BMJ Open

Association of Fatty Liver Index with risk of Incident Type 2 Diabetes by Metabolic Syndrome Status

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026949
Article Type:	Research
Date Submitted by the Author:	28-Sep-2018
Complete List of Authors:	Olubamwo, Olubunmi; Ita-Suomen yliopisto Terveystieteiden tiedekunta, Public Health and Cliical Nutrition Virtanen, Jyrki K.; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition Pihläjämäki, Jussi; Public Health and Clinical Nutrition; Kuopio University Hospital, KYS , Clinical Nutrition and Obesity Center Tuomainen, Tomi-Pekka; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition
Keywords:	EPIDEMIOLOGY, Hepatology < INTERNAL MEDICINE, Diabetes & endocrinology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, PUBLIC HEALTH

Association of Fatty Liver Index with risk of Incident Type 2 Diabetes by Metabolic Syndrome Status

Olubunmi O. Olubamwo [1], Jyrki K. Virtanen[1], Jussi Pihlajamäki[1,2], Tomi-Pekka Tuomainen [1]

[1] Institute of Public Health and Clinical Nutrition, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland.
[2] Clinical Nutrition and Obesity Center, Kuopio University Hospital, KYS Kuopio, Finland.

Running head: FLI and risk of T2D by MS status. Olubamwo et al.

Word count: main text 3864 words, abstract 267 words.

*Corresponding author:

Olubunmi O, Olubamwo
Institute of Public Health and Clinical Nutrition
Faculty of Health Sciences
University of Eastern Finland, Kuopio,
P.O.Box 1627, FI-70211 Kuopio, Finland.
E-mail : olubunmi.olubamwo@uef.fi Tel : +358 403552905.

ABSTRACT

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

Design and Participants: In this prospective epidemiological study, our subjects were 1792 men without diabetes at baseline in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort. Using fatty liver index (FLI), the association of baseline FLD with incident T2D was analyzed in multivariable-adjusted Cox regression models, considering their MS statuses.

Results: During a mean follow-up of 19 years, 375 incident cases of T2D were recorded. In overall analysis, in model adjusted for constitutional, lifestyle and inflammation biomarkers, for the high (FLI≥60) vs low (FLI<30) FLI category, the hazard ratio (HR (95%CI)) was 3.68(2.80-4.82). The association was attenuated, but maintained, with further adjustment for metabolic factors. Including MS status instead of metabolic factors, the HRs (95%CIs) were 2.63(1.92-3.59) for FLI≥60 and 1.77(1.35-2.31) for MS.

In MS-stratified analysis, FLI predicted T2D only among persons without MS.

In unstratified analysis with subjects categorized by FLI-MS, compared with persons with FLI<30 without MS, persons with FLI≥60 without MS had increased risk (HR= 3.19(2.26-4.52)). Persons with FLI<30 and MS had greater risk (HR= 4.31(2.15-8.61)) and, persons with both FLI≥60 and MS had the greatest risk (HR=4.66(3.42-6.35)).

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

KEY WORDS: fatty liver index, fatty liver disease, type 2 diabetes, metabolic syndrome, predictor, metabolic factors, Kuopio Ischaemic Heart Disease Risk Factor Study

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 subjects with respect to viral hepatitis were not established at baseline, although, viral hepatitis have remained low in the Finnish population.

INTRODUCTION / BACKGROUND

There is increasing recognition that fatty liver disease (FLD), also known as hepatic steatosis, is the commonest cause of chronic liver disease worldwide and that it is associated with increased risk of cardiovascular disease and Type 2 diabetes (T2D). Although the prevalence varies in different populations, it is also on the rise. Recent estimates suggest a global prevalence of 25% among adults, with the highest prevalence in the Middle East and South America and lowest in Africa [1]. The prevalence is estimated to be 24% in Europe and more than 30% in developed countries [1]. Approximately one third of FLD patients progress to steatohepatitis with fibrosis, which can progress to cirrhosis, liver failure and hepatocellular carcinoma [1]. In addition, 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].

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

Liver biopsy, which is the gold standard for characterizing liver histology in patients with fatty liver, is expensive and carries some morbidity and very rare mortality risks [6]. The fatty liver index (FLI), an algorithm comprising of body mass index, waist circumference, gamma-glutamyl transferase (GGT) and triglyceride concentrations, was developed by Bedogni et al., to predict the presence of FLD. The algorithm has been widely validated, and is gaining acceptance [7, 8]. There has been reports of association of high FLI (FLD) with incident T2D [9], [10]. However, with FLD being intimately linked with metabolic diseases, it is not known if 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 consisted of participants in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The KIHD study is a prospective population-based study 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. They were enrolled in the baseline examinations between March 1984 and December 1989. The men were 42, 48, 54, or 60 years of age at baseline. The study was approved by the Research Ethics Committee of the University of Kuopio [11], and the subjects gave their written consent.

Data Collection: Data were collected through self-administered questionnaires, interviews, physical examinations, and various blood tests to determine physiological and biochemical parameters [12, 13]. The self-administered questionnaires were used to collect data on medical history, including history of type 2 diabetes, metabolic diseases, liver disease, etc., 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]. Categorization 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].

The 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 4-day food recording [18].

Physical examinations included anthropometric measurements and indices, vital signs, and physiologic measurements. All physical measurements were measured following standard protocol. Waist circumference was calculated as the mean of waist circumference taken at maximal inspiration and that taken at maximal expiration. Body mass index (BMI) was computed as the ratio of weight in Kg to the square of height in meters (kg/m2). Blood pressure, which was measured by two separate nurses, was taken as the mean of, three measurements in supine, one on standing and two in 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 12 hours 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, HDL cholesterol, LDL cholesterol, serum triglycerides), liver function tests including albumin, gamma-glutamyl transferase, 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 subjects at baseline was 2682. Of these, we excluded 40 subjects with history of physician diagnosed liver or pancreas disease, and 162 subjects with history of diabetes. Of the remaining 2480 subjects, 1792 who had complete data for FLI calculation, were included in the analyses.

Measuring the components of the Fatty liver index: We calculated FLI using the algorithm developed by Bedogni et al [7]. The algorithm, incorporates four variables: BMI, waist

circumference, serum triglycerides, and serum gamma-glutamyl transferase (GGT), and is expressed as follows:

$$FLI = \frac{(e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})}{(1 + e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})} \times 100$$

Where triglycerides is in mg/dl, waist circumference in cm, and BMI in Kg/m^2. We categorized FLI in accordance with Bedogni et al's categorization, as low FLI (<30), intermediate FLI (30-<60), and moderate-high FLI (>60), indicating no fatty liver, indeterminate, and fatty liver, respectively.

Defining Metabolic Syndrome Status: MS was defined in accordance with the harmonized criteria for diagnosis of MS [20]. The presence of any 3 of the following 5 risk factors constitutes a diagnosis of metabolic syndrome: 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); 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 ≥7.0mmol/L or 2-h oral glucose tolerance test plasma glucose ≥11.1 mmol/L at re-examination rounds 4, 11, and 20 years after the baseline and; 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. T2D cases that were included were those coded in the Tenth International Classification of Diseases (ICD 10 code numbers from E11.0 to E11.9).

Patient and public involvement: The study was carried out at 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 Version 21.0 for Windows (IBM, Chicago, IL). In all analyses, two-sided alpha <0.05 was considered statistically significant.

Descriptive analyses were performed to summarize 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 Chi-Square 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 2014 [21].

After confirmation of proportionality of hazards, we proceeded with multivariable-adjusted Cox proportional hazards analysis, to analyze the association of baseline FLI with incident T2D in considering metabolic factors and the MS statuses of the subjects as follows:

First, we analyzed the association overall, 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) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count). Model 2- Model 1 variables plus metabolic factors (fasting glucose, insulin, HDL, LDL, systolic blood pressure, diastolic blood pressure). Model 3- Model 1 variables plus MS status.

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

Secondly, in stratified analyses, we stratified our population 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 model 1 and 2 as explained above.

Thirdly, 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 subjects 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, men in the high FLI category had a greater proportion of subjects with family history of diabetes, and a greater proportion with family history of CVD. They consumed less fruit, berries and vegetables, had a higher proportion of heavy alcohol consumers, but a lower proportion of smokers. 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.

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 rates for T2D were 11 cases per 1000 person-years. Significantly lower survival free of incident T2D was noted for participants in high baseline FLI category compared to the low (normal) FLI category 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 Fatty Liver Index and incident T2D

Overall analyses: Table 2 shows the association of FLI with incident T2D. In model 1, the HRs for incident T2D was 98% higher for the intermediate category, and 268% higher for the high FLI category, when compared with the low category. The association was maintained, though attenuated in model 2, and in model 3 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(1.35-2.31)).

Sensitivity analyses - after exclusion of 241 men who were heavy alcohol consumers (Table 3), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 2. 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 analyzed 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. Among those without MS, when compared with those in the low FLI category, high FLI was associated with over 100% increased risk of T2D. Among those with MS, when compared with those in the low FLI category, high FLI was not associated with additional risk.

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

3.77(2.50-5.70) for presence of MS with intermediate FLI category, and 4.66(3.42-6.35) for the presence of MS with high 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 physiologic 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
Leukocyte count $x10^9$/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. Chi-Square linear-by-linear association for categorical variables. bp-blood pressure

DIABETES PREDIDICTION

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)	Model 3 HR (95% CI)
FLI*	1792 (20.9) (11)	1.022(1.018-1.027)	1.013(1.007-1.018)[a]	1.017(1.012-1.022)[b]
FLI category				
≤30 (Ref.)	833 (12.1) (6)	1.000	1.000	1.000
30-<60	552 (22.6) (12)	1.98(1.52-2.59)	1.42(1.07-1.88)	1.81(1.38-2.37)
≥60	407 (36.6) (22)	3.68(2.80-4.82)	2.134(1.56-2.93)[a]	2.63(1.92-3.59)[b]
P-trend	-	<0.001	<0.001	<0.001

*FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 albumin, fibrinogen, and ferritin.

