

Body Composition and Genetic Lipodystrophy Risk Score Associate With Nonalcoholic Fatty Liver Disease and Liver Fibrosis

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Up to 25% of patients with nonalcoholic fatty liver disease (NAFLD) are not obese but may have a fat or muscle composition that predisposes them to NAFLD. Our aim was to determine whether body composition parameters associate with NAFLD and to identify genetic contributors to this association. This study included two cohorts. The first included 2,249 participants from the Framingham Heart Study who underwent a computed tomography scan to evaluate hepatic steatosis, dual-energy x-ray absorptiometry testing to assess body composition, and clinical examination. Body composition parameters were normalized to total body weight. A subset of participants underwent genotyping with an Affymetrix 550K single-nucleotide polymorphism array. The second cohort, Michigan Genomics Initiative, included 19,239 individuals with genotyping on the Illumina HumanCoreExome v.12.1 array and full electronic health record data. Using sex-stratified multivariable linear regression, greater central body fat associated with increased hepatic steatosis while greater lower extremity body fat associated with decreased hepatic steatosis. Greater appendicular lean mass was associated with decreased hepatic steatosis in men but not in women. A polygenic risk score for lipodystrophy (regional or global loss of adipose tissue) was associated with increased hepatic steatosis, increased liver fibrosis, and decreased lower extremity fat mass. **Conclusion:** Greater central body fat associated with increased hepatic steatosis, while greater lower extremity body fat and, in men, greater appendicular lean mass were associated with decreased hepatic steatosis. A genetic risk score for lipodystrophy was associated with NAFLD and liver fibrosis. Our results suggest that buffering of excess energy by peripheral fat and muscle may protect against NAFLD and liver fibrosis in the general population. (*Hepatology Communications* 2019;3:1073-1084).

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive triglyceride accumulation in the liver in the absence of significant alcohol use or other underlying cause.⁽¹⁾ NAFLD is the most common chronic liver disease worldwide, affecting 20%-40% of the general

Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; BMI, body mass index; CI, confidence interval; CT, computed tomography; DXA, dual-energy x-ray absorption; FHS, Framingham Heart Study; LPR, liver:phantom ratio; LPRS, lipodystrophy polygenic risk score; MGI, Michigan Genomics Initiative; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; SNP, single-nucleotide polymorphism.

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population, and is associated with metabolic conditions, such as obesity, diabetes, and dyslipidemia.⁽²⁻⁴⁾ Unfortunately, treatment options remain limited, and a better understanding of the pathophysiology underlying NAFLD will be critical in developing more effective treatments.

While NAFLD is associated with obesity, approximately 25% of patients with NAFLD are not obese.⁽⁵⁾ This finding suggests that not all fat contributes equally to NAFLD risk; it may be regional adiposity rather than overall adiposity that contributes to liver steatosis. Visceral fat is associated with increased risk of NAFLD and progression to hepatic fibrosis.⁽⁶⁻⁸⁾ In contrast, gluteofemoral and lower extremity fat correlate with decreased transaminases and may be protective against hepatic steatosis.⁽⁹⁻¹¹⁾ Despite the importance of different fat depots in NAFLD, the literature on lower extremity fat in NAFLD is limited to studies of a few hundred subjects, mostly in Asian populations.

Skeletal muscle mass may also protect against NAFLD. Sarcopenia, a condition of low skeletal muscle mass, has been linked to increased risk of NAFLD and advanced fibrosis.⁽¹²⁻¹⁴⁾ Skeletal muscle function, too, may influence NAFLD; greater hand grip strength has been linked to decreased NAFLD prevalence.⁽¹⁵⁾ Further, NAFLD is associated with the substitution of adipose tissue in skeletal muscle⁽¹⁶⁾ and increased insulin resistance of skeletal muscle.⁽¹⁷⁾ However, again, the literature on muscle fat and NAFLD risk is limited to small studies of a few hundred subjects and has not been studied in a Western population.

A number of genes have been implicated in NAFLD, and some of these genes also influence

body composition.⁽¹⁸⁾ For example, individuals with NAFLD in the presence of the patatin-like phospholipase domain-containing 3 (PNPLA3) I148M variant are less frequently obese than those with the ancestral allele at *PNPLA3*.^(19,20) More recently, several genetic contributors to lipodystrophy have been identified. Lipodystrophy is characterized by global or selective deficiency of adipose tissue in the absence of malnutrition or a catabolic state.⁽²¹⁾ While most patients with NAFLD are not overtly “lipodystrophic,” NAFLD is itself a form of ectopic fat accumulation and is highly prevalent in patients with familial lipodystrophy.^(21,22) Lipodystrophy was previously viewed primarily through the lens of rare familial diseases, but relative lipodystrophy may also exist in the general population as a continuous trait.⁽²³⁾ Nonfamilial lipodystrophy is heritable, and a reported lipodystrophy polygenic risk score (LPRS) predicts insulin resistance and decreased lower extremity adiposity, a feature of lipodystrophy.⁽²⁴⁾ Whether people with an increased polygenic lipodystrophy score store more fat in the liver is not known.

We hypothesize that NAFLD may be a marker of partial lipodystrophy in the population. We test whether body composition, specifically fat distribution and muscle bulk, strength, and fat content, associate with NAFLD in a large, well-characterized, European ancestry cohort, the Framingham Heart Study (FHS). Further, we test whether individuals with higher lipodystrophy polygenic scores have a higher prevalence of NAFLD and liver fibrosis using the FHS and another cohort, the Michigan Genomics Initiative (MGI).

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Participants and Methods

ETHICS STATEMENT

All FHS participants provided written informed consent approved by the Boston University Institutional Review Board and Hebrew SeniorLife Institutional Review Board. All MGI participants provided written informed consent approved by the institutional review board of the University of Michigan (Ann Arbor, MI), and all research performed in this paper was approved by the institutional review board of the University of Michigan.

