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Association of fatty liver index with risk of incident type 2 diabetes by metabolic syndrome status in an Eastern Finland male cohort: a prospective study

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ABSTRACT

Objective Fatty liver disease (FLD) is increasingly recognised as a predictor of cardiometabolic risk. Our objective was to examine if metabolic syndrome (MS) status affects the association of FLD with incident type 2 diabetes (T2D) in middle-aged men.

Design Prospective epidemiological study.

Setting University affiliated research centre in Kuopio, Eastern Finland.

Participants Our subjects were 1792 Finnish men without diabetes at baseline in the KuopioIschaemicHeart Disease Risk Factor Study cohort.

Outcome measure Using fatty liver index (FLI), the association of baseline FLD with incident T2D was analysed in multivariable-adjusted Cox regression models, considering their MS statuses. The main models were adjusted for constitutional factors, lifestyle factors, biomarkers of inflammation and for high (FLI ≥60) versus low (FLI <30) FLI categories.

Results During a mean follow-up of 19 years, 375 incident cases of T2D were recorded. In the full model, the HR (HR (95% CI)) for T2D was 3.68 (2.80 to 4.82). The association was attenuated, but maintained, with further adjustment for metabolic factors. When MS status was adjusted for in place of metabolic factors, the HRs (95% CIs) were 2.63 (1.92 to 3.59) for FLI ≥60 and 1.77 (1.35 to 2.31) for MS. In MS-stratified analysis, FLI predicted T2D only among persons without MS. In unstratified analysis with subjects categorised by FLI-MS, persons with FLI ≥60 without MS had increased risk for T2D (HR=3.19 (2.26 to 4.52)) compared with persons with FLI <30 without MS. Persons with FLI <30 and MS had greater risk (HR=4.31 (2.15 to 8.61)) and persons with both FLI ≥60 and MS had the greatest risk (HR=4.66 (3.42 to 6.35)).

Conclusion Generally, FLD (FLI ≥60) predicts T2D. It specifically predicted T2D among men without MS but not among men with MS, for whom MS alone already increases the risk. Both FLI and MS can complement each other in screening and surveillance for persons with increased T2D risk.

Strengths and limitations of this study

► The study is population based, and the design is prospective, with long follow-up.
► We adjusted for a range of constitutional factors, lifestyle factors and biomarkers keeping in cognizance the components of both major exposure variables to avoid overadjustment.
► The study population comprised of men only.
► Fatty liver index used as a surrogate of fatty liver does not detect progression of fatty liver disease.
► The statuses of the men with respect to viral hepatitis were not established at baseline, although viral hepatitis has remained low in the Finnish population.

known as hepatic steatosis, FLD is associated with increased risk of cardiovascular disease (CVD) and type 2 diabetes (T2D). The prevalence has been observed to steadily rise, although this varies in different populations. Recent estimates suggest a global prevalence of 25% among adults, but the highest prevalence occurs in the Middle East and South America, while the lowest prevalence is in Africa.[1] The prevalence is estimated to be 24% in Europe and more than 30% in developed countries.[1] Approximately one-third of patients with FLD progress to steatohepatitis with fibrosis, which can thereafter progress to cirrhosis, liver failure and hepatocellular carcinoma.[1] FLD is intimately linked with metabolic diseases, including T2D, and it can be considered a predictor of metabolic diseases, even in the non-obese population.[2]

While FLD is an acknowledged public health problem, there is growing interest in FLD as a predictor of incident T2D.[3] A number of epidemiological studies suggest that non-alcoholic fatty liver disease (NAFLD), diagnosed using either liver enzymes or ultrasound scan, is associated with an increase in T2D incidence.[4 5]

INTRODUCTION/BACKGROUND

There is increasing recognition of the fact that fatty liver disease (FLD) is the most common cause of chronic liver disease worldwide. Also

Liver biopsy is the gold standard for characterising liver histology in patients with fatty liver. The procedure is expensive and carries some morbidity and very rare mortality risks.[6] The fatty liver index (FLI), an algorithm comprising body mass index (BMI), waist circumference, gamma-glutamyl transferase (GGT) and triglyceride concentrations. It was developed by Bedogni *et al* to predict the presence of FLD. The algorithm has been widely validated and has gained increased acceptance.[7 8] There have been reports of an association of high FLI (FLD) with incident T2D.[9 10] However, with FLD being intimately linked with metabolic diseases, it is uncertain whether the predictive ability of FLD is independent of presence of established metabolic syndrome (MS), a known potent predictor of T2D.

