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Prevalence of non-alcoholic fatty liver in the general Dutch population and in groups at increased risk

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Abstract *Background and aim:* Non-alcoholic fatty liver disease (NAFLD) is defined as a liver fat content ≥5.56%. It is of clinical interest to know the prevalence of NAFLD in people with a combination of metabolic risk factors. We aimed to examine the prevalence of NAFLD, including groups with metabolic risk factors.

Methods and results: In this cross-sectional analysis of the Netherlands Epidemiology of Obesity (NEO) study, liver fat content was assessed using proton magnetic resonance spectroscopy (H-MRS). Participants with excessive alcohol consumption or missing values were excluded, leaving a total of 1570 participants for the analyses.

Mean (SD) age of the population was 55 years, BMI 25.9 (4.0) kg/m^2 and 46% were men. The prevalence of NAFLD was 27% (95% CI 24–30). The prevalence of NAFLD was increased in participants with hypertriglyceridemia (57%, 52–63), obesity (62%, 58–66) and diabetes (69%, 61–77). The prevalence of NAFLD was highest in those with diabetes and obesity (79%, 71–87), obesity and hypertriglyceridemia (81%, 76–86) and with diabetes and hypertriglyceridemia (86%, 77–95). NAFLD was also present in 12% (8-16) of participants without overweight.

Conclusions: The prevalence of NAFLD in a middle-aged population in the Netherlands in 2010 was 27%. The prevalence of NAFLD is particularly increased in individuals with diabetes, obesity, and hypertriglyceridemia. This information may help clinicians and general practitioners in the risk stratification of their patients in daily practice.

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index kg/m^2; DHD, Dutch heathy diet; ELF, enhanced liver fibrosis; FFA, free fatty acids; FFQ, food frequency questionnaire (FFQ); FLI, fatty liver index; gGT, gamma glutamyl transferase; HBA1c, glycated haemoglobin; HDL, high density lipoprotein; H-MRS, proton magnetic resonance spectroscopy; HOMA-IR, homeostatic model assessment for insulin resistance; HTGC, hepatic triglyceride content (liver fat content); LDL, low density lipoprotein; LUMC, Leiden University Medical Center; VAT, visceral adipose tissue; MET, *metabolic equivalents of task*; METC, medical ethics committee; MRI, magnetic resonance imaging; NAFLD, Non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NEO, Netherlands Epidemiology of Obesity study; NFS, NAFLD fibrosis score; FIB-4, fibrosis 4 calculator; PNPLA3, patatin-like phospholipase domain-containing protein 3; SNP, single nucleotide polymorphism.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of steatosis (hepatic triglyceride content or liver fat content), not due to excessive alcohol consumption, in more than 5% of the hepatocytes in histological analysis or more than 5.56% assessed by proton magnetic resonance spectroscopy (H-MRS) [1–3]. NAFLD covers a broad clinical spectrum, ranging from pure hepatic steatosis to non-alcoholic steatohepatitis (NASH), and cirrhosis. NAFLD is a leading cause of chronic liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC), and it is associated with type 2 diabetes and cardiovascular disease [4–8]. A worldwide prevalence of NAFLD of 25% was reported in a meta-analysis of more than 8.5 million adult participants in 85 studies from all continents of the world [9]. However, a wide range of prevalences for different countries were reported. In Europe, the overall pooled NAFLD prevalence was 24% varied from 20 to 80%, depending on the country but also on the selection of the studied group, with differences in ethnicity and the presence of metabolic risk factors [8,9].

Multiple factors are involved in the pathogenesis of NAFLD such as metabolic risk factors: hypertension, diabetes mellitus, obesity and dyslipidaemia [10–13]. As a result, the prevalence of NAFLD is increased in groups with these risk factors. In addition, several genetic variants of NAFLD have been detected, with patatin-like phospholipase domain containing 3 gene (PNPLA3-SNPs) most strongly predisposing for NAFLD [14,15].

Whereas the relation between obesity and NAFLD is well established, it is of clinical interest to know the prevalence of NAFLD in people with a combination of metabolic risk factors of NAFLD, such as type 2 diabetes, hypertension, dyslipidaemia and PNPLA3-SNPs. Improved knowledge of which individuals with certain clinical features are at increased risk of NAFLD will contribute to development of clinically applicable and cost-effective strategies to identify subjects at an increased risk of complications due to NAFLD.

The aim of this study was therefore to extensively examine the prevalence of NAFLD in a Dutch middle aged population-based study with direct assessment of liver triglyceride content by H-MRS, and in different groups in this population with known risk factors for NAFLD.

2. Methods

2.1. Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in individuals aged 45–65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. The present study is a cross-sectional analysis of the baseline measurements of the participants. Detailed information about the study design and data collection has been described elsewhere [16]. Between September 2008 and September 2012, men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher, living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. Participants of the NEO study were recruited via three recruitment strategies. First, by general practitioners in the area of Leiden, who sent invitations to their population aged between 45 and 65 years. Men and women with a self-reported BMI$\geq$27 kg/m^2 were invited to participate. Second, through advertisements in local newspapers and posters in Leiden and surroundings. Third, via the registries of three municipalities surrounding Leiden (Katwijk, Leiderdorp and Teylingen). All inhabitants aged between 45 and 65 years were sent an invitation to participate in the NEO study. Inhabitants of Katwijk and Teylingen were invited to participate if they had a self-reported BMI of 27 or higher. All inhabitants aged between 45 and 65 years from the municipality Leiderdorp were invited to participate, irrespective of their BMI, to allow for a reference distribution of BMI. Of the 8229 inhabitants of Leiderdorp within the range 45–65 years who were sent an invitation to participate, 1671 participated in the NEO study (20.3%) [16].