COHORTS

This study included two cohorts. The first was the FHS, a multigenerational prospective cohort study of residents in and around Framingham, MA, characterizing a broad array of phenotypes related to cardiovascular health.⁽²⁵⁾ We included the FHS Offspring and Generation 3 subcohorts. Between 1995 and 1998, 3,492 participants from the Offspring cohort completed the seventh clinical examination (exam 7). Between 2008 and 2011, 3,399 participants from Generation 3 completed the second clinical examination (exam 2). These examinations included a detailed medical history, physical examination, collection of blood specimens, and measurement of anthropometric data, including hand grip strength assessment (for the Offspring cohort, hand grip strength measurements were collected separately).⁽²⁵⁾ Selected subjects participated in substudies that involved additional testing, including multidetector computed tomography (CT) scan, whole-body dual energy x-ray absorptiometry (DXA) scan, and quadriceps strength testing. We excluded participants who reported excess alcohol use (>21 alcoholic drinks per week for men and >14 alcoholic drinks per week for women). The physical activity index is a composite score calculated based on participant responses to questions regarding different levels of physical activity and sleep patterns over a 24-hour period.⁽²⁶⁾ Grip strength and quadriceps strength were measured as described.^(27,28)

In the FHS, a subset of participants underwent genotyping with a 550K single-nucleotide polymorphism (SNP) array (Affymetrix 500K Dual GeneChip and 50K gene-centered molecular inversion probe set).⁽²⁹⁾ Imputation was performed using the 1000

Genomes cosmopolitan panel March 2012(v3) on the Michigan Imputation Server (<https://imputation.server.sph.umich.edu/index.html>).⁽³⁰⁾

The MGI is a prospective cohort with ongoing enrollment; all patients undergoing elective surgery at Michigan Medicine (Ann Arbor, MI) are potentially eligible for enrollment in this cohort. Enrollment involves genotyping of peripheral blood on the Illumina HumanCoreExome v.12.1 array, a genome-wide association study and exome array consisting of >500,000 SNPs.⁽³¹⁾ In addition, full laboratory information and billing codes are available.

HEPATIC STEATOSIS AND MUSCLE ATTENUATION ASSESSMENT

Between 2008 and 2011, multidetector abdominal CT scans (64 slice; General Electric Health Care) were performed, as described.⁽³²⁾ The mean attenuation (Hounsfield units) from three regions in the liver as well as from a calibration control (phantom) was calculated. The liver:phantom ratio (LPR) was calculated by dividing the mean hepatic attenuation by the attenuation of the calibration control ("phantom"). LPR ≤ 0.33 was used to define NAFLD, as reported.⁽³³⁾ Muscle attenuation was measured at the left and right paraspinous muscles at the midabdominal level, as described.⁽³⁴⁾

BODY COMPOSITION ASSESSMENT

Whole-body and regional measures of lean mass and fat mass were obtained by DXA scan (GE Lunar Prodigy fan beam densitometer), as described.^(28,35) For the Offspring cohort, these DXA scans were obtained from 1996 to 2001. For Generation 3, they were obtained in 2010 and 2011. The DXA protocol was the same between the two cohorts. Lower extremity fat mass was a reported measure that combined the fat mass in both legs. Total fat mass was also reported. Appendicular lean mass was calculated by combining bilateral upper and lower extremity lean mass. Central fat mass was calculated by subtracting the bilateral upper and lower extremity fat mass from the whole body total fat mass. These measures were scaled to body weight by dividing the respective values by each participant's weight in kilograms and were reported as a percentage.

CLINICAL AND LABORATORY MEASUREMENTS

The age of the participant documented at the time of the clinical examination was used for the analysis. Body mass index (BMI) was defined as weight (kg)/height (m²). Diabetes was defined by the presence of a fasting glucose ≥ 126 mg/dL, hemoglobin A1c $\geq 6.5\%$, medical history of physician-diagnosed diabetes, or receiving medication for the treatment of diabetes. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, physician-diagnosed hypertension, or receiving anti-hypertensive therapy. Metabolic syndrome was diagnosed based on the National Cholesterol Education Program's Adult Treatment Panel III guidelines.⁽³⁶⁾

In both the FHS and MGI, the aspartate aminotransferase to platelet ratio index (APRI) score was used for noninvasive assessment of liver fibrosis. In the MGI, we defined cirrhosis based on the presence of an International Classification of Diseases, Ninth Revision (ICD-9) code (571.5, 571.2, and 571.6), ICD-10 code (K74.X, K70.2-4, and K71.7), or a text search for cirrhosis. A text search of radiology and pathology reports was performed for the character "cirrho," and participants with that character were flagged as having cirrhosis with the following exceptions: (1) if the word "without" or "no" appeared in the same sentence as "cirrho," subjects were considered to not have cirrhosis; (2) if the words "primary biliary cirrhosis" appeared in a sentence, that sentence was ignored for text-search purposes; and (3) if the words "evaluate," "assess," or "rule out" appeared in a sentence with "cirrho," that sentence was ignored for text-search purposes. A gastroenterologist (V.L.C.) manually reviewed 200 randomly selected text strings and identified no false-positive cirrhosis diagnoses.

STATISTICAL ANALYSIS

Nongenetic Analysis

Differences in characteristics between participants with and without NAFLD were determined using a *t* test for continuous variables and chi-square test for proportions.

For the graphs of % NAFLD versus central fat mass, we computed sex-specific percentiles of central body fat mass and identified the percentage of participants within each percentile with NAFLD, as defined

by LPR < 0.33 . These percentages were then stratified separately by high versus low appendicular lean mass or lower extremity fat mass (i.e., above versus below sex-stratified median). Univariable linear regression was performed on % NAFLD versus central fat mass and graphed as a smoothed linear model. Addition of quadratic terms did not improve the regression ($P > 0.05$ for all comparisons).

