

# Prevalence, risk factors and diagnostic accuracy of non-invasive tests for NAFLD in people with type 1 diabetes

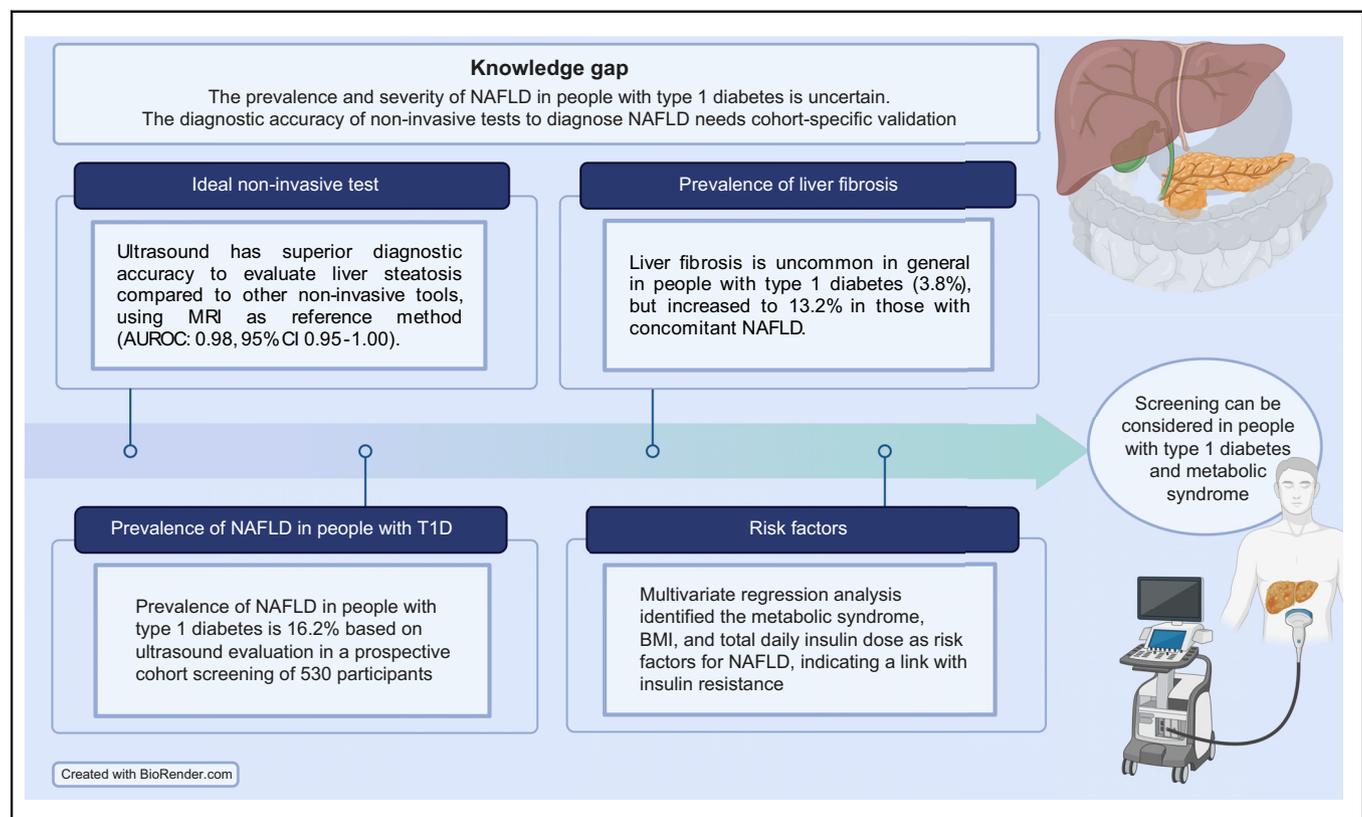
## Authors

Jonathan Mertens, Jonas Weyler, Eveline Dirinck, Luisa Vonghia, Wilhelmus J. Kwanten, Laura Mortelmans, Cedric Peleman, Shivani Chotkoe, Maarten Spinhoven, Floris Vanhevel, Luc F. Van Gaal, Benedicte Y. De Winter, Christophe E.M. De Block, Sven M. Francque

## Correspondence

[jonathan.mertens@uza.be](mailto:jonathan.mertens@uza.be) (J. Mertens), [jonas.weyler@zna.be](mailto:jonas.weyler@zna.be) (J. Weyler), [eveline.dirinck@uza.be](mailto:eveline.dirinck@uza.be) (E. Dirinck), [christophe.deblock@uza.be](mailto:christophe.deblock@uza.be) (C.E.M. De Block), [sven.francque@uza.be](mailto:sven.francque@uza.be) (S.M. Francque).

## Graphical abstract



## Highlights

- There is a knowledge gap regarding whether NAFLD is prevalent in people with type 1 diabetes and how to screen for it.
- Ultrasound is the most accurate screening test for NAFLD, compared with magnetic resonance spectroscopy.
- NAFLD prevalence in type 1 diabetes is 16.2%, and is associated with the metabolic syndrome and increased BMI.
- Elevated LSM ( $\geq 8.0$  kPa), indicating fibrosis, reaches 13.2% when NAFLD is concomitant in people with type 1 diabetes.
- It seems appropriate to only screen for NAFLD in metabolically unhealthy people with type 1 diabetes.

## Impact and Implications

We aimed to report on the prevalence, disease severity, and risk factors of NAFLD in type 1 diabetes (T1D), while also tackling which non-invasive test for NAFLD is the most accurate. We found that ultrasound is the best test to diagnose NAFLD. NAFLD prevalence is 16.2%, and is associated with metabolic syndrome and BMI. Elevated liver stiffness indicating fibrosis is overall not prevalent in people with T1D (3.8%), but it reaches 13.2% in those with T1D and NAFLD.

# Prevalence, risk factors and diagnostic accuracy of non-invasive tests for NAFLD in people with type 1 diabetes



Jonathan Mertens,<sup>1,2,3</sup> Jonas Weyler,<sup>2,3</sup> Eveline Dirinck,<sup>1,2</sup> Luisa Vonghia,<sup>2,3</sup> Wilhelmus J. Kwanten,<sup>2,3</sup> Laura Mortelmans,<sup>3</sup> Cedric Peleman,<sup>2,3</sup> Shivani Chotkoe,<sup>2</sup> Maarten Spinhoven,<sup>4</sup> Floris Vanhevel,<sup>4</sup> Luc F. Van Gaal,<sup>1</sup> Benedicte Y. De Winter,<sup>2</sup> Christophe E.M. De Block,<sup>1,2,†</sup> Sven M. Francque<sup>2,3,†,\*</sup>

<sup>1</sup>Department of Endocrinology, Diabetology & Metabolism, Antwerp University Hospital, Antwerp, Belgium; <sup>2</sup>Laboratory of Experimental Medicine and Paediatrics and member of the Infla-Med Centre of Excellence, University of Antwerp, Faculty of Medicine & Health Sciences, Antwerp, Belgium;

<sup>3</sup>Department of Gastroenterology & Hepatology, Antwerp University Hospital, Antwerp, Belgium; <sup>4</sup>Department of Radiology, Antwerp University Hospital, Antwerp, Belgium

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**Background & Aims:** The epidemiology of non-alcoholic fatty liver disease (NAFLD) in people with type 1 diabetes (T1D) is not yet elucidated. This study aimed to assess the diagnostic accuracy of non-invasive tests for NAFLD, to investigate the prevalence and severity of NAFLD, and to search for factors contributing to NAFLD in people with T1D.

**Methods:** In this prospective cohort study, we consecutively screened 530 adults with T1D from a tertiary care hospital, using ultrasound (US), vibration-controlled transient elastography equipped with liver stiffness measurement (LSM) and controlled attenuation parameter, and the fatty liver index. Magnetic resonance spectroscopy (MRS) was performed in a representative subgroup of 132 individuals to validate the diagnostic accuracy of the non-invasive tests.

**Results:** Based on MRS as reference standard, US identified individuals with NAFLD with an AUROC of 0.98 (95% CI 0.95–1.00, sensitivity: 1.00, specificity: 0.96). The controlled attenuation parameter was also accurate with an AUROC of 0.85 (95% CI 0.77–0.93). Youden cut-off was  $\geq 270$  dB/m (sensitivity: 0.90, specificity: 0.74). The fatty liver index yielded a similar AUROC of 0.83 (95% CI 0.74–0.91), but the conventional cut-off used to rule in ( $\geq 60$ ) had low sensitivity and specificity (0.62, 0.78). The prevalence of NAFLD in the overall cohort was 16.2% based on US. Metabolic syndrome was associated with NAFLD (OR: 2.35 [1.08–5.12],  $p = 0.031$ ). The overall prevalence of LSM  $\geq 8.0$  kPa indicating significant fibrosis was 3.8%, but reached 13.2% in people with NAFLD.

**Conclusions:** NAFLD prevalence in individuals with T1D is 16.2%, with approximately one in 10 featuring elevated LSM. US-based screening could be considered in people with T1D and metabolic syndrome.

**Impact and Implications:** We aimed to report on the prevalence, disease severity, and risk factors of NAFLD in type 1 diabetes (T1D), while also tackling which non-invasive test for NAFLD is the most accurate. We found that ultrasound is the best test to diagnose NAFLD. NAFLD prevalence is 16.2%, and is associated with metabolic syndrome and BMI. Elevated liver stiffness indicating fibrosis is overall not prevalent in people with T1D (3.8%), but it reaches 13.2% in those with T1D and NAFLD.