Model 2: Model 1 plus systolic blood pressure, diastolic blood pressure, insulin, fasting glucose, LDL, and HDL.

Model 3: Model 1 plus metabolic syndrome status.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and glucose.
[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.77(1.35-2.31).

Table 3 – Association of baseline fatty liver index 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)	Model 3 HR (95% CI)
FLI*	1548(20.9) (11)	1.023(1.018-1.027)	1.014(1.008-1.019)	1.018(1.012-1.024)
FLI category				
≤30 (Ref.)	771(12.5) (6)	1.000	1.000	1.000
30-<60	472(23.1) (12)	1.96(1.48-2.60)	1.43(1.06-1.93)	1.78(1.33-2.37)
≥60	305(38.7) (23)	3.61(2.71-4.81)	2.21(1.57-3.10)	2.63(1.89-3.66)
P-trend	-	<0.001	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years
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 albumin, fibrinogen, and ferritin.
Model 2: Model 1 plus systolic blood pressure, diastolic blood pressure, insulin, fasting glucose, LDL, and HDL.
Model 3: Model 1 plus metabolic syndrome status.
[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and fasting glucose.
[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.65(1.24-2.21).

Table 4 – Association of fatty liver index (FLI) with incident type 2 diabetes by metabolic syndrome status (sub-analyses)

No metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	1427(16.7) (9)	1.021 (1.015-1.027)	1.017 (1.009-1.024)[a]
FLI category			
≤30 (Ref.)	803(11.5) (6)	1.00	1.00[b]
30-<60	456(20.0) (11)	1.81(1.33-2.46)	1.40(1.01-1.94)
≥60	168(33.3) (18)	3.07(2.14-4.41)	2.38(1.58-3.58)
p-trend		<0.001	<0.001
Metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	358(37.7)(24)	1.007(0.997-1.016)	1.003(0.992-1.014)[c]
FLI category			
≤30 (Ref.)	29(31.0) (23)	1.000	1.000[d]
30-<60	91(36.3) (21)	0.77(0.35-1.70)	0.95(0.41-2.24)
≥60	238(39.1) (25)	1.02(0.49-2.16)	1.23 (0.54-2.82)
p-trend		0.42	0.42
Category by FLI and MS status	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	

FLI≤30 MS^{-}	803(11.5) (6)	1.000	-
FLI30-<60MS^{-}	456(20.0) (11)	1.79(1.33-2.41)	-
FLI≥60MS^{-}	168(33.3) (18)	3.19(2.26-4.51)	-
FLI≤30 MS^{+}	29(31.0) (23)	4.31(2.15-8.61)	-
FLI30-<60MS^{+}	91(36.3) (21)	3.77(2.50-5.70)	-
FLI≥60MS^{+}	238(39.1) (25)	4.66(3.42-6.35)	-

*FLI uncategorized, Ref – reference. HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

‡Statistically significant at P≤0.05. MS – Metabolic syndrome. MS^{-} - Metabolic syndrome negative. MS^{+} - Metabolic syndrome positive.

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, fasting glucose, LDL, and HDL

[a] Other independent predictors of T2D in the model were serum ferritin and insulin.

[b] Other independent predictors of T2D in the model were serum ferritin and insulin.

[c] Independent predictors of T2D in the model were fasting glucose and insulin.

[d] Independent predictors of T2D in the model were fasting glucose and insulin.

Figure 1 – Graph of FLI and risk of incident T2D.

DISCUSSION

We examined the association of FLI, as a surrogate of fatty liver, with incident T2D in a population of middle-aged men, considering the baseline MS status. Specifically, we found that although FLD assessed by FLI predicts risk of T2D in the whole population, the association was strongest among those without MS at baseline.

Few studies have investigated the association of baseline FLI as categorized by Bedogni et al., with incident T2D [10, 22]. Jager et al.[22] and Onat et al. [10], studied the association of FLI with incident T2D in healthy populations, followed up for eight years. Nishi et al.[23], studied the association of FLI with incident T2D in a population of prediabetic subjects followed up for three 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 categorization 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 analyses, we are careful in our comparison of findings.

Our finding that high FLI (FLI≥60) indicating fatty liver, as compared with low FLI(FLI<30) is associated with increased risk independent of constitutional and lifestyle factors, agrees with findings from previous studies by Jager et al.[22] and Onat et al.[10]. We found a 2 to 3-fold increased risk in our multivariable adjusted models. However, Jager et al., reported 11-fold increase while Onat et al reported a 5-fold increase. Our finding, that FLI 30-<60 is also associated with increased risk of T2D, is also in line with reports by Jager et al.

Our finding that high FLI is associated with incident T2D even after adjusting for metabolic factors, agrees with reports from Onat et al.[10], Balkau et al.[24] and Jung et al. [9], each of which used

different cut-off points in categorizing 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 analyzed 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 metabolic syndrome [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, our finding, in our man analysis, that high FLI category is associated with increased risk of incident T2D after adjusting for of MS status agrees with 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, the relative contribution of heavy ethanol intake in the pathogenesis of fatty liver is still uncertain [26].

It is remarkable that, when we stratified by MS status, the association of high FLI with incident T2D did not reach statistical significance among 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 to 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 hyperinsulinemia and, together with visceral obesity, promotes the development of FLD [28]. In return, the insulin resistant fatty liver overproduces glucose and VLDL. 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 of 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 co-presence 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. Firstly, we show that FLI, a surrogate of hepatic steatosis, predicts risk of incident T2D especially in persons who are negative for MS. Secondly, 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.

Our study does have a number of limitations. Firstly, FLI as a surrogate of fatty liver does not detect progression of FLD. Therefore, we are unable to differentiate the contribution of 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 (Karvonen 2016) (Safreed-Harmon 2018). Also, our study population comprised of men only. There are reports that suggest that lower FLI cut off values may apply to women [30]. We are unable to explore the influence of gender on the predictability of T2D using FLI. Nevertheless, Bedogni et al., concluded that the influence of gender in FLI is related to insulin and skinfold thickness, and probably insignificant [7].

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, especially in subjects without MS. Persons with high FLI should be further evaluated for FLD and, if found with FLD, 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.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

OOO and T-PT conceived the study. OOO and T-PT designed the study. OOO performed the statistical analyses. OOO wrote the first draft of the manuscript. JV , JP and T-PT contributed towards development of this manuscript. All authors reviewed the final draft of the manuscript.

Data sharing statement:

No additional data are available.

Acknowledgements:

OOO received grants from the Finnish Cultural Foundation Central, Finland, in partial support for the conduct of this study.

REFERENCES

1 Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology* 2016;64:73-84.

2 Kwon Y, Oh S, Hwang S, et al. Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults, *Am J Gastroenterol* 2012;107:1852.

3 Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives, *Nature Reviews Endocrinology* 2012;8:228-36.

4 Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease, *Dig Dis* 2010;28:210-3.

5 Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity, *Ann Med* 2011;43:617-49.

6 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association, *Hepatology* 2012;55:2005-23.

7 Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population, *BMC Gastroenterol* 2006;6:33.

8 Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease, *Aliment Pharmacol Ther* 2014;40:1209-22.

9 Jung C, Lee W, Hwang J, et al. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population, *Diabetic Med* 2013;30:428-35.

10 Onat A, Can G, Kaya A, et al. Fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality, *World J Gastroenterol* 2015;21:13555-65.

11 Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study, *Ann Clin Res* 1988;20:46-50.

12 Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men, *Circulation* 1995;91:645-55.

13 RĀSĀNEN J, Kauhanen J, Lakka T, et al. Religious affiliation and all-cause mortality: a prospective population study in middle-aged men in eastern Finland, *Int J Epidemiol* 1996;25:1244-9.

14 Laukkanen JA, Pukkala E, Rauramaa R, et al. Cardiorespiratory fitness, lifestyle factors and cancer risk and mortality in Finnish men, *Eur J Cancer* 2010;46:355-63.

15 Willenbring ML, Massey SH, Gardner MB. Helping Patients Who Drink Too Much: An Evidence-Based Guide for Primary Care Physicians. *Am Fam Physician* 2009;80.

16 McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011, *Adv Nutr* 2011;2:293-4.

17 Fawehinmi TO, Ilomäki J, Voutilainen S, et al. Alcohol consumption and dietary patterns: the FinDrink study, *PLoS One* 2012;7:e38607.

18 Virtanen JK, Mursu J, Tuomainen TP, et al. Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study, *Arterioscler Thromb Vasc Biol* 2014;34:2679-87.

19 Kauhanen J, Kaplan GA, Goldberg DE, et al. Pattern of alcohol drinking and progression of atherosclerosis, *Arterioscler Thromb Vasc Biol* 1999;19:3001-6.

20 Alberti K. International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; Wo, *Circulation* 2009;120:1640-5.

21 Cheema JR. Some general guidelines for choosing missing data handling methods in educational research, *Journal of Modern Applied Statistical Methods* 2014;13:3.

22 Jäger S, Jacobs S, Kröger J, et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam Study, *PloS one* 2015;10:e0124749.

23 Nishi T, Babazono A, Maeda T, et al. Evaluation of the fatty liver index as a predictor for the development of diabetes among insurance beneficiaries with prediabetes, *Journal of diabetes investigation* 2015;6:309-16.

24 Balkau B, Lange C, Vol S, et al. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study, *BMC Gastroenterol* 2010;10:56,230X-10-56.

25 Shibata M, Kihara Y, Taguchi M, et al. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men, *Diabetes Care* 2007;30:2940-4.