Therefore, using FLI as a surrogate for FLD, we examined whether MS status affects the association of FLD, with incident T2D in middle-aged men.

METHODS
Study population
Our study population comprised participants in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The KIHD study is a prospective population-based study. It was designed to investigate risk factors for CVDs and related outcomes, in middle-aged and ageing men, from Eastern Finland. The original study population consisted of an age-stratified sample of 2682 men. These were enrolled at baseline between March 1984 and December 1989. The men were 42, 48, 54 or 60 years of age at baseline.[11]

Data collection
Data were collected using self-administered questionnaires, interviews, physical examinations and various blood tests that aimed to elucidate physiological and biochemical parameters.[12 13] The self-administered questionnaire was used to collect data on medical history, including history of T2D, metabolic diseases, liver disease and so on, medication history, family history of diabetes and family history of CVD.[12] Data on lifestyle, including physical activity, history of smoking habit, history of alcohol consumption and diet, were also collected.[14] Categorisation of alcohol consumption was done according to standard guidelines by the National Institute of Alcohol Abuse and Alcoholism[15] and Dietary Guidelines for Americans 2010[16] as already published.[17]

A family history of CVD or diabetes was defined as positive if the father, mother, sister or brother of the subject had a history of CVD or diabetes.[12] A subject was defined as a smoker if he had ever smoked on a regular basis and had smoked cigarettes, cigars or pipe within the previous 30 days. Dietary intakes including fruit, berry and vegetable consumption were assessed with a 4-day food recording.[18]

Physical examinations included anthropometric indices, vital signs and physiological measurements. All measurements were made following standard protocols. Waist circumference was calculated as the mean of waist circumferences taken at maximal inspiration and maximal expiration. BMI was computed as the ratio of weight in kg to the square of height in metres (kg/m^2). Blood pressure was taken as the mean of measurements taken in the supine, standing and sitting position with 5 min intervals.[19]

Specimen collection and laboratory measurements
Blood samples were collected between 08:00 and 10:00 hours after 3 days of abstinence from alcohol ingestion and a 12-hour abstinence from smoking and eating. Data on complete blood count, serum electrolytes, homeostatic model assessment of insulin resistance (HOMA1-IR), fasting glucose, lipoprotein fractions (including total cholesterol, high-density cholesterol (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and serum triglycerides), liver function tests including albumin, GGT, fibrinogen, ferritin and biomarkers like C reactive protein (CRP), were each determined from appropriately collected and processed samples. Detailed description of the KIHD has been published elsewhere.[11]

Included and excluded subjects
The initial number of men at baseline was 2682. Of these, we excluded 40 men with history of physician diagnosed liver or pancreas disease, and 162 men with history of diabetes. Of the remaining 2480 men, 1792 who had complete data for FLI calculation, were included in the analyses.

Measuring the components of the FLI
We calculated FLI using the algorithm developed by Bedogni *et al.*[7] The algorithm incorporates four variables: BMI, waist circumference, serum triglycerides and serum GGT and is expressed as follows:

$$FLI = \frac{(e^{0.953 \times In(triglycerides) + 0.139 \times BMI + 0.718 \times In(ggt) + 0.053 \times waist\ circumference - 15.754})}{(1 + e^{0.953 \times In(triglycerides) + 0.139 \times BMI + 0.718 \times In(ggt) + 0.053 \times waist\ circumference - 15.754})} \times 100$$

where triglycerides is in mg/dL, waist circumference in cm and BMI in kg/m^2. We categorised FLI in accordance with Bedogni's categorisation, as low FLI (<30), intermediate FLI (30–<60) and moderate to high FLI (>60), indicating no fatty liver, indeterminate and fatty liver, respectively.