Prior to the study visit, participants completed a questionnaire about demographic and clinical information. At the study site, participants completed a screening form, asking about anything that might create a health risk or interfere with MRI (most notably metallic devices or claustrophobia). A body circumference of more than 1.70 m was an additional contraindication for undergoing MRI. Approximately 35% of the participants who were eligible for MRI were randomly selected to undergo MRI. For the present analysis, we included participants with available measurement of hepatic fat content, and excluded participants who reported to use more than 30 g/day alcohol for men and 20 g/day alcohol for women and those with missing data [1].

The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent [16].

2.2. Data collection

Ethnicity was self-reported and then grouped into white and other. Education level was grouped as low (none, primary school, or lower vocational education) and high education (intermediate secondary education, middle-level vocational education, higher secondary education, higher professional education, university). Tobacco smoking was categorized into three categories: current smokers, former smokers and those who never smoked.

Brachial blood pressure was measured in a seated position on the right arm using a validated automatic oscillometric device (OMRON, Model M10-IT, Omron Health Care Inc, IL, USA). The mean systolic and diastolic blood pressure were calculated over 3 blood pressure measurements after 5 min of rest. Body weight and height were measured in the morning after an overnight fast. Body weight and percent body fat were estimated using the Tanita bio impedance balance (TBF-310, Tanita,

International division, UK) without shoes and 1 kg was subtracted from the body weight for clothes. Body Mass Index (BMI) was calculated by dividing the body weight in kilograms by the height in meters squared [16].

Habitual dietary intake of all participants was estimated using a semi-quantitative 125-item food frequency questionnaire [17–19]. Dietary intake of nutrients and alcohol was estimated using the Dutch Food Composition Table (NEVO-2011) [19]. Alcohol consumption was expressed in grams/day. Based on the food frequency questionnaire (FFQ) the Dutch heathy diet (DHD) index 2015 was calculated, with a higher score representing better adherence to the Dutch Guidelines for Healthy Diet of 2015 [18–20]. Participants reported the frequency and duration of their habitual physical activity during leisure time, which was expressed in hours per week of metabolic equivalents of task (MET-h/week). [21,22].

2.3. Blood sampling and analyses

Fasting blood samples were drawn from the antecubital vein after an overnight fast of at least 10 h after 5 min rest of the participant. Fasting serum total cholesterol and triglyceride concentrations were measured with enzymatic colorimetric assays (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany) and fasting serum HDL concentrations with third-generation homogenous HDL methods (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany). Fasting LDL concentrations were calculated using the Friedewald equation [23]. Concentrations of aspartate amino transferase (AST), alanine amino transferase (ALT) and gamma glutamyl transferase (gGT) were measured using Cobas Integra 800 analyzer, Roche Diagnostics. Fasting plasma glucose concentrations were determined by enzymatic colorimetric methods (CV <5%, Roche Modular Analytics P800; Roche Diagnostics, Mannheim, Germany).

Fasting serum insulin concentrations were determined by an immunometric method (CV <5%, Siemens Immulite 2500; Siemens Healthcare Diagnostics, Breda, the Netherlands). HbA1c concentrations were measured by HPLC boronate affinity chromatography (CV <3%, Primus Ultra; Siemens Healthcare Diagnostics, Breda, the Netherlands). Using fasting glucose and insulin concentrations, the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 was calculated.

Genomic DNA was extracted from venous blood samples obtained from the antecubital vein. Genotyping was performed in Centre National de Génotypage (Evry Cedex, France), using the Illumina HumanCoreExome-24 BeadChip (Illumina, San Diego, CA). The detailed quality-control process has previously been described [14]. Genotypes were further imputed to the 1000 Genome Project reference panel (version 3, 2011) using IMPUTE (version2.2) software. The PNPLA3 variant rs738409, which was not directly genotyped (imputation quality (Rsq): 0.94), was hard-called with PLINK using an uncertainty threshold of 0.2 [24–27]. Because of the small proportion of PNPLA3 GG (7%) we merged those with PNPLA3 CG into one group.

2.4. Hepatic triglyceride content and definition of NAFLD

Hepatic triglyceride content was assessed using H-MRS. A 8 ml voxel was visually positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages with water suppression were collected. Spectra were obtained with an echo time of 26 ms and a repetition time of 3000 ms. Data points [1,024] were collected using a 1000 Hz spectral line. Spectra without water suppression, with a repetition time of 10 s and without changing any parameters, were obtained as internal reference. H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium). Hepatic triglyceride content relative to water was calculated as the sum of signal amplitudes of methyl and methylene divided by the signal amplitude of water and then multiplied by 100 [28]. A fat percentage of 5.56% or higher was defined as presence of NAFLD [1,2].

In the same subsample, visceral adipose tissue was assessed by MRI (1.5 T magnetic resonance imaging, Philips Medical Systems, Best, the Netherlands) using a turbo spin echo imaging protocol. Three transverse images with a slice thickness of 10 mm were obtained at the level of the 5th lumbar vertebra during a breath-hold. Visceral adipose tissue was quantified by converting the number of pixels to centimeters squared using in house developed software (MASS, Medis, Leiden, the Netherlands). In the analyses, the average of the three slices was used [16].

2.5. Definitions of risk groups

Participants were divided into two age groups of 45–55 years and 55–65 years of age. In addition, the participants were divided in three BMI classes: normal weight, defined as BMI <25 kg/m^2, overweight as BMI 25–30 kg/m^2, and obese as BMI >30 kg/m^2. Moreover, participants were categorized according to visceral adipose tissue: <100 cm^2 or ≥100 cm^2. Diabetes was defined as self-reported diabetes, use of glucose lowering medication and/or high fasting plasma glucose concentration (≥7 mmol/l)). Impaired glucose metabolism was defined as fasting plasma glucose concentration 6.1–7.0 mmol/l, and normal glucose metabolism as fasting plasma glucose concentration <6.1 mmol/l.