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) affects one-quarter of the adult population, with incidence rates expected to rise proportionally alongside the obesity pandemic.<sup>1</sup> NAFLD is characterised by the accumulation of lipid-laden vacuoles in hepatocytes in the absence of other causes of macrovesicular

steatosis or chronic liver disease and encompasses both isolated steatosis and non-alcoholic steatohepatitis (NASH). The latter is considered the more aggressive form and can lead to fibrosis potentially culminating into cirrhosis.<sup>2,3</sup> The presence of excess liver fat can be detected using several non-invasive tests (NITs) including risk scores, abdominal ultrasound (US), controlled attenuation parameter (CAP) as part of a liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE), and magnetic resonance imaging (MRI) such as 1-H magnetic resonance spectroscopy (MRS). The gold standard is liver biopsy, because it is the only reliable technique to simultaneously evaluate steatosis, hepatic necro-inflammation and fibrosis.<sup>2,3</sup> However, liver biopsy is invasive, making it unsuited for epidemiological purposes. MRI is highly accurate to evaluate liver fat content (LFC), and is therefore considered the non-

Keywords: Type 1 diabetes mellitus; NAFLD; Transient elastography; MRI; Liver fibrosis; Metabolic syndrome.

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<sup>†</sup> These authors share last authorship.

\* Corresponding author. Address: Antwerp University Hospital, Drie eikenstraat 655, B-2650 Edegem, Belgium; Tel.: +32 3 821 45 72, fax: +32 3 821 44 78

E-mail addresses: [jonathan.mertens@uza.be](mailto:jonathan.mertens@uza.be) (J. Mertens), [jonas.weyler@zna.be](mailto:jonas.weyler@zna.be) (J. Weyler), [eveline.dirinck@uza.be](mailto:eveline.dirinck@uza.be) (E. Dirinck), [christophe.deblock@uza.be](mailto:christophe.deblock@uza.be) (C.E.M. De Block), [sven.francque@uza.be](mailto:sven.francque@uza.be) (S.M. Francque).



invasive gold standard to assess liver steatosis with increasing implementation in observational studies and pharmaceutical trials.<sup>4</sup> Nonetheless, population-based screening using MRI is logistically unrealistic.

NAFLD has a bidirectional relation with metabolic syndrome (MetS) and type 2 diabetes (T2D), and poses not only a hepatic, but also a cardiovascular health burden, the latter more notable in people with concomitant diabetes.<sup>5–7</sup> Although the risk of cardiovascular disease (CVD) is associated with NAFLD severity, even isolated steatosis imposes an increased CVD risk.<sup>5</sup>

Individuals with type 1 diabetes (T1D) are increasingly affected by overweight/obesity, insulin resistance (IR), and MetS.<sup>8–11</sup> Overall mortality in T1D is increased compared with the general population, mainly attributable to CVD.<sup>12</sup> Because of the negative synergy of hyperglycaemia and MetS regarding CVD, diabetes management includes both tight glycaemic control and efforts to prevent or reduce features of MetS.<sup>13–15</sup> NAFLD is currently not screened for in individuals with T1D. A recent meta-analysis reported a NAFLD prevalence of 22% in adults with T1D.<sup>16</sup> The meta-analysis, however, included highly heterogeneous and mostly retrospective studies, with significant variation in prevalence, ranging from 0.0 to 64.7%.

The aim of the current study was to determine the prevalence of NAFLD in people with T1D and to identify associated factors. We aimed first at assessing the diagnostic accuracy of several NITs to diagnose NAFLD using MRS as the reference standard, and subsequently at determining the prevalence of NAFLD in the overall cohort. Thirdly, we aimed at evaluating the distribution of disease severity, that is the presence of significant fibrosis, based on LSM. Finally, we analysed characteristics associated with NAFLD in this specific population.

## Patients and methods

### Study design

In this observational study conducted between 2018 and 2022, adults with T1D (disease duration >2 years) visiting the diabetes clinic of the Antwerp University Hospital were consecutively screened for NAFLD. Individuals were excluded in case of alpha-1 antitrypsin deficiency, viral hepatitis, Wilson's disease, autoimmune hepatitis, significant alcohol use ( $\geq 3$  units of alcohol per day for males,  $\geq 2$  units per day for females), steatogenic medication use (corticosteroids, amiodarone, tamoxifen, methotrexate, nucleoside reverse transcriptase inhibitors), active pregnancy or pancreas transplantation. We aimed to enrol minimally 50% of all outpatient individuals attending the clinic to obtain an unbiased sample size and adequate power. This study was conducted in accordance with the amended Declaration of Helsinki. All individuals signed an informed consent form. The research protocol was approved by the ethics committee of the University Hospital (18/32/361). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.<sup>17</sup>

### Data collection

Data were collected on demographics, diabetes duration, alcohol consumption, smoking, medication use, and comorbidities. The total daily dose of insulin per kg bodyweight (TDI, U/kg) was calculated. Clinical variables included height (cm), weight (kg), BMI, and waist circumference (WC, cm). Blood pressure was taken after resting for 10 min. The lowest of minimally three

measurements was used. MetS was present if more than two of the following criteria were met, as all individuals were considered to feature hyperglycaemia: (1) increased WC (men  $\geq 102$  cm, women  $\geq 88$  cm), (2) hypertriglyceridaemia ( $\geq 150$  mg/dl = 1.7 mmol/L) or fibrates use, (3) low HDL (men  $< 40$  mg/dl = 1.03 mmol/L, women  $< 50$  mg/dl = 1.29 mmol/L), and (4) hypertension (blood pressure  $\geq 130/85$  mmHg or antihypertensive drug use), based on the 2005 revised National Cholesterol Education Program Adult Treatment Panel III definition.<sup>18</sup> The following laboratory parameters were obtained (fasted): glycated haemoglobin A1c (HbA1c, measured with high-performance liquid chromatography), creatinine, total cholesterol, HDL, LDL, triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and platelet count. Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. Screening for viral hepatitis was done by determining hepatitis B surface antigen and antibodies against hepatitis C. Alpha-1 antitrypsin, IgG, autoantibodies, copper, and ceruloplasmin were determined to screen for antitrypsin deficiency, autoimmune hepatitis and Wilson's disease, respectively.

### Evaluation of liver steatosis and fibrosis

All participants had US/VCTE performed after an overnight fast by trained staff, supervised by a single experienced physician (JM). All investigators were *a priori* certified by Echosens. Steatosis was detected based on the observation of diffuse hyperechogenicity of the liver parenchyma in comparison to the kidney, attenuation of the ultrasonographic beam, and loss of visualisation of the diaphragm and peripheral portal vessels, resulting in an ultrasound score grading 0–3.<sup>19</sup> Liver parenchyma had to be hyperechogenic  $\geq$  grade 1 on ultrasound to be diagnosed as steatosis. VCTE was performed with FibroScan© 502 (Echosens, Paris, France). The Fibroscan featured an M and XL probe, with the probe choice provided by the Automatic Probe Selection algorithm of the device, dependent on the skin–liver capsule distance. In agreement with the European guideline, we explored the prevalence of steatosis based on CAP using a CAP  $> 275$  dB/m (sensitivity and PPV  $> 90\%$ ), irrespective of the probe.<sup>20–22</sup> This proposed cut-off for  $S \geq S1$  steatosis was then internally compared with our MRS data for cohort-specific validation. We used LSM  $< 8.0$  kPa to rule out significant fibrosis ( $\geq F2$  fibrosis).<sup>20</sup> VCTE results were considered reliable if at least 10 valid measurements, a valid/invalid measurement ratio  $> 60\%$  and an interquartile range/median ratio for LSM  $\leq 30\%$  were obtained.<sup>23</sup> The fatty liver index score (FLI) and Fibrosis-4 (FIB-4) scores were calculated and applied in accordance with the guideline, and then compared with MRS and LSM, respectively.<sup>20,24</sup>

### MRS

A subgroup underwent additional MRS to evaluate LFC. As accuracy indices depend on disease prevalence, patients were recruited in a 1:5 NAFLD/no-NAFLD ratio (based on US) to reflect the prevalence previously described in the literature.<sup>16</sup> A 1.5T MAGNETOM Aera (Siemens Healthcare, Erlangen, Germany) magnetic resonance tomograph was used, equipped with syngo MR E11 software running the clinical application Liverlab. We measured LFC in three regions of interest. A mean LFC  $> 5.56\%$  defined NAFLD.<sup>25</sup> Magnetic imaging was assessed by a single certified expert radiologist (MS) blinded from US/VCTE results.

**Statistical analysis**

Data are mean ± SD, median (IQR), or frequencies (percentage), when appropriate. Groups were compared with independent samples *t* test for normally distributed variables, Mann–Whitney *U* test for skewed variables, and  $\chi^2$  test or Fisher’s exact test for categorical variables. Areas under the receiver–operator curve (AUROCs) were determined to evaluate diagnostic accuracy of the variable NITs. AUROCs were compared using DeLong test. Youden’s index was used to determine cut-offs. Multivariable logistic regression was performed to determine factors associated with NAFLD. Odds ratio (OR) with 95% CI were expressed as indicated. A two-sided value of *p* <0.05 was considered significant. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) 28.0 (IBM Corp., Armonk, N.Y., USA) and R Statistical Software (v4.2.2; R Core Team 2021, R Foundation for Statistical Computing, Vienna, Austria).