Multivariable linear regression analysis was performed to determine the relationship between liver steatosis (as measured by negative LPR) and measures of body composition and strength. For these analyses, negative LPR (increased liver steatosis) was treated as the dependent (outcome) variable. Note that a higher LPR is associated with decreased liver steatosis so that positive beta values for covariates actually imply decreased liver steatosis. Because this is counterintuitive, to increase readability we used negative LPR as the dependent variable so that positive beta values imply increased liver steatosis. The primary independent (exposure) variables were (1) appendicular lean mass, (2) lower extremity fat mass, (3) central fat mass, (4) grip strength, (5) quadriceps strength, and (6) muscle steatosis (negative muscle attenuation for reasons similar to those for LPR, as above). These were inverse normally transformed in order to improve interpretability and treated as continuous independent variables.⁽¹⁸⁾ β values for body composition parameters were reported as the effect of one rank unit (one sixth of the total variation of that trait) on LPR. In sensitivity analyses, we ran these regressions with nontransformed covariates and the results were qualitatively the same (data not shown). Regression analyses were stratified by sex. Proportion of variation explained by variables was estimated by comparing sums of squares for individual variables in the model with the total sum of squares.

Analyses were performed using R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org) with the tidyverse package (www.tidyverse.org). A two-sided *P* value of 0.05 was used to determine statistical significance.

Genetic Analyses

Only participants of European ancestry were included in the genetic analyses. First, principal components were calculated based on LASER/TRACE (<https://laser.sph.umich.edu>), using the World imputed reference panel.⁽³⁷⁾ To exclude individuals who did

not cluster with the European group, individuals with $|Z \text{ score}| > 3$ for any of the first three principal components were removed. Then, the principal components were recalculated on the remaining individuals, using the European panel.

LPRS was calculated as reported.⁽²⁴⁾ In brief, LPRS was the total number of disease-causing alleles at each of 53 reported SNPs each individual carried. These SNPs were selected based on being associated with increased serum insulin, decreased high-density lipoprotein cholesterol, and increased triglycerides; they were tested and shown to associate with decreased lower extremity fat indicative of lipodystrophy.⁽²⁴⁾ In cases when the genotype at that SNP was imputed rather than directly genotyped, we used dose, i.e., probability of having that given genotype at the SNP. First, we calculated the percentage of participants with NAFLD in the FHS (defined as $LPR \leq 0.33$ ⁽³⁸⁾) as a function of LPRS and performed logistic regression using the proportion of NAFLD as the dependent

variable and the number of risk alleles as the independent variable. This was graphed as a smoothed linear model. There was no improvement in the model after addition to quadratic terms for the number of risk alleles. Next, LPRS was used as an independent variable for phenotypes, including the continuous traits of hepatic steatosis (negative LPR) and APRI as well as the binary traits of cirrhosis or NAFLD. These models were adjusted for age, age², and the first 10 principal components (to account for ethnic differences) and either stratified by or adjusted for sex as well.

Results

STUDY POPULATION

FHS participant selection for this study is illustrated in Fig. 1. Data on CT-measured hepatic steatosis, whole body DXA scan, quadriceps and hand

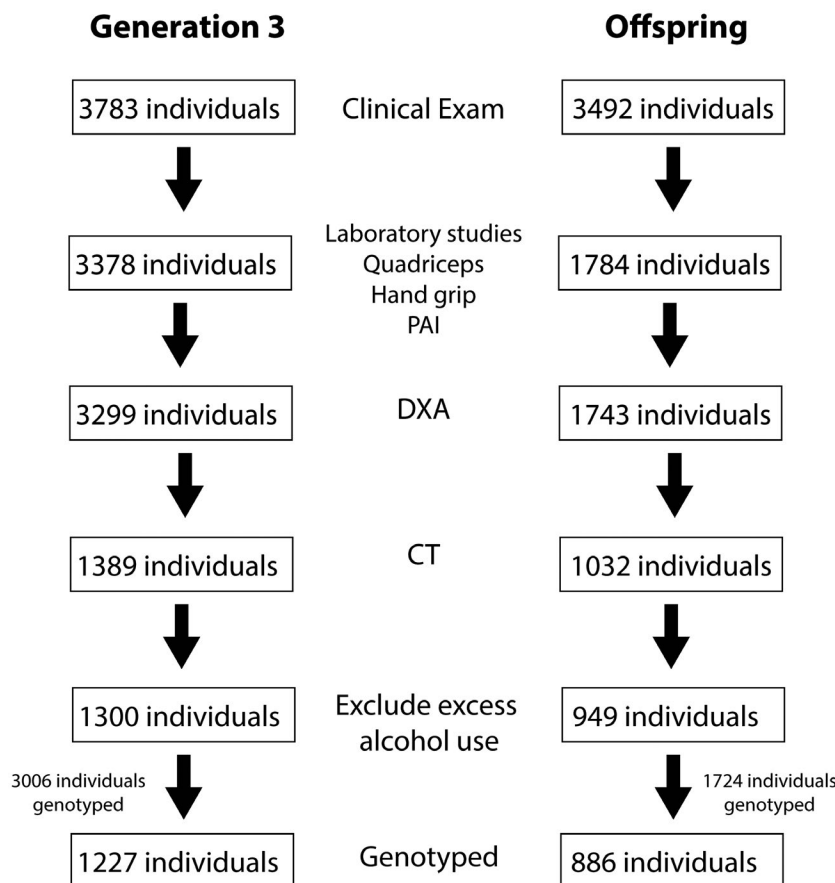


FIG. 1. Study design flowchart. Abbreviation: PAI, physical activity index.

grip strength, physical activity index, and clinical examination were available from 1,389 individuals from Generation 3 and 1,032 from Offspring. After excluding individuals with excess alcohol intake, 1,300 individuals from Generation 3 and 949 individuals from Offspring remained for a total of 2,249 individuals.

Overall, the cohort was 49% male participants with a mean age of 58.5 ± 11.8 years (Supporting Table S1). Prevalence of NAFLD was 28.3%. Clinical parameters stratified by presence versus absence of NAFLD are shown in Table 1. Participants with NAFLD were older and more frequently men and had a higher prevalence of diabetes, hypertension, and metabolic syndrome as well as expected differences in biochemical profiles ($P < 0.05$ for all comparisons).