Hypertension was defined as a systolic blood pressure ≥135 mmHg, diastolic blood pressure ≥85 mmHg diastolic, or the use of antihypertensive medication. PNPLA3 genotype was divided in two groups: CC and CG + GG genotype. Hypertriglyceridemia was defined as serum triglyceride concentration ≥1.7 mmol/l. A low HDL concentration was defined as <1.05 mmol/l for women and <1.25 mmol/l for men.

2.6. Statistical analyses

In the NEO study, individuals with a BMI ≥27 kg/m^2 or higher were overrepresented [29]. To correct for this oversampling, all analyses were weighted towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI

distribution of the general Dutch population [30,31]. Therefore, the results apply to a general population without oversampling of individuals with a BMI >27 kg/m^2.

Characteristics of the study population were expressed as mean (standard deviation (SD)), or as percentages (95% confident interval (CI), stratified by the presence of NAFLD. We assessed the prevalence of NAFLD with 95% confidence intervals for the overall population and predefined risk groups with a specific risk factor or combination of risk factors. We constructed a scatterplot of BMI and liver fat content and explored non-linearity of the association. For this, we transformed liver fat content to the natural logarithm. Finally, we used logistic regression analysis to calculate odds ratios with 95% CI for having NAFLD for the following groups compared with the reference: age ≥55 years (reference: <55 years), men (reference: women), BMI 25–30 and BMI ≥30 kg/m^2 (reference: BMI <25 kg/m^2). Visceral adipose tissue ≥100 cm^2 (reference: <100 cm^2), impaired glucose metabolism and diabetes (reference: normal glucose metabolism), hypertension (reference: normotension), hypertriglyceridemia (reference: normal or low triglycerides), and low HDL (reference: normal or high HDL). All statistical analyses were performed with STATA statistical software, version 12 (StataCorp LP, college station, TX, U.S.A.).

3. Results

3.1. Characteristics of the study population

In total, 2083 participants underwent H-MRS of liver fat content. After exclusion of participants with excessive alcohol consumption (men >30 g/day (n = 343 participants) and women >20 g/day (n = 116 participants)) and with missing values (missing concentrations of glucose (n = 13), insulin (n = 6), ALT (n = 3), HbA1c (n = 10), missing information on education (n = 11), missing history of cardiovascular disease (n = 5) and visceral adipose tissue (n = 6)), 1570 participants were included in the analyses. For the analyses on PNPLA3 participants from non-European ancestry (n = 208) were additionally excluded.

The mean (SD) age of the study population was 55 years (6.0), mean BMI was 25.9 kg/m^2 (4.0) and 46% were men (Table 1). The overall prevalence (95% CI) of NAFLD in the total population was 27% (24–30). Men and smokers more often had NAFLD (Table 1). NAFLD was also more frequently present in participants with diabetes, cardiovascular disease, use of antihypertensive, lipid lowering and glucose lowering drugs (Table 1). Within this population without excessive alcohol consumption, alcohol

Table 1 Characteristics of the study population, 45–65 years of age, stratified by the presence of NAFLD.

	Total	Without NAFLD	NAFLD
Proportion (%)	100	73 (70–76)	27 (24–30)
Age (year)	55 (6.0)	55 (6.0)	57 (5.7)
Sex (% men)	46 (44–48)	41 (38–44)	59 (55–63)
BMI (kg/m^2)	25.9 (4.0)	24.9 (3.4)	28.5 (4.2)
Education level (% high)	44 (41–45)	44 (41–47)	42 (38–46)
Etnicity (% white)	95 (94–96)	95 (94–96)	95 (93–97)
Tobacco smoking (% never)	44 (42–46)	47 (44–50)	38 (34–41)
Alcohol intake (g/d)	8.6 (8.0)	8.2 (7.7)	9.5 (8.5)
Physical activity (MET hr/week)	37.9 (32.5)	39.6 (32.7)	33.3 (31.4)
Dutch healthy diet index	73.1 (14.2)	74.3 (14.2)	70.0 (13.6)
Medical history			
Diabetes (%)	4 (3–5)	2 (1–3)	11 (9–13)
Cardiovascular disease (%)	4 (3–5)	4 (3–5)	6 (4–8)
Medication			
Antihypertensive drugs (%)	19 (17–21)	15 (13–17)	29 (26–32)
Lipid lowering drugs (%)	8 (7–9)	6 (4–8)	15 (12–18)
Glucose lowering drugs (%)	2 (1–3)	1 (0–2)	5 (3–7)
Liver fat and visceral fat			
Liver fat content (%)	5.4 (5.0–5.8)	2.1 (2.0–2.2)	14.1 (13.2–15.0)
Visceral adipose tissue (cm^2)	86.1 (53.3)	70.9 (42.4)	126.6 (58.1)
Serum concentrations			
Triglycerides (mmol/l)	1.2 (0.8)	1.0 (0.6)	1.7 (1.1)
LDL cholesterol (mmol/l)	3.6 (1.0)	3.6 (0.9)	3.7 (1.0)
HDL cholesterol (mmol/l)	1.5 (0.5)	1.6 (0.5)	1.3 (0.4)
Fasting insulin (IU/l)	9.7 (7.9)	8.1 (6.1)	14.1 (10.1)
Fasting glucose (mmol/l)	5.4 (1.0)	5.2 (0.7)	5.9 (1.5)
HBA1c (mmol/mol)	36 (0.5)	34 (0.3)	38 (0.7)
AST (IU/l)	24.3 (6.9)	23.5 (6.2)	26.5 (8.0)
ALT (IU/l)	24.6 (10.9)	22.1 (7.7)	31.4 (14.6)
gGT (U/l)	25.8 (21.0)	22.1 (15.5)	35.4 (29.0)
Other			
HOMA-IR	2.4 (2.6)	1.9 (1.5)	3.8 (4.0)
PNPLA3 CC* (%)	61.2 (59–64)	64.0 (61–67)	53.9 (50–58)
PNPLA3 CG* (%)	32.0 (30–34)	30.7 (28–34)	35.4 (32–39)
PNPLA3 GG* (%)	6.8 (6–8)	5.3 (4–7)	10.7 (8–13)

Data are shown as mean with standard deviation or proportion with 95% confident interval. Results are based on analyses weighted towards the BMI distribution of the general population (n = 1570). *n = 1362.

consumption was higher in those with NAFLD (9.5 g/day (SD 8.5)), than in participants without NAFLD (8.2 g/day (7.7)). All markers of lipid and glucose metabolism (LDL, triglycerides, insulin, glucose concentration, HOMA-IR) were highest in those with NAFLD. In addition, AST, ALT and gGT concentration were higher in participants with NAFLD than in those without.