26 Bedogni G, Bellentani S. Fatty liver: how frequent is it and why, *Ann Hepatol* 2004;3:63-5.

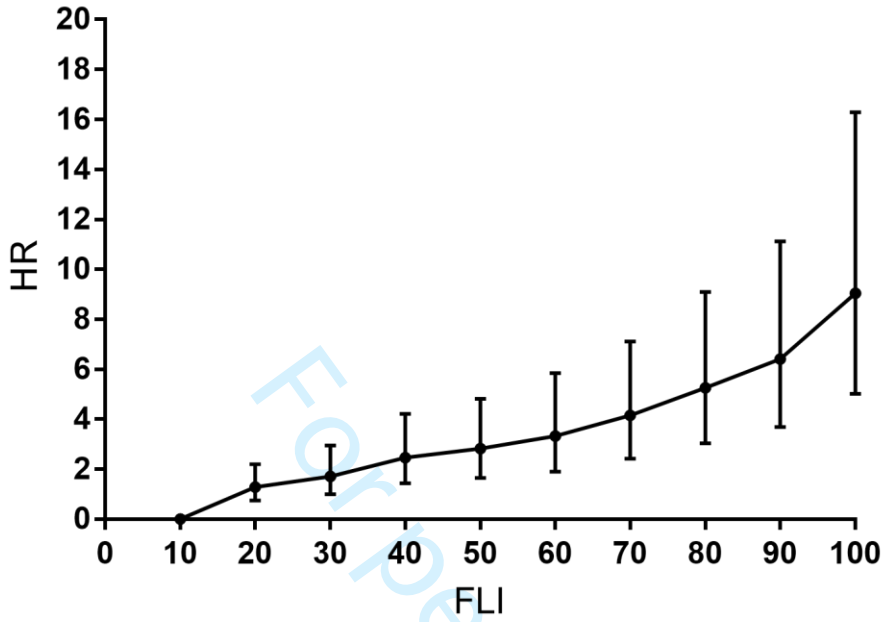
27 Karajamaki AJ, Bloigu R, Kauma H, et al. Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term outcomes, *Metabolism-Clinical and Experimental* 2017;66:55-63.

28 Valenti L, Bugianesi E, Pajvani U, et al. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? *Liver International* 2016;36:1563-79.

29 Targher G, Byrne CD. Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications, *The Journal of Clinical Endocrinology & Metabolism* 2013;98:483-95.

30 Yang BL, Wu WC, Fang KC, et al. External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan, *PLoS One* 2015;10:e0120443.

Figure 1. Variation of hazard ratio of incident type 2 diabetes with baseline fatty liver index



HR= Hazard ratio; FLI= Fatty liver index

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	2, 5
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	7-8
Bias	9	Describe any efforts to address potential sources of bias	7, 9-10
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	9
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	9-10
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	7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, 9
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, Tables 2 & 3
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	9-11, Tables 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	9-11, Tables 2 & 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, Table 4, Figure 1
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
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 cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.

BMJ Open

Association of Fatty Liver Index with risk of Incident Type 2 Diabetes by Metabolic Syndrome Status in an Eastern Finland Male Cohort

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026949.R1
Article Type:	Research
Date Submitted by the Author:	21-Mar-2019
Complete List of Authors:	Olubamwo, Olubunmi; Ita-Suomen yliopisto Terveystieteiden tiedekunta, Public Health and Cliical Nutrition Virtanen, Jyrki K.; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition Pihläjämäki, Jussi; Public Health and Clinical Nutrition; Kuopio University Hospital, KYS , Clinical Nutrition and Obesity Center Tuomainen, Tomi-Pekka; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Gastroenterology and hepatology, Public health
Keywords:	EPIDEMIOLOGY, Hepatology < INTERNAL MEDICINE, Diabetes & endocrinology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, PUBLIC HEALTH

Association of Fatty Liver Index with risk of Incident Type 2 Diabetes by Metabolic Syndrome Status in an Eastern Finland Male Cohort

Olubunmi O. Olubamwo [1], Jyrki K. Virtanen[1], Jussi Pihlajamäki[1,2], Tomi-Pekka Tuomainen [1]

[1] Institute of Public Health and Clinical Nutrition, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland.
[2] Clinical Nutrition and Obesity Center, Kuopio University Hospital, KYS Kuopio, Finland.

Running head: FLI and risk of T2D by MS status. Olubamwo et al.

Word count: main text 3897 words, abstract 288 words.

*Corresponding author:

Olubunmi O, Olubamwo
Institute of Public Health and Clinical Nutrition
Faculty of Health Sciences
University of Eastern Finland, Kuopio,
P.O.Box 1627, FI-70211 Kuopio, Finland.
E-mail : olubunmi.olubamwo@uef.fi Tel : +358 403552905.

ABSTRACT

Objective: Fatty liver disease (FLD) is increasingly recognized 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 center in Kuopio, Eastern Finland.

Participants: our subjects were 1792 Finnish men without diabetes at baseline in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort.

Outcome Measure: Using fatty liver index (FLI), the association of baseline FLD with incident T2D was analyzed 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) vs 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 hazard ratio (HR (95%CI)) for T2D was 3.68(2.80-4.82). 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(1.92-3.59) for FLI≥60 and 1.77(1.35-2.31) for MS.

In MS-stratified analysis, FLI predicted T2D only among persons without MS. In unstratified analysis with subjects categorized by FLI-MS, persons with FLI≥60 without MS had increased risk for T2D (HR= 3.19(2.26-4.52)) compared with persons with FLI<30 without MS. Persons with FLI<30 and MS had greater risk (HR= 4.31(2.15-8.61)) and, persons with both FLI≥60 and MS had the greatest risk (HR=4.66(3.42-6.35)).

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.

KEY WORDS: fatty liver index, fatty liver disease, type 2 diabetes, metabolic syndrome, predictor, metabolic factors, Kuopio Ischaemic Heart Disease Risk Factor Study

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 have remained low in the Finnish population.

INTRODUCTION / BACKGROUND

There is increasing recognition of the fact that fatty liver disease (FLD) is the commonest cause of chronic liver disease worldwide. Also 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 FLD patients progress to steatohepatitis with fibrosis, which can thereafter progress to cirrhosis, liver failure and hepatocellular carcinoma [1]. Fatty Liver Disease 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 (USS), is associated with an increase in T2D incidence [4, 5].

Liver biopsy is the gold standard for characterizing 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 of body mass index, 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. The study was approved by the Research Ethics Committee of the University of Kuopio [11], and the subjects gave their written consent.

Data Collection: Data were collected using self-administered questionnaires, interviews, physical examinations, and various blood tests which aimed to elucidate physiological and biochemical parameters [12, 13]. The self-administered questionnaire was used to collect data on medical history, including history of type 2 diabetes, metabolic diseases, liver disease, etc., 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 physiologic 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. Body mass index (BMI) was computed as the ratio of weight in Kg to the square of height in meters (kg/m2). Blood pressure, was taken as the mean of measurements taken in the supine, standing and sitting position with 5-minute 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, HDL cholesterol, LDL cholesterol, serum triglycerides), liver function tests including albumin, gamma-glutamyl transferase, 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 Fatty liver index: We calculated FLI using the algorithm developed by Bedogni et al [7]. The algorithm, incorporates four variables: BMI, waist circumference, serum triglycerides, and serum gamma-glutamyl transferase (GGT), and is expressed as follows:

$$FLI = \frac{(e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})}{(1 + e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})} \times 100$$

Where triglycerides is in mg/dl, waist circumference in cm, and BMI in Kg/m^2. We categorized FLI in accordance with Bedogni's categorization, as low FLI (<30), intermediate FLI (30-<60), and moderate-high FLI (>60), indicating no fatty liver, indeterminate, and fatty liver, respectively.

Defining Metabolic Syndrome Status: MS was defined in accordance with the harmonized criteria for diagnosis of MS [20]. The presence of any three of the following five risk factors constitutes a diagnosis of metabolic syndrome: 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); 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 ≥7.0mmol/L or 2-h 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 Tenth International Classification of Diseases (ICD 10 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 Version 21.0 for Windows (IBM, Chicago, IL). 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 Chi-Square 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 2014 [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 analyzed 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) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and metabolic factors (fasting glucose, insulin, HDL, LDL, systolic blood pressure, 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,

berries and vegetables) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and MS status.

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

Secondly, we performed sub-group 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 model 1 and 2 as explained above, but excluding fasting glucose in model 2.

Thirdly, 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 112mg/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 rates for T2D were 11 cases per 1000 person-years. Significantly lower survival free of incident T2D was noted for participants in high baseline FLI category compared to the low (normal) FLI category 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 Fatty Liver Index 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(1.35-2.31)).

Sensitivity analyses - after exclusion of 241 men who were heavy alcohol consumers (Table 3), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 2. Similarly, after exclusion of 571 men who were smokers (Appendix), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 1 of the 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 analyzed 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(2.26-4.51)). Having normal FLI with MS was associated with 331% increased risk (the HR (95%CI) was 4.31(2.15-8.61)) and, persons having high FLI and MS were at greatest risk, with 366% increase in risk (HR (95%CI) 4.66(3.42-6.35)). The presence of MS was associated with greater risk in intermediate and high FLI categories (the HRs (95%CI) were 3.77(2.50-5.70) for presence of MS with intermediate FLI category, and 4.66(3.42-6.35) for the presence of MS with high 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 physiologic 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
Leukocyte count x10^9/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. Chi-Square linear-by-linear association for categorical variables. bp-blood pressure

DIABETES PREDIDICTION

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(1.007-1.018)[a]	1.017(1.012-1.022)[b]
FLI category			
≤30 (Ref.)	833 (12.1) (6)	1.000	1.000
30-<60	552 (22.6) (12)	1.42(1.07-1.88)	1.81(1.38-2.37)
≥60	407 (36.6) (22)	2.13(1.56-2.93)[a]	2.63(1.92-3.59)[b]
P-trend	-	<0.001	<0.001

*FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 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.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.77(1.35-2.31).

