Non-alcoholic fatty liver disease in The Rotterdam Study: about muscle mass, sarcopenia, fat mass and fat distribution

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J Bone Miner Res. 2019 Jul;34(7):1254–1263

Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease worldwide. Obesity is a major risk factor for NAFLD and recently, low skeletal muscle mass emerged as additional risk factor for NAFLD. However, the different contributions of BMI to the risk of NAFLD are not yet well-known. We therefore studied body composition and muscle function with NAFLD in an elderly population-based study. Participants of European descent underwent dual X-ray absorptiometry and hepatic ultrasonography. NAFLD was defined as liver steatosis in absence of secondary causes for steatosis. Skeletal muscle index (SMI) was defined as appendicular lean mass/height² and (pre)sarcopenia was defined using the EWGSOP-consensus guidelines. All analyses were stratified by sex and BMI (cut-point: 25kg/m²) and adjusted for age, weight, height, HOMA-IR, triglycerides, and android-fat-to-gynoid-fat ratio (AGR). We included 4609 participants of whom 1623 had NAFLD (n=161 normal-weight and n=1462 overweight). Pre-sarcopenia and sarcopenia prevalence was low (5.9% and 4.5% respectively) and both were not associated with NAFLD. SMI was associated with less NAFLD in normal-weight women (OR 0.48, 95%CI 0.29–0.80). A similar association for SMI and NAFLD was seen in normal-weight men, but significance dissipated after adjustment for AGR (OR 0.63, 95%CI 0.39–1.02). Generally, fat mass was a better predictor for NAFLD than lean mass. In particular, android fat mass was associated with all NAFLD subgroups (OR from 1.77 in overweight men to 8.34 in normal-weight women, P_{max}=0.001), whereas substitution of gynoid fat mass for other body components had a significant protective association with NAFLD in every subgroup, but normal-weight men. Likewise, AGR was the best performing predictor for NAFLD prevalence (OR from 1.97 in normal-weight men to 4.81 in normal-weight women, P_{max}<0.001). In conclusion, both high fat mass and low SMI were associated with normal-weight NAFLD. However, fat distribution (as assessed by AGR) could best predict NAFLD prevalence.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease today and it parallels the epidemic of obesity and diabetes mellitus.[6,13] NAFLD constitutes a major public health threat as it leads not only to an increased risk of liver-specific,[22] but also cardiovascular morbidity and mortality.

Obesity is strongly related to NAFLD.[13] That said, certainly not all obese individuals have NAFLD and not every NAFLD patient is obese. In fact, about 1 in 6 of the NAFLD patients have a normal body mass index (BMI).[242] Therefore, the accuracy of BMI as the all-encompassing measure of adiposity is debated.[366] Presence of excess (visceral) fat mass is a well-established risk factor for NAFLD, independent of BMI.[367,368] Recently, emerging evidence suggested that low skeletal muscle mass (or pre-sarcopenia) also contributes to the risk of NAFLD.[369-371] Indeed, as skeletal muscle mass is the primary tissue responsible for insulin-mediated glucose disposal, skeletal muscle plays an important role in glucose homeostasis and insulin resistance, which are key in the pathogenesis of NAFLD.[372]

However, evidence on the association between skeletal muscle mass and NAFLD predominantly originates from young Asian populations with a high BMI.[12,370] This gives room for thought whether it is a true shortage of muscle mass, or a relative excess of fat mass, or both that is associated with NAFLD. Interestingly, a recent population-based study found that the two body components, lean body mass and fat mass, both explained the relation between BMI and mortality.[373] To the best of our knowledge, there are no studies that have examined the role of both components, independent of each other, in relation to NAFLD. But considering the above, there are arguments to think that both contributions of BMI, i.e. (lean) muscle mass and fat mass, are risk factors for NAFLD.

We therefore studied the independent association of the different components of the body with NAFLD, stratified by sex and BMI, in a large elderly European population. We were particularly interested in the association between skeletal muscle mass with (sarcopenia) or without (pre-sarcopenia) loss of muscle function, and NAFLD. In addition, we assessed which body composition parameter could best predict NAFLD prevalence.

Participants and methods

Study Population

This cross-sectional analysis included participants from The Rotterdam Study, a large ongoing population-based cohort of participants aged 45 years or older in the Netherlands. The design and rationale of this population-based study have been described in detail previously.[248] In short, the study commenced in 1989 and comprises three cohorts. Hepatic

imaging has been part of the physical work-up since 2009. There are no specific eligibility criteria for The Rotterdam Study, except for the minimum age and residential area (ZIP-codes). As body composition differs amongst ethnicities[12] and The Rotterdam Study is predominantly of European background, the low number of non-European participants were excluded to examine a homogenous population. Ethnicity was determined using genome-wide genotypes into HapMap CEU release 22 (build 36). The genotype data was pruned in order to end up with variants in linkage equilibrium and the ancestry component for each individual was estimated on the basis of the maximum likelihood using the ADMIXTURE software.[248] The Rotterdam Study is approved by the institutional review board of the Erasmus MC University Medical Centre Rotterdam and by the review board of The Netherlands Ministry of Health, Welfare and Sports. Written informed consent was obtained from all participants.

Dual X-ray Absorptiometry Scanning

Body components were assessed using dual X-ray absorptiometry (DXA)-scans with iDXA total body fan-beam densitometer (GE Lunar Corp, Madison, WI, USA). All scans were analysed using enCORE software, which divides scan-results into total lean mass, total fat mass and bone/organ mass. Total lean mass is the sum of trunk lean mass and appendicular lean mass (ALM; the sum of lean tissue from the arms and legs), and total body fat mass is the sum of android fat mass (localized around the waist), gynoid fat mass (localized around the breasts, hips and thighs), and fat mass not otherwise specified. The latter includes mainly trunk fat mass and a small proportion of appendicular fat mass, but as this was not further specified, we refer to this remaining component as trunk fat mass. The relative proportion (or fraction) of each component to the body was expressed as percentage of total body weight. For instance, ALM-fraction was calculated as ALM/total body weight*100.
Skeletal muscle mass was estimated by the skeletal muscle index (SMI) using ALM divided by squared body height (kg/m²) in order to adjust for variation in skeletal size. Low SMI was defined as ≤7.25 kg/m² in men and ≤5.67 kg/m² in women, based on cut-off values suggested by the European Working Group on Sarcopenia in Older People (EWGSOP).[374]
Fat distribution was assessed using the standardized android-fat-to-gynoid-fat ratio (AGR).

Pre-sarcopenia and Sarcopenia

Grip strength (proxy for overall muscle strength) was examined using a hydraulic hand dynamometer (Fabrication Enterprises Inc., USA). The maximum grip strength was defined as the maximum value (in kg) out of three serial attempts using the non-dominant hand. EWGSOP cut-off values for low grip strength were ≤29kg for BMI≤24kg/m², ≤30kg for BMI 24.1–28kg/m², or ≤32kg for BMI>28kg/m² in men; and ≤17kg for BMI≤23kg/m²,

≤17.3kg for BMI 23.1–26kg/m², ≤18kg for BMI 26.1–29kg/m², or ≤21kg for BMI>29kg/m² in women.[374]

Gait speed (measure of physical performance)[375] was examined using the GAITRite walkway (CIR Systems, Inc., Sparta, New Jersey), a 5.79 meters long electronical walkway. Again, EWGSOP cut-offs were applied for the definition of low gait speed, i.e. for men:<0.65m/s if height≤173cm, or <0.76m/s if height>173cm; and for women:<0.65m/s if height≤159cm, or <0.76m/s if height>159cm.[374]

Pre-sarcopenia and Sarcopenia were defined according to the EWGSOP consensus guideline.[374,376] Pre-sarcopenia was defined as presence of low SMI, and sarcopenia as low SMI plus either low muscle strength or low physical performance.

Hepatic Imaging

Hepatic steatosis was assessed using abdominal ultrasound (US), which was carried out by a certified and experienced technician (Hitachi HI VISION 900). Images were stored digitally. Diagnosis of steatosis was determined dichotomously as presence of a hyperechogenic liver parenchyma.[104] Participants with secondary causes for steatosis were excluded from this study, i.e. those with 1) excessive alcohol consumption (men>30g/day and women>20g/day); 2) use of steatogenic drugs, i.e. amiodarone, systemic corticosteroids, methotrexate, or tamoxifen (extracted from automated pharmacy linkage); and 3) viral hepatitis, based on hepatitis B surface antigen and anti-hepatitis C virus, measured by an automatic immunoassay (Roche Diagnostic GmbH). The remainder participants with steatosis were considered to have NAFLD.