**Results**

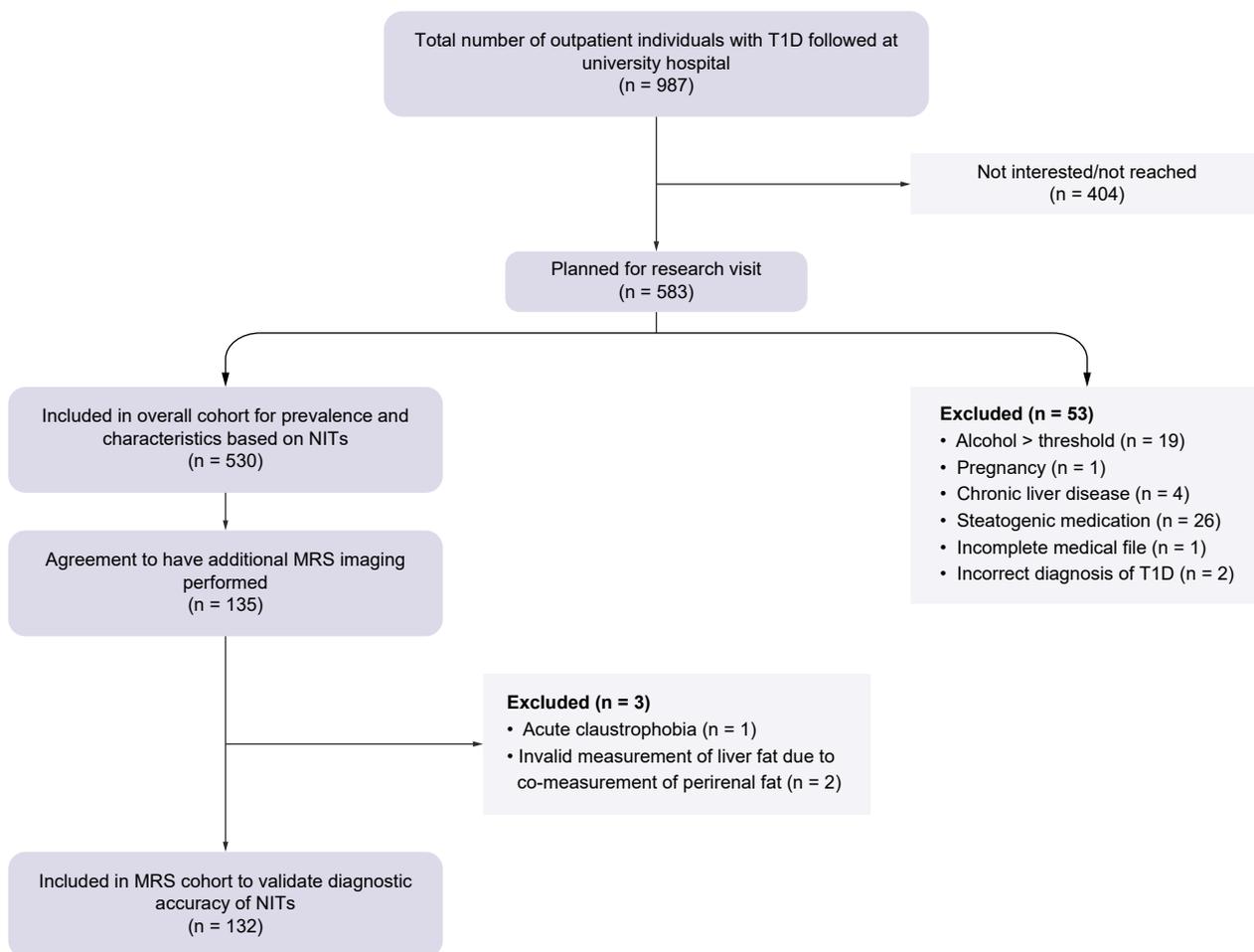
Between October 2018 and March 2022, 583 out of a total population of 987 adults consented to participate (59%). Fifty-three individuals (9.0%) were excluded for reasons summarised in

Fig. 1. Ultimately, 530 individuals were eligible for final analysis. Additional MRS imaging studies were performed in 135 individuals, of which 132 were successful.

**NAFLD diagnosis and characteristics in the MRS cohort**

The median age was 50 (33–61) years, and mean diabetes duration was 29 ± 14 years. Seventy-four individuals (56.1%) were male. Mean BMI was 26.9 ± 4.4 kg/m<sup>2</sup>, with a 29.5% obesity rate (Table 1). Median LFC was 3.2 (2.6–4.4). Twenty-one individuals (15.9%) had NAFLD according to MRS, whereas 25 had NAFLD according to US. This implies that, as the cohort was composed starting from a 1:5 US-based NAFLD ratio, 21/25 were correctly classified as having NAFLD, whereas 0/107 were falsely ruled out by US (see below). LSM ≥8.0 kPa, indicative of fibrosis, was present in five individuals, (3.9%).

Individuals with NAFLD were more often male, and more often obese. Male individuals with NAFLD had an increased WC, whereas the difference in females failed to reach statistical significance. People with NAFLD had more (systolic) hypertension and MetS was more prevalent. Markers of steatosis (FLI and CAP) were significantly higher in the NAFLD group, as were liver enzymes and lipids related to MetS. An increased LSM was



**Fig. 1. Flowchart of study recruitment process showing exclusion criteria for the main and MRS cohort.** Alcohol thresholds >three daily standardised alcoholic drinks for men, >two daily standardised alcoholic drinks for women; chronic liver disease: alpha-1 antitrypsin deficiency, viral hepatitis, Wilson’s disease, autoimmune hepatitis; steatogenic medication: corticosteroids, amiodarone, tamoxifen, methotrexate, nucleoside reverse transcriptase inhibitors; incorrect diagnosis of T1D: absence of autoimmune markers or absence of low/undetectable C-peptide. One person discontinued MRS because of acute claustrophobia. NIT, non-invasive test; MRS, magnetic resonance spectroscopy; T1D, type 1 diabetes.

**Table 1. Baseline characteristics of the MRS cohort and stratification by presence/absence of NAFLD.**

N	MRS cohort	NAFLD	No NAFLD	p value
	132	21	111	
LFC, %	50 [33–6]	8.2 [6.8–17.7]	3.0 [2.5–3.6]	<b>&lt;0.001</b>
Age, years	50 [33–61]	57 [29–65]	49 [35–59]	0.289
Male sex, n (%)	74 (56.1)	16 (76.2)	58 (52.3)	<b>0.043</b>
Caucasian, n (%)	124 (93.9)	19 (90.5)	105 (94.6)	0.468
Alcohol abstinence, n (%)	45 (34.1)	6 (28.6)	39 (35.1)	0.561
Active smoking, n (%)	8 (6.1)	3 (14.3)	5 (4.5)	0.115
Diabetes duration, years	29 ± 14	28 ± 12	29 ± 14	0.915
CSII, n (%)	38 (28.8)	4 (19.0)	34 (30.6)	0.282
TDI, U/kg per 24 h*	0.63 [0.47–0.85]	0.92 [0.80–1.14]	0.60 [0.45–0.75]	<b>&lt;0.001</b>
Biguanide use, n (%)	12 (9.1)	4 (19.0)	8 (7.2)	0.083
GLP-1 RA use, n (%)	8 (6.1)	0 (0.0)	8 (7.2)	0.354
BMI, kg/m <sup>2</sup>	26.9 ± 4.39	29.3 ± 4.1	26.5 ± 4.3	<b>0.007</b>
Obesity, n (%)	39 (29.5)	10 (47.6)	29 (26.1)	<b>0.048</b>
WC, cm				
Males	98.4 ± 12.3	107.6 ± 12.8	95.8 ± 11.0	<b>&lt;0.001</b>
Females	86.2 ± 13.1	94.5 ± 12.0	85.4 ± 13.1	0.139
Blood pressure, mm Hg				
SBP	129 ± 12	134 ± 10	128 ± 12	<b>0.027</b>
DPB	75 ± 9	76 ± 11	75 ± 9	0.705
Antihypertensive drug use, n (%)	52 (39.4)	13 (61.9)	39 (35.1)	<b>0.021</b>
MetS # elements NCEP ATPIII, n (%)				<b>&lt;0.001</b>
1	49 (37.1)	0 (0)	49 (44.1)	
2	41 (31.1)	6 (28.6)	35 (31.5)	
3	27 (20.5)	8 (38.1)	19 (17.1)	
4	12 (9.1)	6 (28.6)	6 (5.4)	
5	3 (2.3)	1 (4.8)	2 (1.8)	
MetS NCEP ATPIII, n (%)	42 (31.8)	15 (71.4)	27 (24.3)	<b>&lt;0.001</b>
Creatinine, mg/dl	0.76 [0.68–0.90]	0.89 [0.62–0.97]	0.76 [0.68–0.86]	0.378
eGFR, mL/min per 1.73 m <sup>2</sup>	100.9 ± 23.5	99.6 ± 28.8	101.1 ± 22.5	0.826
HbA1c, %	7.4 ± 0.9	7.6 ± 0.9	7.4 ± 0.9	0.265
HbA1c, mmol/mol	57 ± 10	60 ± 10	57 ± 10	0.265
Albumin, g/L	41.7 ± 3.1	42.2 ± 3.2	41.5 ± 3.1	0.385
AST, IU/L	24 [20–29]	27 [21–40]	24 [20–28]	<b>0.025</b>
ALT, IU/L	22 [16–31]	32 [20–50]	22 [15–29]	<b>0.007</b>
GGT, IU/L	20 [14–30]	30 [17–51]	19 [13–28]	<b>0.007</b>
TG, mg/dl	85 [64–109]	124 [94–249]	79 [62–95]	<b>&lt;0.001</b>
Total cholesterol, mg/dl	172 ± 35	158 ± 33	175 ± 35	<b>0.048</b>
HDL, mg/dl				
Males	51 ± 12	45 ± 9	52 ± 13	<b>0.038</b>
Females	67 ± 18	44 ± 10	69 ± 18	<b>0.003</b>
LDL, mg/dl	102 [81–120]	99 [76–113]	102 [81–122]	0.383
Statin use, n (%)	64 (48.5)	11 (52.4)	53 (47.7)	0.697
US-NAFLD, n (%)	25 (18.9)	21 (100.0)	4 (3.6)	<b>&lt;0.001</b>
M probe, n (%)	106 (83.5)	14 (70.0)	92 (86.0)	0.100
CAP, dB/m <sup>†</sup>	254 ± 54	310 ± 44	244 ± 49	<b>&lt;0.001</b>
CAP >275 dB/m, n (%) <sup>†</sup>	43 (33.9)	16 (80.0)	27 (25.2)	<b>&lt;0.001</b>
FLI	37 [12–62]	68 [47–86]	33 [9–56]	<b>&lt;0.001</b>
FLI categories, n (%)				<b>0.003</b>
Low risk	55 (41.7)	3 (15.0)	52 (46.4)	
Medium risk	43 (32.6)	6 (30.0)	37 (33.0)	
High risk	34 (25.8)	11 (55.0)	23 (20.5)	
LSM, kPa <sup>†</sup>	5.3 [4.4–6.1]	5.9 [5.3–7.1]	5.1 [4.3–6.0]	<b>0.008</b>
LSM ≥8.0 kPa, n (%) <sup>†</sup>	5 (3.9)	3 (15.0)	2 (1.9)	<b>0.010</b>