BODY COMPOSITION AND MUSCLE STRENGTH

Body composition and muscle strength in FHS participants with or without NAFLD in univariate analyses are depicted in Table 1. In the overall cohort,

participants with NAFLD had greater amounts of total fat and central fat and smaller amounts of total lean mass and appendicular (i.e., arms and legs) lean mass (Table 1; $P < 0.0001$ for all). There was no difference in lower extremity fat mass ($P = 0.26$). Grip strength was greater in participants with NAFLD ($P = 0.007$), while there was no difference in quadriceps strength based on NAFLD status ($P = 0.92$; Table 1). Sex-stratified analysis was fairly similar overall. However, among women, grip strength no longer differed based on NAFLD status (Supporting Table S2). Among men, lower extremity fat was higher and grip strength lower in those with NAFLD (Supporting Table S3).

EFFECT OF BODY COMPOSITION ON NAFLD AND FIBROSIS

Next, we sought to identify whether differences in body composition associated with increased risk of NAFLD in the FHS. Because central fat, lower extremity fat, and appendicular lean mass are correlated, we investigated whether they independently affected hepatic steatosis after adjustment for one another. The percentage of participants with

TABLE 1. CLINICAL AND LABORATORY CHARACTERISTICS

Characteristic	Overall (n = 2,249)	No NAFLD (n = 1,613)	NAFLD (n = 636)	P Value for No NAFLD vs. NAFLD
Age (years)	58.5 (11.8)	58.1 (11.8)	59.3 (11.6)	0.028
Male (%)	48.6%	44.8%	58.2%	<0.0001
Hypertension (%)	31.8%	25.6%	47.3%	<0.0001
Diabetes (%)	14.9%	9.8%	27.5%	<0.0001
Metabolic syndrome (%)	26.1%	15.2%	50.9%	<0.0001
BMI (kg/m ²)	28.1 (5.0)	26.9 (4.4)	31.1 (5.1)	<0.0001
Systolic blood pressure (mm Hg)	120.2 (15.9)	118.5 (15.8)	124.4 (15.3)	<0.0001
Diastolic blood pressure (mm Hg)	74.3 (9.2)	73.3 (8.9)	76.9 (9.6)	<0.0001
Hemoglobin (g/dL)	13.9 (1.3)	13.8 (1.3)	14.0 (1.3)	0.001
Platelets (10 ⁹ /L)	239.4 (61.2)	239.0 (62.1)	240.3 (59.2)	0.67
Hemoglobin A1c (%)	5.5 (0.5)	5.5 (0.4)	5.8 (0.8)	<0.0001
Creatinine (mg/dL)	0.91 (0.23)	0.90 (0.23)	0.93 (0.23)	0.007
Fasting glucose (mg/dL)	99.8 (19.1)	97.0 (15.9)	106.8 (24.0)	<0.0001
Total cholesterol (mg/dL)	187.1 (36.0)	188.4 (35.0)	183.9 (38.0)	0.01
Triglycerides (mg/dL)	117.0 (75.3)	103.6 (54.7)	150.9 (104.2)	<0.0001
High-density lipoprotein (mg/dL)	60.0 (18.4)	62.7 (18.5)	53.0 (16.2)	<0.0001
Alanine aminotransferase (U/L)	24.2 (14.7)	21.9 (13.2)	29.9 (16.7)	<0.0001
Aspartate aminotransferase (U/L)	22.5 (10.3)	21.8 (10.4)	24.1 (9.8)	<0.0001
Total bilirubin (mg/dL)	0.49 (0.27)	0.49 (0.26)	0.50 (0.30)	0.26
Gamma-glutamyltransferase (U/L)	29.7 (33.7)	25.5 (23.5)	40.3 (49.6)	<0.0001
Albumin (g/dL)	4.5 (0.3)	4.5 (0.3)	4.5 (0.3)	0.63
LPR	0.34 (0.06)	0.37 (0.03)	0.27 (0.07)	<0.0001

Data are reported as mean (standard deviation) or proportion.