NAFLD severity was assessed using transient elastography (FibroScan®, EchoSens, Paris, France). Practical implementation of this examination has been described in detail previously.[78] Liver stiffness measurements (LSM, in kilopascals [kPa]) were available for a subset of the study population (from January 2011 onwards). A single operator obtained 10 serial measurements using either the M or XL-probe dependent on the thickness of the subcutaneous fat layer. We excluded participants with 1) unreliable measurements (i.e., interquartile range/median LSM>0.3 and LSM≥7.1kPa)[101], 2) failure of assessment, or 3) presence of an intracardial device. For this study, non-alcoholic steatohepatitis (NASH), or advanced NAFLD, was defined as presence of steatosis and LSM≥8.0 kPa, a proxy for fibrosis.[54]

Biochemistry and additional covariates

Fasting blood lipids, platelet count, glucose, alanine aminotransferase (ALT), aspartate aminotransferase, and gamma-glutamyl transferase were measured using automatic enzyme procedures (Roche Diagnostic GmbH, Mannheim, DE). Insulin was determined using an automatic immunoassay (Roche Diagnostic GmbH).

Data on demographics, physical activity, smoking behaviour, educational level, and comorbid conditions were obtained during an extensive home interview. Daily energy intake in kilocalories and alcohol intake in grams was assessed using a 389-item semi-quantitative food frequency questionnaire.[98] We excluded unreliable energy consumption of <500 or >7500 kcal/day. Blood pressure measurements were obtained using two subsequent measurements in upright position. BMI was calculated as weight/height² (kg/m²) and dichotomized into normal-weight:<25kg/m² and overweight:≥25kg/m².
Insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose (mmol/dl) times fasting insulin (mU/L) divided by 22.5).[106] The metabolic syndrome was diagnosed when three out of five metabolic traits were present: 1) abdominal obesity, i.e. waist circumference>102cm in men and >88cm in women; 2) serum triglycerides ≥130mg/dl or drug treatment for elevated triglycerides; 3) serum high-density lipoprotein cholesterol (HDL-C) <40mg/dl in men and <50mg/dl in women or drug treatment for low HDL-C; 4) Blood pressure ≥130/85mmHg or drug treatment for elevated blood pressure; 5) fasting plasma glucose ≥100mg/dl or drug treatment for elevated blood glucose.[28] Hypertension was diagnosed if either systolic (≥140mmHg) or diastolic (≥90mmHg) blood pressure was increased and/or if the participant was on anti-hypertensive medication. Diabetes was diagnosed as fasting glucose above 7.0mmol/L and/or drug treatment for elevated blood glucose.

Statistical analyses

To reduce bias due to missing data, missing values were imputed using multiple imputation (fully conditioned specification). Details on this imputation process can be found in the *Supplementary Methods*. Continuous data were presented as mean ± standard deviation (SD) or median with 25th or 75th percentile (P25-P75). Categorical data were presented as percentage. Chi-square test, one-way analyses of variance, or Kruskall-Wallis test were used to assess differences by strata.
Associations between body composition and NAFLD were examined using logistic regression models stratified by BMI and sex, because of the known sex-differences in body composition.[377] We examined the association between 1) the different body components, 2) SMI, and 3) (pre)sarcopenia with NAFLD. We evaluated weight, height, weight and height, BMI, and body fat fraction as adjustment for body composition using the Akaike Information Criterion (AIC).[378] After evaluation, models adjusted for weight and height performed best. Moreover, without weight as factor in the model, all body components were associated with higher prevalence of NAFLD. If body fat fraction was put in the model together with ALM (or SMI), the beta for ALM could reflect two scenarios. First, the beta could reflect an increase in ALM and subsequent increase in total body weight. In this case, total body fat would increase too (as body fat fraction is set and total weight increases).

Second, the beta could reflect an increase of ALM at a set weight, while body fat fraction remains the same (weight is set) and bone and organ mass hardly varies. Thus an increase of ALM would then be at the expense of trunk lean mass. The latter scenario reflects the substitution of one component of the body at the expense of another. We performed such a substitution analyses[255,379], which is often used in nutritional epidemiology to assess the relative replacement of one nutrient by another for a given caloric intake. A similar formula can be applied in order to assess the relative replacement of one body component with another for a given body weight. For example:

NAFLD ~ total lean mass(%) + bone/organ mass(%) + total body weight.

In this example, only total fat mass (%) is not included in this formula and hence, the beta for total lean mass reflects the increase of total lean mass at the expense of total body fat mass (in % body weight).

In addition to weight and height, all analyses were adjusted for age and study cohorts as well. Potential confounding of a nested set of sociodemographic, lifestyle, and metabolic predictor variables (based on the literature[6]) was tested, taking potential overfitting into account.

Furthermore, in order to assess which parameter had the best performance for NAFLD (i.e. explained more variation of the outcome and thus resulted in a better model fit), we compared SMI, ALM-fraction, AGR, body fat fraction, (pre)sarcopenia, grip, and speed using the AIC.

To test the robustness of our conclusions we performed three sensitivity analyses. First, we explored ALM-fraction as alternative proxy for skeletal muscle mass to facilitate comparison with previous reports.[370,371] Second, as gait speed measurements were performed on a separate day at the research facility, this measurement was missing in 32.6% of individuals. In the main analysis, we assumed that if one of both proxies for physical functioning was normal, there would be no sarcopenia. In the sensitivity analysis, we used imputed grip strength and gait speed data to re-classify sarcopenia. Third, we analysed the association of SMI with NASH in order to assess whether SMI was also associated with NAFLD severity.

We checked all analyses for potential multicollinearity using the variance inflation factor. In addition we corrected for the inflated type I error that arises due to multiple testing. We applied the adapted method proposed by Sidák,[256,257] using the effective number of tests instead of the actual number of tests (n=7). This adaptation took into account that the different body components are strongly interrelated and, hence, are not independent from each other. The corrected significance level was $P<0.010$. All analyses were performed using R version 3.5.1 (R core team, Vienna, Austria).

Results

Study population

The flowchart of the study is illustrated in *Figure 1*. In total, 5967 participants underwent abdominal US. We excluded 253 participants (4.2%) because of unreliable food questionnaires, missing data on DXA-scans or outlier values. Second, 887 participants (15.5%) were excluded while having secondary causes for steatosis. Lastly, 218 participants were excluded because of non-European background. Hence, the total number of eligible study participants was 4609. Mean age was 69.3±9.2 years, 57.0% was female and mean BMI was 27.5±4.2 kg/m² (range 15.0–47.2). Both original and imputed data of the total population are depicted in *Supplementary Table 1*.

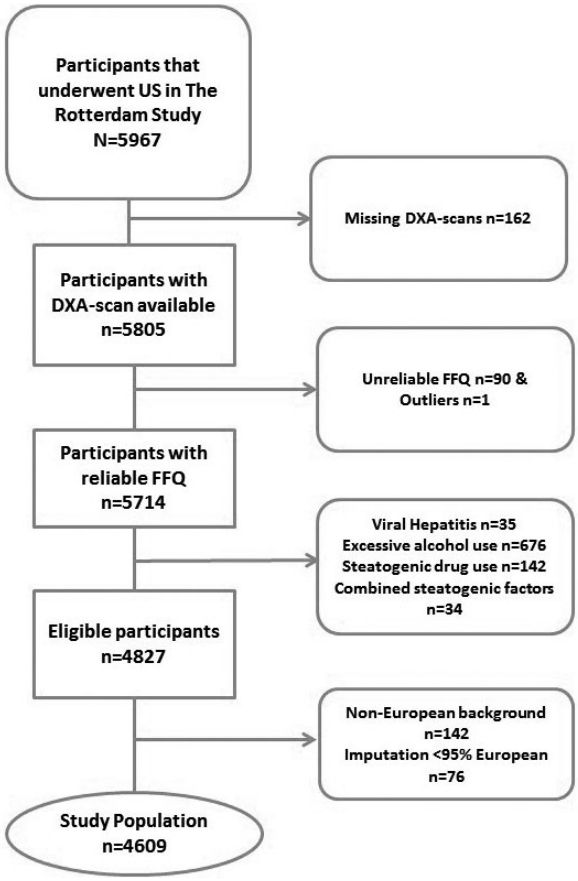

Figure 1: Flowchart of the study
Abbreviations: DXA: dual X-ray absorptiometry, FFQ: food frequency questionnaire, US: ultrasound

NAFLD characteristics

The overall prevalence of NAFLD in this study population was 35.2% (n=1623). Of those, 161 individuals were normal-weight (10%) and 1462 were overweight (90%). Data on demographics, biochemistry and comorbidities are given, stratified by BMI, in *Supplementary Table 2*. Differences between NAFLD strata were most pronounced in the overweight group. However, ALT, gamma-glutamyl transferase, insulin resistance and triglycerides were significantly different in both NAFLD subgroups.