Results are given as mean ± SD, median [IQR] or N (%). Comparison between groups with independent samples *t* test for normally distributed variables, Mann–Whitney *U* test for skewed variables, and  $\chi^2$  test or Fisher’s exact test for categorical variables. The significance level was set at *p* < 0.05. Values in bold denote significance.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSII, continuous subcutaneous insulin infusion; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FLI, fatty liver index; GGT, gamma-glutamyl transferase; GLP-1 RA, glucagon-like peptide receptor agonist; HbA1c, haemoglobin A1c; LFC, liver fat content; LSM, liver stiffness measurement; MetS, metabolic syndrome; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III; SBP, systolic blood pressure; TDI, total daily dose of insulin; TG, triglycerides; Tot Chol, total cholesterol; US-NAFLD, ultrasound-determined NAFLD; WC, waist circumference.

\* TDI available in 105 individuals.

<sup>†</sup> VCTE results available in 127 individuals, 20 with NAFLD, 107 without.

proportionally more prevalent in those with NAFLD (15.0 vs. 1.9%) (Table 1).

Multivariate regression analysis showed that MetS and TDI were strongly associated with the presence of NAFLD, whereas

age, sex, HbA1c or diabetes duration were not. When MetS was broken down into its components, elevated TG levels and hypertension were associated with NAFLD (Table 2, panel A). Liver enzymes were not independently associated with NAFLD.

**Table 2. Regression analysis of factors associated with NAFLD.**

Panel A: MRS cohort				
Variable	B	Odds ratio	95% CI	p value
Model 1 (Nagelkerke R <sup>2</sup> = 0.437)				
Age, years	0.017	1.02	0.97–1.07	0.488
Male sex	0.477	1.61	0.31–8.29	0.568
HbA1c (%)	0.117	1.12	0.55–2.31	0.750
<b>MetS</b>	<b>1.453</b>	<b>4.28</b>	<b>1.01–18.05</b>	<b>0.048</b>
<b>TDI (U/kg)</b>	<b>3.570</b>	<b>35.51</b>	<b>2.98–422.98</b>	<b>0.005</b>
Diabetes duration, years	–0.026	0.98	0.91–1.04	0.435
BMI, kg/m <sup>2</sup>	0.111	1.12	0.91–1.37	0.278
Model 2 (Nagelkerke R <sup>2</sup> = 0.330)				
Age, years	0.022	1.02	0.968–1.07	0.347
Male sex	0.833	2.30	0.70–7.59	0.172
HbA1c (%)	0.041	1.04	0.58–1.87	0.890
Increased WC	0.756	2.13	0.67–6.81	0.202
<b>Increased TG</b>	<b>1.401</b>	<b>4.06</b>	<b>1.05–15.64</b>	<b>0.042</b>
Decreased HDL	1.123	3.07	0.82–11.53	0.096
Hypertension	1.331	3.79	1.01–14.33	0.050
Diabetes duration, years	–0.033	0.97	0.92–1.02	0.251
Panel B: US cohort				
Model 1 (Nagelkerke R <sup>2</sup> = 0.371)				
Age, years	0.002	1.00	0.98–1.03	0.846
Male sex	0.104	1.11	0.57–2.15	0.757
HbA1c (%)	–0.052	0.95	0.67–1.34	0.768
<b>MetS</b>	<b>0.952</b>	<b>2.59</b>	<b>1.20–5.58</b>	<b>0.015</b>
<b>TDI, U/kg</b>	<b>1.825</b>	<b>6.20</b>	<b>2.13–18.05</b>	<b>&lt;0.001</b>
Diabetes duration, years	–0.001	1.00	0.97–1.03	0.923
<b>BMI, kg/m<sup>2</sup></b>	<b>0.202</b>	<b>1.22</b>	<b>1.13–1.33</b>	<b>&lt;0.001</b>
Model 2 (Nagelkerke R <sup>2</sup> = 0.317)				
Age, years	–0.003	1.000	0.97–1.02	0.828
Male sex	0.030	1.03	0.55–1.94	0.926
HbA1c (%)	–0.120	0.89	0.64–1.24	0.481
<b>Increased WC</b>	<b>1.95</b>	<b>7.05</b>	<b>3.55–13.99</b>	<b>&lt;0.001</b>
<b>Increased TG</b>	<b>0.853</b>	<b>2.35</b>	<b>1.01–5.48</b>	<b>0.048</b>
Decreased HDL	–0.527	0.59	0.24–1.45	0.252
Hypertension	0.570	1.77	0.84–3.74	0.136
<b>TDI, U/kg</b>	<b>1.969</b>	<b>7.16</b>	<b>2.45–20.90</b>	<b>&lt;0.001</b>
Diabetes duration, years	–0.10	0.99	0.96–1.02	0.485
Model 3 (Nagelkerke R <sup>2</sup> = 0.392)				
Age, years	0.003	1.00	0.98–1.03	0.838
Male sex	0.021	1.02	0.51–2.03	0.953
HbA1c (%)	–0.032	0.97	0.68–1.38	0.860
<b>MetS</b>	<b>0.856</b>	<b>2.35</b>	<b>1.08–5.12</b>	<b>0.031</b>
<b>TDI, U/kg</b>	<b>1.901</b>	<b>6.70</b>	<b>2.20–20.42</b>	<b>&lt;0.001</b>
Diabetes duration, years	–0.006	0.99	0.97–1.02	0.994
<b>BMI, kg/m<sup>2</sup></b>	<b>0.212</b>	<b>1.24</b>	<b>1.14–1.34</b>	<b>&lt;0.001</b>
ALT, IU/L	–0.016	0.99	0.95–1.02	0.423
AST, IU/L	0.039	1.04	0.98–1.10	0.173
<b>GGT, IU/L</b>	<b>0.014</b>	<b>1.01</b>	<b>1.00–1.03</b>	<b>0.043</b>

Logistic regression analysis to determine factors associated with the presence of NAFLD. The significance level was set at  $p < 0.05$ . Values in bold denote significance. Panel A: Model includes all significant covariates derived from univariate analyses and variables based on clinical reasoning. Increased WC: men  $\geq 102$  cm, women  $\geq 88$  cm; increased TG:  $\geq 150$  mg/dl [1.7 mmol/L] or fibrate use; decreased HDL: men  $< 40$  mg/dl [1.03 mmol/L], women  $< 50$  mg/dl [1.29 mmol/L]; hypertension: blood pressure  $\geq 130/85$  mmHg or antihypertensive drug use. TDI was not included in the second model because of missing data and thus lack of power in the MRS cohort. BMI was not included in the second model because of multicollinearity with WC. Liver enzymes are not included in panel A, since panel A aims to identify risk factors. Panel B: Models 1 and 2 include the same factors as the MRS model. Model 3 includes liver enzymes AST, ALT, and GGT to explore their potential as first-line biomarkers. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HbA1c, haemoglobin A1c; MetS, metabolic syndrome; TDI, total daily dose of insulin; TG, triglycerides; WC, waist circumference.