Defining MS status
MS was defined in accordance with the harmonised criteria for diagnosis of MS.[20] The presence of any three of the following five risk factors constitutes a diagnosis of MS: waist circumference ≥120 cm; serum triglycerides ≥150 mg/dL (1.7 mmol/L) (or drug treatment for elevated triglycerides); HDL cholesterol <40 mg/

dL (1.0 mmol/L) (or drug treatment for reduced HDL cholesterol); blood pressure with systolic ≥130 and/or diastolic ≥85 mm Hg (or antihypertensive drug treatment in a patient with a history of hypertension); and fasting glucose ≥100 mg/dL (or drug treatment of elevated glucose).[20]

Outcome definitions

We defined incident T2D outcomes as self-reported physician-set diagnosis of T2D and/or fasting plasma glucose ≥7.0 mmol/L or 2-hour oral glucose tolerance test plasma glucose ≥11.1 mmol/L at re-examination rounds 4, 11 and 20 years after the baseline; and T2D information derived by record linkage to either the national hospital discharge registers or to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for T2D. Detection of T2D by self-report of physician-diagnosed T2D was followed by either detection via the hospital discharge registers or national drug reimbursement register. The proportion of the data obtained by the record linkage are as follows: hospital discharge registers: 42% and national drug reimbursement register: 58%. T2D cases that were included were those coded in the International Classification of Diseases, 10th Revision (code numbers from E11.0 to E11.9).

Patient and public involvement

The study was carried out in a non-patient research facility. All the study participants were volunteers. Neither the study participants nor the public were involved in the design of the study.

Statistical methods

All statistical analyses were performed using SPSS software V.21.0 for Windows. In all analyses, two-sided alpha <0.05 was considered statistically significant.

Descriptive analyses were performed to summarise baseline characteristics of participants according to baseline FLI categories. For continuous variables, we used Jonckheere trend test to test for linear trend across FLI categories. For categorical variables, we used χ^2 test to test for linear association across FLI categories. To make up for missing 0.4% values (spread across 50% of the variables and 13.4% of subjects), we used a regression-based multiple imputation method (40 iterations) according to guideline by Cheema.[21]

After confirmation of proportionality of hazards, we implemented a multivariable-adjusted Cox proportional hazards model to examine the relationship between baseline FLI and incident T2D considering metabolic factors and the MS statuses of the subjects as follows:

First, we analysed the overall association, adjusting for MS status. The models were as follows: model 1: examination year, constitutional factors (age and family history of T2D), lifestyle factors (smoking pack years, alcohol consumption, physical activity and consumption of fruits, berries and vegetables), inflammatory markers (C reactive protein, leucocyte count and thrombocyte count)

and metabolic factors (fasting glucose, insulin, HDL, LDL, systolic blood pressure and diastolic blood pressure); model 2: examination year, constitutional factors (age and family history of T2D), lifestyle factors (smoking pack years, alcohol consumption, physical activity and consumption of fruits and berries and vegetables) and inflammatory markers (C reactive protein, leucocyte count and thrombocyte count) and MS status.

In sensitivity analyses, we excluded men with a high weekly alcohol consumption of ≥168 g[17] before analysing the overall association of FLI with T2D in multivariable adjusted Cox proportional hazards as explained above. In addition, we excluded smokers before analysing the overall association of FLI with T2D in multivariable adjusted Cox proportional hazards.

Second, we performed subgroup analyses in which we stratified our study sample by MS status. We then performed multivariable-adjusted Cox proportional hazards analysis with adjustment for covariates to observe if the association of FLI with incident T2D differs by MS status in models 1 and 2 as explained above but excluding fasting glucose in model 2.

Third, for clearer understanding of the relation of the associations considering both FL and MS statuses, using the combination of FLI category and MS status as a composite variable, we performed multivariable-adjusted Cox proportional hazards analysis on the study population with adjustment for covariates as in model 1 above to elaborate the variation of the association by MS status.

RESULTS

Characteristics of the study population

The baseline characteristics of the study population (1792 men) according to FLI categories are shown in table 1. In general, the mean values and proportions for men in the intermediate FLI category were in between estimates for the lowest (reference) FLI category and estimates for the highest FLI category. Compared with the low FLI category, the high FLI category had a greater proportion of men with family history of diabetes and a greater proportion with family history of CVD. Men in the high FLI category consumed less fruit, berries and vegetables and more likely to be heavy alcohol consumers. They had higher mean waist circumference and mean BMI, and they were more likely to be hypertensive. They also had higher GGT levels, higher triglyceride, higher fasting insulin, higher blood glucose, lower HDL cholesterol and higher levels of markers of systemic inflammation. Generally, the range of values of the fasting blood glucose for the eligible men was between 3.1 mmol/L (minimum) and 6.2 mmol/L (or 112 mg/dL) (maximum).