Table 3 – Association of baseline fatty liver index 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(1.008-1.019)	1.018(1.012-1.024)
FLI category			
≤30 (Ref.)	771(12.5) (6)	1.000	1.000
30-<60	472(23.1) (12)	1.43(1.06-1.93)	1.78(1.33-2.37)
≥60	305(38.7) (23)	2.21(1.57-3.10)	2.63(1.89-3.66)
P-trend	-	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 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.[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.65(1.24-2.21).

Table 4 – Association of fatty liver index (FLI) with incident type 2 diabetes by metabolic syndrome status (sub-analyses)

No metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	1427(16.7) (9)	1.021 (1.015-1.027)	1.017 (1.010-1.025)[a]
FLI category			
≤30 (Ref.)	803(11.5) (6)	1.00	1.00[b]
30-<60	456(20.0) (11)	1.81(1.33-2.46)	1.58(1.14-2.19)
≥60	168(33.3) (18)	3.07(2.14-4.41)	2.38(1.58-3.58)
p-trend		<0.001	<0.001
Metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	358(37.7)(24)	1.007(0.997-1.016)	0.996(0.992-1.000)[c]
FLI category			
≤30 (Ref.)	29(31.0) (23)	1.000	1.000[d]
30-<60	91(36.3) (21)	0.77(0.35-1.70)	0.80(0.59-1.09)
≥60	238(39.1) (25)	1.02(0.49-2.16)	0.79 (0.58-1.06)
p-trend		0.42	0.22
Category by FLI and MS status	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	
FLI≤30 MS^-	803(11.5) (6)	1.000	-
FLI30-<60MS^-	456(20.0) (11)	1.79(1.33-2.41)	-

FLI≥60MS^{-}	168(33.3) (18)	3.19(2.26-4.51)	-
FLI≤30 MS^{+}	29(31.0) (23)	4.31(2.15-8.61)	-
FLI30-<60MS^{+}	91(36.3) (21)	3.77(2.50-5.70)	-
FLI≥60MS^{+}	238(39.1) (25)	4.66(3.42-6.35)	-

*FLI uncategorized, Ref – reference. HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

‡Statistically significant at P≤0.05. MS – Metabolic syndrome. MS^{-} - Metabolic syndrome negative. MS^{+} - Metabolic syndrome positive.

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

[a] Other independent predictors of T2D in the model were serum ferritin and insulin.

[b] Other independent predictors of T2D in the model were serum ferritin and insulin.

[c] Independent predictors of T2D in the model were fasting glucose and insulin.

[d] Independent predictors of T2D in the model were fasting glucose and insulin.

Figure 1 – Graph of FLI and risk of incident T2D.

DISCUSSION

We examined the association of FLI, a surrogate of fatty liver disease, 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 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 categorized by Bedogni et al., with incident T2D [10, 22]. Jager et al.[22] and Onat et al. [10], studied the association of FLI with incident T2D in healthy populations, followed up for eight years. Nishi et al.[23], studied the association of FLI with incident T2D in a population of prediabetic subjects followed up for three 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 categorization 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 Jager et al.[22] and Onat et al.[10]. We found a 2 to 3-fold increased risk in our multivariable adjusted models. However, Jager et al., reported 11-fold increase while Onat et al reported a 5-fold increase. Our finding, that intermediate FLI is also associated with increased risk of T2D, is also in line with reports by Jager et al.

Our finding that high FLI is associated with incident T2D even after adjusting for metabolic factors, agrees with other reports.[10],[24] [9], each of which used different cut-off points in categorizing 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 re-analyzed 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 metabolic syndrome [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.

It is remarkable that, 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 to 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 hyperinsulinemia 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 of 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 co-presence 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. Firstly, we show that FLI, a surrogate of hepatic steatosis, predicts risk of incident T2D especially in persons who are negative for MS. Secondly, 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. Firstly, FLI as a surrogate of fatty liver does not detect progression of FLD. Therefore, we are unable to differentiate the contribution of 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 (Karvonen 2016) (Safreed-Harmon 2018). Also, our study population comprised of men only. There are reports that suggest that lower FLI cut off values may apply to women [31]. We are unable to explore the influence of gender on the predictability of T2D using FLI. Nevertheless, Bedogni et al., concluded that the influence of gender in FLI is related to insulin and skinfold thickness, and probably insignificant [7].

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, especially 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.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

OOO and T-PT conceived the study. OOO and T-PT designed the study. OOO performed the statistical analyses. OOO wrote the first draft of the manuscript. JV , JP and T-PT contributed towards development of this manuscript. All authors reviewed the final draft of the manuscript.

Data sharing statement:

No additional data are available.

Acknowledgements:

OOO received study grant from the Finnish Cultural Foundation Central, Finland (Suomen Kulttuurirahasto), in partial support for the conduct of this study.

Language editing was by Enembe O Okokon MBBch., MSc., FMCPH., PhD.

REFERENCES

1 Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology* 2016;64:73-84.

2 Kwon Y, Oh S, Hwang S, et al. Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults, *Am J Gastroenterol* 2012;107:1852.

3 Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives, *Nature Reviews Endocrinology* 2012;8:228-36.

4 Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease, *Dig Dis* 2010;28:210-3.

5 Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity, *Ann Med* 2011;43:617-49.

6 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association, *Hepatology* 2012;55:2005-23.

7 Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population, *BMC Gastroenterol* 2006;6:33.

8 Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease, *Aliment Pharmacol Ther* 2014;40:1209-22.

9 Jung C, Lee W, Hwang J, et al. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population, *Diabetic Med* 2013;30:428-35.

10 Onat A, Can G, Kaya A, et al. Fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality, *World J Gastroenterol* 2015;21:13555-65.

11 Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study, *Ann Clin Res* 1988;20:46-50.

12 Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men, *Circulation* 1995;91:645-55.

13 Räsänen J, Kauhanen J, Lakka T, et al. Religious affiliation and all-cause mortality: a prospective population study in middle-aged men in eastern Finland, *Int J Epidemiol* 1996;25:1244-9.

14 Laukkanen JA, Pukkala E, Rauramaa R, et al. Cardiorespiratory fitness, lifestyle factors and cancer risk and mortality in Finnish men, *Eur J Cancer* 2010;46:355-63.

15 Willenbring ML, Massey SH, Gardner MB. Helping Patients Who Drink Too Much: An Evidence-Based Guide for Primary Care Physicians. *Am Fam Physician* 2009;80.

16 McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011, *Adv Nutr* 2011;2:293-4.

17 Fawehinmi TO, Ilomäki J, Voutilainen S, et al. Alcohol consumption and dietary patterns: the FinDrink study, *PLoS One* 2012;7:e38607.

18 Virtanen JK, Mursu J, Tuomainen TP, et al. Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study, *Arterioscler Thromb Vasc Biol* 2014;34:2679-87.

19 Kauhanen J, Kaplan GA, Goldberg DE, et al. Pattern of alcohol drinking and progression of atherosclerosis, *Arterioscler Thromb Vasc Biol* 1999;19:3001-6.

20 Alberti K. International Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; Wo, *Circulation* 2009;120:1640-5.

21 Cheema JR. Some general guidelines for choosing missing data handling methods in educational research, *Journal of Modern Applied Statistical Methods* 2014;13:3.

22 Jäger S, Jacobs S, Kröger J, et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam Study, *PloS one* 2015;10:e0124749.

23 Nishi T, Babazono A, Maeda T, et al. Evaluation of the fatty liver index as a predictor for the development of diabetes among insurance beneficiaries with prediabetes, *Journal of diabetes investigation* 2015;6:309-16.

24 Balkau B, Lange C, Vol S, et al. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study, *BMC Gastroenterol* 2010;10:56,230X-10-56.

25 Shibata M, Kihara Y, Taguchi M, et al. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men, *Diabetes Care* 2007;30:2940-4.

26 Bedogni G, Bellentani S. Fatty liver: how frequent is it and why, *Ann Hepatol* 2004;3:63-5.

27 Karajamaki AJ, Bloigu R, Kauma H, et al. Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term outcomes, *Metabolism-Clinical and Experimental* 2017;66:55-63.

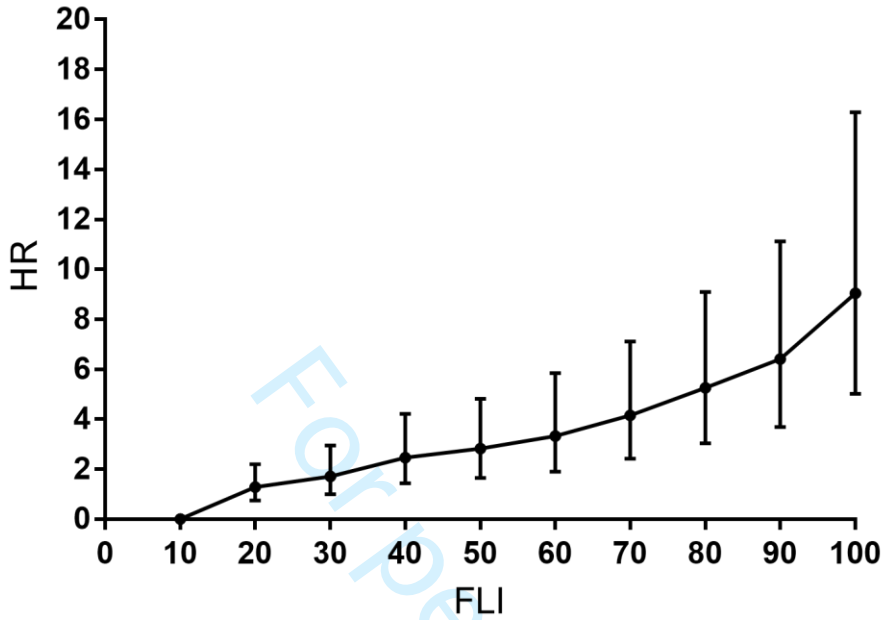
28 Valenti L, Bugianesi E, Pajvani U, et al. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? *Liver International* 2016;36:1563-79.

29 Targher G, Byrne CD. Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications, *The Journal of Clinical Endocrinology & Metabolism* 2013;98:483-95.