Body composition and NAFLD

Table 1 depicts the data on body composition stratified by sex and BMI. Fat mass parameters were higher in every NAFLD subgroup, whereas lean mass parameters were lower mostly in the normal-weight NAFLD subgroups. Interestingly, ALM-fraction was the only parameter

Table 1: Study Characteristics of participants for different NAFLD strata

	Normal weight n=1339			Overweight n=3270		
	Men N=1980					
	No NAFLD n=432	NAFLD n=67	*P*-value*	No NAFLD n=822	NAFLD n=659	*P*-value*
Height (m)	177.0 (7.1)	176.8 (7.8)	0.796	176 (7.1)	176 (6.9)	0.703
Weight (kg)	72.9 (7.0)	74.8 (7.6)	0.041	86.8 (10.1)	93.2 (12.9)	<0.001
Total fat mass (kg)	19.1 [16.5, 21.8]	21.0 [18.6, 24.1]	<0.001	26.6 [23.3, 30.5]	30.9 [26.6, 36.3]	<0.001
Body fat fraction (%)	26.0 (4.6)	28.6 (4.3)	<0.001	31.4 (4.6)	34.3 (4.6)	<0.001
Android fat mass (kg)	1.70 [1.28, 2.08]	2.01 [1.75, 2.42]	<0.001	2.73 [2.21, 3.24]	3.40 [2.82, 4.08]	<0.001
Trunk/Appendicular fat mass (kg)	14.9 [12.8, 16.8]	16.2 [14.6, 18.5]	<0.001	20.4 [18.0, 23.3]	23.6 [20.5, 27.7]	<0.001
Gynoid fat mass (kg)	2.56 [2.19, 2.95]	2.71 [2.35, 3.08]	0.028	3.38 [2.94, 3.98]	3.86 [3.22, 4.65]	<0.001
AGR	0.66 [0.54, 0.76]	0.76 [0.68, 0.84]	0.028	0.79 [0.69, 0.89]	0.87 [0.79, 0.97]	<0.001
Total lean mass (kg)	51.0 [47.4, 54.8]	49.9 [47.0, 54.5]	0.371	55.7 [51.8, 59.9]	57.0 [52.9, 61.2]	<0.001
Trunk lean mass (kg)	27.6 [26.0, 29.6]	26.9 [25.5, 28.9]	0.209	29.5 [27.6, 31.6]	30.1 [28.0, 32.4]	0.001
Appendicular lean mass (kg)	23.3 [21.1, 25.4]	23.1 [20.8, 25.2]	0.998	26.0 [24.0, 28.7]	26.9 [24.7, 29.4]	<0.001
SMI (kg/m^2)	7.43 (0.74)	7.41 (0.69)	0.769	8.45 (0.80)	8.70 (0.91)	<0.001
ALM-fraction (%)	31.9 [30.3, 33.9]	31.2 [29.1, 33.2]	0.015	30.5 [28.7, 32.3]	29.4 [27.7, 31.0]	<0.001
Normal SMI	58.8	58.2		94.3	94.7	
Presarcopenia	24.5	20.9	0.630	3.0	2.0	0.337
Sarcopenia	16.7	20.9		2.7	3.3	
Gait speed (m/s)	1.26 [1.11, 1.40]	1.27 [1.13, 1.38]	0.727	1.24 [1.09, 1.35]	1.23 [1.09, 1.34]	0.990
Hand grip strength (kg)	35.4 (8.5)	33.0 (9.2)	0.036	36.8 (8.9)	36.7 (9.1)	0.841

Table 1 (*continued*)

	Women N=2629					
	No NAFLD n=746	**NAFLD n=94**	***P*-value***	**No NAFLD n=986**	**NAFLD n=803**	***P*-value***
Height (m)	164.0 (6.6)	163.4 (6.4)	0.420	162.5 (6.7)	162.7 (6.4)	0.435
Weight (kg)	61.0 (6.6)	63.1 (5.6)	0.003	75.8 (10.5)	82.6 (11.7)	<0.001
Total fat mass (kg)	20.9 [17.7, 23.4]	23.5 [21.1, 25.0]	<0.001	29.9 [26.5, 34.5]	34.8 [30.3, 40.8]	<0.001
Body fat fraction (%)	34.1 (4.9)	37.4 (3.6)	<0.001	41.8 (4.4)	44.3 (4.3)	<0.001
Android fat mass (kg)	1.48 [1.12, 1.81]	1.95 [1.70, 2.29]	<0.001	2.47 [2.06, 2.99]	3.20 [2.67, 3.92]	<0.001
Trunk/Appendicular fat mass (kg)	16.1 [13.7, 17.9]	17.9 [16.4, 19.0]	<0.001	22.7 [20.2, 26.4]	26.5 [23.1, 30.9]	<0.001
Gynoid fat mass (kg)	3.33 [2.81, 3.83]	3.46 [2.97, 3.86]	0.080	4.67 [3.99, 5.48]	5.16 [4.35, 6.13]	<0.001
AGR	0.43 [0.35, 0.51]	0.56 [0.50, 0.65]	0.080	0.53 [0.46, 0.61]	0.62 [0.55, 0.71]	<0.001
Total lean mass (kg)	37.3 [34.7, 40.1]	37.1 [34.4, 38.7]	0.118	40.3 [37.6, 43.7]	42.3 [39.3, 45.8]	<0.001
Trunk lean mass (kg)	20.8 [19.4, 22.3]	20.5 [19.2, 21.8]	0.135	21.9 [20.6, 23.7]	22.9 [21.3, 24.8]	<0.001
Appendicular lean mass (kg)	16.5 [15.0, 18.1]	16.2 [15.2, 17.3]	0.109	18.3 [16.8, 20.2]	19.3 [17.6, 21.3]	<0.001
SMI (kg/m²)	6.14 (0.60)	6.03 (0.45)	0.088	7.01 (0.71)	7.36 (0.84)	<0.001
ALM-fraction (%)	27.7 [25.8, 29.4]	26.2 [24.7, 27.5]	<0.001	25.2 [23.7, 26.6]	24.2 [22.9, 25.5]	<0.001
Normal SMI	79.9	76.6		98.8	98.8	
Pre-sarcopenia	12.2	14.9	0.727	0.5	0.7	0.691
Sarcopenia	7.9	8.5		0.7	0.5	
Gait speed (m/s)	1.24 [1.11, 1.36]	1.21 [1.08, 1.31]	0.994	1.17 [1.03, 1.28]	1.15 [1.02, 1.27]	0.570
Hand grip strength (kg)	21.8 (6.0)	20.9 (5.1)	0.176	21.77 (5.75)	21.61 (5.66)	0.553

Pooled data based on 74 imputations is presented as mean (SD), median [P25-P75] or percentage. Data is presented stratified by sex and BMI. *P-value is calculated using analyses of variance, Kruskall-Wallis, or Chi-squared test.
Abbreviations: AGR: android-fat-to-gynoid-fat ratio; ALM: appendicular lean mass; BMI: body mass index; NAFLD: non-alcoholic fatty liver disease; SMI; skeletal muscle index.

that was lower in *every* NAFLD subgroup. Also, overall prevalence of pre-sarcopenia and sarcopenia was low (5.9% and 4.5% respectively) and not associated with NAFLD (*Table 1*). We observed no clear effect modification, but the association between body components and NAFLD was most pronounced in normal-weight women (*Table 2*). Again, fat mass was generally associated with higher NAFLD and lean mass with lower NAFLD prevalence. In both sexes, android fat mass remained associated with NAFLD. In addition, ALM remained independently associated with normal-weight women (OR 0.75, 95%CI 0.62–0.91, *P*=0.003; *Table 2*).

Supplementary Table 3 shows the substitution analyses. Substituting ALM for fat mass was associated with lower prevalence of NAFLD, except when replaced for gynoid fat mass. Indeed, replacing components by gynoid fat mass was associated with lower NAFLD prevalence, whereas the opposite was true for android fat mass.