Multivariable proportional hazards model analyses

During a mean (SD) follow-up of 18.8 (6.6) years, there were 375 cases of incident T2D. The incidence rate for T2D was 11 cases per 1000 person-years. Significantly lower survival free of incident T2D was noted for participants in high baseline FLI category compared with the low (normal) FLI category

Table 1 Baseline characteristics of 1792 men according to fatty liver index (FLI) categories

Characteristic	FLI <30 Mean (SD) or n (%) n=833	FLI =30–<60 Mean (SD) or n (%) n=552	FLI ≥60 Mean (SD) or n (%) n=407	P trend*
FLI	16.2 (7.7)	43.3 (8.1)	76.8 (10.6)	<0.001
Constitutional factors				
Age in years	52.6 (5.6)	53.4 (5.5)	52.5 (5.6)	0.251
Family history of diabetes	212 (25.5%)	145 (27.8%)	108 (26.5%)	0.651
Family history of CVD	667 (80.1%)	459 (83.2%)	341 (83.8%)	0.072
Lifestyle factors				
Smoking pack years	7.5 (16.0)	8.4 (16.8)	6.8 (13.6)	0.785
Alcohol consumption (g/week)	55 (89)	78 (117)	116 (165)	<0.001
Physical activity (energy exp.) (kcal/day)	136(156)	147(175)	129(192)	0.36
Fruit, berry and vegetable consumption (g/day)	265 (171)	261 (148)	233 (147)	0.023
Anthropometrics and physiological measurements				
Mean waist circumference (cm)	83.9 (6.1)	92.7 (5.2)	101.8 (8.1)	<0.001
BMI (kg/m^2)	24.4 (2.0)	27.3 (1.9)	30.7 (3.1)	<0.001
Mean systolic bp	135.5 (17.0)	135.7 (17.8)	135.9 (18.1)	<0.001
Mean diastolic bp	89.4 (10.5)	88.5 (10.6)	89.6 (11.1)	<0.001
Hypertension	259 (31.1%)	275 (49.8%)	261 (64.1%)	<0.001
Biomarkers				
Insulin	8.3 (3.0)	11.2 (4.4)	16.6 (9.7)	<0.001
Glucose (mmol/L)	4.5 (0.4)	4.6 (0.5)	4.8 (0.5)	<0.001
HOMA1-IR insulin resistance	1.86 (0.71)	2.60 (1.10)	3.91 (2.30)	<0.001
Total cholesterol (mmol/L)	5.71 (1.07)	5.93 (1.02)	6.05 (1.00)	<0.001
HDL cholesterol (mmol/L)	1.38 (0.32)	1.25 (0.26)	1.20 (0.27)	<0.001
LDL cholesterol (mmol/L)	3.91 (1.01)	4.09 (0.97)	4.01 (0.93)	0.04
Triglycerides (mmol/L)	0.94 (0.40)	1.35 (0.62)	1.93 (1.02)	<0.001
Gamma-glutamyl transferase (U/L)	18 (11)	28 (20)	51 (47)	<0.001
Albumin	42 (4)	42 (4)	43 (3)	<0.001
C reactive protein (m/L)	1.86 (4.46)	2.61 (4.54)	3.15 (4.26)	<0.001
Ferritin (µg/L)	128 (100)	172 (157)	235 (186)	<0.001
Fibrinogen g/L	2.92 (0.58)	3.06 (0.57)	3.10 (0.55)	<0.001
Leucocyte count ×10^9/L	5.4 (1.6)	5.7 (1.6)	5.9 (1.6)	<0.001
Metabolic syndrome and medication use history				
Metabolic syndrome	29 (3.5%)	91 (16.5%)	238 (58.5%)	<0.001
Drug for high cholesterol	7 (0.84%)	2 (0.36%)	6 (1.47%)	0.509
Drug for hypertension	111 (13.32%)	127 (23.05%)	141 (34.56%)	<0.001

*Jonckheere trend test for continuous variable. χ^2 linear-by-linear association for categorical variables.
BP, blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA1-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

at baseline (log-rank <0.001). Subjects in intermediate FLI category also separated clearly from those with low FLI for incident T2D.

Relation between baseline FLI and incident T2D
Overall analyses
Table 2 shows the association of FLI with incident T2D. In model 1, the HRs for incident T2D was 42% higher for the intermediate category, and 113% higher for the high FLI category, when compared with the low category. The association was maintained in model 2 with MS where high FLI category was associated with 163% increased risk. MS was also independently associated with incident T2D in the model with 77% increased risk (HR (95% CI): 1.77 (1.35 to 2.31)).