**Validation of NITs compared with MRS in the MRS cohort**

Results including predictive values and likelihood ratios are summarised in Table 3. US yielded an excellent AUROC of 0.98 (0.95–1.00), with a sensitivity of 1.00 and a specificity of 0.96 for  $\geq S1$ . CAP yielded an AUROC of 0.85 (0.77–0.93). The optimal CAP threshold to detect  $\geq S1$ , regardless of the probe, was  $\geq 270$  dB/m, (sensitivity 0.90, specificity 0.74). FLI had an AUROC of 0.83 (0.74–0.91). Youden cut-off was  $\geq 45$  (sensitivity 0.91, specificity 0.64). The conventional cut-off ( $\geq 60$ ) had a sensitivity of 0.62 and a specificity of 0.78. FLI  $< 30$  resulted in a sensitivity of 0.95 and a

specificity of 0.47. DeLong pairwise comparisons showed that the AUROC of US was significantly different from both CAP and FLI ( $p < 0.001$ ), whereas the difference in AUROC between CAP and FLI was not significant ( $p = 0.684$ ). Combining US + CAP or US + FLI did not increase diagnostic accuracy.

**Prevalence and characteristics of NAFLD according to NITs in the overall cohort**

The acquired cut-offs from the accuracy analysis described above were used to further investigate the epidemiology of NAFLD in

**Table 3. Diagnostic accuracy results of NITs compared with MRS.**

N	MRS	US	Conventional CAP according to EASL	CAP determined by Youden index	Conventional FLI	FLI determined by Youden index	US + CAP	US + FLI
	132	132	127	127	132	132	131*	132
AUROC	Ref.	0.98	0.85	0.85	0.83	0.83	0.93	0.93
Cut-off	Ref.	≥1 criterion	>275 dB/m	≥270 dB/m	≥60	≥45	US ≥1 criterion and CAP ≥270 dB/m	US ≥1 criterion and FLI ≥45
NAFLD prevalence, N (%)	21 (15.9)	25 (18.9)	43 (33.9)	46 (36.2)	37 (28.0)	59 (44.7)	21 (16.0)	23 (17.4)
Sensitivity	Ref.	1.00	0.80	0.90	0.62	0.91	0.91	0.91
Specificity	Ref.	0.96	0.75	0.74	0.78	0.64	0.98	0.96
PPV	Ref.	0.84	0.37	0.39	0.35	0.32	0.91	0.83
NPV	Ref.	1.00	0.95	0.98	0.92	0.97	0.98	0.98
+LR	Ref.	25	3.20	3.46	2.81	2.53	45.5	22.8
-LR	Ref.	0.001†	0.27	0.14	0.48	0.14	0.91	0.09

Accuracy analysis of NITs compared with MRS >5.56%. Conventional CAP and FLI cut-offs according to current EASL guidelines. Prevalence shown as N (%). Cut-off determined by Youden's index (sensitivity + specificity - 1). Diagnostic indices shown as percentages. The significance level was set at *p* <0.05.

AUROC, area under receiver-operator curve; CAP, controlled attenuation parameter; FLI, fatty liver index; +LR, positive likelihood ratio; -LR, negative likelihood ratio; MRS, (1H) magnetic resonance spectroscopy; NITs, non-invasive tests; NPV, negative predictive value; PPV, positive predictive value; US, ultrasound.

\* One subject had a positive US, but failed VCTE.

† Equation performed with sensitivity of 0.999.

the overall cohort. The median age of the overall cohort (*n* = 530) was 47 (31–59) years, with a diabetes duration of 26 ± 14 years (Table 4). The majority of individuals were Caucasian (92.1%), and 55.8% were males. Mean BMI was 26.2 ± 4.7 kg/m<sup>2</sup>, 20.6% were obese. MetS was present in 30%. Mean HbA1c level was 7.5 ± 1.0% or 58.0 ± 10.4 mmol/mol. Median TDI was 0.59 U/kg per 24 h. VCTE exams data were successful in 505 individuals. VCTE failed in 3.0% of men compared with 6.8% of women (*p* = 0.041). BMI was higher, for both sexes, in people with failed VCTE, but females with failed VCTE had distinctively higher BMI compared with their male equivalents (36.1 ± 5.8 vs. 25.4 ± 4.7 [females], 31.9 ± 5.0 vs. 26.2 vs. 3.8 kg/m<sup>2</sup> [males], *p* <0.001 for both). Significant fibrosis according to LSM was present in 19 individuals (3.8%).

We stratified the individuals in our main cohort according to US (Table 4). Additional analyses according to CAP and the combination of US + CAP can be found in the Supplementary material.

US-based NAFLD prevalence was 16.2%. Those with NAFLD were older and more often obese with larger WC, higher TDI, and a higher rate of hypertension. Furthermore, HbA1c, liver enzymes, and TG were higher, whereas HDL levels were lower. There were no significant differences in sex distribution, creatinine, or LDL levels. The prevalence of MetS and the percentage of individuals treated with antihypertensive or statins was higher in the NAFLD group. Urinary albuminuria rate and prevalence of clinical microalbuminuria were higher in those with NAFLD. Thirty-nine percent of people with MetS presented with NAFLD, whereas only 6.5% of people without MetS had NAFLD (*p* <0.001). Regression analysis (Table 2, panel B) confirmed the associations found in the MRS subcohort, with NAFLD being associated with MetS, BMI, and TDI. GGT was associated with NAFLD, whereas AST and ALT were not.

### Evaluation of fibrosis based on LSM

As mentioned, 19 persons in the overall cohort had LSM ≥8.0 kPa. Furthermore, elevated LSM was more prevalent in the NAFLD group (US), with an in-group prevalence of 13.2% (Table 4).

Individuals with elevated LSM had a larger WC (98.3 ± 13.4 vs. 90.1 ± 13.3 cm, *p* = 0.008) and more often MetS (52.6 vs. 26.5%, *p* = 0.013). CAP, FLI, and FIB-4 were significantly higher (270 ± 78 vs. 229 ± 56 dB/m, *p* = 0.033; 49 ± 29 vs. 31 ± 27, *p* = 0.004; 0.94

[0.70–1.41] vs. 0.73 [0.48–1.08]), *p* = 0.036). Half of people with significant fibrosis had a CAP above threshold. Age, diabetes duration, HbA1c, TDI, systolic blood pressure and diastolic blood pressure were not different. BMI, liver enzymes and lipids were higher, while thrombocytes and HDL were lower, but this failed to reach statistical significance. Logistic regression showed an OR for significant fibrosis of 7.07 (2.77–18.05, *p* <0.001) when NAFLD was present. No other variables showed an association with fibrosis. Only five of the 19 individuals (sensitivity: 0.26) with elevated LSM had a FIB-4 score ≥1.3 (2.0 for elderly). However, 415 of 485 (specificity: 0.86) without elevated LSM values were correctly ruled out by the FIB-4.

### Screening proposal for people with T1D

We provided an algorithm to screen for NAFLD in people with T1D with MetS and/or obesity, based on our data and the European management guideline for NAFLD.<sup>2</sup> We recommend screening based on US, with referral to more advanced imaging based on the combination of steatosis and fibrosis assessment by VCTE, to detect those individuals at risk for NAFLD-fibrosis. People with steatosis should be rescreened on a regular basis, to intercept progression towards advanced fatty liver disease (Fig. 2).

### Discussion

In this prospective cohort study we report a NAFLD prevalence of 16.2% in people with T1D. The prevalence of significant fibrosis according to an increased LSM ≥8.0 kPa in people with concomitant NAFLD was 13.2% whereas the general prevalence was low (3.2%). MetS was strongly associated with NAFLD, with associations found with its individual components. BMI, TDI, and GGT were also associated with NAFLD. This study is the first to validate commonly used NITs for NAFLD in T1D using MRS as the reference standard, and demonstrates that US has excellent accuracy and outperforms other NITs. We thus propose to use US for screening. When unavailable, CAP or FLI are alternatives, bearing in mind the higher rate of false positives. Although AUROCs were similar, FLI seems to be less able to identify true positives compared with CAP. Furthermore, the ability of FLI to rule in steatosis was lower in people with T1D compared with reports in other populations.<sup>24,26</sup>

**Table 4. Baseline characteristics of the overall cohort and stratification by presence/absence of US-determined NAFLD.**