Table 2: Association of different parts of body with NAFLD

	Men			
	Normal-weight n=499	***P*-Value**	**Overweight n=1481**	***P*-Value**
		Model 1		
Total lean mass	**0.87 (0.80 – 0.96)**	**0.003**	**0.93 (0.90 – 0.96)**	**<0.001**
Appendicular lean mass	**0.80 (0.69 – 0.93)**	**0.003**	**0.91 (0.86 – 0.96)**	**0.001**
Trunk lean mass	0.86 (0.74 – 1.00)	0.045	**0.90 (0.85 – 0.95)**	**<0.001**
Total body fat	**1.16 (1.06 – 1.27)**	**0.001**	**1.08 (1.05 – 1.12)**	**<0.001**
Trunk fat mass	**1.22 (1.08 – 1.37)**	**0.001**	**1.11 (1.06 – 1.17)**	**<0.001**
Gynoid fat mass	1.46 (0.81 – 2.63)	0.203	0.97 (0.80 – 1.17)	0.720
Android fat mass	**3.49 (1.95 – 6.22)**	**<0.001**	**2.49 (2.00 – 3.11)**	**<0.001**
		Model 2		
Total lean mass	0.90 (0.82 – 0.99)	0.026	0.96 (0.93 – 1.00)	0.038
Appendicular lean mass	0.84 (0.72 – 0.97)	0.020	0.95 (0.89 – 1.01)	0.105
Trunk lean mass	0.90 (0.77 – 1.06)	0.199	0.94 (0.89 – 1.00)	0.065
Total body fat	1.13 (1.03 – 1.24)	0.011	1.04 (1.01 – 1.08)	0.022
Trunk fat mass	1.17 (1.04 – 1.33)	0.012	1.06 (1.01 – 1.11)	0.030
Gynoid fat mass	1.32 (0.71 – 2.42)	0.377	0.96 (0.78 – 1.18)	0.685
Android fat mass	**2.94 (1.59 – 5.45)**	**0.001**	**1.77 (1.40 – 2.25)**	**<0.001**
		Model 3		
Total lean mass	0.94 (0.85 – 1.04)	0.202	0.97 (0.94 – 1.01)	0.147
Appendicular lean mass	0.87 (0.74 – 1.01)	0.071	0.97 (0.91 – 1.03)	0.296
Trunk lean mass	0.99 (0.83 – 1.17)	0.867	0.96 (0.90 – 1.02)	0.169
Total body fat	1.09 (0.99 – 1.20)	0.094	1.03 (1.00 – 1.07)	0.094
	Women			
	Normal-weight n=840	***P*-Value**	**Overweight n=1789**	***P*-Value**
		Model 1		
Total lean mass	**0.79 (0.71 – 0.87)**	**<0.001**	0.97 (0.93 – 1.01)	0.098
Appendicular lean mass	**0.68 (0.57 – 0.81)**	**<0.001**	0.95 (0.88 – 1.02)	0.146
Trunk lean mass	**0.76 (0.64 – 0.87)**	**0.001**	0.97 (0.91 – 1.02)	0.227
Total body fat	**1.22 (1.10 – 1.35)**	**<0.001**	1.04 (1.00 – 1.08)	0.034
Trunk fat mass	**1.30 (1.14 – 1.49)**	**<0.001**	1.06 (1.01 – 1.12)	0.020
Gynoid fat mass	0.73 (0.47 – 1.12)	0.150	**0.63 (0.54 – 0.73)**	**<0.001**
Android fat mass	**12.5 (6.28 – 24.9)**	**<0.001**	**3.40 (2.71 – 4.27)**	**<0.001**
		Model 2		
Total lean mass	**0.80 (0.71 – 0.89)**	**<0.001**	0.96 (0.92 – 1.00)	0.074
Appendicular lean mass	**0.69 (0.57 – 0.83)**	**<0.001**	0.98 (0.90 – 1.06)	0.545
Trunk lean mass	**0.76 (0.64 – 0.90)**	**0.001**	0.93 (0.88 – 1.00)	0.035
Total body fat	**1.20 (1.08 – 1.33)**	**0.001**	1.05 (1.01 – 1.10)	0.020

Table 2 (*continued*)

	Women			
	Normal-weight n=840	***P*-Value**	**Overweight n=1789**	***P*-Value**
Trunk fat mass	**1.26 (1.09 – 1.44)**	**0.001**	1.07 (1.01 – 1.13)	0.021
Gynoid fat mass	1.00 (0.62 – 1.61)	0.996	0.85 (0.73 – 1.00)	0.050
Android fat mass	**8.34 (3.95 – 17.6)**	**<0.001**	**2.16 (1.69 – 2.76)**	**<0.001**
	Model 3			
Total lean mass	**0.84 (0.75 – 0.94)**	**0.002**	0.97 (0.93 – 1.01)	0.170
Appendicular lean mass	**0.75 (0.62 – 0.91)**	**0.003**	1.01 (0.94 – 1.10)	0.772
Trunk lean mass	0.82 (0.69 – 0.98)	0.029	0.93 (0.87 – 0.99)	0.025
Total body fat	1.16 (1.03 – 1.29)	0.011	1.06 (1.01 – 1.10)	0.010

Results are given as OR (95%CI) for NAFLD as outcome stratified by sex and BMI. Results in bold reflect significant findings with a P-value <0.010. Model 1: adjusted for age, study cohorts, weight, and height. Model 2: in addition: HOMA-IR, and triglycerides. Model 3: in addition AGR. Additional adjustments for confounding by education level, physical activity, alcohol intake, energy intake, ALT, and smoking resulted in negligible changes in odds ratio (<5%).
Abbreviations: AGR: android-fat-to-gynoid-fat ratio; BMI: body mass index; CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease; OR: odds ratio.

Comparing the performance of the different body composition parameters for NAFLD, fat mass parameters explained more variation of the outcome (resulted in a better model fit) than lean mass parameters (as indicated by a lower AIC). AGR was the predictor with the overall best model fit (*Supplementary Table 4*). Interestingly, SMI and ALM-fraction performed equally with regard to the model fit in the analysis for NAFLD.

SMI & NAFLD

Table 3 shows the association between our exposure variable of interest, SMI, and NAFLD. SMI was associated with less NAFLD prevalence in normal-weight men, but significance dissipated after correction for multiple testing and adjustment for AGR. In normal-weight women, however, SMI remained associated with less NAFLD after full adjustment (OR 0.48, 95%CI 0.29–0.80, *P*=0.005. *Table 3*). Using ALM-fraction (instead of SMI) provided similar conclusions, underscoring the robustness of our results (*Supplementary Table 5*). No independent association was found between SMI and overweight NAFLD. However, SMI was relatively high in this population. The prevalence of low SMI (i.e. ≤7.25 kg/m² or ≤5.67 kg/m²) was only 5% in overweight individuals against 28% in the normal-weight. Prevalence of (pre)sarcopenic obesity (i.e. BMI≥30kg/m²) was even lower, in fact, only five out of the 1091 *obese* individuals had a low SMI. Interestingly, four of them had NAFLD.
The analysis of pre-sarcopenia and sarcopenia is given in *Supplementary Table 6*. In model 1, sarcopenia was associated with NAFLD in men and pre-sarcopenia with NAFLD in

Table 3: SMI & NAFLD

	Men			
	Normal-weight (n=499)	***P*-value**	**Overweight (n=1481)**	***P*-value**
Model 1				
SMI	**0.50 (0.32 – 0.79)**	**0.003**	**0.75 (0.63 – 0.90)**	**0.002**
Model 2				
SMI	0.57 (0.36 – 0.91)	0.018	0.88 (0.72 – 1.06)	0.169
Model 3				
SMI	0.63 (0.39 – 1.02)	0.061	0.92 (0.76 – 1.12)	0.401
	Women			
	Normal-weight (n=840)	***P*-value**	**Overweight (n=1789)**	***P*-value**
Model 1				
SMI	**0.37 (0.23 – 0.59)**	**<0.001**	0.90 (0.75 – 1.09)	0.291
Model 2				
SMI	**0.39 (0.23 – 0.64)**	**<0.001**	0.98 (0.80 – 1.20)	0.846
Model 3				
SMI	**0.48 (0.29 – 0.80)**	**0.005**	1.08 (0.87 – 1.33)	0.485

Results are given as OR (95%CI) for NAFLD as outcome stratified by sex and BMI. Results in **bold** reflect significant findings with a *P*-value <0.010. **Model 1:** adjusted for age, study cohorts, weight and height. **Model 2:** in addition: HOMA-IR, and triglycerides. **Model 3:** in addition: AGR. **Additional adjustments** for confounding by education level, physical activity, alcohol intake, energy intake, ALT, and smoking resulted in negligible changes in odds ratio (<5%).

Abbreviations: AGR: android-fat-to-gynoid-fat ratio; BMI: body mass index; CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease; OR: odds ratio; SMI; skeletal muscle index.

women, nonetheless these findings did not hold after further adjustment (*Supplementary Table 6*). In total, 1548 participants (33.6%) had either missing data on grip strength or gait speed. None of the participants had missing data on both variables. Sensitivity analysis using imputed values on grip strength and gait speed resulted in reclassification of 24 participants and yielded similar results to the original data (data not shown).

Of the 1623 individuals with NAFLD, 1126 (69.4%) had data available on LSM. NASH prevalence was low (total population: (n=115) 10.2%, normal-weight: (n=10) 8.5%, and overweight: (n=105) 10.4%). The small number of cases in the normal-weight (3 in men and 7 in women) hampered the possibility to stratify by BMI. *Supplementary Table 7* shows that SMI was associated with lower NASH prevalence in women, but this was no longer significant after correction for multiple testing (OR 0.48, 95%CI 0.25–0.92, *P*=0.027).

