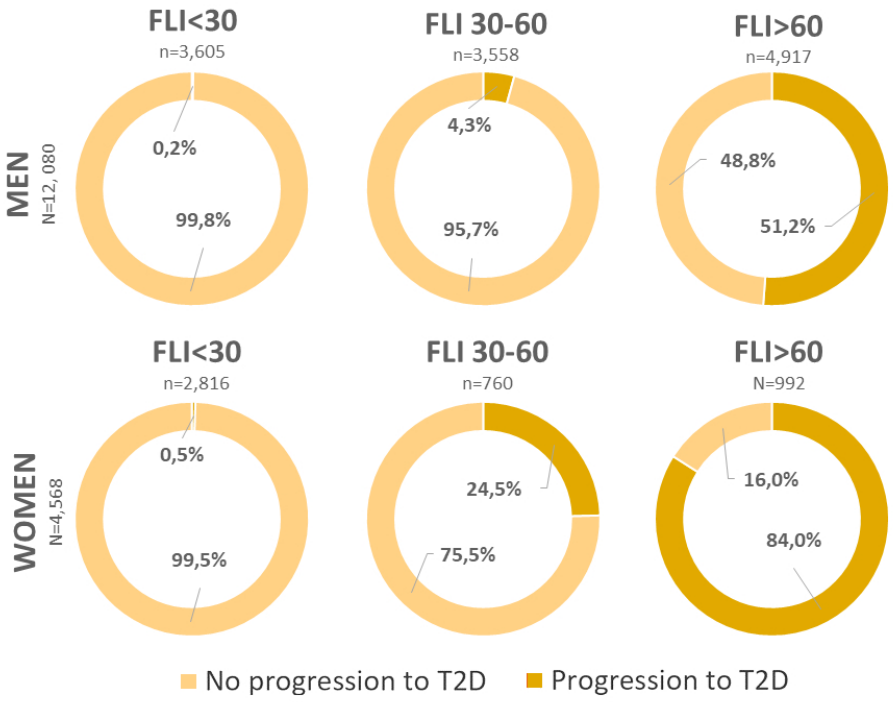

Figure 2

254x190mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	14
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	-

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.