N	Overall cohort	NAFLD	No NAFLD	p value
	530	86	444	
Age, years	47 [31–59]	52 [35–62]	45 [30–58]	<b>0.029</b>
Male sex, n (%)	296 (55.8)	52 (59.8)	244 (55.1)	0.420
Caucasian, n (%)	488 (92.1)	75 (87.2)	413 (93.0)	0.068
Alcohol abstinence, n (%)	177 (33.4)	27 (31.4)	150 (33.8)	0.667
Active smoking, n (%)	53 (10.0)	15 (17.4)	38 (8.6)	<b>0.038</b>
Diabetes duration, years	26 ± 14	27 ± 13	26 ± 14	0.307
CSII, n (%)	122 (23.0)	17 (19.8)	105 (23.6)	0.434
TDI, U/kg per 24 h*	0.59 [0.46–0.77]	0.77 [0.56–1.03]	0.56 [0.45–0.73]	<b>&lt;0.001</b>
Biguanide use, n (%)	43 (8.2)	15 (17.6)	28 (6.3)	<b>&lt;0.001</b>
GLP-1 RA use, n (%)	11 (2.1)	4 (4.7)	7 (1.6)	0.086
BMI, kg/m <sup>2</sup>	26.2 ± 4.7	30.1 ± 4.5	25.3 ± 4.1	<b>&lt;0.001</b>
Obesity, n (%)	109 (20.6)	51 (59.3)	58 (13.1)	<b>&lt;0.001</b>
WC, cm				
Males	95.4 ± 12.9	108.2 ± 11.2	92.7 ± 11.6	<b>&lt;0.001</b>
Females	86.7 ± 14.6	105.1 ± 15.6	83.5 ± 11.9	<b>&lt;0.001</b>
Blood pressure, mm Hg				
SBP	127 ± 13	133 ± 13	126 ± 13	<b>&lt;0.001</b>
DBP	74 ± 9	75 ± 9	74 ± 9	0.136
Antihypertensive drug use, n (%)	5.1 ± 1.6	45 (52.3)	148 (33.3)	<b>&lt;0.001</b>
Hypertension, n (%)	303 (57.1)	66 (75.9)	237 (53.5)	<b>&lt;0.001</b>
MetS # elements NCEP ATPIII, n (%)				<b>&lt;0.001</b>
1	167 (31.5)	4 (4.7)	163 (36.7)	
2	204 (38.5)	20 (23.3)	184 (41.4)	
3	111 (20.9)	40 (46.5)	71 (16.0)	
4	36 (6.8)	17 (19.8)	19 (4.3)	
5	12 (2.3)	5 (5.8)	7 (1.6)	
MetS, N (%)	159 (30.0)	62 (72.1)	97 (21.8)	<b>&lt;0.001</b>
Creatinine, mg/dl	0.76 [0.67–0.86]	0.78 [0.56–1.03]	0.76 [0.66–0.86]	0.222
eGFR, mL/min per 1.73 m <sup>2</sup>	104 [92–119]	99 [88–115]	107 [93–119]	0.057
HbA1c, %	7.5 ± 1.0	7.6 ± 0.9	7.4 ± 1.0	<b>0.045</b>
HbA1c, mmol/mol	58 ± 10	60 ± 10	58 ± 11	<b>0.045</b>
Albumin, g/L	41.9 ± 3.1	41.0 ± 3.2	42.0 ± 4.1	<b>0.044</b>
AST, IU/L	21 [17–26]	23 [18–28]	21 [16–25]	<b>0.018</b>
ALT, IU/L	22 [16–29]	27 [19–36]	21 [16–28]	<b>&lt;0.001</b>
GGT, IU/L	21 [15–30]	94 [73–138]	20 [15–28]	<b>&lt;0.001</b>
TG, mg/dl	75 [58–99]	94 [73–138]	73 [56–93]	<b>&lt;0.001</b>
Total cholesterol, mg/dl	172 ± 33	175 ± 34	172 ± 33	0.386
HDL, mg/dl				
Males	55 ± 14	50 ± 11	56 ± 14	<b>0.001</b>
Females	69 ± 18	62 ± 18	71 ± 17	<b>0.008</b>
LDL, mg/dl	95 [79–117]	100 [82–120]	94 [78–115]	0.122
Statin use, n (%)	27 ± 22	49 (57.0)	162 (36.6)	<b>&lt;0.001</b>
Use of M probe, n (%)	405 (78.6)	41 (51.2)	364 (83.7)	<b>&lt;0.001</b>
CAP, dB/m <sup>†</sup>	230 ± 58	290 ± 58	220 ± 51	<b>&lt;0.001</b>
FLI	34 ± 28	69 ± 24	28 ± 24	<b>&lt;0.001</b>
LSM, kPa <sup>†</sup>	4.9 [4.0–5.9]	5.5 [4.2–6.6]	4.8 [4.0–5.8]	<b>0.019</b>
LSM ≥8 kPa, n (%) <sup>†</sup>	19 (3.8)	10 (13.2)	9 (2.1)	<b>&lt;0.001</b>
FIB-4	0.74 [0.49–1.08]	0.78 [0.50–1.09]	0.73 [0.49–1.06]	0.802
FIB-4 <1.3 (<2.0 if >65 years)	488 (92.1)	78 (90.7)	410 (92.3)	0.605
Urinary albuminuria rate, µg/min	3.8 [2.1–8.0]	5.0 [2.5–11.1]	3.5 [2.0–7.0]	<b>0.004</b>
Microalbuminuria, n (%)	52 (9.9)	14 (16.5)	38 (8.6)	<b>0.026</b>

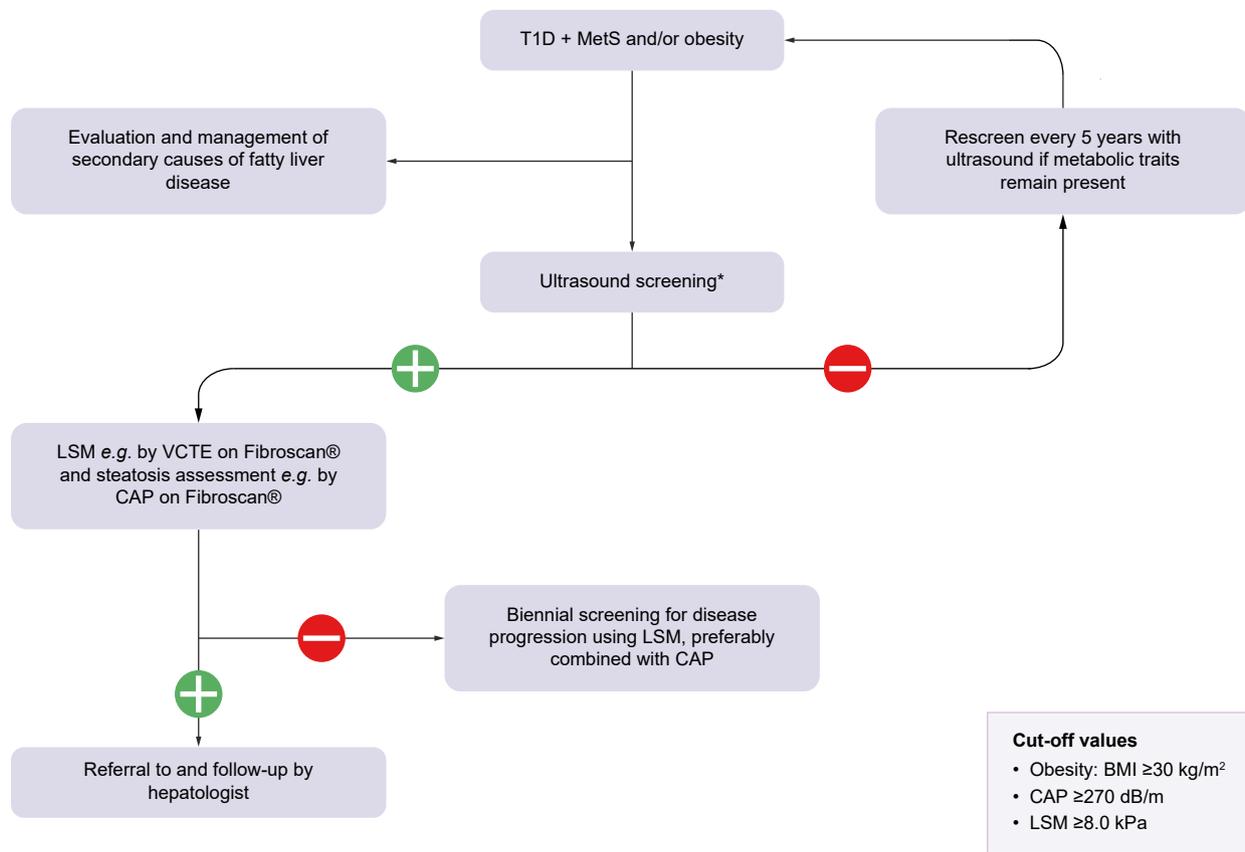
Results are given as mean ± SD, median [IQR] or N (%). Comparison between groups with independent samples *t* test for normally distributed variables, Mann–Whitney *U* test for skewed variables, and  $\chi^2$  test or Fisher's exact test for categorical variables. The significance level was set at *p* < 0.05. Values in bold denote significance.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CSII, continuous subcutaneous insulin infusion; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FIB-4, Fibrosis-4; FLI, fatty liver index; GGT, gamma-glutamyl transferase; GLP-1 RA, glucagon-like peptide receptor agonist; HbA1c, haemoglobin A1c; LFC, liver fat content; LSM, liver stiffness measurement; MetS, metabolic syndrome; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III; SBP, systolic blood pressure; TDI, total daily dose of insulin; TG, triglycerides; Tot Chol, total cholesterol level; WC, waist circumference.

\* Available in 415 individuals.

† VCTE available in 505 individuals, 76 with NAFLD, 429 without.

Our prevalence report aligns with a recent Dutch study in a smaller, comparable cohort. That study found a prevalence of 20% with NAFLD defined as CAP ≥274 dB/m.<sup>27</sup> Prevalence of NAFLD in our study based on CAP ≥270 dB/m was 22.4% (Supplementary material). A Brazilian study reported a prevalence of 12.6% based on US, and 16.8% based on CAP ≥248 dB/m.