Discussion

In this large Western population-based study we examined the association between muscle mass, fat mass, fat distribution and NAFLD, using DXA-scans and hepatic imaging, both highly reliable measuring methods. Moreover, this is the first study that examines the sarcopenia definition, as proposed by the EWGSOP consensus, in association with NAFLD. We made several novel observations. First, we showed that incremental skeletal muscle mass was consistently associated with lower NAFLD prevalence and severity in normal-weight women (using different approaches). This association was independent of metabolic confounders, and importantly, independent of fat distribution. A similar association was seen in normal-weight men, but significance did not hold after correction for multiple testing. Prevalence of pre-sarcopenia and sarcopenia was low, but most prevalent in normal-weight NAFLD. However, in multivariable analysis neither sarcopenia nor pre-sarcopenia was associated with NAFLD, most likely because of the relative high SMI in this elderly community dwelling population. Second, high fat mass appeared a better predictor for NAFLD prevalence than low muscle mass. In particular, android fat mass had a significant association with higher NAFLD prevalence in all subgroups, whereas gynoid fat tended to be associated with lower NAFLD prevalence. Likewise, AGR as proxy for fat distribution was the strongest predictor for NAFLD.

Recently, low skeletal muscle mass emerged as potential risk factor for steatosis independent of age and BMI.[380-384] Most of these studies were carried out in Korean populations.[380-382,384] However, as ethnicity is important in the evaluation of body composition,[12] it is difficult to generalize these results to Western populations. Asian individuals carry proportionately higher fat mass for a given BMI than Caucasians. To date, only two studies have been performed in Western populations.[371,385] Both studies found that the prevalence of pre-sarcopenia increased with fibrosis severity in NAFLD. But direct comparison to these studies is hampered by the different study populations (abovementioned studies included advanced steatohepatitis patients with cirrhosis prevalence of 33.3% and 15.6% without healthy control group).[371,385] This is in contrast to our study in which we targeted a different study population, i.e. an unselected presumably healthy population in which only 10% of the participants with steatosis had coinciding elevated LSM. Furthermore, low skeletal muscle mass alone has been systematically referred to as *sarcopenia* in these previous reports. However, loss of strength or performance is strictly needed to make the actual diagnosis of sarcopenia.[374] A recent small study examined actual sarcopenia univariately with NAFLD and found that sarcopenia was actually associated with lower instead of higher NAFLD prevalence.[386] Yet, this finding is in line with our univariate results in which SMI was also associated with higher prevalence of NAFLD. While the previous study did not perform a multivariable analysis, we found that this association changed after adjustment for weight.

Another commonly used proxy for skeletal muscle mass is ALM-fraction.[370,371,380-384] We chose to use SMI as advised by the EWGSOP consensus guidelines, but we did perform additional analyses on ALM-fraction, and found that both ALM fraction and SMI were equally good in predicting the prevalence of NAFLD if only adjusted for weight and height. We hypothesize that the explanation lies in the fact that most phenotypes associated with sarcopenia, are typically related to frailty and poor nutritional status (and hence to a low BMI),[374] whereas NAFLD is typically related to adiposity and over-nutrition (and thus to a high BMI).[13] We would therefore like to pose that ALM-fraction might be more clinically applicable than SMI for the (univariate) assessment of pre-sarcopenia in NAFLD as it already takes into account the confounder weight. In our study, neither pre-sarcopenia nor sarcopenia was independently associated with NAFLD. We believe this is due to the low number of (pre)sarcopenic cases. Indeed, the median SMI was relatively high in our population. As a consequence, (pre)sarcopenia in the obese and overweight was rare (prevalence of 0.4% and 5% respectively). This could explain the lack of SMI being associated with overweight NAFLD. As for muscle function, grip strength has been analysed previously in a large Asian cohort that found an inverse association between grip strength (relative to body weight) and NAFLD.[387] In our study, incremental grip strength was associated with lower NAFLD prevalence in men only, however this association did not hold significance after multivariable adjustment.

Interestingly, the association between SMI and NAFLD was more pronounced in women than in men. This is in contrast to previous studies in which associations are generally more pronounced in men. However, there were fewer men than women in this study (n=1980 vs n=2629) and, as said before, SMI was relatively high in our community-dwelling population. Therefore, we cannot fully exclude the possibility that differences in sex-stratified analyses resulted, in part, from power issues. Nevertheless, there are obvious differences in sex hormones between men and women that could have affected the association between SMI with NAFLD. As our study concerns an elderly population, there is a presumed relative reduced testosterone, a strong anabolic hormone, in men.[388] Nonetheless, absolute testosterone in elderly men is generally still higher than in women.[389]Also, relative oestrogen is decreased in women because of the menopause,[388] and fat mass-to-fat free mass ratio is much higher in women than in men.[377] Furthermore, apart from the differences in sex hormones, other signalling hormones such as growth hormone could have also affected the relation between body composition and NAFLD.[317] In addition, inflammation has been suggested to play a major role in sarcopenia; myokines secreted by skeletal muscle mass, for instance, antagonize the pro-inflammatory and metabolic effects of adipocytes from fat tissue.[383,390] This could explain the hypothesized synergistic effect of low skeletal muscle mass and adiposity together. Moreover, it is known that visceral fat, in particular, impairs adipocyte function and adipocytokine secretion, which can lead to an increase in pro-inflammatory cytokines such as interleukin-6 and tumour necrosis factor-alpha.[391]

This could explain the attenuating effect of AGR between body components with NAFLD, which is particularly interesting as AGR was much higher in men than in women.

A major strength of our study is the use of a large population-based cohort with access to a great number of reliably measured traits. Additionally, body composition was determined by the gold standard (DXA-scan) and we used the recommended EWGSOP definition to determine the presence of pre-sarcopenia and sarcopenia.[374] Moreover, we included only individuals with a European background to exclude bias due to racial differences in body composition. And lastly, we performed several sensitivity analyses to test the robustness of our conclusions. Nonetheless, several limitations need mentioning. First, the cross-sectional design of this study makes it impossible to draw conclusions on the cause-effect relation between body composition and NAFLD. Second, the gold standard for diagnosis of NAFLD is liver biopsy rather than US. However, performing an invasive liver biopsy in presumed healthy individuals is unethical. Moreover, US is a widely used screening tool that yields high sensitivity and specificity for moderate and severe steatosis.[50] Third, missing data on gait speed was substantial (32.6%). Yet, grip strength, as indicator for muscle strength, was present in almost all participants (>99%). Moreover, sensitivity analyses using imputed data on gait speed showed similar results to the main non-imputed analysis, suggesting no under or overestimation of sarcopenia. Fourth, we had no information on possible fat infiltration in the muscle, which could have affected the quality of muscle mass. Previous studies have suggested that, in older women particularly, there is quite a proportion of fat within the quadriceps muscle, this infiltration was related to gait speed.[392] However, in our study was gait speed not associated with NAFLD, neither in men nor in women.

In summary, skeletal muscle mass as assessed by multiple proxies was consistently associated with NAFLD in normal-weight women. However, fat mass was a better predictor for NAFLD probability in both sexes. In particular, android-fat-to-gynoid-fat ratio was strongly associated with NAFLD. This is in line with android fat being associated with higher odds and gynoid fat being associated with lower odds for NAFLD. These findings, if confirmed by others, add to the rationale of resistance training in order to replace fat mass by lean mass (regardless of simultaneous weight loss) as an easily accessible, inexpensive, and targeted approach for individuals with NAFLD.[393]

Supplementary Methods: details on the imputation process

Missing values of analysis variables were 0.04–18.1%. Following the advice of White et al.,[394] 74 imputed datasets were created using the R-package mice.[323] The analyses were performed in each dataset. The results presented are pooled over the multiple analyses using Rubin's rules in order to take into account the added uncertainty due to the missing data.[254] Imputation was performed before exclusion of subjects due to alcohol abuse or non-European background. Participants with missing values on these 'exclusion variables' were included only if the imputed values fulfilled the inclusion criteria for alcohol consumption in at least >80% of the imputed datasets, and ethnicity was imputed as European background in at least >95% of the imputed datasets. The difference in cut-off is based on the fact that ethnicity is purely an exclusion criterion, whereas alcohol consumption was evaluated as predictor in the regression models.

Supplementary Methods: details on the multiple imputation process

	Multiple imputation
Software used	R version 3.4.2
Imputation method and key settings	Fully conditional specification (Markov chain Monte Carlo method); maximum iterations: 50
No. of imputed data sets created	74
Analyses variables	diabetes mellitus, hypertension, smoking status, total cholesterol, diastolic and systolic blood pressure, high density lipoprotein cholesterol, energy intake, platelets, waist circumference, haemoglobin, haematocrit, heart rate, waist/hip ratio, aspartate transaminase; alanine transaminase; homeostasis model assessment of insulin resistance, triglycerides, insulin, gamma-glutamyl transferase, alkaline phosphatase, bilirubin, alcohol consumption, creatinine, glomerular filtration rate, grip strength, physical activity, gait speed, spleen size, glucose, education level, and ancestry, liver stiffness measurements, IQR of liver stiffness
Auxiliary variables	android and gynoid fat mass, height, skeletal muscle index, weight, age, amiodarone, antihypertensives, systemic corticosteroid use, sex, Rotterdam Study cohorts, steatosis, and tamoxifen
Treatment of not normally distributed variables	Predictive mean matching
Treatment of normally distributed variables	Linear regression
Treatment of binary/categorical variables	(Proportional odds) Linear Regression
Population	For the imputation we excluded outliers, unreliable FFQs and missing data on DXA-scans. Imputed population (n=5.714).