Sensitivity analyses
After exclusion of 241 men who were heavy alcohol consumers (table 3), the results were similar to those

Table 2 General association of baseline fatty liver index (FLI) with incident type 2 diabetes

FLI	Number of subjects (% with T2D) (IR)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
FLI	1792 (20.9) (11)	1.013 (1.007 to 1.018)*	1.017 (1.012 to 1.022)†
FLI category			
≤30 (Ref.)	833 (12.1) (6)	1.000	1.000
30–<60	552 (22.6) (12)	1.42 (1.07 to 1.88)	1.81 (1.38 to 2.37)
≥60	407 (36.6) (22)	2.13 (1.56 to 2.93)*	2.63 (1.92 to 3.59)†
P trend	–	<0.001	<0.001

Model 1: FLI, age, examination date, family history of diabetes, smoking pack years, alcohol consumption per week, physical activity, fruit–berry–vegetable consumption, C reactive protein, leucocytes albumin, fibrinogen and ferritin, systolic blood pressure, diastolic blood pressure, insulin, fasting glucose, LDL and HDL.
Model 2: FLI, age, examination date, family history of diabetes, smoking pack years, alcohol consumption per week, physical activity, fruit–berry–vegetable consumption, C reactive protein, leukocytes albumin, fibrinogen and ferritin and metabolic syndrome status.
*FLI uncategorised.
†Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin and glucose.
‡Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome=1.77(1.35 to 2.31).
HDL, high-density lipoprotein; IR, incidence rate per 1000 person-years; LDL, low-density lipoprotein; T2D, type 2 diabetes.

obtained in the analyses with the whole sample, as shown in table 2. Similarly, after exclusion of 571 men who were smokers (online supplementary appendix), the results were similar to those obtained in the analyses with the whole sample, as shown in table 1 of the online supplementary appendix.

Further exploration of the association of FLI with incident T2D across FLI categories of 10 (see figure 1) reveals steady increase in HR across the categories without any threshold areas. When we analysed our data with FLI as continuous variable, a unit increase in FLI was associated with 1.7% increase in HR (in the analyses with the whole sample) and 1.8% increase (after exclusion heavy alcohol consumers) as shown in tables 2 and 3.

Stratified analyses
Table 4 shows the results of Cox regression analysis when we stratified by MS status. In the stratus without MS, high FLI was associated with over 100% increased risk of T2D when compared with those in the low FLI category. Among those with MS, high FLI was not associated with additional risk when compared with those in the low FLI category.

Analysis with composite FLI-MS variable
In additional sensitivity analyses, with the combination of FLI category and MS status as composite exposure variable, when compared with subjects having neither fatty liver nor MS, having high FLI with no MS was associated with 219% increase in risk (the HR (95% CI) was 3.19

Table 3 Association of baseline fatty liver index (FLI) with incident type 2 diabetes after excluding men with high alcohol intake

FLI	Number of subjects (% with T2D) (IR)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1548 (20.9) (11)	1.014 (1.008 to 1.019)	1.018 (1.012 to 1.024)
FLI category			
≤30 (Ref.)	771 (12.5) (6)	1.000	1.000
30–<60	472 (23.1) (12)	1.43 (1.06 to 1.93)	1.78 (1.33 to 2.37)
≥60	305 (38.7) (23)	2.21 (1.57 to 3.10)	2.63 (1.89 to 3.66)
P trend	–	<0.001	<0.001