3.2. BMI and NAFLD

In Fig. 1, a scatterplot of the relation between BMI and liver fat content is shown. NAFLD was present in 12% (8–16) of participants with a BMI <25 kg/m^2, 38% (34–42) of the participants with a BMI 25–30 kg/m^2 and 62% (58–66) of participants with a BMI ≥30 kg/m^2 NAFLD (Table 2).

The majority of participants with a BMI<25 kg/m^2 and NAFLD were men. Also, the use of medication, alcohol consumption and the presence of diabetes mellitus were more frequent in this group, whereas the degree of physical activity was lower than in those with BMI<25 kg/m^2 without NAFLD (Table 2). In addition, concentrations of all markers of lipid and glucose metabolism were higher in those with NAFLD than in those without NAFLD, except for HDL concentration.

Participants with a BMI≥30 kg/m^2 and NAFLD were slightly older, less physically active, more often had diabetes and cardiovascular diseases and more often used medication than those with obesity without NAFLD. All concentrations of biomarkers of lipid (except for HDL) and glucose metabolism, as well as liver enzymes were higher in participants with NAFLD and BMI≥30 kg/m^2 than in those without NAFLD.

3.3. Prevalence of NAFLD in groups with metabolic risk factors

In Fig. 2A is shown that NAFLD was more frequently present in participants of 55–65 years, men, participants with BMI 25–30 kg/m^2 or ≥30 kg/m^2, visceral adipose tissue ≥100 cm^2, impaired glucose metabolism or diabetes, hypertension, PNPLA3-SNPs CG and GG genotype, serum hypertriglyceridemia or low HDL concentrations. The prevalence of NAFLD was highest 69%, (95% CI 61–77) in participants with diabetes (Fig. 2A).

In Table 3, crude odds ratios of NAFLD are shown for the different risk groups compared with the appropriate reference categories. Risk groups with diabetes, BMI≥30 kg/m^2, excess visceral adipose tissue, and hypertriglyceridemia had the highest prevalence odd ratio of NAFLD.

Table 4 shows that the prevalence of NAFLD was highest in individuals with a combination of risk factors, namely 79% (95% CI71-78) in those with diabetes and obesity, 81% (76–86) in those with obesity and hypertriglyceridemia, and 86% (77–95) in those with diabetes and hypertriglyceridemia. NAFLD was more prevalent in women (68%, 56–80) than in men (45% (40–50) with low HDL concentrations. In all other groups, women had a lower prevalence of NAFLD than men. The combination of the risk factors in Table 4 with the PNPLA3CGGG genotype resulted in a prevalence of NAFLD ranging between 40 and 77%.

4. Discussion

In this population-based study with direct assessment of liver fat content by H-MRS, the prevalence of NAFLD in the middle-aged Dutch population was 27%. The prevalence of

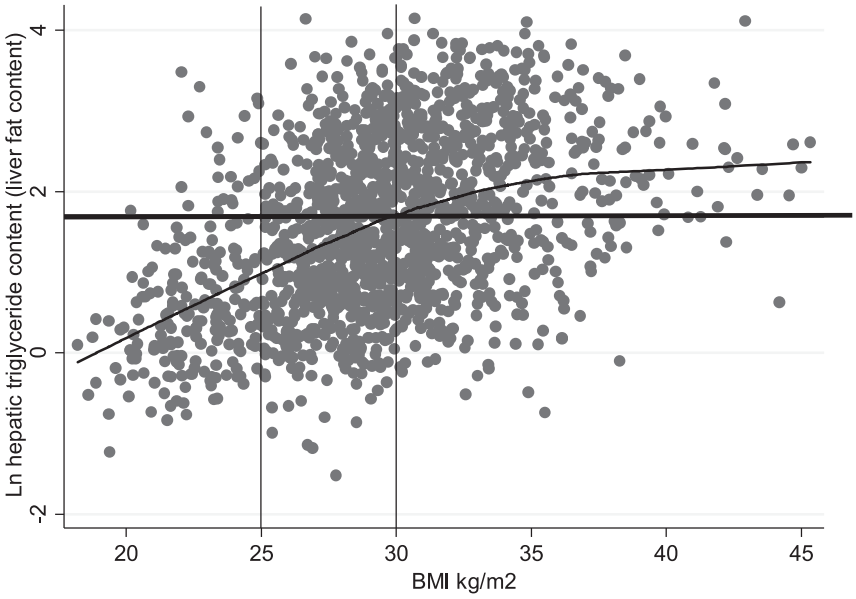

Figure 1 Crude scatterplot of the relation of liver fat content. The vertical lines presents BMI 25 kg/m^2 and BMI 30 kg/m^2. The bold line represents a hepatic triglyceride content of 5.56%, values > 5.56% represent NAFLD. NAFLD was present in 12% of the participants with a BMI below 25 kg/m^2 had NAFLD. 38% of participants with a BMI 25–30 kg/m^2 and 62% of participants with a BMI>30 kg/m^2 had NAFLD. The curved line represents the nonlinear relation between BMI and lnhepatic triglyceride content (liver fat content). The results are based on unweighted NEO population (n = 1570).