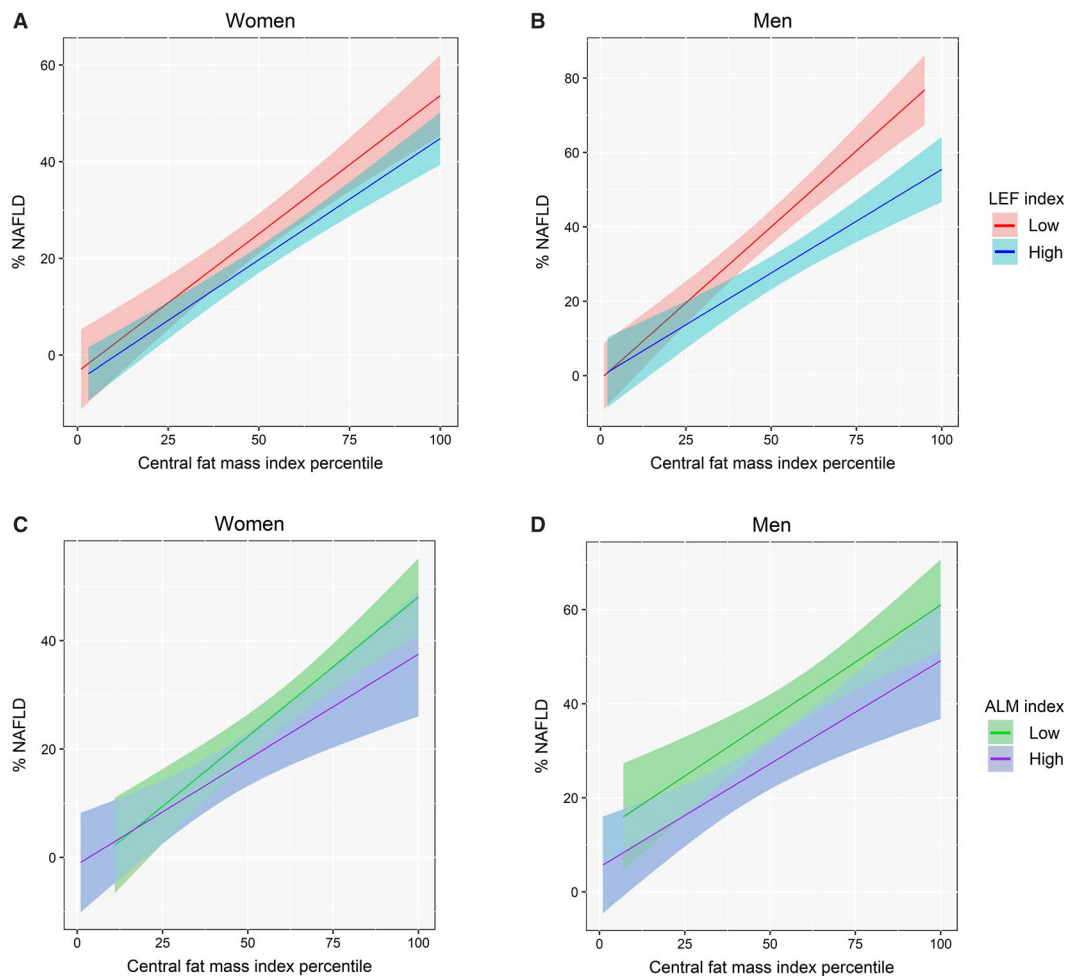


FIG. 2. Effect of central body fat, lower extremity body fat, and appendicular lean mass on NAFLD prevalence. Percentage of participants with NAFLD based on sex-specific percentile of central fat mass. (A,B) Stratified based on lower extremity fat mass above or at the median (“high”) versus below the median (“low”) for (A) women and (B) men. (C,D) Stratified based on appendicular lean mass above or at the median (“high”) versus below the median (“low”) for (C) women and (D) men. Shaded areas represent 95% CIs. Abbreviations: ALM, appendicular lean mass; LEF, lower extremity fat.

NAFLD as a function of percentile of central fat mass stratified by sex and either lower extremity fat mass or appendicular lean mass status is shown in Fig. 2. In all analyses, higher central body fat associated with greater NAFLD prevalence ($P < 0.0001$). High lower extremity body fat associated with lower NAFLD prevalence in both men and women ($P < 0.05$). High appendicular lean mass associated with lower NAFLD prevalence in men ($P < 0.05$) but not in women ($P = 0.16$).

We then performed multivariable linear regressions to determine whether body composition parameters independently associated with hepatic steatosis as a continuous variable in the FHS. We used age, physical

activity, alcoholic drinks per week, and cohort as minimal covariates in all models. On multivariable analysis, greater central fat mass associated with more liver steatosis in both men and women while greater lower extremity fat mass associated with less liver steatosis (Table 2; Supporting Table S4). In men but not in women, greater appendicular lean mass associated with less hepatic steatosis (Table 2; Supporting Table S4). In both sexes, greater paraspinal muscle fat associated with increased hepatic fat (Table 2; Supporting Table S4). These findings persisted in models adjusting for minimal covariates, central fat mass, lower extremity fat mass, appendicular lean mass, and hand grip strength, quadriceps strength, or muscle attenuation

TABLE 2. BODY COMPOSITION AND STRENGTH METRICS

Characteristic	Overall (n = 2,249)	No NAFLD (n = 1,613)	NAFLD (n = 636)	P Value for No NAFLD vs. NAFLD
Total body fat (kg)	27.5 (10.2)	25.4 (9.1)	33.0 (10.7)	<0.0001
Central body fat (kg)	15.8 (5.7)	14.4 (5.1)	19.6 (5.8)	<0.0001
Lower extremity fat (kg)	8.4 (3.6)	8.0 (3.4)	9.4 (4.1)	<0.0001
Total lean mass (kg)	48.2 (11.5)	46.7 (11.1)	52.0 (11.7)	<0.0001
Appendicular lean mass (kg)	21.4 (5.9)	20.8 (5.8)	23.2 (6.1)	<0.0001
Total body fat/weight (kg/kg × 100%)	34.4% (9.3)	33.5% (9.4)	36.9% (8.5)	<0.0001
Central body fat/weight (kg/kg × 100%)	19.7% (4.8)	18.9% (4.9)	21.8% (4.0)	<0.0001
Lower extremity fat/weight (kg/kg × 100%)	10.5% (3.8)	10.6% (3.8)	10.4% (3.7)	0.26
Total lean mass/weight (kg/kg × 100%)	61.1% (9.5)	62.1% (9.6)	58.6% (8.8)	<0.0001
Appendicular lean mass/weight (kg/kg × 100%)	27.0% (4.8)	27.4% (4.8)	26.1% (4.5)	<0.0001
Hand grip strength (kg)	35.6 (12.3)	35.1 (12.2)	36.7 (12.4)	0.007
Quadriceps muscle strength (kg)	25.5 (8.6)	25.5 (8.5)	25.5 (9.0)	0.92
Muscle attenuation (Hounsfield units)	49.2 (7.3)	50.0 (6.8)	47.4 (7.9)	<0.0001

Data are reported as mean (standard deviation) or proportion.

(Supporting Table S4). In no model did quadriceps strength or hand grip strength associate with hepatic steatosis (Supporting Table S4).

For reference among women, 1 SD of liver steatosis corresponds to an LPR of 0.06. Thus, the fact that in women each rank unit of central fat mass was associated with a change in liver steatosis of 0.02 implies significant explanatory power. Among men, the liver steatosis SD was 0.07, and the β values associated in men with each inverse-normalized unit of central fat mass (0.03), lower extremity fat (−0.01), and appendicular lean mass (−0.01) were relatively large. The combination of central fat, lower extremity fat, appendicular lean mass, and muscle attenuation accounted for 14.4% of variation in liver steatosis in women and 18.1% in men.

We also tested whether these fat depots associated with the APRI, a noninvasive marker of fibrosis, in the FHS. In men, greater central body fat associated with increased APRI; each rank unit increase was associated with a 0.031 increase in APRI (95% confidence interval [CI], 0.003–0.061). There were no other associations between body composition and APRI in men. In women, there was no association between body composition and APRI.