30 Olubamwo OO, Virtanen JK, Voutilainen A, et al. Association of fatty liver index with the risk of incident cardiovascular disease and acute myocardial infarction, *Eur J Gastroenterol Hepatol* 2018;30:1047-54.

31 Yang BL, Wu WC, Fang KC, et al. External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan, *PLoS One* 2015;10:e0120443.

Figure 1. Variation of hazard ratio of incident type 2 diabetes with baseline fatty liver index



HR= Hazard ratio; FLI= Fatty liver index

APPENDIX

Table 1 – Association of baseline fatty liver index with incident type 2 diabetes after excluding smokers

FLI	Number of subjects (% with T2D) (IR)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1221(22.4) (11)	1.014(1.008-1.021)	1.016(1.009-1.022)
FLI category			
≤30 (Ref.)	549 (13.5) (7)	1.000	1.000
30-<60	380(23.9) (12)	1.43(1.02-1.99)	1.69(1.23-2.33)
≥60	292(37.0) (21)	2.24(1.54-3.26)	2.38(1.65-3.44)
P-trend	-	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

Model 1: FLI, age, examination date, family history of diabetes, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes 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, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes albumin, fibrinogen, and ferritin, metabolic syndrome status.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and metabolic syndrome status. HR metabolic syndrome = 1.76(1.28-2.41).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	2, 5
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	7-8
Bias	9	Describe any efforts to address potential sources of bias	7, 9-10
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	9
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	9-10
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	7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, 9
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, Tables 2 & 3
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	9-11, Tables 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	9-11, Tables 2 & 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, Table 4 &5, Figure 1
Discussion			
Key results	18	Summarise key results with reference to study objectives	20
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	23-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	23-24
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	25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.

Association of Fatty Liver Index with risk of Incident Type 2 Diabetes by Metabolic Syndrome Status in an Eastern Finland Male Cohort

Olubunmi O. Olubamwo [1], Jyrki K. Virtanen[1], Jussi Pihlajamäki[1,2], Tomi-Pekka Tuomainen [1]

[1] Institute of Public Health and Clinical Nutrition, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland.
[2] Clinical Nutrition and Obesity Center, Kuopio University Hospital, KYS Kuopio, Finland.

Running head: FLI and risk of T2D by MS status. Olubamwo et al.

Word count: main text 3864 words, abstract 267 words.

*Corresponding author:

Olubunmi O, Olubamwo
Institute of Public Health and Clinical Nutrition
Faculty of Health Sciences
University of Eastern Finland, Kuopio,
P.O.Box 1627, FI-70211 Kuopio, Finland.
E-mail : olubunmi.olubamwo@uef.fi Tel : +358 403552905.

ABSTRACT

Objective: Fatty liver disease (FLD) is increasingly recognized 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 center in Kuopio, Eastern Finland.

Participants: our subjects were 1792 Finnish men without diabetes at baseline in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort.

Outcome Measure: Using fatty liver index (FLI), the association of baseline FLD with incident T2D was analyzed 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) vs 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 hazard ratio (HR (95%CI)) for T2D was 3.68(2.80-4.82). 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(1.92-3.59) for FLI≥60 and 1.77(1.35-2.31) for MS.

In MS-stratified analysis, FLI predicted T2D only among persons without MS. In unstratified analysis with subjects categorized by FLI-MS, persons with FLI≥60 without MS had increased risk for T2D (HR= 3.19(2.26-4.52)) compared with persons with FLI<30 without MS. Persons with FLI<30 and MS had greater risk (HR= 4.31(2.15-8.61)) and, persons with both FLI≥60 and MS had the greatest risk (HR=4.66(3.42-6.35)).

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 at with increased T2D risk.

KEY WORDS: fatty liver index, fatty liver disease, type 2 diabetes, metabolic syndrome, predictor, metabolic factors, Kuopio Ischaemic Heart Disease Risk Factor Study

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 have remained low in the Finnish population.

INTRODUCTION / BACKGROUND

There is increasing recognition of the fact that fatty liver disease (FLD), also known as hepatic steatosis, is the commonest cause of chronic liver disease worldwide. and that Also known as hepatic steatosis, it FLD is associated with increased risk of cardiovascular disease (CVD) and Type 2 diabetes (T2D). Although the The prevalence has been observed to steadily rise; although, this varies in different populations, it is also on the rise. Recent estimates suggest a global prevalence of 25% among adults, with but the highest prevalence occurs in the Middle East and South America and 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 FLD patients progress to steatohepatitis with fibrosis, which can thereafter progress to cirrhosis, liver failure and hepatocellular carcinoma [1]. In addition, FLD Fatty Liver Disease 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].

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

Liver biopsy, which is the gold standard for characterizing 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 of body mass index, 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 is has gained increased acceptance [7, 8]. There has 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 not known uncertain whether if 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 consisted of 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. They These were enrolled in at baseline examinations between March 1984 and December 1989. The men were 42, 48, 54, or 60 years of age at baseline. The study was approved by the Research Ethics Committee of the University of Kuopio [11], and the subjects gave their written consent.

Data Collection: Data were collected through using self-administered questionnaires, interviews, physical examinations, and various blood tests which aimed to elucidate determine physiological and biochemical parameters [12, 13]. The self-administered questionnaires were was used to collect data on medical history, including history of type 2 diabetes, metabolic diseases, liver disease, etc., 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].

The 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 measurements and indices, vital signs, and physiologic measurements. All physical measurements were measured made following standard protocols. Waist circumference was calculated as the mean of waist circumferences taken at maximal inspiration and that taken at maximal expiration. Body mass index (BMI) was computed as the ratio of weight in Kg to the square of height in meters (kg/m2). Blood pressure, which was measured by two separate nurses, was taken as the mean of three measurements taken in the supine, one on standing and two in sitting position with 5-minute 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-hours 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, HDL cholesterol, LDL cholesterol, serum triglycerides), liver function tests including albumin, gamma-glutamyl transferase, 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 Fatty liver index: We calculated FLI using the algorithm developed by Bedogni et al [7]. The algorithm, incorporates four variables: BMI, waist circumference, serum triglycerides, and serum gamma-glutamyl transferase (GGT), and is expressed as follows:

$$FLI = \frac{(e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})}{(1 + e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})} \times 100$$

Where triglycerides is in mg/dl, waist circumference in cm, and BMI in Kg/m^2. We categorized FLI in accordance with Bedogni et al's categorization, as low FLI (<30), intermediate FLI (30-<60), and moderate-high FLI (>60), indicating no fatty liver, indeterminate, and fatty liver, respectively.

Defining Metabolic Syndrome Status: MS was defined in accordance with the harmonized criteria for diagnosis of MS [20]. The presence of any 3 three of the following 5 five risk factors constitutes a diagnosis of metabolic syndrome: 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); 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 ≥7.0mmol/L or 2-h 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 Tenth International Classification of Diseases (ICD 10 code numbers from E11.0 to E11.9).

Patient and public involvement: The study was carried out at 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 Version 21.0 for Windows (IBM, Chicago, IL). 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 Chi-Square 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 2014 [21].

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

First, we analyzed the overall association overall, 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) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and metabolic factors (fasting glucose, insulin, HDL, LDL, systolic blood pressure, 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, berries and vegetables) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and MS status.

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

Secondly, we performed sub-group analyses in which we stratified our study sample by MS status. in stratified analyses, we stratified our population 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 model 1 and 2 as explained above, but excluding fasting glucose in model 2.

Thirdly, 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, men in 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 They consumed less fruit, berries and vegetables, and had a higher proportion of more likely to be heavy alcohol consumers. but a lower proportion of smokers. 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 112mg/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 rates for T2D were 11 cases per 1000 person-years. Significantly lower survival free of incident T2D was noted for participants in high baseline FLI category compared to the low (normal) FLI category 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 Fatty Liver Index 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, though

attenuated in model 2, and. 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(1.35-2.31)).

Sensitivity analyses - after exclusion of 241 men who were heavy alcohol consumers (Table 3), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 2. Similarly, after exclusion of 571 men who were smokers (Appendix), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 1 of the 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 analyzed 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. Among those 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, high FLI was associated with over 100% increased risk of T2D. Among those with MS, high FLI was not associated with additional risk when compared with those in the low FLI category, high FLI was not associated with additional risk.

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

presence of MS with intermediate FLI category, and 4.66(3.42-6.35) for the presence of MS with high 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 physiologic 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
Leukocyte count $x10^9$/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. Chi-Square linear-by-linear association for categorical variables. bp-blood pressure

DIABETES PREDIDICTION

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 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1792 (20.9) (11)	1.022(1.018-1.027)	1.013(1.007-1.018)[a]	1.017(1.012-1.022)[b]
FLI category				
≤30 (Ref.)	833 (12.1) (6)	1.000	1.000	1.000
30-<60	552 (22.6) (12)	1.98(1.52-2.59)	1.42(1.07-1.88)	1.81(1.38-2.37)
≥60	407 (36.6) (22)	3.68(2.80-4.82)	2.13(1.56-2.93)[a]	2.63(1.92-3.59)[b]
P-trend	-	<0.001	<0.001	<0.001

*FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 albumin, fibrinogen, and ferritin.

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 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.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.77(1.35-2.31).

Table 3 – Association of baseline fatty liver index 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 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1548(20.9) (11)	1.023(1.018-1.027)	1.014(1.008-1.019)	1.018(1.012-1.024)
FLI category				
≤30 (Ref.)	771(12.5) (6)	1.000	1.000	1.000
30-<60	472(23.1) (12)	1.96(1.48-2.60)	1.43(1.06-1.93)	1.78(1.33-2.37)
≥60	305(38.7) (23)	3.61(2.71-4.81)	2.21(1.57-3.10)	2.63(1.89-3.66)
P-trend	-	<0.001	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 albumin, fibrinogen, and ferritin.

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 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.[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.65(1.24-2.21).