*FLI, FLI uncategorised.
Model 1: FLI, age, examination date, family history of diabetes, smoking pack years, alcohol consumption per week, physical activity, fruit–berry–vegetable consumption, C reactive protein, leucocytes albumin, fibrinogen and ferritin, systolic blood pressure, diastolic blood pressure, insulin, fasting glucose, LDL and and HDL.
Model 2: FLI, age, examination date, family history of diabetes, smoking pack years, alcohol consumption per week, physical activity, fruit–berry–vegetable consumption, C reactive protein, leucocytes albumin, fibrinogen and ferritin and metabolic syndrome status.
*Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin and fasting glucose.
†Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome=1.65 (1.24 to 2.21).
HDL, high-density lipoprotein; IR, incidence rate per 1000 person-years; LDL, low-density lipoprotein.
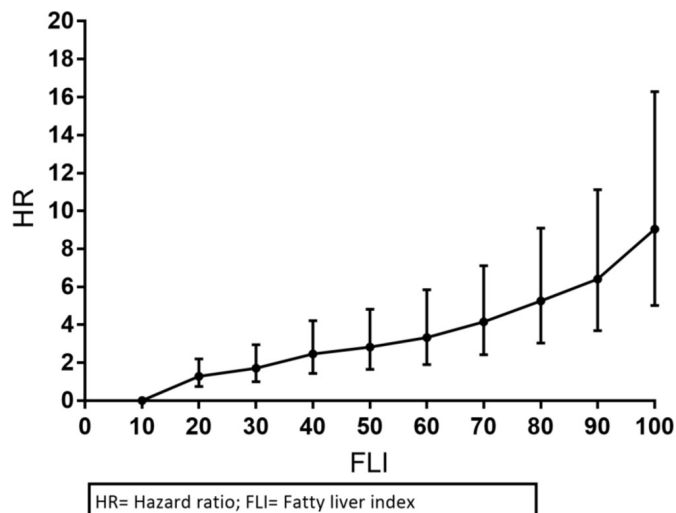
HR= Hazard ratio; FLI= Fatty liver index

Figure 1 Graph of FLI and risk of incident type 2 diabetes.

(2.26 to 4.51)). Having normal FLI with MS was associated with 331% increased risk (the HR (95% CI) was 4.31 (2.15 to 8.61)), and persons having high FLI and MS were at greatest risk, with 366% increase in risk (HR (95% CI) 4.66 (3.42 to 6.35)). The presence of MS was associated with greater risk in intermediate and high FLI categories (the HRs (95% CI) were 3.77 (2.50 to 5.70) for presence of MS with intermediate FLI category and 4.66 (3.42 to 6.35) for the presence of MS with high FLI category).

DIABETES PREDICTION
Discussion
We examined the association of FLI, a surrogate of FLD, in relation to incident T2D in a population of middle-aged men while taking the baseline MS status into account. We found that although FLD assessed by FLI predicts the risk

Table 4 Association of fatty liver index (FLI) with incident type 2 diabetes by metabolic syndrome status (subanalyses)

No metabolic syndrome	Number of subjects (% with T2D) (IR)	Model 1 hour (95% CI)	Model 2 hour (95% CI)
FLI*	1427 (16.7) (9)	1.021 (1.015 to 1.027)	1.017 (1.010 to 1.025)†
FLI category			
≤30 (Ref.)	803 (11.5) (6)	1.00	1.00‡
30–<60	456 (20.0) (11)	1.81 (1.33 to 2.46)	1.58 (1.14 to 2.19)
≥60	168 (33.3) (18)	3.07 (2.14 to 4.41)	2.38 (1.58 to 3.58)
P trend		<0.001	<0.001
Metabolic syndrome			
FLI*	358 (37.7) (24)	1.007 (0.997 to 1.016)	0.996(0.992-1.000)§
FLI category			
≤30 (Ref.)	29 (31.0) (23)	1.000	1.000¶
30–<60	91 (36.3) (21)	0.77 (0.35 to 1.70)	0.80 (0.59 to 1.09)
≥60	238 (39.1) (25)	1.02 (0.49 to 2.16)	0.79 (0.58 to 1.06)
P trend		0.42	0.22
Category by FLI and MS status			
FLI ≤30 MS	803 (11.5) (6)	1.000	–
FLI 30–<60 MS	456 (20.0) (11)	1.79 (1.33 to 2.41)	–
FLI ≥60 MS	168 (33.3) (18)	3.19 (2.26 to 4.51)	–
FLI ≤30 MS+	29 (31.0) (23)	4.31 (2.15 to 8.61)	–
FLI 30–<60MS+	91 (36.3) (21)	3.77 (2.50 to 5.70)	–
FLI ≥60MS+	238 (39.1) (25)	4.66 (3.42 to 6.35)	–

Model 1: FLI, age, examination date, family history of diabetes, smoking pack years, alcohol consumption per week, physical activity, fruit–berry–vegetable consumption, C reactive protein, leukocytes, thrombocytes, fibrinogen and ferritin.
Model 2: Model 1 plus systolic blood pressure, diastolic blood pressure, insulin, LDL and HDL.
*FLI uncategorised.IR – incidence rate per 1000 person-years
†Other independent predictors of T2D in the model were serum ferritin and insulin.
‡Other independent predictors of T2D in the model were serum ferritin and insulin.
§Independent predictors of T2D in the model were fasting glucose and insulin.
¶Independent predictors of T2D in the model were fasting glucose and insulin.
Statistically significant at p≤0.05.
IR, incidence access rate per 1000 person-years; MS, metabolic syndrome; MS⁻, metabolic syndrome negative; MS⁺, metabolic syndrome positive; T2D, type 2 diabetes.

of T2D in the study population, the association was strongest among persons without MS at baseline.