Supplementary Table 1: Imputation Characteristics

	Original Data (n=4609)	Imputed data* (n=4609)
Demographics		
Age (years)	69.3 (9.2)	-
Female (%)	57.0	-
Education Level (%)		
Low	49.2	49.3
Intermediate	29.6	29.6
High	21.1	21.1
Smoking status (%)		
Never	35.1	34.5
Past/Current	64.9	65.5
Alcohol (units/d)	0.47 [0.06, 1.22]	0.50 [0.07, 1.28]
Physical Activity	39.8 [15.8, 76.5]	38.9 [15.5, 75.6]
Caloric Intake (kcal/day)	2114 (730)	2132 (737)
Physical examination		
Height (cm)	168.8 (9.5)	-
Weight (kg)	78.5 (14.4)	-
BMI (kg/m^2)	27.5 (4.2)	-
underweight	0.3	-
normal	28.7	
overweight	47.3	
obese	18.4	
morbid obese	5.3	
Gait speed (m/s)	1.21 (0.20)	1.19 (0.21)
Hand grip strength (kg)	28.0 (10.3)	28.0 (10.3)
Android fat mass (kg)	2.5 [1.8, 3.2]	-
Gynoid fat mass (kg)	3.8 [3.1, 4.8]	-
Android/Gynoid ratio		
men	0.79 [0.69, 0.91]	-
women	0.53 [0.45, 0.63]	-
Total fat mass (kg)	27.3 [22.2, 33.1]	-
Body fat (%)	36.3 (7.4)	-
ALM (kg)	21.5 (5.0)	-
SMI (kg/m^2)	7.5 (1.2)	-
Biochemistry		
AST (U/L)	24 [21, 28]	24 [21, 28]
ALT (U/L)	18 [15, 24]	18 [15, 24]
GGT (U/L)	23 [17, 34]	23 [17, 34]
Platelets ($*10^9$/L)	261 [222, 305]	261 [222, 306]
HOMA-IR	2.6 [1.7, 4.1]	2.6 [1.7, 4.1]

Supplementary Table 1 (*continued*)

	Original Data (n=4609)	Imputed data* (n=4609)
Total Cholesterol (mmol/l)	5.4 (1.1)	5.4 (1.1)
HDL-C (mmol/l)	1.5 (0.4)	1.5 (0.4)
Triglycerides (mmol/l)	1.3 [1.0, 1.7]	1.3 [1.0, 1.7]
Comorbidities		
Metabolic Syndrome (%)	53.6	53.6
- WC	44.2	44.2
- Triglycerides	46.8	46.5
- HDL-C	45.9	45.6
- Blood pressure	83.4	83.4
- Fasting Plasma Glucose	47.2	47.2
Diabetes Mellitus (%)	13.2	13.1
Hypertension (%)	72.8	72.8
NAFLD	35.2	-

* Imputed data is based on pooled data from 74 imputations. Data is presented as mean (SD), median [P25-P75] or percentage. ¥ Physical activity in metabolic equivalent task hours/week. – Represents no missing values.

Supplementary Table 2: Population Characteristics across NAFLD strata in normal and overweight individuals

	Total population			*Normal-weight*			*Overweight*		
	No NAFLD n=2986	NAFLD n=1623	*P*-value*	No NAFLD n=1178	NAFLD n=161	*P*-value*	No NAFLD n=1808	NAFLD n=1462	*P*-value*
Demographics									
Age (years)	69.3 (9.5)	69.3 (8.5)	0.887	69.2 (9.9)	68.8 (8.5)	0.615	69.5 (9.3)	69.36 (8.51)	0.787
Female (%)	58.0	55.3	0.078	63.3	58.4	0.259	54.5	54.9	0.852
Education Level (%)									
Low	46.4	54.5	<0.001	46.0	48.4	0.117	46.6	55.2	
Intermediate	30.3	28.4		27.9	32.8		31.9	27.9	<0.001
High	23.3	17.1		26.1	18.8		21.5	16.9	
Smoking status (%)									
Never	36.9	30.3	<0.001	38.7	35.1	0.038	35.7	29.7	<0.001
Past/Current	63.1	69.7		61.3	64.9		64.3	70.3	
Alcohol (units/d)	0.50 [0.07, 1.26]	0.50 [0.07, 1.28]	0.986	0.49 [0.05, 1.22]	0.61 [0.17, 1.57]	0.243	0.52 [0.09, 1.33]	0.49 [0.06, 1.27]	0.996
Physical activity¥	42.5 [16.8, 79.7]	32.7 [13.5, 67.3]	<0.001	45.5 [18.7, 81.6]	38.4 [18.1, 80.2]	0.919	39.7 [15.3, 77.0]	31.8 [13.0, 66.2]	0.001
Caloric Intake (kcal/day)	2154 (740)	2092 (729)	0.015	2180 (746)	2205 (707)	0.656	2137 (736)	2080 (730)	0.044
Biochemistry									
AST (U/L)	24.0 [21.0, 28.0]	25.0 [21.0, 29.0]	<0.001	24.0 [21.0, 28.0]	25.0 [21.0, 29.0]	0.101	24.0 [21.0, 27.0]	25.0 [21.0, 29.0]	<0.001

Supplementary Table 2 (*continued*)

	Total population			*Normal-weight*			*Overweight*		
	No NAFLD n=2986	**NAFLD n=1623**	***P*-value***	**No NAFLD n=1178**	**NAFLD n=161**	***P*-value***	**No NAFLD n=1808**	**NAFLD n=1462**	***P*-value***
ALT (U/L)	17.0 [14.0, 22.0]	22.0 [17.0, 29.0]	<0.001	17.0 [14.0, 21.0]	20.0 [16.0, 27.0]	<0.001	18.0 [14.0, 22.0]	22.0 [17.0, 29.0]	<0.001
GGT (U/L)	21.0 [15.0, 30.0]	28.0 [20.0, 41.0]	<0.001	19.0 [14.0, 27.0]	26.0 [19.0, 37.0]	<0.001	22.0 [16.0, 32.0]	28.0 [20.0, 41.0]	<0.001
Platelets (*10^9/L)	259 [221, 304]	266 [225, 308]	0.040	265 [229, 312]	274 [230, 315]	0.371	255 [218, 298]	265 [224, 308]	0.002
HOMA-IR	2.15 [1.50, 3.10]	4.03 [2.74, 6.09]	<0.001	1.72 [1.27, 2.41]	2.66 [1.73, 4.22]	<0.001	2.48 [1.76, 3.53]	4.20 [2.87, 6.26]	<0.001
Total Cholesterol	5.46 (1.10)	5.36 (1.13)	0.003	5.53 (1.08)	5.54 (1.04)	0.919	5.42 (1.11)	5.34 (1.13)	0.055
HDL-C	1.54 (0.42)	1.32 (0.37)	<0.001	1.65 (0.45)	1.42 (0.40)	<0.001	1.46 (0.39)	1.31 (0.36)	<0.001
Triglycerides	1.16 [0.90, 1.53]	1.55 [1.17, 2.06]	<0.001	1.07 [0.83, 1.38]	1.29 [0.93, 1.86]	<0.001	1.23 [0.96, 1.61]	1.57 [1.20, 2.08]	<0.001
Comorbidities									
Metabolic Syndrome (%)	41.9	75.2	<0.001	25.9	44.7	<0.001	52.3	78.5	<0.001
- WC	29.9	70.4	<0.001	3.7	11.8	<0.001	47.0	76.9	<0.001
- Triglycerides	39.1	60.1	<0.001	31.2	48.4	<0.001	44.7	61.6	<0.001
- HDL-C	39.0	57.8	<0.001	32.0	48.4	<0.001	43.9	59.1	<0.001
- Blood Pressure	79.4	90.6	<0.001	72.4	78.9	0.100	84.0	91.9	<0.001
- Fasting plasma glucose	36.7	66.5	<0.001	25.5	44.0	<0.001	43.8	69.0	<0.001
Diabetes Mellitus (%)	7.6	17.7	<0.001	5.2	14.5	<0.001	9.3	24.3	<0.001
Hypertension (%)	67.5	82.3	<0.001	59.3	67.1	0.069	72.9	84.0	<0.001

Pooled data based on 74 imputations is presented as mean (SD), median [P25-P75] or percentage. *P-value is calculated using analyses of variance, Kruskall-Wallis, or Chi-squared test. ¥ Physical activity in metabolic equivalent task hours/week.