Table 4 – Association of fatty liver index (FLI) with incident type 2 diabetes by metabolic syndrome status (sub-analyses)

No metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	1427(16.7) (9)	1.021 (1.015-1.027)	1.017 (1.010-1.025)[a]
FLI category			
≤30 (Ref.)	803(11.5) (6)	1.00	1.00[b]
30-<60	456(20.0) (11)	1.81(1.33-2.46)	1.58(1.14-2.19)
≥60	168(33.3) (18)	3.07(2.14-4.41)	2.38(1.58-3.58)
p-trend		<0.001	<0.001
Metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	358(37.7)(24)	1.007(0.997-1.016)	0.996(0.992-1.000)[c]
FLI category			
≤30 (Ref.)	29(31.0) (23)	1.000	1.000[d]
30-<60	91(36.3) (21)	0.77(0.35-1.70)	0.80(0.59-1.09)
≥60	238(39.1) (25)	1.02(0.49-2.16)	0.79 (0.58-1.06)
p-trend		0.42	0.22
Category by FLI and MS status	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	
FLI≤30 MS^-	803(11.5) (6)	1.000	-
FLI30-<60MS^-	456(20.0) (11)	1.79(1.33-2.41)	-

FLI≥60MS^{-}	168(33.3) (18)	3.19(2.26-4.51)	-
FLI≤30 MS^{+}	29(31.0) (23)	4.31(2.15-8.61)	-
FLI30-<60MS^{+}	91(36.3) (21)	3.77(2.50-5.70)	-
FLI≥60MS^{+}	238(39.1) (25)	4.66(3.42-6.35)	-

*FLI uncategorized, Ref – reference. HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

‡Statistically significant at P≤0.05. MS – Metabolic syndrome. MS^{-} - Metabolic syndrome negative. MS^{+} - Metabolic syndrome positive.

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, fasting glucose, LDL, and HDL

[a] Other independent predictors of T2D in the model were serum ferritin and insulin.

[b] Other independent predictors of T2D in the model were serum ferritin and insulin.

[c] Independent predictors of T2D in the model were fasting glucose and insulin.

[d] Independent predictors of T2D in the model were fasting glucose and insulin.

Figure 1 – Graph of FLI and risk of incident T2D.

DISCUSSION

We examined the association of FLI, as a surrogate of fatty liver disease, with in relation to incident T2D in a population of middle-aged men, considering while taking the baseline MS status into account. Specifically, we We found that although FLD assessed by FLI predicts the risk of T2D in the whole study population, the association was strongest among those persons without MS at baseline.

Few studies have investigated the association of baseline FLI as categorized by Bedogni et al., with incident T2D [10, 22]. Jager et al.[22] and Onat et al. [10], studied the association of FLI with incident T2D in healthy populations, followed up for eight years. Nishi et al.[23], studied the association of FLI with incident T2D in a population of prediabetic subjects followed up for three 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 categorization 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 analyses models, we are careful in our comparison of findings.

Our finding that high FLI (FLI≥60) indicating fatty liver, as compared with low FLI(FLI<30) is associated with increased risk independent of constitutional and lifestyle factors, agrees with findings from previous studies findings by Jager et al.[22] and Onat et al.[10]. We found a 2 to 3-fold increased risk in our multivariable adjusted models. However, Jager et al., reported 11-fold increase while Onat et al reported a 5-fold increase. Our finding, that FLI 30-<60 intermediate FLI is also associated with increased risk of T2D, is also in line with reports by Jager et al.

Our finding that high FLI is associated with incident T2D even after adjusting for metabolic factors, agrees with other reports from Onat et al.[10], Balkau et al.[24] and Jung et al. [9], each of which used different cut-off points in categorizing 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 re-analyzed 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 metabolic syndrome [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, our finding, in our man analysis, that high FLI category is associated with increased risk of incident T2D after adjusting for of MS status agrees with 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.

It is remarkable that, when we stratified stratification by MS status did not reveal significant association of high FLI with incident T2D in subjects with MS, the association of high FLI with incident T2D did not reach statistical significance among 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 to 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 hyperinsulinemia and, together with visceral obesity, promotes the development of FLD [28]. In return, the insulin resistant fatty liver overproduces glucose and VLDL 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 of 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 co-presence 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. Firstly, we show that FLI, a surrogate of hepatic steatosis, predicts risk of incident T2D especially in persons who are negative for MS. Secondly, 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. Firstly, FLI as a surrogate of fatty liver does not detect progression of FLD. Therefore, we are unable to differentiate the contribution of 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 (Karvonen 2016) (Safreed-Harmon 2018). Also, our study population comprised of men only. There are reports that suggest that lower FLI cut off values may apply to women [31]. We are unable to explore the influence of gender on the predictability of T2D using FLI. Nevertheless, Bedogni et al., concluded that the influence of gender in FLI is related to insulin and skinfold thickness, and probably insignificant [7]. 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, especially in men without MS. Persons with high FLI should be further evaluated for FLD and, if found with 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.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

OOO and T-PT conceived the study. OOO and T-PT designed the study. OOO performed the statistical analyses. OOO wrote the first draft of the manuscript. JV , JP and T-PT contributed towards development of this manuscript. All authors reviewed the final draft of the manuscript.

Data sharing statement:

No additional data are available.

Acknowledgements:

OOO received study grant from the Finnish Cultural Foundation Central, Finland (Suomen Kulttuurirahasto), in partial support for the conduct of this study.

Language editing was by Enembe O Okokon MBBch., MSc., FMCPH., PhD.

REFERENCES

1 Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology* 2016;64:73-84.

2 Kwon Y, Oh S, Hwang S, et al. Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults, *Am J Gastroenterol* 2012;107:1852.

3 Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives, *Nature Reviews Endocrinology* 2012;8:228-36.

4 Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease, *Dig Dis* 2010;28:210-3.

5 Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity, *Ann Med* 2011;43:617-49.

6 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association, *Hepatology* 2012;55:2005-23.

7 Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population, *BMC Gastroenterol* 2006;6:33.

8 Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease, *Aliment Pharmacol Ther* 2014;40:1209-22.

9 Jung C, Lee W, Hwang J, et al. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population, *Diabetic Med* 2013;30:428-35.

10 Onat A, Can G, Kaya A, et al. Fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality, *World J Gastroenterol* 2015;21:13555-65.

11 Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study, *Ann Clin Res* 1988;20:46-50.

12 Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men, *Circulation* 1995;91:645-55.

13 Räsänen J, Kauhanen J, Lakka T, et al. Religious affiliation and all-cause mortality: a prospective population study in middle-aged men in eastern Finland, *Int J Epidemiol* 1996;25:1244-9.

14 Laukkanen JA, Pukkala E, Rauramaa R, et al. Cardiorespiratory fitness, lifestyle factors and cancer risk and mortality in Finnish men, *Eur J Cancer* 2010;46:355-63.

15 Willenbring ML, Massey SH, Gardner MB. Helping Patients Who Drink Too Much: An Evidence-Based Guide for Primary Care Physicians. *Am Fam Physician* 2009;80.

16 McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011, *Adv Nutr* 2011;2:293-4.

17 Fawehinmi TO, Ilomäki J, Voutilainen S, et al. Alcohol consumption and dietary patterns: the FinDrink study, *PLoS One* 2012;7:e38607.

18 Virtanen JK, Mursu J, Tuomainen TP, et al. Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study, *Arterioscler Thromb Vasc Biol* 2014;34:2679-87.

19 Kauhanen J, Kaplan GA, Goldberg DE, et al. Pattern of alcohol drinking and progression of atherosclerosis, *Arterioscler Thromb Vasc Biol* 1999;19:3001-6.

20 Alberti K. International Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; Wo, *Circulation* 2009;120:1640-5.

21 Cheema JR. Some general guidelines for choosing missing data handling methods in educational research, *Journal of Modern Applied Statistical Methods* 2014;13:3.

22 Jäger S, Jacobs S, Kröger J, et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam Study, *PloS one* 2015;10:e0124749.

23 Nishi T, Babazono A, Maeda T, et al. Evaluation of the fatty liver index as a predictor for the development of diabetes among insurance beneficiaries with prediabetes, *Journal of diabetes investigation* 2015;6:309-16.

24 Balkau B, Lange C, Vol S, et al. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study, *BMC Gastroenterol* 2010;10:56,230X-10-56.

25 Shibata M, Kihara Y, Taguchi M, et al. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men, *Diabetes Care* 2007;30:2940-4.

26 Bedogni G, Bellentani S. Fatty liver: how frequent is it and why, *Ann Hepatol* 2004;3:63-5.

27 Karajamaki AJ, Bloigu R, Kauma H, et al. Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term outcomes, *Metabolism-Clinical and Experimental* 2017;66:55-63.

28 Valenti L, Bugianesi E, Pajvani U, et al. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? *Liver International* 2016;36:1563-79.

29 Targher G, Byrne CD. Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications, *The Journal of Clinical Endocrinology & Metabolism* 2013;98:483-95.

30 Olubamwo OO, Virtanen JK, Voutilainen A, et al. Association of fatty liver index with the risk of incident cardiovascular disease and acute myocardial infarction, *Eur J Gastroenterol Hepatol* 2018;30:1047-54.

31 Yang BL, Wu WC, Fang KC, et al. External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan, *PLoS One* 2015;10:e0120443.

APPENDIX

Table 5 – Association of baseline fatty liver index with incident type 2 diabetes after excluding smokers

FLI	Number of subjects (% with T2D) (IR)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1221(22.4) (11)	1.014(1.008-1.021)	1.016(1.009-1.022)
FLI category			
≤30 (Ref.)	549 (13.5) (7)	1.000	1.000
30-<60	380(23.9) (12)	1.43(1.02-1.99)	1.69(1.23-2.33)
≥60	292(37.0) (21)	2.24(1.54-3.26)	2.38(1.65-3.44)
P-trend	-	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

Model 1: FLI, age, examination date, family history of diabetes, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes 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, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes albumin, fibrinogen, and ferritin, metabolic syndrome status.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and metabolic syndrome status. HR metabolic syndrome = 1.76(1.28-2.41).