Few studies have investigated the association of baseline FLI as categorised by Bedogni *et al*, with incident T2D.[10 22] Jäger *et al*[22] and Onat *et al*[10] studied the association of FLI with incident T2D in healthy populations, followed up for 8 years. Nishi *et al*[23] studied the association of FLI with incident T2D in a population of prediabetic subjects followed up for 3 years.[23]

A few studies, Balkau *et al*[24] and Jung *et al*,[9] also reported the association of FLI with incident T2D using FLI categorisation different from that proposed by Bedogni *et al*.[9 24] Because previous studies on the association of FLI with incident T2D have adjusted for different groups of variables in their multivariable models, we are careful in our comparison of findings.

Our finding that high FLI (FLI≥60) indicating fatty liver is associated with increased risk independent of constitutional and lifestyle factors agrees with findings from previous findings by Jäger *et al*[22] and Onat *et al*.[10] We found a 2–3-fold increased risk in our multivariable adjusted models. However, Jäger *et al* reported 11-fold increase, while Onat *et al* reported a fivefold increase. Our finding that intermediate FLI is also associated with increased risk of T2D is also in line with reports by Jäger *et al*.

Our finding that high FLI is associated with incident T2D even after adjusting for metabolic factors agrees with other reports,[9 10 24] each of which used different cut-off points in categorising FLI. Balkau *et al* used FLI <20 and FLI ≥70 as lower and upper cut-off points and adjusted for glucose, insulin and hypertension. Jung *et al* used FLI <20 and FLI ≥60 as lower and upper cut-off points. When we reanalysed our data using these cut-off points, the results (data not shown) did not differ markedly from what we present here.

Indeed, ultrasound diagnosed NAFLD has been shown to be associated with incident T2D, and the association is not affected by adjustment for MS.[5] However, the predictability of T2D independent of MS using FLI needs to be clarified.

We are unable to compare our findings on association of FLI with incident T2D in view of MS status of the subjects, with previous studies on the association between FLI and incident T2D because previous studies on the association did not consider the MS status of the subjects. However, this finding corroborates the report by Shibata *et al*.[25] Shibata *et al* found that the presence of fatty liver, as diagnosed by ultrasonography, is associated with increased risk of T2D when compared with those without fatty liver after adjusting for age, BMI, smoking status, physical activity and MS status.[25]

The finding of similar results, after excluding men who were heavy consumers of alcohol, indicates that our findings are also applicable to NAFLD. However, despite the multifactorial nature of the aetiology of FLD, the relative contribution of heavy ethanol intake in the pathogenesis of fatty liver is still uncertain.[26] Therefore, we did not exclude men with high alcohol intake in our main analysis. The finding of similar results after excluding smokers proves further that smoking is not a confounder in this target population.

Stratification by MS status did not reveal significant association of high FLI with incident T2D in subjects with MS, despite the fact that increasing proportions of subjects with MS developed T2D across the FLI categories. This suggests that among persons with MS, which is already a cluster of risk factors for T2D (including hyperglycaemia), high FLI is likely not associated with significantly higher risk than that due to positive MS status alone. It also suggests that the MS status was an effect modifier in the overall analysis.

Notwithstanding, our findings from the analysis with FLI-MS composite variable are noteworthy. It appears that FLI predicts risk of T2D in a dose-dependent manner among subjects without MS, but among subjects with MS, it does not predict risk of T2D in a dose-dependent manner. The additional risk associated with high FLI appears less than that associated with MS positive status. Therefore, the greatest risk was in subjects with both fatty liver and MS positivity. Our finding that the presence of MS appears to be associated with higher risks than high FLI may be consistent with the finding by Käräjämäki *et al*.[27] However, Käräjämäki *et al* observed from their data that, in the absence of MS, fatty liver does not tend to pose a higher risk for development of T2D in comparison with healthy subjects.[27] Our finding that, compared with healthy subjects (persons with normal FLI and no MS), persons having high FLI and negative MS status were at increased risk disagrees with their observation.