**Fig. 2. Diagnostic flowchart to assess and monitor disease severity in the presence of type 1 diabetes combined with metabolic risk factors.** Rescreening intervals at level 1 (negative ultrasound) and level 2 (non-elevated LSM) are derived from the European Association for the Study of the Liver NAFLD management guideline.<sup>2</sup> \*If screening is initially performed with combined ultrasound + VCTE imaging, proceeding immediately to level 2 is recommended. CAP, controlled attenuation parameter; LSM, liver stiffness measurement; MetS, metabolic syndrome; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NIT, non-invasive test; T1D, type 1 diabetes; VCTE, vibration controlled transient elastography.

m.<sup>28</sup> A Finnish study linking NAFLD based on MRI with body fat distribution found a prevalence of 11.3%.<sup>29</sup> These studies were all prospective in design. One meta-analysis reported an overall prevalence rate of NAFLD in T1D (22%). However, the majority of included studies were retrospective analyses of US data with hence selection/referral bias leading to high heterogeneity of the included studies.<sup>16</sup> Indeed, the included US studies reported a pooled prevalence of 27.1%, compared with 8.6% in MRI studies, the latter less prone to retrospective designs<sup>30–32</sup> Our cohort was older, had a higher BMI and had a longer diabetes duration, whereas metabolic control (reflected by the mean HbA1c range of 7.6–12.7%) was globally better compared with populations described in prior studies. Furthermore, the largest MRI-based study involved a *post hoc* analysis of people preselected for a clinical trial of insulin, thus with restrictions on high HbA1c level and BMI.<sup>27,32</sup> Therefore, our study provides currently the largest, unbiased estimate of the true prevalence of NAFLD in T1D.

Until now, accuracy of NITs to report NAFLD in T1D was unproven. Our study is the first to combine several NITs, and validate them to MRS, the gold standard to quantify LFC. Our study compared NITs to diagnose an LFC >5.56%, indicating S ≥ S1.<sup>25</sup> The prevalence of NAFLD varies according to the used NIT,

with US being the most accurate. It is known that the accuracy of US is less in mild steatosis,<sup>19</sup> but our study showed excellent sensitivity and specificity to diagnose even S1. False positivity for NAFLD on US encompassed four patients. This could be explained by the technical difficulty in obtaining adequate imaging of the liver vs. the kidney, leading to false hyper-echogenicity. Indeed, these individuals were obese, rendering imaging more difficult. Glycogenic hepatopathy is recognised as a mimicker of hepatic steatosis on US, however, this is a rare condition reserved for extremely poorly controlled T1D.<sup>33</sup> This was not the case. More importantly, US did not produce any false negatives.

The AUROC and +LR of CAP were statistically lower compared with US. Our CAP cut-offs, derived from our own MRS dataset, aligned strongly with the proposed cut-off >275 dB/m for ≥S1 of the European guideline.<sup>20,21</sup> In CAP-based analysis, contrasting US-based analysis, males were more affected than females (Supplementary material). As VCTE had a higher failure rate in females than in males, possibly because of the distribution of adipose tissue, we possibly missed more cases of females with NAFLD leading to a male predominance in CAP-based assessment.

The AUROC of FLI was significantly lower compared with US, but not different from CAP. However, FLI produced a higher rate of false positives compared to CAP. Our study showed that the +LR for the conventional cut-off  $\geq 60$  (+LR: 2.81), and the alternative cut-off  $\geq 45$  (+LR: 2.53) are significantly lower in people with T1D compared to the reported general population (+LR: 4.3).<sup>24</sup> The -LR of the alternative cut-off matches that of CAP (-LR: 0.14). Globally, this makes FLI less useful for screening directly compared to CAP, not only because of the lower +LR, but also because CAP is part of VCTE thus providing a wider hepatic assessment in a single investigation. We showed that BMI, WC, TG, and GGT were all associated with NAFLD in multivariate regression, whereas AST and ALT were not. The latter is known and indicates that normal transaminase levels do not rule out NAFLD.<sup>34,35</sup>

The prevalence of fibrosis based on LSM we found in T1D resembles previous reports describing a prevalence of 1.8–6.7%, although different cut-offs were used.<sup>27,28,36,37</sup> The recent Dutch study used  $\geq 8.2$  kPa for  $\geq F2$  fibrosis, resulting in a cohort prevalence of elevated LSM of 6.7%, and a prevalence of NAFLD-fibrosis (defined as having both NAFLD and fibrosis) of 3.3%, which is a lower rate of NAFLD-fibrosis compared to us.<sup>27</sup> The Brazilian study reported a NAFLD-fibrosis rate of  $\geq F2$  of 8.4%, with cut-off  $\geq 7.0$  kPa.<sup>28</sup> Given the sample size and probable low overall prevalence, our study has adequate power to estimate overall and NAFLD-related fibrosis based on LSM.

The association of NAFLD with MetS, BMI, and TDI implies a correlation with IR. Screening for NAFLD in individuals with co-existing MetS, as a proxy for clinically relevant IR, could be considered. IR is a pivotal driver for NAFLD, as it contributes to hepatic fat accumulation directly by increasing *de novo* lipogenesis and indirectly by increasing free fatty acid (FFA) flux towards the liver due to decreased inhibition of adipose tissue lipolysis.<sup>38</sup> As adipose tissue dysfunction leading to IR is the pathophysiological driver of T2D, this can explain why NAFLD is more prevalent in T2D compared with T1D. As insulin suppresses peripheral lipolysis, insulin therapy leads to lower FFA availability. Indeed, one study compared people with T1D, insulin-naïve T2D, and insulin-treated T2D and documented that prevalence of NAFLD was lowest in those with T1D, but also distinctively lower in those with insulin-treated T2D compared with insulin-naïve individuals.<sup>32</sup> Furthermore, insulin dynamics are altered in people with T1D, where insulin is administered subcutaneously leading to an altered peripheral-portal insulin gradient, which could also protect against hepatic steatosis.<sup>33</sup> However, alterations associated with T1D can upregulate several transcription factors such as sterol regulatory element-binding proteins (SREBPs) affecting lipogenesis. Thus, the exact mechanism behind NAFLD in T1D is largely unexplored, although IR remains the most probable driver. It is important to notice that the average BMI is increasing in people with T1D, which could

lead to NAFLD rates in T1D closing in on prevalence rates in the general population/T2D.<sup>9</sup>

Both NAFLD (especially in those with concomitant IR) and T1D (even with tight glycaemic control) are strongly related to CVD.<sup>5,39</sup> In addition, some studies have already linked NAFLD with CVD and renal disease in T1D.<sup>38</sup> We demonstrated that patients with T1D and NAFLD have unfavourable cardiometabolic profiles with higher BMI, larger WC and dyslipidaemia, regardless of associated fibrosis, indicating that even early NAFLD reflects an unhealthy metabolism. Whether NAFLD independently contributes to the risk of CVD in people with T1D still needs further study. Nevertheless, given the unfavourable cardiometabolic profile, consideration of more in-depth investigation of metabolic comorbidities and treating them appropriately with comprehensive lifestyle interventions in people with T1D and NAFLD should be advocated. Adjuvant anti-diabetic therapies leading to weight loss, such as GLP-1 receptor agonists or novel dual GIP/GLP-1 receptor agonists are currently not registered for T1D.

Our study has strengths and limitations. This study is the largest study to date using NITs and MRS prospectively in people with T1D. The combined use allows for validation of NITs, tailored to people with T1D. A first limitation is the representativeness of the MRS cohort for the overall T1D population. However, this cohort featured comparable clinical characteristics compared with the overall cohort, and the proportion of NAFLD seen on US was similar in the MRS and the overall cohort, both supporting that the MRS cohort was representative. A second limitation is the unavailability of histology. MRS is considered equally accurate as histology to assess steatosis, but it cannot evaluate the presence of fibrosis.<sup>4</sup> LSM is an adequate surrogate for fibrosis assessment, but is not the gold standard, and the true fibrosis rate is probably lower, owing to the number of false positives that are seen with LSM compared with histology.<sup>2,20</sup> Therefore, it is likewise that the overall presence of significant fibrosis in people with T1D is low, but histological or magnetic resonance elastography data are needed to address this question. Nevertheless, we have shown that elevated LSM values indicating significant fibrosis are present in one out of ten cases with concomitant NAFLD, prompting subsequent investigation of fibrosis when steatosis is detected. Thirdly, as the study population consisted mostly of Caucasian people, data cannot be extrapolated to other ethnicities. Lastly, we could not explore the added value of sequential screening by VCTE or risk scores after performing US to reduce the rate of type I errors, owing to the small sample size of false positives.

In conclusion, this study showed that NAFLD is present in approximately one-sixth of people with T1D, with one-tenth of cases showing elevated LSM. We suggest screening people with T1D and co-existent MetS, preferably with US. Further assessment could benefit from the addition of VCTE, to co-evaluate steatosis and possible fibrosis.

## Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver-operator characteristics curve; CAP, controlled attenuation parameter; CSII, continuous subcutaneous insulin infusion; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR,

estimated glomerular filtration rate; FFA, free fatty acid; FLI, fatty liver index; GGT, gamma-glutamyl transferase; GLP1-RA, glucagon-like peptide 1 receptor agonist; HbA1c, glycated haemoglobin A1c; IR, insulin resistance; LFC, liver fat content; LR, likelihood ratio; LSM, liver stiffness measurement; MRI, magnetic resonance imaging; MRS, (1-H) magnetic

resonance spectroscopy; Mets, metabolic syndrome; NAFLD/NASH, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis; NIT, non-invasive test; OR, odds ratio; SBP, systolic blood pressure; TDI, total daily dose of insulin; TG, triglycerides; T1D, type 1 diabetes; T2D, type 2 diabetes; US, ultrasound; VCTE, vibration-controlled transient elastography; WC, waist circumference.