Supplementary Table 3: Substitution Analyses

	Men			
	Normal-weight n=499	***P*-value**	**Overweight n=1481**	***P*-Value**
Total lean mass *instead of variables left-below*				
Total body fat	**0.88 (0.81 – 0.94)**	**0.001**	**0.92 (0.89 – 0.95)**	**<0.001**
Trunk fat	0.92 (0.72 – 1.18)	0.505	1.02 (0.93 – 1.11)	0.748
Gynoid fat	1.62 (0.74 – 3.56)	0.231	**1.45 (1.11 – 1.88)**	**0.006**
Android fat	0.36 (0.15 – 0.85)	0.021	**0.31 (0.22 – 0.44)**	**<0.001**
Appendicular lean mass				
Trunk lean mass	0.89 (0.73 – 1.09)	0.267	1.02 (0.93 – 1.12)	0.694
Total body fat	**0.83 (0.73 – 0.94)**	**0.004**	0.93 (0.88 – 0.99)	0.018
Trunk fat	0.86 (0.66 – 1.13)	0.282	1.06 (0.95 – 1.18)	0.273

Supplementary Table 3 (*continued*)

	Men			
	Normal-weight n=499	***P*-value**	**Overweight n=1481**	***P*-Value**
Gynoid fat	1.58 (0.72 – 3.48)	0.255	**1.47 (1.12 – 1.91)**	**0.005**
Android fat	**0.30 (0.12 – 0.74)**	**0.009**	**0.32 (0.22 – 0.44)**	**<0.001**
Trunk lean mass				
Appendicular lean mass	1.12 (0.92 – 1.37)	0.267	0.98 (0.89 – 1.08)	0.694
Total body fat	0.92 (0.82 – 1.04)	0.197	**0.91 (0.87 – 0.97)**	**0.002**
Trunk fat	1.03 (0.79 – 1.34)	0.845	1.03 (0.93 – 1.14)	0.589
Gynoid fat	1.88 (0.84 – 4.22)	0.125	**1.42 (1.09 – 1.86)**	**0.010**
Android fat	0.36 (0.15 – 0.86)	0.022	**0.31 (0.22 – 0.43)**	**<0.001**
Total body fat				
Total lean mass	**1.14 (1.06 – 1.23)**	**0.001**	**1.08 (1.05 – 1.12)**	**<0.001**
Trunk lean mass	1.08 (0.96 – 1.22)	0.197	**1.09 (1.03 – 1.16)**	**0.002**
Appendicular lean mass	**1.21 (1.06 – 1.38)**	**0.004**	1.07 (1.01 – 1.14)	0.018
Trunk fat				
Gynoid fat	1.72 (0.65 – 4.55)	0.277	1.39 (1.00 – 1.92)	0.049
Android fat	0.38 (0.13 – 1.11)	0.077	**0.30 (0.20 – 0.45)**	**<0.001**
Total lean mass	1.06 (0.83 – 1.36)	0.640	0.96 (0.88 – 1.05)	0.371
Trunk lean mass	0.97 (0.74 – 1.27)	0.845	0.97 (0.88 – 1.08)	0.589
Appendicular lean mass	1.16 (0.89 – 1.51)	0.282	0.94 (0.85 – 1.05)	0.273
Gynoid fat				
Trunk fat	0.58 (0.22 – 1.55)	0.277	0.72 (0.52 – 1.00)	0.049
Android fat	**0.22 (0.09 – 0.56)**	**0.002**	**0.22 (0.15 – 0.32)**	**<0.001**
Total lean mass	0.62 (0.28 – 1.36)	0.231	**0.69 (0.53 – 0.90)**	**0.006**
Trunk lean mass	0.53 (0.24 – 1.19)	0.125	0.70 (0.54 – 0.92)	0.010
Appendicular lean mass	0.63 (0.29 – 1.39)	0.255	**0.68 (0.52 – 0.89)**	**0.005**
Android fat				
Trunk fat	2.66 (0.90 – 7.85)	0.077	**3.34 (2.21 – 5.05)**	**<0.001**
Gynoid fat	**4.55 (1.78 – 11.7)**	**0.002**	**4.64 (3.18 – 6.77)**	**<0.001**
Total lean mass	2.82 (1.17 – 6.76)	0.021	**3.21 (2.28 – 4.51)**	**<0.001**
Trunk lean mass	2.78 (1.16 – 6.68)	0.022	**3.28 (2.31 – 4.64)**	**<0.001**
Appendicular lean mass	**3.31 (1.34 – 8.15)**	**0.009**	**3.17 (2.25 – 4.47)**	**<0.001**
	Women			
	Normal-weight n=840	***P*-value**	**Overweight n=1789**	***P*-Value**
Total lean mass *instead of variables left-below*				
Total body fat	**0.85 (0.80 – 0.91)**	**<0.001**	**0.95 (0.92 – 0.98)**	**0.001**
Trunk fat	0.89 (0.78 – 1.00)	0.058	0.94 (0.89 – 1.00)	0.034
Gynoid fat	**1.82 (1.25 – 2.63)**	**0.002**	**1.65 (1.42 – 1.90)**	**<0.001**
Android fat	**0.23 (0.14 – 0.38)**	**<0.001**	**0.34 (0.28 – 0.42)**	**<0.001**

Supplementary Table 3 (*continued*)

	Women			
	Normal-weight n=840	***P*-value**	**Overweight n=1789**	***P*-Value**
Appendicular lean mass				
Trunk lean mass	0.95 (0.79 – 1.14)	0.573	0.99 (0.91 – 1.07)	0.743
Total body fat	**0.83 (0.74 – 0.93)**	**0.002**	0.94 (0.89 – 1.00)	0.037
Trunk fat	0.87 (0.74 – 1.01)	0.074	1.01 (0.93 – 1.09)	0.859
Gynoid fat	**1.73 (1.18 – 2.54)**	**0.005**	**1.71 (1.46 – 2.00)**	**<0.001**
Android fat	**0.21 (0.13 – 0.36)**	**<0.001**	**0.35 (0.28 – 0.42)**	**<0.001**
Trunk lean mass				
Appendicular lean mass	1.05 (0.88 – 1.27)	0.573	1.01 (0.93 – 1.10)	0.743
Total body fat	0.87 (0.79 – 0.97)	0.012	0.95 (0.91 – 1.00)	0.040
Trunk fat	0.94 (0.81 – 1.10)	0.459	0.94 (0.89 – 1.01)	0.079
Gynoid fat	**1.89 (1.29 – 2.76)**	**0.001**	**1.60 (1.38 – 1.86)**	**<0.001**
Android fat	**0.23 (0.14 – 0.39)**	**<0.001**	**0.32 (0.26 – 0.40)**	**<0.001**
Total body fat				
Total lean mass	**1.17 (1.10 – 1.25)**	**<0.001**	**1.06 (1.02 – 1.09)**	**0.001**
Trunk lean mass	1.15 (1.03 – 1.27)	0.012	1.05 (1.00 – 1.10)	0.040
Appendicular lean mass	**1.21 (1.07 – 1.36)**	**0.002**	1.06 (1.00 – 1.13)	0.037
Trunk fat				
Gynoid fat	**2.01 (1.28 – 3.14)**	**0.002**	**1.70 (1.42 – 2.04)**	**<0.001**
Android fat	**0.25 (0.14 – 0.45)**	**<0.001**	**0.35 (0.28 – 0.44)**	**<0.001**
Total lean mass	1.11 (0.97 – 1.25)	0.125	1.04 (0.98 – 1.10)	0.229
Trunk lean mass	1.06 (0.91 – 1.24)	0.459	1.06 (0.99 – 1.13)	0.079
Appendicular lean mass	1.16 (0.99 – 1.36)	0.074	0.99 (0.92 – 1.08)	0.859
Gynoid fat				
Trunk fat	**0.50 (0.32 – 0.78)**	**0.002**	**0.59 (0.49 – 0.70)**	**<0.001**
Android fat	**0.13 (0.07 – 0.23)**	**<0.001**	**0.21 (0.16 – 0.26)**	**<0.001**
Total lean mass	**0.55 (0.38 – 0.80)**	**0.002**	**0.61 (0.53 – 0.70)**	**<0.001**
Trunk lean mass	**0.53 (0.36 – 0.77)**	**0.001**	**0.62 (0.54 – 0.73)**	**<0.001**
Appendicular lean mass	**0.58 (0.39 – 0.85)**	**0.005**	**0.59 (0.50 – 0.68)**	**<0.001**
Android fat				
Trunk fat	**4.00 (2.22 – 7.20)**	**<0.001**	**2.85 (2.27 – 3.58)**	**<0.001**
Gynoid fat	**8.02 (4.42 – 14.6)**	**<0.001**	**4.85 (3.82 – 6.16)**	**<0.001**
Total lean mass	**4.42 (2.66 – 7.35)**	**<0.001**	**2.95 (2.41 – 3.60)**	**<0.001**
Trunk lean mass	**4.30 (2.58 – 7.16)**	**<0.001**	**3.09 (2.50 – 3.82)**	**<0.001**
Appendicular lean mass	**4.69 (2.78 – 7.92)**	**<0.001**	**2.90 (2.37 – 3.55)**	**<0.001**

Results are presented as OR (95%CI). OR is for substituting body composition parameter (bold and centred) for the left-mentioned parameters (exchange in fraction, %). Results in bold reflect significant findings with a P-value <0.010. Adjusted for age, study cohorts, weight and height (model 1).