Comparison of risks with FLI <10 as the reference reveals steady increase in risk across FLI (figure 1). This supports the suggestion that, even among subjects with intermediate FLI, the risk of incident T2D increases with increasing FLI values.

Our findings can be explained in the light of current knowledge. It is thought that an initial development of insulin resistance results in compensatory hyperinsulinaemia and, together with visceral obesity, promotes the development of FLD.[28] In return, the insulin-resistant fatty liver overproduces glucose and very low-density lipoprotein. This boosts mechanisms that lead to exhaustion of pancreatic beta cell reserve, eventually leading to the development of T2D.[28] Steatotic and inflamed liver secretes hepatokines such as fetuin-A, fetuin-B, angiopoietin-like proteins, fibroblast growth factor 21 and selenoprotein P that have endocrine function at extrahepatic sites to cause insulin resistance and other adverse effects on glucose homeostasis.[29] Hence, the association of high FLI with T2D.

Our finding that MS positive status is associated with higher risk than high FLI and the co-occurrence of MS with fatty liver is associated with the greatest risk raises the suspicion that the MS phenomenon represents a more advanced stage than FLD does, in the pathogenesis of T2D, as proposed by Shibata *et al*[25] and suggested in

recent epidemiological studies.[27] However, this does not explain the population of persons with normal liver (low FLI) among people with MS.

The novel finding in our study is that although high FLI (FLD) is associated with increased risk incident T2D, MS phenomenon, which may occur regardless of FLD, modifies this association. However, the association is more clearly demonstrated when the reference group comprises subjects with normal liver and no MS. MS positive status can also predict T2D independent of FLI. In addition, from our data, MS status is associated with higher risk than presence of fatty liver (FLI ≥60). However, FLI predicts T2D in subjects without MS. Although FLI appears to be a less efficient predictor of T2D among subjects with MS, the copresence of fatty liver and MS positive status is associated with higher risk than that associated with MS alone. The reason why FLI did not predict T2D among MS subjects is unclear. Our data revealed that among subjects with MS, the association conferred by GGT and BMI (components variables of FLI that are not in included in MS) is not significant when compared with that conferred by insulin resistance and hyperglycaemia. However, this finding of disparate association of FLD with T2D, by MS status, needs to be studied further.

Our current study findings have clinical implications. First, we show that FLI, a surrogate of hepatic steatosis, predicts risk of incident T2D especially in persons who are negative for MS. Second, the association can be affected by metabolic factors or MS status. This suggests that FLD can also play a role in the pathogenesis of T2D. Therefore, both FLD and MS are useful for screening risk of incident T2D. From health systems perspective, because high FLI has also been associated with increased risk of CVD[30] and it appears to be detectable before MS may be apparent, screening with FLI may be more cost-effective in asymptomatic persons. The finding of FLI in high category should then prompt further evaluation for T2D and CVD.

Our study does have a number of limitations. First, FLI as a surrogate of fatty liver does not detect progression of FLD. Therefore, we are unable to differentiate the contribution of non-alcoholic steatohepatitis (NASH) and fibrosis to the observed association. Another limitation of the study is that the hepatitis B and hepatitis C statuses of the subjects were not established at baseline. The prevalence rate of hepatitis B and hepatitis C, however, have remained low in the Finnish population.[31 32] Also, our study population comprised of men only. There are reports that suggest that lower FLI cut-off values may apply to women.[33] We are unable to explore the influence of gender on the predictability of T2D using FLI. Nevertheless, Bedogni et al[7], concluded that the influence of gender in FLI is related to insulin and skinfold thickness and probably insignificant.

The strength of our study lies in the prospective design. With this, we are able to demonstrate the ability of FLI, a surrogate of hepatic steatosis, to predict incident occurrence of T2D. We have also adjusted for a range of constitutional factors, lifestyle factors and biomarkers keeping in cognizance the components of both major exposure variables to control for the possible confounding factors in the predictability of T2D using FLI.

CONCLUSION

In conclusion, our data show that high FLI category (FLD) is associated with increased risk of incident T2D in men without MS. Persons with high FLI should be further evaluated for FLD, and if FLD is present, they should be evaluated and monitored for T2D. FLD assessed using FLI can be used as additional screening tool for persons at increased risk of incident T2D in the general population. Both FLI and MS are useful and can complement each other in screening and surveillance of persons at increased risk of T2D. In such persons, appropriate preventive or treatment measures should be instituted to improve their prognoses.

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