Table 2 Characteristics of 6 subgroups according to BMI and the presence of NAFLD.

	BMI <25 without NAFLD	BMI <25 with NAFLD	BMI 25–30 without NAFLD	BMI 25–30 with NAFLD	BMI ≥30 without NAFLD	BMI ≥30 with NAFLD
Proportion of entire cohort (%)	11 (6–16)	2 (-3-7)	28 (22–32)	17 (12–22)	16 (12–20)	26 (22–30)
Proportion NAFLD in BMI subgroup	–	12 (8–16)	–	38 (34–42)	–	62 (58–66)
Age (year)	54.9 (6.0)	58.2 (5.1)	55.2 (6.1)	56.4 (5.7)	54.6 (6.2)	56.1 (5.9)
Sex (% men)	35 (28–42)	56 (37–75)	52 (47–57)	68 (62–74)	33 (27–39)	53 (48–58)
BMI (kg/m^2)	22.4 (1.5)	23.5 (1.2)	27.7 (1.4)	28.2 (1.2)	32.7 (2.5)	33.5 (3.0)
Education level (% high)	50 (42–58)	52 (32–72)	38 (33–43)	44 (38–50)	28 (22–34)	31 (25–35)
Ethnicity (% white)	96 (93–99)	96 (88–104)	96 (94–98)	96 (94–98)	93 (90–96)	95 (93–97)
Tobacco smoking (% never)	50 (43–57)	40 (21–59)	47 (42–52)	39 (33–45)	36 (30–42)	35 (30–40)
Alcohol intake (g/d)	7.8 (7.0)	9.3 (8.5)	8.6 (8.2)	10.8 (8.6)	6.5 (7.5)	7.8 (8.3)
Physical activity (MET hr/week)	41.6 (33.7)	36.5 (42.7)	39.1 (35.6)	32.4 (26.7)	33.8 (31.1)	33.3 (31.5)
Dutch healthy diet index	76.3 (14.1)	75.4 (10.3)	72.7 (13.7)	69.0 (13.2)	72.5 (14.2)	68.2 (14.4)
Medical history						
Diabetes (%)	1 (0–2)	8 (-3-19)	3 (1–5)	7 (4–10)	8 (5–11)	18 (14–22)
Cardiovascular disease (%)	3 (1–5)	0 (0)	4 (2–6)	5 (2–8)	4 (2–6)	8 (5–11)
Medication						
Antihypertensives (%)	11 (7–16)	28 (10–46)	20 (16–23)	24 (19–29)	31 (25–36)	41 (36–46)
Lipid lowering drugs (%)	2 (0–4)	12 (-1-25)	11 (8–13)	12 (8–16)	15 (11–19)	21 (17–25)
Glucose lowering drugs (%)	1 (-1-2)	4 (-4-12)	2 (0–3)	3 (1–5)	4 (2–7)	10 (7–13)
Liver fat and visceral fat						
Liver fat content (%)	1.7 (1.1)	12.5 (7.3)	2.5 (1.3)	13.4 (8.6)	3.0 (1.3)	17.4 (11.0)
Visceral adipose tissue (cm^2)	51.2 (30.4)	88.5 (40.4)	94.6 (42.9)	127.1 (47.4)	120.2 (49.1)	165.3 (62.7)
Concentrations						
Triglycerides (mmol/l)	0.9 (0.4)	1.6 (1.2)	1.2 (0.8)	1.7 (1.2)	1.3 (0.6)	1.8 (1.0)
LDL (mmol/l)	3.5 (0.9)	3.9 (1.1)	3.6 (0.9)	3.8 (1.0)	3.6 (1.0)	3.5 (1.0)
HDL (mmol/l)	1.7 (0.5)	1.4 (0.4)	1.5 (0.4)	1.3 (0.3)	1.4 (0.4)	1.2 (0.3)
Fasting insulin (IU/l)	6.7 (5.5)	9.4 (4.5)	9.4 (6.3)	13.2 (6.7)	11.9 (7.9)	19.1 (13.5)
Fasting glucose (mmol/l)	5.1 (0.6)	6.0 (2.4)	5.4 (0.7)	5.7 (0.9)	5.5 (0.7)	6.1 (1.4)
HbA1c (mmol/mol)	34 (0.3)	38 (1.1)	34 (0.3)	37 (0.5)	36 (0.4)	39 (0.7)
AST (IU/l)	23.8 (6.4)	25.8 (5.3)	23.1 (5.8)	26.8 (8.9)	23.4 (6.8)	27.3 (8.9)
ALT (IU/l)	20.9 (6.7)	27.9 (8.8)	23.1 (8.6)	32.8 (15.2)	23.6 (9.7)	34.3 (16.5)
gGT (U/l)	19.8 (14.2)	28.4 (15.9)	24.3 (16.2)	39.5 (42.2)	27.9 (21.8)	38.0 (23.8)
Other						
HOMA-IR	1.5 (1.3)	2.5 (1.6)	2.3 (1.6)	3.4 (1.9)	3.0 (2.4)	5.4 (6.1)
PNPLA3 CG* (%)	32.9 (26–40)	25.0 (8–42)	26.5 (22–31)	41.3 (35–48)	31.6 (25–38)	37.7 (33–43)
PNPLA3 GG* (%)	7.9 (4–12)	8.3 (3–19)	2.9 (1–5)	10.0 (6–14)	0.5 (0–1)	10.3 (7–13)

Data are shown in mean with SD or proportion (%) with 95% CI. The results are based on unweighted NEO population (n = 1570). * (n = 1362).

NAFLD was particularly increased in participants with hypertriglyceridemia (57%), obesity (62%) and diabetes (69%). NAFLD prevalence was highest in individuals with a combination of these risk factors, namely 79% in those with diabetes and obesity, 81% in those with obesity and hypertriglyceridemia, and 86% in participants with diabetes and hypertriglyceridemia.