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026949.R2
Article Type:	Research
Date Submitted by the Author:	30-May-2019
Complete List of Authors:	Olubamwo, Olubunmi; Ita-Suomen yliopisto Terveystieteiden tiedekunta, Public Health and Cliical Nutrition Virtanen, Jyrki K.; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition Pihlajamaki, Jussi; University of Eastern Finland School of Medicine, Public health and clinical nutrition; Clinical nutrition and obesity center, Kuopio university hospital, Kys, Kuopio, Tuomainen, Tomi-Pekka; Ita-Suomen yliopisto Kansanterveystiede ja kliininen ravitsemustiede, Public Health and Clinical Nutrition
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Gastroenterology and hepatology, Public health
Keywords:	EPIDEMIOLOGY, Hepatology < INTERNAL MEDICINE, Diabetes & endocrinology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, PUBLIC HEALTH

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

Olubunmi O. Olubamwo [1], Jyrki K. Virtanen[1], Jussi Pihlajamäki[1,2], Tomi-Pekka Tuomainen [1]

[1] Institute of Public Health and Clinical Nutrition, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland.
[2] Clinical Nutrition and Obesity Center, Kuopio University Hospital, KYS Kuopio, Finland.

Running head: FLI and risk of T2D by MS status. Olubamwo et al.

Word count: main text 3897 words, abstract 288 words.

*Corresponding author:

Olubunmi O, Olubamwo
Institute of Public Health and Clinical Nutrition
Faculty of Health Sciences
University of Eastern Finland, Kuopio,
P.O.Box 1627, FI-70211 Kuopio, Finland.
E-mail : olubunmi.olubamwo@uef.fi Tel : +358 403552905.

ABSTRACT

Objective: Fatty liver disease (FLD) is increasingly recognized 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 center in Kuopio, Eastern Finland.

Participants: our subjects were 1792 Finnish men without diabetes at baseline in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort.

Outcome Measure: Using fatty liver index (FLI), the association of baseline FLD with incident T2D was analyzed 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) vs 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 hazard ratio (HR (95%CI)) for T2D was 3.68(2.80-4.82). 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(1.92-3.59) for FLI≥60 and 1.77(1.35-2.31) for MS.

In MS-stratified analysis, FLI predicted T2D only among persons without MS. In unstratified analysis with subjects categorized by FLI-MS, persons with FLI≥60 without MS had increased risk for T2D (HR= 3.19(2.26-4.52)) compared with persons with FLI<30 without MS. Persons with FLI<30 and MS had greater risk (HR= 4.31(2.15-8.61)) and, persons with both FLI≥60 and MS had the greatest risk (HR=4.66(3.42-6.35)).

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.

KEY WORDS: fatty liver index, fatty liver disease, type 2 diabetes, metabolic syndrome, predictor, metabolic factors, Kuopio Ischaemic Heart Disease Risk Factor Study

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 have remained low in the Finnish population.

INTRODUCTION / BACKGROUND

There is increasing recognition of the fact that fatty liver disease (FLD) is the commonest cause of chronic liver disease worldwide. Also 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 FLD patients progress to steatohepatitis with fibrosis, which can thereafter progress to cirrhosis, liver failure and hepatocellular carcinoma [1]. Fatty Liver Disease 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 (USS), is associated with an increase in T2D incidence [4, 5].

Liver biopsy is the gold standard for characterizing 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 of body mass index, 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. The study was approved by the Research Ethics Committee of the University of Kuopio [11], and the subjects gave their written consent.

Data Collection: Data were collected using self-administered questionnaires, interviews, physical examinations, and various blood tests which aimed to elucidate physiological and biochemical parameters [12, 13]. The self-administered questionnaire was used to collect data on medical history, including history of type 2 diabetes, metabolic diseases, liver disease, etc., 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 physiologic 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. Body mass index (BMI) was computed as the ratio of weight in Kg to the square of height in meters (kg/m2). Blood pressure, was taken as the mean of measurements taken in the supine, standing and sitting position with 5-minute 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, HDL cholesterol, LDL cholesterol, serum triglycerides), liver function tests including albumin, gamma-glutamyl transferase, 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 Fatty liver index: We calculated FLI using the algorithm developed by Bedogni et al [7]. The algorithm, incorporates four variables: BMI, waist circumference, serum triglycerides, and serum gamma-glutamyl transferase (GGT), and is expressed as follows:

$$FLI = \frac{(e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})}{(1 + e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745})} \times 100$$

Where triglycerides is in mg/dl, waist circumference in cm, and BMI in Kg/m^2. We categorized FLI in accordance with Bedogni's categorization, as low FLI (<30), intermediate FLI (30-<60), and moderate-high FLI (>60), indicating no fatty liver, indeterminate, and fatty liver, respectively.

Defining Metabolic Syndrome Status: MS was defined in accordance with the harmonized criteria for diagnosis of MS [20]. The presence of any three of the following five risk factors constitutes a diagnosis of metabolic syndrome: 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); 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 ≥7.0mmol/L or 2-h 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 Tenth International Classification of Diseases (ICD 10 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 Version 21.0 for Windows (IBM, Chicago, IL). 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 Chi-Square 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 2014 [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 analyzed 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) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and metabolic factors (fasting glucose, insulin, HDL, LDL, systolic blood pressure, 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,

berries and vegetables) and, inflammatory markers (C-reactive protein, leukocyte count, thrombocyte count), and MS status.

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

Secondly, we performed sub-group 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 model 1 and 2 as explained above, but excluding fasting glucose in model 2.

Thirdly, 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 to the low (normal) FLI category 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 Fatty Liver Index 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(1.35-2.31)).

Sensitivity analyses - after exclusion of 241 men who were heavy alcohol consumers (Table 3), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 2. Similarly, after exclusion of 571 men who were smokers (Appendix), the results were similar to those obtained in the analyses with the whole sample, as shown in Table 1 of the 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 analyzed 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(2.26-4.51)). Having normal FLI with MS was associated with 331% increased risk (the HR (95%CI) was 4.31(2.15-8.61)) and, persons having high FLI and MS were at greatest risk, with 366% increase in risk (HR (95%CI) 4.66(3.42-6.35)). The presence of MS was associated with greater risk in intermediate and high FLI categories (the HRs (95%CI) were 3.77(2.50-5.70) for presence of MS with intermediate FLI category, and 4.66(3.42-6.35) for the presence of MS with high 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 physiologic 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
Leukocyte count x10^9/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. Chi-Square linear-by-linear association for categorical variables. bp-blood pressure

DIABETES PREDIDICTION

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(1.007-1.018)[a]	1.017(1.012-1.022)[b]
FLI category			
≤30 (Ref.)	833 (12.1) (6)	1.000	1.000
30-<60	552 (22.6) (12)	1.42(1.07-1.88)	1.81(1.38-2.37)
≥60	407 (36.6) (22)	2.13(1.56-2.93)[a]	2.63(1.92-3.59)[b]
P-trend	-	<0.001	<0.001

*FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 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.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.77(1.35-2.31).

Table 3 – Association of baseline fatty liver index 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(1.008-1.019)	1.018(1.012-1.024)
FLI category			
≤30 (Ref.)	771(12.5) (6)	1.000	1.000
30-<60	472(23.1) (12)	1.43(1.06-1.93)	1.78(1.33-2.37)
≥60	305(38.7) (23)	2.21(1.57-3.10)	2.63(1.89-3.66)
P-trend	-	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

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 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.[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, hypertension, serum insulin, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin and metabolic syndrome status. HR metabolic syndrome = 1.65(1.24-2.21).

Table 4 – Association of fatty liver index (FLI) with incident type 2 diabetes by metabolic syndrome status (sub-analyses)

No metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	1427(16.7) (9)	1.021 (1.015-1.027)	1.017 (1.010-1.025)[a]
FLI category			
≤30 (Ref.)	803(11.5) (6)	1.00	1.00[b]
30-<60	456(20.0) (11)	1.81(1.33-2.46)	1.58(1.14-2.19)
≥60	168(33.3) (18)	3.07(2.14-4.41)	2.38(1.58-3.58)
p-trend		<0.001	<0.001
Metabolic syndrome	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	**Model 2 HR(95%CI)**
FLI*	358(37.7)(24)	1.007(0.997-1.016)	0.996(0.992-1.000)[c]
FLI category			
≤30 (Ref.)	29(31.0) (23)	1.000	1.000[d]
30-<60	91(36.3) (21)	0.77(0.35-1.70)	0.80(0.59-1.09)
≥60	238(39.1) (25)	1.02(0.49-2.16)	0.79 (0.58-1.06)
p-trend		0.42	0.22
Category by FLI and MS status	**Number of subjects (% with T2D) (IR)**	**Model 1 HR(95%CI)**	
FLI≤30 MS^-	803(11.5) (6)	1.000	-
FLI30-<60MS^-	456(20.0) (11)	1.79(1.33-2.41)	-

FLI≥60MS^{-}	168(33.3) (18)	3.19(2.26-4.51)	-
FLI≤30 MS^{+}	29(31.0) (23)	4.31(2.15-8.61)	-
FLI30-<60MS^{+}	91(36.3) (21)	3.77(2.50-5.70)	-
FLI≥60MS^{+}	238(39.1) (25)	4.66(3.42-6.35)	-

*FLI uncategorized, Ref – reference. HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

‡Statistically significant at P≤0.05. MS – Metabolic syndrome. MS^{-} - Metabolic syndrome negative. MS^{+} - Metabolic syndrome positive.

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

[a] Other independent predictors of T2D in the model were serum ferritin and insulin.

[b] Other independent predictors of T2D in the model were serum ferritin and insulin.

[c] Independent predictors of T2D in the model were fasting glucose and insulin.