GENETIC LIPODYSTROPHY RISK SCORE

We further explored whether genetic predisposition to partial lipodystrophy influences liver-related phenotypes in the FHS and MGI, using the LPRS.

Mean LPRS was 52.8 (SD, 4.3) in the FHS and 55.1 (SD, 4.6) in the MGI. Participants with NAFLD had a higher LPRS than those without NAFLD (53.2 vs. 52.7; $P = 0.006$). We validated that, consistent with conferring a partial lipodystrophy phenotype, a higher LPRS associated with dyslipidemia and insulin resistance and decreased lower extremity fat ($P < 0.001$ for all) but did not affect central fat or overall BMI (Fig. 3A). These findings held when men and women were analyzed separately (Supporting Figs. S1 and S2).

Unadjusted NAFLD prevalence increased significantly with increasing LPRS in the overall cohort (Fig. 3B; $P < 0.005$). This association remained in women ($P < 0.005$) but not in men ($P = 0.16$; Supporting Figs. S1 and S2). On multivariable linear regression, higher LPRS was associated with increased liver steatosis in the overall FHS cohort (Fig. 3A) and among women (Supporting Fig. S1A). In men, LPRS did not associate with liver steatosis but the trend was in the same direction as in women (Supporting Fig. S2A). Adjusted odds ratio (OR) for NAFLD per allele of LPRS in the overall cohort was 1.04 (95% CI, 1.01–1.06); overall, individuals in the ninetieth percentile for LPRS were 36% more likely to have NAFLD than those in the tenth percentile (Table 3).

Finally, we examined the effect of LPRS on liver fibrosis. We first performed linear regression with APRI as the dependent and LPRS as the independent variable in the FHS, but the association was not significant. Therefore, we tested this hypothesis in the hospital-based MGI cohort (n = 19,239). In the

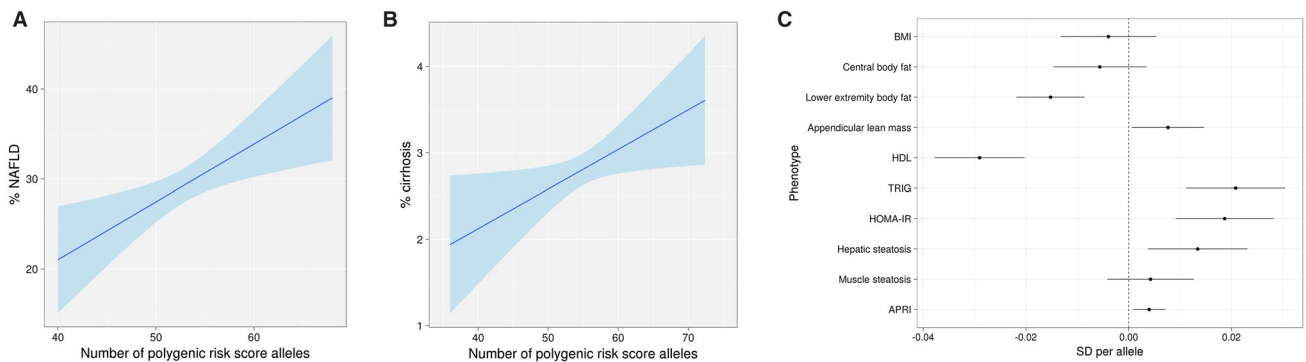


FIG. 3. Effect of LPRS on multiple traits. (A) Percentage of FHS participants with NAFLD based on number of LPRS alleles. (B) Percentage of MGI participants with cirrhosis based on number of LPRS alleles. Shaded area represents 95% CI. (C) Forest plot of associations between LPRS and multiple traits. Scale on the x axis is the allele effect size (β) of one LPRS allele divided by the SD of the specific parameter, i.e., what proportion of SD is accounted for by each additional allele of LPRS. Error bars depict 95% CI. Liver steatosis represents negative LPR. Muscle steatosis represents negative muscle attenuation in Hounsfield units. All traits except APRI were measured in the FHS; APRI was measured in the MGI. Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostatic model of insulin resistance; LDL, low-density lipoprotein; TRIG, triglycerides.

TABLE 3. LINEAR REGRESSION ON HEPATIC STEATOSIS*

Parameter	Beta coefficient	
	Women	Men
Central fat index	0.0193 [†]	0.0272 [†]
Lower extremity fat index	-0.0072 [†]	-0.012 [†]
Appendicular lean mass index	-0.0038 NS	-0.0106 [†]
Muscle steatosis	0.0013 [†]	0.0009 [†]

*Linear regression on hepatic steatosis, as defined as LPR (see Participants and Methods for details). Muscle steatosis was defined as negative muscle attenuation (Hounsfield units). All four traits were inverse normalized. Beta coefficients correspond to the effect of one rank unit (approximately one sixth of the total variation). Analysis was stratified by sex. Covariates were age, age², physical activity index, drinks per week, and cohort (i.e., Offspring versus Generation 3) and the four parameters in the above table.

[†] $P < 0.05$

Abbreviation: NS, not significant.

overall MGI cohort, each allele of LPRS was associated with an OR of 1.02 for cirrhosis diagnosis (95% CI, 1.00-1.03; $P = 0.03$; Fig. 3C). Individuals in the ninetieth percentile of LPRS were 22% more likely to have cirrhosis than those in the tenth percentile. Adjusted OR among men was 1.02 (95% CI, 1.00-1.04; $P = 0.03$; Supporting Fig. S2C), and among women there was no significant association although a consistent direction of effect (OR, 1.01; 95% CI, 0.99-1.03; Supporting Fig. S1C). After adjustment, a higher LPRS was associated with a greater APRI

(Fig. 3A), indicating increased fibrosis. While the association was not significant when men and women were analyzed separately, the directions of effect trended in the same direction ($P = 0.06$ and 0.10 in women and men, respectively; Supporting Figs. S1 and S2).