Whereas the majority of epidemiological studies merely rely on non-invasive scoring system of NAFLD like the fatty liver index, we directly assessed liver fat content with H-MRS in a large population. The prevalence of NAFLD in our study is in line with that reported in a previous worldwide study [8]. The global prevalence of NAFLD in patients with diabetes was estimated 56% with the highest prevalence of 68% in patients with diabetes and an average BMI of 27 kg/m^2 in a large meta-analysis [32]. In our study, the prevalence of NAFLD was 69% in participants with diabetes and 79% in those with both diabetes and obesity. The prevalence of NAFLD in those with hypertension in our study (46%) was similar to the prevalence that was previously reported in hypertensive participants of 49.5% (44.9–54.1) [33]. Whereas previous studies have reported associations between high triglyceride concentration and low HDL concentration with NAFLD, the prevalence of NAFLD in these groups is not well known [34–36]. We observed a NAFLD prevalence of 57% in those with hypertriglyceridemia and 47% in those with low HDL concentrations. Whereas in general men more often have NAFLD than women [37], we observed that NAFLD was more frequently present in women than in men with low HDL

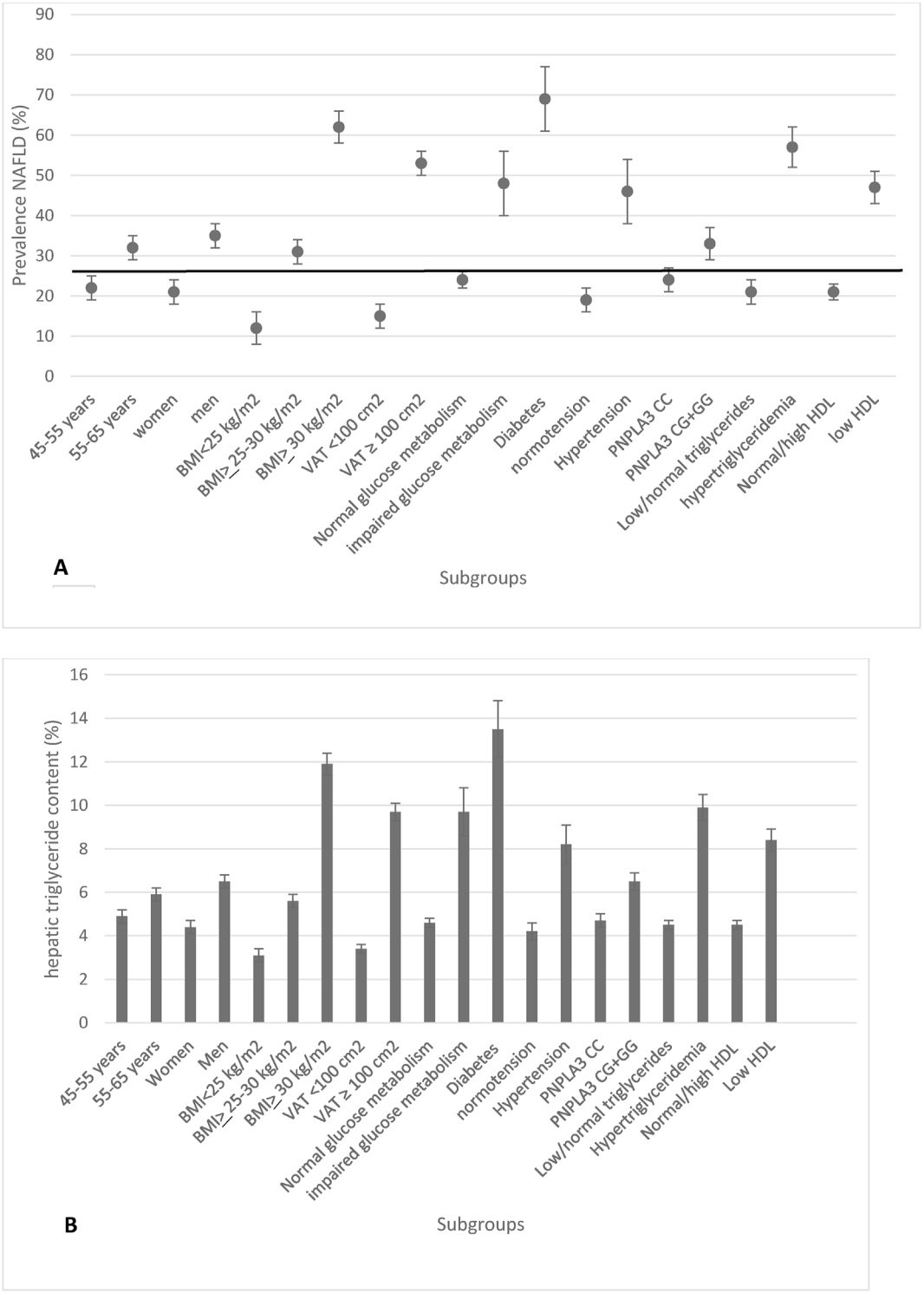

Figure 2 NAFLD prevalence (A) and amount of liver fat content (B). The bold line in Fig. 2A represented the overall NAFLD prevalence in the Netherlands. Data are shown as proportion with 95% CI. Results are based on analysis weighted toward BMI distribution of the general population (n = 1570). Mean PNPLA3 CG and GG polymorphism are based on n = 1362.

concentrations. This observation may be a result of changed hormonal status in these women of whom the majority was postmenopausal [38,39].

When considering the PNPLA3 polymorphism, we observed a prevalence of NAFLD of 24% in the CC subgroup and 33% in CG + GG group, which is lower than the reported frequencies of NAFLD of 47% CG and 69% CG + GG subgroups in a Brazilian biopsy-proven NAFLD population, which could be a result of a differently selected population and ethnic background [40]. Our results only apply to

Table 3 Odds ratios with 95% confidence intervals in the different subgroups.