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### Conflicts of interest

ED has served as a consultant for Novo Nordisk, Ely Lilly, and Boehringer Ingelheim. WK is co-inventor of a patent on the use of lipopigment imaging for disease filed by MIT/MGH. LVG declares to be member of the Advisory Board and/or Speakers Bureau of AstraZeneca, Boehringer Ingelheim, Eli Lilly, MSD, and Novo Nordisk. CDB reports consulting fees and honoraria for speaking for Abbott, AstraZeneca, Boehringer-Ingelheim, A. Menarini Diagnostics, Eli Lilly, Medtronic, Novo Nordisk, and Roche, and research support from AstraZeneca, Boehringer-Ingelheim, Indigo Diabetes, and Novo Nordisk. SF has received grants from Astellas, Falk Pharma, Genfit, Gilead Sciences, GlympsBio, Janssens Pharmaceutica, Inventiva, Merck Sharp & Dome, Pfizer, and Roche. He has acted as consultant for Abbvie, Actelion, Aelin Therapeutics, Aligos Therapeutics, Allergan, Astellas, Astra Zeneca, Bayer, Boehringer Ingelheim, Bristol-Meyers Squibb, CSL Behring, Coherus, Echosens, Eisai, Enyo, Galapagos, Galmed, Genetech, Genfit, Gilead Sciences, Intercept, Inventiva, Janssens Pharmaceutica, Julius Clinical, Madrigal, Medimmune, Merck Sharp & Dome, NGM Bio, Novartis, Novo Nordisk, Promethera, and Roche. He has been lecturer for Abbvie, Allergan, Bayer, Eisai, Genfit, Gilead Sciences, Janssens Cilag, Intercept, Inventiva, Merck Sharp & Dome, Novo Nordisk, and Promethera. All other authors have nothing to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

### Authors' contributions

Conceptualised and designed the screening protocol: JM, JW, BDW, CDB, SF. Performed and supervised the hepatological screening visits: JM, JW, CP, LM, SC, LV, SF. Collected the metabolic data from the diabetes outpatient clinic: CDB, ED, LVG. Performed all MRS protocols: MS, FVH. Wrote the initial manuscript and analysed the data: JM. All authors reviewed the manuscript, provided feedback, and agreed with the final version. CDB and SF are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

### Data availability statement

The datasets generated during and/or analysed in the current study are available from the corresponding author upon reasonable request.

### Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhepr.2023.100753>.

### References

Author names in bold designate shared co-first authorship

- [1] Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11–20.
- [2] EASL-EASD-EASO. Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388–1402.
- [3] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328–357.
- [4] Wang XM, Zhang XJ, Ma L. Diagnostic performance of magnetic resonance technology in detecting steatosis or fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. *Medicine (Baltimore)* 2018;97:e10605.
- [5] Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, et al. NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021;110:921–937.
- [6] Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: pathophysiological mechanisms and implications. *J Hepatol* 2016;65:425–443.
- [7] Morrison AE, Zaccardi F, Khunti K, Davies MJ. Causality between non-alcoholic fatty liver disease and risk of cardiovascular disease and type 2 diabetes: a meta-analysis with bias analysis. *Liver Int* 2019;39:557–567.
- [8] Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, et al. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care* 2005;28:2019–2024.
- [9] Van der Schueren B, Ellis D, Faradji RN, Al-Ozairi E, Rosen J, Mathieu C. Obesity in people living with type 1 diabetes. *Lancet Diabetes Endocrinol* 2021;9:776–785.
- [10] De Block CE, De Leeuw IH, Van Gaal LF. Impact of overweight on chronic microvascular complications in type 1 diabetic patients. *Diabetes Care* 2005;28:1649–1655.
- [11] Corbin KD, Driscoll KA, Pratley RE, Smith SR, Maahs DM, Mayer-Davis EJ. Obesity in type 1 diabetes: pathophysiology, clinical impact, and mechanisms. *Endocr Rev* 2018;39:629–663.
- [12] Huxley RR, Peters SA, Mishra GD, Woodward M. Risk of all-cause mortality and vascular events in women versus men with type 1 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2015;3:198–206.
- [13] Orchard TJ, Olson JC, Erbey JR, Williams K, Forrest KY, Smithline Kinder L, et al. Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 2003;26:1374–1379.
- [14] Holt RIG, DeVries JH, Hess-Fischl A, Hirsch IB, Kirkman MS, Klupa T, et al. The management of type 1 diabetes in adults. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2021:1–44.
- [15] Chillarón JJ, Flores Le-Roux JA, Benaiges D, Pedro-Botet J. Type 1 diabetes, metabolic syndrome and cardiovascular risk. *Metabolism* 2014;63:181–187.
- [16] de Vries M, Westerink J, Kaasjager K, de Valk HW. Prevalence of non-alcoholic fatty liver disease (NAFLD) in patients with type 1 diabetes mellitus: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2020;105:3842–3853.
- [17] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370:1453–1457.
- [18] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American heart association/national heart, lung, and blood Institute scientific statement. *Circulation* 2005;112:2735–2752.
- [19] **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011;54:1082–1090.
- [20] EASL. Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. *J Hepatol* 2021;75:659–689.
- [21] Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2019;156:1717–1730.
- [22] Chan WK, Nik Mustapha NR, Mahadeva S, Wong VW, Cheng JY, Wong GL. Can the same controlled attenuation parameter cut-offs be used for M and XL probes for diagnosing hepatic steatosis? *J Gastroenterol Hepatol* 2018;33:1787–1794.
- [23] Gaia S, Carenzi S, Barilli AL, Bugianesi E, Smedile A, Brunello F, et al. Reliability of transient elastography for the detection of fibrosis in non-

- alcoholic fatty liver disease and chronic viral hepatitis. *J Hepatol* 2011;54:64–71.
- [24] Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;6:33.
- [25] Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462–E468.
- [26] Cuthbertson DJ, Weickert MO, Lythgoe D, Sprung VS, Dobson R, Shojaee-Moradie F, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. *Eur J Endocrinol* 2014;171:561–569.
- [27] de Vries M, Westerink J, El-Morabit F, Kaasjager H, de Valk HW. Prevalence of non-alcoholic fatty liver disease (NAFLD) and its association with surrogate markers of insulin resistance in patients with type 1 diabetes. *Diabetes Res Clin Pract* 2022;186:109827.
- [28] Barros BSV, Monteiro FC, Terra C, Gomes MB. Prevalence of non-alcoholic fatty liver disease and its associated factors in individuals with type 1 diabetes: a cross-sectional study in a tertiary care center in Brazil. *Diabetol Metab Syndr* 2021;13:33.
- [29] Parente EB, Dahlström EH, Harjutsalo V, Inkeri J, Mutter S, Forsblom C, et al. The relationship between body fat distribution and nonalcoholic fatty liver in adults with type 1 diabetes. *Diabetes Care* 2021 Jul;44(7):1706–1713. <https://doi.org/10.2337/dc20-3175>. Epub 2021 May 24. PMID: 34031143.
- [30] Regnell SE, Peterson P, Trinh L, Broberg P, Leander P, Lernmark Å, et al. Magnetic resonance imaging reveals altered distribution of hepatic fat in children with type 1 diabetes compared to controls. *Metabolism* 2015;64:872–878.
- [31] Petit JM, Pedro L, Guiu B, Duvillard L, Bouillet B, Jooste V, et al. Type 1 diabetes is not associated with an increased prevalence of hepatic steatosis. *Diabet Med* 2015;32:1648–1651.
- [32] Cusi K, Sanyal AJ, Zhang S, Hartman ML, Bue-Valleskey JM, Hoogwerf BJ, et al. Non-alcoholic fatty liver disease (NAFLD) prevalence and its metabolic associations in patients with type 1 diabetes and type 2 diabetes. *Diabetes Obes Metab* 2017;19:1630–1634.
- [33] Mertens J, De Block C, Spinhoven M, Driessen A, Francque SM, Kwanten WJ. Hepatopathy associated with type 1 diabetes: distinguishing non-alcoholic fatty liver disease from glycogenic hepatopathy. *Front Pharmacol* 2021;12:768576.
- [34] Ma X, Liu S, Zhang J, Dong M, Wang Y, Wang M, et al. Proportion of NAFLD patients with normal ALT value in overall NAFLD patients: a systematic review and meta-analysis. *BMC Gastroenterol* 2020;20:10.
- [35] Francque SM, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, et al. Noninvasive assessment of nonalcoholic fatty liver disease in obese or overweight patients. *Clin Gastroenterol Hepatol* 2012;10:1162–1168.
- [36] de Lédinghen V, Vergniol J, Gonzalez C, Foucher J, Maury E, Chemineau L, et al. Screening for liver fibrosis by using FibroScan<sup>®</sup> and FibroTest in patients with diabetes. *Dig Liver Dis* 2012;44:413–418.
- [37] Marjot T, Sbardella E, Moolla A, Hazlehurst JM, Tan GD, Ainsworth M, et al. Prevalence and severity of non-alcoholic fatty liver disease are underestimated in clinical practice: impact of a dedicated screening approach at a large university teaching hospital. *Diabet Med* 2018;35:89–98.
- [38] Mertens J, Van Gaal LF, Francque SM, De Block C. NAFLD in type 1 diabetes: overrated or underappreciated? *Ther Adv Endocrinol Metab* 2021;12:20420188211055557.
- [39] Schofield J, Ho J, Soran H. Cardiovascular risk in type 1 diabetes mellitus. *Diabetes Ther* 2019;10:773–789.