Discussion

In summary, we show that greater central fat mass associated with increased hepatic steatosis while greater lower extremity fat mass and appendicular lean mass associated with less hepatic steatosis. In addition, greater paraspinal muscle fat was associated with increased hepatic steatosis. Overall, these four body composition parameters accounted for a substantial proportion of variation in hepatic steatosis (14% in women and 18% in men). Finally, higher LPRS led to increased hepatic steatosis and fibrosis in the population, with a 36% and 22% increased risk, respectively, in individuals with high versus low LPRS.

Our findings suggest that NAFLD may be a marker of partial lipodystrophy in the population. Lipodystrophy is classically thought of as a rare monogenic disease, but partial lipodystrophy (or even differences in fat depot distribution) may exist as a continuous trait in the population.^(21,24) We found that LPRS associates with increased hepatic steatosis and fibrosis. Further, among participants with NAFLD, nonobese

participants had less appendicular fat than did obese subjects. Together, these findings imply that inadequate appendicular adipose tissue may contribute to NAFLD and fibrosis.

Interestingly, we found the LPRS associates with increased hepatic steatosis in women but not men. This may be because men have less lower extremity fat (7.7% vs. 13.2%) and consequently greater total lean mass (67% vs. 56%) and appendicular lean mass (31% vs. 24%) than women. We note that while there was no statistical association between appendicular lean mass and hepatic steatosis in women, the direction of effect was the same in both men and women, suggesting that muscle mass is protective against NAFLD in both sexes. Thus, it may be that muscle may be able to buffer excess calories more in men than in women, who have less mass and thus use fat to buffer excess calories. A genetic decrease in lower extremity fat may therefore confer a proportionally greater risk for NAFLD in women than in men, who already have a small amount of lower extremity fat depot.

We also found that muscle steatosis associates with NAFLD, likely because when excess energy cannot be buffered by adipose tissue, it may be stored in ectopic fat depots, such as muscle and liver. Unlike muscle steatosis, however, muscle strength as measured by quadriceps and hand grip strength did not correlate with NAFLD in this study. Thus, while it appears that muscle fat is associated with increased hepatic steatosis and muscle mass with decreased steatosis, muscle strength does not appear to associate with NAFLD.

Consistent with earlier findings, we found that greater central (visceral) adiposity associates with increased prevalence of NAFLD while greater lower extremity adiposity associates with decreased NAFLD prevalence.^(10,39) The mechanisms underlying these differences in disease risk based on fat location remain incompletely characterized but may relate to differences in macrophage and cytokine profiles in visceral fat and direct blood flow from visceral fat to the liver through the portal circulation.^(40,41) In both men and women, body composition metrics explained a substantial proportion of variation in hepatic steatosis (18% and 14%, respectively). Additional studies will be required to better understand the biology underlying these relationships.

Our study is limited by including only participants of European ancestry. The association between hepatic steatosis and LPRS may only reflect an association with

one particular form of lipodystrophy and may not be generalizable to all lipodystrophy. Finally, DXA cannot distinguish between subcutaneous lower extremity fat and deeper lower extremity fat layers or between muscle and other lean tissues, such as skin and connective tissue, although there is no clear pathophysiologic reason nonmuscle lean tissue would be related to NAFLD.

Strengths of the study include that it is a large population-based study, which increases the generalizability of our findings. In addition, CT and DXA are excellent quantitative noninvasive measurements of hepatic steatosis and body composition, respectively, allowing rigorous testing of how body composition relates to NAFLD. Full genotypic information was available for genetic analysis. We also were able to assess for effects of the LPRS on fibrosis using two independent methods.

In conclusion, we demonstrated a novel association between partial lipodystrophy and liver steatosis and fibrosis in the population and report a connection between CT-measured hepatic steatosis and muscle steatosis. To our knowledge, this is the first such report in a Caucasian population. These results suggest that interventions directed at increasing muscle quantity, decreasing overall fat burden, or shifting fat distribution toward appendicular fat may be beneficial in reducing NAFLD and preventing its complications.

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REFERENCES

- 1) Rinella ME, Sanyal AJ. NAFLD in 2014: Genetics, diagnostics and therapeutic advances in NAFLD. *Nat Rev Gastroenterol Hepatol* 2015;12:65-66.
- 2) Eguchi Y, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, et al.; JSG-NAFLD. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2009;2012(47):586-595.
- 3) Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013;178:38-45.
- 4) Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged

- population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011;140:124-131.
- 5) Zhou Y, Oresic M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, et al. Noninvasive detection of nonalcoholic steatohepatitis using clinical markers and circulating levels of lipids and metabolites. *Clin Gastroenterol Hepatol* 2016;14:1463-1472.e1466.
 - 6) Speliotes EK, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, et al. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology* 2010;51:1979-1987.
 - 7) van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008;48:449-457.
 - 8) Petta S, Amato MC, Di Marco V, Camma C, Pizzolanti G, Barcellona MR, et al. Visceral adiposity index is associated with significant fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2012;35:238-247.
 - 9) Jun DW, Han JH, Kim SH, Jang EC, Kim NI, Lee JS, et al. Association between low thigh fat and non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2008;23:888-893.
 - 10) Perlemuter G, Naveau S, Belle-Croix F, Buffet C, Agostini H, Laromiguiere M, et al. Independent and opposite associations of trunk fat and leg fat with liver enzyme levels. *Liver Int* 2008;28:1381-1388.
 - 11) Ruhl CE, Everhart JE. Trunk fat is associated with increased serum levels of alanine aminotransferase in the United States. *Gastroenterology* 2010;138:1346-1356, 1356.e1341-e1343.
 - 12) Hong HC, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study. *Hepatology* 2014;59:1772-1778.
 - 13) Petta S, Ciminnisi S, Di Marco V, Cabibi D, Camma C, Licata A, et al. Sarcopenia is associated with severe liver fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017;45:510-518.
 - 14) Koo BK, Kim D, Joo SK, Kim JH, Chang MS, Kim BG, et al. Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis. *J Hepatol* 2017;66:123-131.
 - 15) Meng G, Wu H, Fang L, Li C, Yu F, Zhang Q, et al. Relationship between grip strength and newly diagnosed non-alcoholic fatty liver disease in a large-scale adult population. *Sci Rep* 2016;6:33255.
 - 16) Kitajima Y, Hyogo H, Sumida Y, Eguchi Y, Ono N, Kuwashiro T, et al; Japan Nonalcoholic Fatty Liver Disease Study Group (JSG-NAFLD). Severity of non-alcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle. *J Gastroenterol Hepatol* 2013;28:1507-1514.
 - 17) Kato K, Takeshita Y, Misu H, Zen Y, Kaneko S, Takamura T. Liver steatosis is associated with insulin resistance in skeletal muscle rather than in the liver in Japanese patients with non-alcoholic fatty liver disease. *J Diabetes Investig* 2015;6:158-163.
 - 18) Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al.; NASH CRN; GIANT Consortium; MAGIC Investigators; GOLD Consortium. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7:e1001324.
 - 19) Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. GIANT Consortium; MIGen Consortium; NASH CRN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010;52:904-912.
 - 20) Park JH, Cho B, Kwon H, Prilutsky D, Yun JM, Choi HC, et al. I148M variant in PNPLA3 reduces central adiposity and metabolic disease risks while increasing nonalcoholic fatty liver disease. *Liver Int* 2015;35:2537-2546.
 - 21) Brown RJ, Araujo-Vilar D, Cheung PT, Dunger D, Garg A, Jack M, et al. The diagnosis and management of lipodystrophy syndromes: a multi-society practice guideline. *J Clin Endocrinol Metab* 2016;101:4500-4511.
 - 22) Ajluni N, Meral R, Neidert AH, Brady GF, Buras E, McKenna B, et al. Spectrum of disease associated with partial lipodystrophy: lessons from a trial cohort. *Clin Endocrinol (Oxf)* 2017;86:698-707.
 - 23) Haghghi A, Kavehmanesh Z, Haghghi A, Salehzadeh F, Santos-Simarro F, Van Maldergem L, et al. Congenital generalized lipodystrophy: identification of novel variants and expansion of clinical spectrum. *Clin Genet* 2016;89:434-441.
 - 24) Lotta LA, Gulati P, Day FR, Payne F, Ongen H, van de Bunt M, et al.; EPIC-InterAct Consortium; Cambridge FPLD1 Consortium. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat Genet* 2017;49:17-26.
 - 25) Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, et al. The third generation cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am J Epidemiol* 2007;165:1328-1335.
 - 26) Tan ZS, Spartano NL, Beiser AS, DeCarli C, Auerbach SH, Vasan RS, et al. Physical activity, brain volume, and dementia risk: The Framingham Study. *J Gerontol A Biol Sci Med Sci* 2017;72:789-795.
 - 27) McLean RR, Mangano KM, Hannan MT, Kiel DP, Sahni S. Dietary protein intake is protective against loss of grip strength among older adults in the Framingham offspring cohort. *J Gerontol A Biol Sci Med Sci* 2016;71:356-361.
 - 28) Mangano KM, Sahni S, Kiel DP, Tucker KL, Dufour AB, Hannan MT. Dietary protein is associated with musculoskeletal health independently of dietary pattern: The Framingham Third Generation Study. *Am J Clin Nutr* 2017;105:714-722.
 - 29) Rivadeneira F, Styrkársdóttir U, Estrada K, Halldórsson BV, Hsu YH, Richards JB, et al.; Genetic Factors for Osteoporosis (GEFOS) Consortium. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat Genet* 2009;41:1199-1206.
 - 30) Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. *Nat Genet* 2016;48:1284-1287.
 - 31) Dey R, Schmidt EM, Abecasis GR, Lee S. A fast and accurate algorithm to test for binary phenotypes and its application to PheWAS. *Am J Hum Genet* 2017;101:37-49.
 - 32) Roseman DA, Hwang SJ, Manders ES, O'Donnell CJ, Upadhyay A, Hoffmann U, et al. Renal artery calcium, cardiovascular risk factors, and indexes of renal function. *Am J Cardiol* 2014;113:156-161.
 - 33) Mellinger JL, Pencina KM, Massaro JM, Hoffmann U, Seshadri S, Fox CS, et al. Hepatic steatosis and cardiovascular disease outcomes: an analysis of the Framingham Heart Study. *J Hepatol* 2015;63:470-476.
 - 34) Therikelsen KE, Pedley A, Speliotes EK, Massaro JM, Murabito J, Hoffmann U, et al. Intramuscular fat and associations with metabolic risk factors in the Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2013;33:863-870.
 - 35) Visser M, Harris TB, Langlois J, Hannan MT, Roubenoff R, Felson DT, et al. Body fat and skeletal muscle mass in relation to physical disability in very old men and women of the Framingham Heart Study. *J Gerontol A Biol Sci Med Sci* 1998;53:M214-M221.
 - 36) Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart

- Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
- 37) Fuchsberger C, Flannick J, Teslovich TM, Mahajan A, Agarwala V, Gaulton KJ, et al. The genetic architecture of type 2 diabetes. *Nature* 2016;536:41-47.
- 38) Speliotes EK, Massaro JM, Hoffmann U, Foster MC, Sahani DV, Hirschhorn JN, et al. Liver fat is reproducibly measured using computed tomography in the Framingham Heart Study. *J Gastroenterol Hepatol* 2008;23:894-899.
- 39) Chung GE, Kim D, Kwark MS, Kim W, Yim JY, Kim YJ, et al. Visceral adipose tissue area as an independent risk factor for elevated liver enzyme in nonalcoholic fatty liver disease. *Medicine (Baltimore)* 2015;94:e573.

- 40) Item F, Konrad D. Visceral fat and metabolic inflammation: the portal theory revisited. *Obes Rev* 2012;13(Suppl 2):30-39.
- 41) du Plessis J, van Pelt J, Korf H, Mathieu C, van der Schueren B, Lannoo M, et al. Association of adipose tissue inflammation with histologic severity of nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:635-648.e614.

Supporting Information

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