Subgroup	Odds ratio	95% CI
Age≥55 year (reference <55 year)	1.8	1.3–2.4
Men (reference women)	2.1	1.5–2.8
BMI 25–30 (reference BMI<25)	3.3	2.1–5.2
BMI ≥30 kg/m^2 (reference BMI<25)	11.6	7.4–18.1
Visceral adipose tissue ≥100 cm^2 (reference <100 cm^2)	6.4	4.7–8.8
Impaired glucose metabolism (reference normal glucose metabolism)	2.9	1.8–4.8
Diabetes (reference normal glucose metabolism)	7.0	3.5–13.8
Hypertension (reference normotension)	2.5	1.5–4.0
PNPLA3CG + GG (reference PNPLA3 CC)	1.5	1.1–2.1
Hypertriglyceridemia (reference low/normal triglycerides)	5.1	3.6–7.2
Low HDL (reference normal/high HDL)	3.3	2.4–4.5

Results are based on analysis weighted towards BMI distribution of the general population (n = 1570). * Mean PNPLA3 CG and GG polymorphism are based on n=1362.

Table 4 Combinations of two risk factors and the prevalence of NAFLD, liver fat content and liver enzymes.

	NAFLD %	Liver fat content %	AST (U/l)	ALT (U/l)	gGT (U/l)
Hypertension					
Men	48 (42–58)	14.3 (0.7)	28.7 (10.3)	37.9 (18.1)	44.3 (25.3)
Women	28 (22–34)	14.1 (1.1)	24.5 (6.5)	27.6 (11.3)	30.1 (19.6)
BMI>30 kg/m^2	75 (68–78)	17.0 (1.3)	27.7 (11.4)	35.8 (21.4)	38.6 (18.6)
High triglycerides	66 (59–73)	15.3 (1.0)	26.9 (8.1)	34.8 (15.3)	43.3 (24.6)
Low HDL	62 (55–69)	14.6 (0.9)	28.3 (10.7)	37.7 (18.6)	41.8 (21.3)
Diabetes	57 (36–78)	23.3 (4.0)	33.0 (19.6)	47.2 (36.9)	47.2 (19.3)
PNPLA3CGGG*	40 (24–56)	15.5 (1.6)	26.1 (6.0)	30.8 (9.3)	35.4 (16.6)
BMI > 30 kg/m^2					
Men	72 (67–77)	18.0 (0.7)	29.3 (10.0)	39.4 (18.7)	43.3 (22.1)
Women	53 (48–58)	16.6 (0.8)	25.0 (6.9)	28.6 (11.3)	32.1 (24.3)
High triglycerides	81 (76–86)	18.9 (0.8)	27.4 (8.0)	35.4 (15.5)	44.1 (24.9)
Low HDL	77 (72–82)	18.3 (0.8)	28.5 (10.5)	37.7 (18.3)	41.4 (21.5)
Hypertension	75 (68–78)	17.0 (1.3)	27.7 (11.4)	35.8 (21.4)	38.6 (18.6)
Diabetes	79 (71–87)	21.7 (1.5)	29.2 (12.5)	39.4 (23.7)	48.2 (33.4)
PNPLA3CGGG*	72 (66–78)	18.2 (11.6)	27.8 (8.2)	35.3 (14.8)	38.1 (20.6)
Diabetes					
Men	76 (66–86)	16.8 (1.5)	30.2 (14.1)	42.1 (26.1)	54.9 (73.1)
Women	57 (44–70)	20.9 (1.9)	26.7 (8.1)	34.2 (14.0)	48.5 (42.2)
BMI>30 kg/m^2	79 (71–87)	21.7 (1.5)	29.2 (12.5)	39.4 (23.7)	48.2 (33.4)
High triglycerides	86 (77–95)	21.3 (1.9)	28.9 (9.8)	39.2 (18.8)	58.3 (77.5)
Low HDL	80 (70–90)	17.9 (14.9)	29.2 (13.7)	40.0 (25.0)	42.7 (22.7)
Hypertension	57 (36–78)	23.3 (4.0)	33.0 (19.6)	47.2 (36.9)	47.2 (19.3)
PNPLA3CGGG*	77 (66–90)	18.1 (2.3)	26.8 (8.8)	34.2 (14.4)	43.0 (24.6)
Low HDL					
Men	45 (40–50)	14.1 (0.7)	28.2 (9.6)	37.4 (17.4)	40.7 (22.4)
Women	68 (56–80)	18.5 (3.7)	23.8 (7.4)	27.1 (9.3)	36.0 (23.6)
BMI>30 kg/m^2	77 (72–82)	18.3 (0.8)	28.5 (10.5)	37.7 (18.3)	41.4 (21.5)
Hypertension	62 (55–69)	14.6 (0.9)	28.3 (10.7)	37.7 (18.6)	41.8 (21.3)
Diabetes	80 (70–90)	17.9 (14.9)	29.2 (13.7)	40.0 (25.0)	42.7 (22.7)
PNPLA3CGGG*	54 (46–62)	16.2 (1.4)	28.1 (9.0)	36.9 (16.1)	38.3 (20.8)
High triglycerides					
Men	59 (53–65)	14.8 (0.9)	28.4 (8.3)	38.5 (17.2)	48.7 (44.8)
Women	54 (46–62)	15.6 (1.5)	25.4 (7.8)	28.3 (11.4)	36.4 (24.9)
BMI>30 kg/m^2	81 (76–86)	18.9 (0.8)	27.4 (8.0)	35.4 (15.5)	44.1 (24.9)
Hypertension	66 (59–73)	15.3 (1.0)	26.9 (8.1)	34.8 (15.3)	43.3 (24.6)
Diabetes	86 (77–95)	21.3 (1.9)	28.9 (9.8)	39.2 (18.8)	58.3 (77.5)
PNPLA3CGGG*	58 (50–66)	16.5 (1.4)	27.7 (7.6)	35.5 (15.5)	39.8 (19.4)

The prevalence of NAFLD (95% CI), mean liver fat content and liver enzymes (SD) in those with two risk factors. Results are based on analysis weighted towards the BMI distribution of the general population (n=1570). *Analyses with PNPLA3 polymorphism are based on n=1362.

those with European ancestry, because the PNPLA3 genotyping was performed in this population.