[d] Independent predictors of T2D in the model were fasting glucose and insulin.

Figure 1 – Graph of FLI and risk of incident T2D.

DISCUSSION

We examined the association of FLI, a surrogate of fatty liver disease, 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 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 categorized by Bedogni et al., with incident T2D [10, 22]. Jager et al.[22] and Onat et al. [10], studied the association of FLI with incident T2D in healthy populations, followed up for eight years. Nishi et al.[23], studied the association of FLI with incident T2D in a population of prediabetic subjects followed up for three 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 categorization 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 Jager et al.[22] and Onat et al.[10]. We found a 2 to 3-fold increased risk in our multivariable adjusted models. However, Jager et al., reported 11-fold increase while Onat et al reported a 5-fold increase. Our finding, that intermediate FLI is also associated with increased risk of T2D, is also in line with reports by Jager et al.

Our finding that high FLI is associated with incident T2D even after adjusting for metabolic factors, agrees with other reports.[10],[24] [9], each of which used different cut-off points in categorizing 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 re-analyzed 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 metabolic syndrome [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 to 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 hyperinsulinemia 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 of 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 co-presence 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. Firstly, we show that FLI, a surrogate of hepatic steatosis, predicts risk of incident T2D especially in persons who are negative for MS. Secondly, 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. Firstly, FLI as a surrogate of fatty liver does not detect progression of FLD. Therefore, we are unable to differentiate the contribution of 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 (Karvonen 2016) (Safreed-Harmon 2018). Also, our study population comprised of men only. There are reports that suggest that lower FLI cut off values may apply to women [31]. We are unable to explore the influence of gender on the predictability of T2D using FLI. Nevertheless, Bedogni et al., concluded that the influence of gender in FLI is related to insulin and skinfold thickness, and probably insignificant [7].

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.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

OOO and T-PT conceived the study. OOO and T-PT designed the study. OOO performed the statistical analyses. OOO wrote the first draft of the manuscript. JV, JP and T-PT contributed towards development of this manuscript. All authors reviewed the final draft of the manuscript.

Data sharing statement:

No additional data are available. The raw data are not available for distribution because they contain sensitive health information, which cannot be distributed.

Acknowledgements:

OOO received study grant from the Finnish Cultural Foundation Central, Finland (Suomen Kulttuurirahasto), in partial support for the conduct of this study. The foundation had no role in the design, conduct or publication of the study.

Language editing was by Enembe O Okokon MBBch., MSc., FMCPH., PhD.

REFERENCES

1 Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology* 2016;64:73-84.

2 Kwon Y, Oh S, Hwang S, et al. Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults, *Am J Gastroenterol* 2012;107:1852.

3 Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives, *Nature Reviews Endocrinology* 2012;8:228-36.

4 Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease, *Dig Dis* 2010;28:210-3.

5 Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity, *Ann Med* 2011;43:617-49.

6 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association, *Hepatology* 2012;55:2005-23.

7 Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population, *BMC Gastroenterol* 2006;6:33.

8 Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease, *Aliment Pharmacol Ther* 2014;40:1209-22.

9 Jung C, Lee W, Hwang J, et al. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population, *Diabetic Med* 2013;30:428-35.

10 Onat A, Can G, Kaya A, et al. Fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality, *World J Gastroenterol* 2015;21:13555-65.

11 Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study, *Ann Clin Res* 1988;20:46-50.

12 Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men, *Circulation* 1995;91:645-55.

13 Räsänen J, Kauhanen J, Lakka T, et al. Religious affiliation and all-cause mortality: a prospective population study in middle-aged men in eastern Finland, *Int J Epidemiol* 1996;25:1244-9.

14 Laukkanen JA, Pukkala E, Rauramaa R, et al. Cardiorespiratory fitness, lifestyle factors and cancer risk and mortality in Finnish men, *Eur J Cancer* 2010;46:355-63.

15 Willenbring ML, Massey SH, Gardner MB. Helping Patients Who Drink Too Much: An Evidence-Based Guide for Primary Care Physicians. *Am Fam Physician* 2009;80.

16 McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011, *Adv Nutr* 2011;2:293-4.

17 Fawehinmi TO, Ilomäki J, Voutilainen S, et al. Alcohol consumption and dietary patterns: the FinDrink study, *PLoS One* 2012;7:e38607.

18 Virtanen JK, Mursu J, Tuomainen TP, et al. Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study, *Arterioscler Thromb Vasc Biol* 2014;34:2679-87.

19 Kauhanen J, Kaplan GA, Goldberg DE, et al. Pattern of alcohol drinking and progression of atherosclerosis, *Arterioscler Thromb Vasc Biol* 1999;19:3001-6.

20 Alberti K. International Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; Wo, *Circulation* 2009;120:1640-5.

21 Cheema JR. Some general guidelines for choosing missing data handling methods in educational research, *Journal of Modern Applied Statistical Methods* 2014;13:3.

22 Jäger S, Jacobs S, Kröger J, et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam Study, *PloS one* 2015;10:e0124749.

23 Nishi T, Babazono A, Maeda T, et al. Evaluation of the fatty liver index as a predictor for the development of diabetes among insurance beneficiaries with prediabetes, *Journal of diabetes investigation* 2015;6:309-16.

24 Balkau B, Lange C, Vol S, et al. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study, *BMC Gastroenterol* 2010;10:56,230X-10-56.

25 Shibata M, Kihara Y, Taguchi M, et al. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men, *Diabetes Care* 2007;30:2940-4.

26 Bedogni G, Bellentani S. Fatty liver: how frequent is it and why, *Ann Hepatol* 2004;3:63-5.

27 Karajamaki AJ, Bloigu R, Kauma H, et al. Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term outcomes, *Metabolism-Clinical and Experimental* 2017;66:55-63.

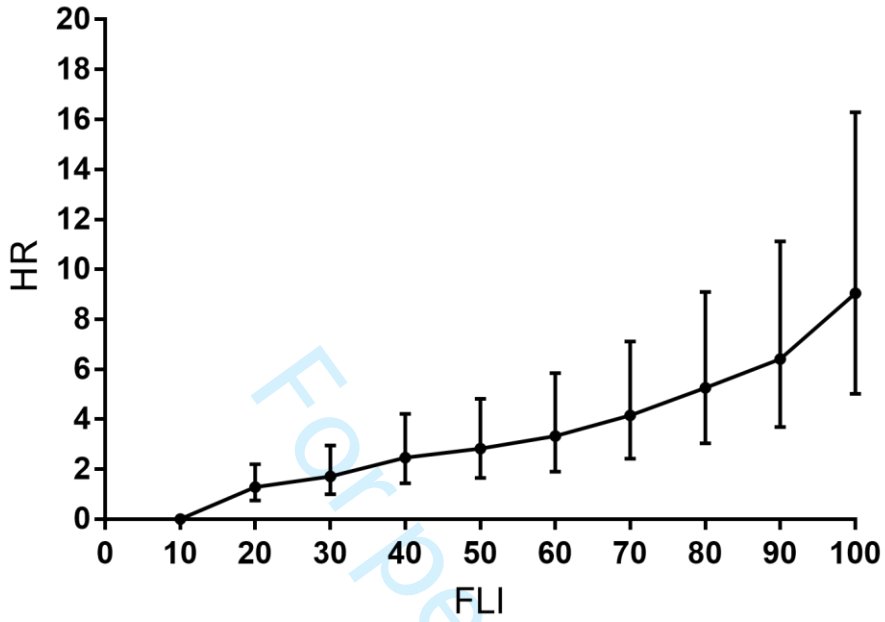
28 Valenti L, Bugianesi E, Pajvani U, et al. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? *Liver International* 2016;36:1563-79.

29 Targher G, Byrne CD. Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications, *The Journal of Clinical Endocrinology & Metabolism* 2013;98:483-95.

30 Olubamwo OO, Virtanen JK, Voutilainen A, et al. Association of fatty liver index with the risk of incident cardiovascular disease and acute myocardial infarction, *Eur J Gastroenterol Hepatol* 2018;30:1047-54.

31 Yang BL, Wu WC, Fang KC, et al. External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan, *PLoS One* 2015;10:e0120443.

Figure 1. Variation of hazard ratio of incident type 2 diabetes with baseline fatty liver index



HR= Hazard ratio; FLI= Fatty liver index

APPENDIX

Table 1 – Association of baseline fatty liver index with incident type 2 diabetes after excluding smokers

FLI	Number of subjects (% with T2D) (IR)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
FLI*	1221(22.4) (11)	1.014(1.008-1.021)	1.016(1.009-1.022)
FLI category			
≤30 (Ref.)	549 (13.5) (7)	1.000	1.000
30-<60	380(23.9) (12)	1.43(1.02-1.99)	1.69(1.23-2.33)
≥60	292(37.0) (21)	2.24(1.54-3.26)	2.38(1.65-3.44)
P-trend	-	<0.001	<0.001

FLI – fatty liver index, FLI* - FLI uncategorized, HR – hazard ratio. CI – confidence interval. T2D – type 2 diabetes, IR – Incidence rate per 1000 person-years

Model 1: FLI, age, examination date, family history of diabetes, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes 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, alcohol consumption per week, physical activity, fruit-berry-vegetable consumption, C-reactive protein, leukocytes albumin, fibrinogen, and ferritin, metabolic syndrome status.

[a] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and fasting glucose.

[b] Other independent predictors of T2D in the model were family history of diabetes, serum ferritin, alcohol consumption, and metabolic syndrome status. HR metabolic syndrome = 1.76(1.28-2.41).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	2, 5
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	7-8
Bias	9	Describe any efforts to address potential sources of bias	7, 9-10
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	9
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	9-10
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	7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, 9
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, Tables 2 & 3
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	9-11, Tables 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	9-11, Tables 2 & 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, Table 4 &5, Figure 1
Discussion			
Key results	18	Summarise key results with reference to study objectives	20
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	23-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	23-24
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	25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.