Supplementary Table 4: Comparing body composition parameter in normal and overweight across sex

	Men	
	Normal-weight n=499	**Overweight n=1481**
SMI	**0.50 (0.32 – 0.79)**	**0.75 (0.63 – 0.90)**
AIC	378	1857
ALM-fraction	**0.85 (0.76 – 0.95)**	**0.91 (0.87 – 0.96)**
AIC	379	1854
(Pre)sarcopenia		
Normal SMI	ref (1)	ref (1)
Presarcopenia	1.73 (0.84 – 3.59)	1.28 (0.63 – 2.59)
Sarcopenia	2.88 (1.26 – 6.58)	2.11 (1.12 – 3.98)
AIC	383	1864
Grip	**0.93 (0.89 – 0.96)**	0.99 (0.98 – 1.01)
AIC	373	1865
Speed	0.85 (0.15 – 4.97)	1.41 (0.71 – 2.82)
AIC	387	1864
Body fat fraction	**1.12 (1.05 – 1.20)**	**1.08 (1.05 – 1.12)**
AIC	376	1841
AGR*	**1.97 (1.41 – 2.76)**	**1.98 (1.69 – 2.32)**
AIC	372	1790
	Women	
	Normal-weight n=840	**Overweight n=1789**
SMI	**0.37 (0.23 – 0.59)**	0.90 (0.75 – 1.09)
AIC	546	2270
ALM-fraction	**0.79 (0.71 – 0.88)**	0.95 (0.89 – 1.00)
AIC	546	2267
(Pre)sarcopenia		
Normal SMI	ref (1)	ref (1)
Presarcopenia	**2.48 (1.25 – 4.95)**	2.49 (0.74 – 8.41)
Sarcopenia	1.54 (0.66 – 3.62)	0.99 (0.28 – 3.52)
AIC	560	2271
Grip	0.96 (0.91 – 1.00)	1.00 (0.98 – 1.02)
AIC	561	2271
Speed	0.78 (0.18 – 3.38)	1.60 (0.80 – 3.20)
AIC	564	2270
Body fat fraction	**1.15 (1.08 – 1.23)**	**1.05 (1.02 – 1.08)**
AIC	545	2261
AGR*	**4.81 (3.19 – 7.26)**	**3.42 (2.83 – 4.14)**
AIC	501	2083

Results are presented as OR (95%CI). Results in bold reflect significant findings with a P-value <0.010.The lowest AIC is highlighted and reflects the best predictive parameter. Adjusted for age, study cohorts, weight and height. *AGR is standardised (increase is per 1SD). Abbreviations: AGR: android-to-gynoid fat ratio; AIC: akaike information criterion; ALM: appendicular lean mass; SMI: skeletal muscle index.

Supplementary Table 5: ALM-fraction & NAFLD

	Men			
	Normal-weight (n=499)	***P*-value**	**Overweight (n=1481)**	***P*-value**
Model 1				
ALM-fraction	**0.85 (0.76 – 0.95)**	**0.003**	**0.91 (0.87 – 0.96)**	**<0.001**
Model 2				
ALM-fraction	0.88 (0.78 – 0.98)	0.021	0.95 (0.90 – 1.01)	0.090
Model 3				
ALM-fraction	0.90 (0.80 – 1.01)	0.070	0.97 (0.92 – 1.03)	0.279
	Women			
	Normal-weight (n=840)	***P*-value**	**Overweight (n=1789)**	***P*-value**
Model 1				
ALM-fraction	**0.79 (0.71 – 0.88)**	**<0.001**	0.95 (0.89 – 1.00)	0.050
Model 2				
ALM-fraction	**0.80 (0.71 – 0.90)**	**<0.001**	0.97 (0.91 – 1.03)	0.298
Model 3				
ALM-fraction	**0.84 (0.75 – 0.95)**	**0.006**	1.00 (0.94 – 1.06)	0.908

Results are presented as OR (95%CI). Results in bold reflect significant findings with a P-value <0.010. Model 1: adjusted for age, study cohorts, weight and height Model 2: in addition to model 1; HOMA-IR and triglycerides. Model 3: in addition to model 2; AGR. Additional adjustments for confounding by education level, physical activity, alcohol intake, energy intake, ALT, and smoking resulted in negligible changes in odds ratio (<5%). Abbreviations: AGR: android/Gynoid ratio; ALM: appendicular lean mass; CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease; OR: odds ratio.

Supplementary Table 6: Presarcopenia, sarcopenia & NAFLD

	Men			
	Normal-weight (n=499)	***P*-value**	**Overweight (n=1481)**	***P*-value**
Model 1				
normal SMI	ref (1)		ref (1)	
presarcopenia	1.73 (0.84 – 3.59)	0.139	1.28 (0.63 – 2.59)	0.495
sarcopenia	2.88 (1.26 – 6.58)	0.012	2.11 (1.12 – 3.98)	0.022
Model 2				
normal SMI	ref (1)		ref (1)	
presarcopenia	1.51 (0.72 – 3.19)	0.276	1.19 (0.56 – 2.52)	0.657
sarcopenia	2.34 (1.00 – 5.45)	0.050	2.07 (1.06 – 4.05)	0.033
Model 3				
normal SMI	ref (1)		ref (1)	
presarcopenia	1.36 (0.63 – 2.89)	0.433	1.09 (0.50 – 2.33)	0.836
sarcopenia	2.20 (0.94 – 5.13)	0.069	1.88 (0.95 – 3.72)	0.068

Supplementary Table 6 (*continued*)

	Women			
	Normal-weight (n=840)	***P*-value**	**Overweight (n=1789)**	***P*-value**
Model 1				
normal SMI	ref (1)		ref (1)	
presarcopenia	**2.48 (1.25 – 4.95)**	**0.010**	2.49 (0.74 – 8.41)	0.143
sarcopenia	1.54 (0.66 – 3.62)	0.321	0.99 (0.28 – 3.52)	0.986
Model 2				
normal SMI	ref (1)		ref (1)	
presarcopenia	1.68 (0.79 – 3.58)	0.182	2.88 (0.74 – 11.19)	0.127
sarcopenia	1.31 (0.53 – 3.22)	0.561	0.75 (0.18 – 3.09)	0.692
Model 3				
normal SMI	ref (1)		ref (1)	
presarcopenia	1.20 (0.55 – 2.64)	0.641	2.54 (0.62 – 10.44)	0.196
sarcopenia	1.23 (0.49 – 3.07)	0.654	0.57 (0.14 – 2.41)	0.446

In men: presarcopenia: n=158 & sarcopenia n=130 & in women: presarcopenia: n=116 & sarcopenia: n=78. Results are presented as OR (95%CI). Results in bold reflect significant findings with a P-value <0.010. Model 1: adjusted for age, study cohorts, weight and height. Model 2: in addition to model 1; HOMA-IR and triglycerides. Model 3: in addition to model 2; AGR. Abbreviations: AGR: android-to-gynoid fat ratio; CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease.

Supplementary Table 7: SMI & NASH

	Men		**Women**	
	Total (n=539)	**P-value**	**Total (n=587)**	**P-value**
Model 1				
SMI	1.00 (0.64 – 1.55)	0.982	0.52 (0.28 – 0.96)	0.038
Model 2				
SMI	1.02 (0.65 – 1.61)	0.922	0.47 (0.25 – 0.90)	0.023
Model 3				
SMI	1.03 (0.65 – 1.61)	0.909	0.48 (0.25 – 0.92)	0.027

In men: n=71 NASH and in women: n=44 NASH. Results are presented as OR (95%CI). Results in bold reflect significant findings with a P-value <0.010. Model 1: adjusted for age, study cohorts, weight and height. Model 2: in addition to model 1; HOMA-IR and triglycerides. Model 3: in addition to model 2; AGR. Abbreviations: AGR: android-to-gynoid fat ratio; CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance; NASH: non-alcoholic steatohepatitis; OR: odds ratio; SMI: skeletal muscle index.