Obesity is strongly related with NAFLD [9,15], which is supported by our study where we observed a higher prevalence of NAFLD in those with obesity than in those without obesity. It must be noted that obesity alone does not always result in NAFLD, as 38% of participants with obesity did not have NAFLD. This group with obesity but

without NAFLD was slightly younger and had a somewhat healthier lifestyle than the participants with obesity and NAFLD, as appeared from the level of alcohol consumption, smoking behaviour, diet quality and physical activity. Moreover, 12% of the participants without overweight had so-called lean NAFLD [41–44]. In the U.S.A., the prevalence of lean NAFLD in adolescents was estimated 8%, based on alanine aminotransferase >25.8 U/l for men and >22.1 U/l for women [45]. In a large Dutch population-based study, a prevalence of lean NAFLD of 8.8% was observed. However, it must be noted that this was based on the less accurate fatty liver index [46]. In contrast, we estimated the prevalence of NAFLD based on directly assessed liver fat content with H-MRS. The 12% of lean NAFLD in our population is somewhat higher than reported in a meta-analysis of 93 studies from 24 countries, in which an overall prevalence of 12% non-obese (BMI<30 kg/m^2) NAFLD and 5% lean NAFLD (BMI<25 kg/m^2) in the general population was observed [47]. We observed that participants in the lean NAFLD group in our population were more often men, with a higher alcohol consumption and less physical activity, than lean participants without NAFLD. In addition, there was more frequent use of medication, and they had more visceral adipose tissue than those without NAFLD. Moreover, all blood concentrations of lipid and glucose metabolism were also increased in this group compared with the group of BMI<25 kg/m^2 without NAFLD. This confirms that not only high body weight is an important risk factor but also diabetes, dyslipidemia, hypertension, male sex and alcohol consumption contribute to the development of NAFLD in a normal weight population.

Whereas in individuals with overweight or obesity the PNPLA3 CG and GG genotypes were more prevalent in those with NAFLD than in those without, this was not the case in the BMI group<25. In the group with BMI<25, the prevalence of the PNPLA3 CG and GG genotypes was higher in those without NAFLD. This may suggest that the PNPLA3 polymorphism may not play a large role in lean NAFLD in people from European ancestry [48]. Notably, the combination of the studied risk factors with the PNPLA3CGGG genotype did not result in the highest risk increases of NAFLD.

The presence of more than one metabolic risk factor resulted in an increased prevalence of NAFLD up to 86%. This suggests that participants with more than one metabolic risk factor are at high risk of having NAFLD. In the European Association for the Study of the Liver (EASL) guidelines it is suggested that every patient with metabolic risk factors should undergo a liver ultrasound or liver function tests to assess the presence of NAFLD with or without fibrosis (using serum fibrosis markers). Based on these results, it should be decided whether patients require follow up after 2 or 3–5 years or referral to a medical specialist [1,2]. In the American Association for the Study of Liver Disease (AASLD) guidelines, no routine NAFLD screening is recommended because of uncertainties regarding diagnostic tests sensitivity and treatment options, but also because of the lack of proof regarding the long term benefits and cost effectiveness of screening [3]. However, this guideline suggests that for groups with a high risk of NAFLD, such as patients with diabetes type 2, transient elastography and serum fibrosis markers can be used to determine the risk of fibrosis [3]. Currently, the main treatment of NAFLD is to advice a healthy life style, weight loss, and abstaining from alcohol consumption [1–3]. By identifying high risk groups of NAFLD, the risk in these groups may be lowered by lifestyle advice and optimized treatment of diabetes, hypertension and dyslipidaemia. Most of these patients will be treated by general practitioners. We would advise that patients with any combination of obesity, type 2 diabetes, and hypertriglyceridemia should be regularly tested using serum fibrosis markers, ultrasound or transient elastography. When NASH is suspected, patients should be referred to a medical specialist [1–3,49].

An important strength of this population-based study is the large sample size with directly assessed liver fat content with H-MRS, which allowed estimation of the prevalence of NAFLD in several predefined risk groups. One of the limitations of this study is that a prognostically important complication of NAFLD, namely liver fibrosis, was not assessed. In addition, our study population mainly consisted of individuals from European ancestry and our findings need to be confirmed in other ethnic groups, where the amount of liver fat and related risks may differ. It should be noted that the prevalence of NAFLD in our population were estimated using baseline measurements of the NEO study collected between 2008 and 2013. With the progressively increasing prevalence of overweight and obesity, it is likely that the prevalence of NAFLD in the Dutch population also increased since.

In conclusion, The prevalence of NAFLD in the middle-aged Dutch population was 27%, as assessed by H-MRS. The prevalence of NAFLD was particularly increased in people with diabetes, obesity, and hypertriglyceridemia, combining subgroups results in even higher prevalence of NAFLD. When assessing patients in an outpatient clinic or at the general practitioner, the high prevalence of NAFLD in these high risk groups should be considered. Future studies are needed to investigate to what extent these high risk groups develop complications of NAFLD.

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Ethics approval and patient consent statement

The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Data availability statement

Due to the privacy of the participants of the NEO study and legal reasons, we cannot publicly deposit the data. Data will be made available upon request according to the NEO research procedure. Data requests should be sent to the NEO Executive Board which can be contacted via https://www.lumc.nl/org/neo-studie/contact/.the NEO Executive Board which can be contacted via https://www.lumc.nl/org/neo-studie/contact/.

Declaration of competing interest

None.

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